
**UNIVERSIDADE FEDERAL DO PAMPA**

**MARIELE DA SILVA HERNANDEZ**

# EFEITO DO TREINAMENTO FÍSICO AERÓBICO E DA SUPLEMENTAÇÃO COM CAFEÍNA SOBRE PARÂMETROS BIOQUÍMICOS E COGNITIVOS EM RATOS COM SÍNDROME METABÓLICA.

**URUGUAIANA**

**2020**

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Dissertação de mestrado apresentada ao programa de Pós-graduação *Stricto sensu* em Bioquímica da Universidade Federal do Pampa, como requisito para obtenção do Grau de Mestre em Bioquímica.

Orientador: Prof. Dr. Leonardo Magno Rambo

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## Dedicatória

À memória do meu pai, Claudio Hernandez, que dedicou toda sua vida para os filhos e para a realização dos nossos sonhos.
Independentemente de onde tu esteja quero que saiba que você é responsável por esta conquista. Te amo

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Pai, saudade eterna!

“O mundo está nas mãos daqueles que tem coragem de sonhar, e correr o risco de viver seus sonhos.”

Paulo Coelho

**RESUMO**

A Síndrome Metabólica é um distúrbio, que ao longo dos anos, vem crescendo na população mundial. Pouco se sabe sobre sua fisiopatologia, no entanto acredita-se que a mudança de hábitos e a mudança na alimentação diária tenham um impacto sobre o aumento desta doença. Sendo assim, a busca por tratamentos não medicamentosos e de baixo custo tem sido uma alternativa para o seu tratamento. Neste sentido, o objetivo deste estudo é investigar o efeito do treinamento aeróbico de natação e da suplementação com cafeína sobre parâmetros neuroquímicos e cognitivos de ratos previamente tratados com frutose. Para avaliar o efeito do treinamento e da suplementação com cafeína foram necessários 8 grupos para contemplar todas as variáveis, o tempo do experimento foi de 10 semanas, o treinamento físico durou 6 semanas, onde a cafeína era administrada após os dias de treinamento. Nossos resultados mostram que os animais tratados com frutose ingerem menos comida, bebem mais água, mas ingeriram mais caloria durante todo o experimento, no que se refere às análises antropométricas foram observados que o consumo de frutose aumentou a gordura visceral, no entanto não apresentou aumento no peso corporal e do músculo sóleo, já os animais treinados tiveram o aumento no peso do músculo e diminuição do peso corporal e gordura visceral e a suplementação de cafeína foi capaz de diminuir o ganho da massa de gordura visceral. Na depuração de glicose foi possível observar que o treinamento e a suplementação de cafeína puderam reverter o efeito da frutose diminuindo a depuração de glicose ao longo do tempo, no perfil lipídico da mesma forma, o fator de treinamento físico foi capaz de reverter o efeito da frutose diminuindo o colesterol total, HDL e triglicerídeos, mas não nos níveis de LDL. A análise estatística mostrou que a administração de frutose por 10 semanas prejudicou a memória de curto prazo e memória espacial, mas não a memória de longo prazo, no entanto o treinamento físico melhorou a memória de curto prazo, longo prazo e espacial e pode reverter o efeito da frutose na memória de curto prazo. A administração de frutose induziu os ratos a passar mais tempo em braços fechados na tarefa de labirinto em cruz elevada, o que nos leva a crer que estes animais estavam mais ansiosos. O treinamento físico diminuiu o tempo gasto nos braços fechados e reverteu o efeito da frutose no tempo gasto nos braços fechados e por fim o treinamento físico aumentou os níveis de NRF2 em comparação com animais sedentários. Dessa forma, foi possível concluir que o alto consumo de frutose desenvolve desordens metabólicas como: o aumento de gordura visceral, aumento nos níveis lipídicos, diminuição na depuração da glicose e que além disso, gera danos cognitivos prejudicando a memória e causando ansiedade. Por outro lado o treinamento físico e a cafeína puderam reverter boa parte dos danos causados pela frutose, sendo assim, pode-se considerar o treinamento físico e a ingestão de cafeína como uma terapia alternativa e não medicamentosa para auxiliar na diminuição dos fatores de risco associados à síndrome metabólica.

Palavras-chave: frutose; cafeína; exercício físico; síndrome metabólica.

## ABSTRACT

Metabolic syndrome is a disorder that has been growing in the world population. Changes in life style and the in dietary habits is believed to have an impact on the increased prevalence of this disease. Therefore, the search for non-medication and low-cost therapies for the treatment of this disorder is of great interest. In this sense, the objective of this study is to investigate the effect of aerobic exercise on the neurochemical and cognitive parameters of rats submitted to a model of fructose-induced metabolic syndrome . To assess the effect of exercise training and caffeine supplementation, animals were assigned to 8 different groups . Animals received either fructose or vehicle for 10 weeks. The swimming training started in the fourth week after the start of fructose administration. During the period of exercise animals were also administered with caffeine. Our results show that fructose consumption increased visceral fat, however there did not increase the body weight and neither chaged the weight of the soleus muscle. Animals that were exposed to physical exercise showed an increase in muscle weight and a decrease in total body weight as well as visceral fat. Caffeine supplementation was able to prevent against the gain of visceral fat induced by the fructose treatment. Furthermore, both the caffeine treatment and the physical exercise were also able to decrease the glucose clearance over time. Physical exercise also lowered the total cholesterol, HDL and triglycerides, but not LDL levels induced by the fructose treatment. Statistical analysis showed that the administration of fructose for 10 weeks impaired short-term memory and localization, but not long-term memory, however physical training improved short-term, long-term and localization memory and reverted the effect fructose in short-term memory. Physical training increased the levels of NRF2 compared to sedentary animals. Thus, it was possible to conclude that the high consumption of fructose caused animals to develop metabolic disorders such as: the increase of visceral fat, increase in lipid levels, increase in the clearance of glucose and that, in addition, generates cognitive damages impairing memory and causing anxiety, on the other hand, physical training and caffeine reverted a some of the damages caused by fructose, therefore, one can consider physical training and caffeine intake as an alternative and non-medication therapy to help reduce the risk factors associated with metabolic syndrome.

Key-words: fructose; caffeine; physical exercise; metabolic syndrome.

## LISTA DE ABREVIATURAS E SIGLAS

Sistema nervoso central - SNC

Lipoproteína de alta densidade - HDL

Síndrome metabólica - SM

Espécies reativas de oxigênio - EROs

Adenilato ciclase - AMPc

**SUMÁRIO**

## 1 INTRODUÇÃO

O estilo de vida atual, trazido principalmente pela evolução da tecnologia, causa preocupação com o aumento do estilo sedentário, que por sua vez, vem acompanhado pelo aumento de ingesta calórica através dos alimentos processados, que nestes estão embutidos uma grande quantidade de frutose (Choi *et al*., 2005; Dale *et al*., 2014). Foi demonstrado que a inatividade física associada ao alto consumo de frutose está relacionada a várias disfunções metabólicas, como obesidade (Bluher, 2019), intolerância à glicose, aumento da pressão arterial, hipertrigliceridemia, baixos níveis de lipoproteína de alta densidade (HDL) e aterosclerose (Bidwell, 2017). Quando existe o acúmulo de pelo menos três dos distúrbios metabólicos mencionados, é considerado uma síndrome metabólica (SM) (Swarup *et al*., 2019). Atualmente, a SM é considerada um problema de saúde pública, de difícil manejo clínico, apresentando, entre outros, quadro de inflamação sistêmica de baixo grau e aumento de estresse oxidativo (Devries et al., 2008; Farinha et al., 2015; Rogowski et al., 2008). Mais do que uma doença sistêmica, a SM apresenta várias consequências em nível de sistema nervoso central (SNC). Nesse sentido, foi demonstrado que pessoas com síndrome metabólica apresentam redução do fluxo sanguíneo cerebral, causando o declínio cognitivo, déficits de memória e demência (Livingston et al., 2020; Yates et al., 2012; Birdsill et al., 2013). O menor fluxo de sangue altera o metabolismo energético no cérebro, dessa forma causa aumento nas espécies reativas de oxigênio (EROs) e neuroinflamação (Zafar et al., 2018) Considerando a patogênese multifatorial desta síndrome (Scaglione et al., 2010), é difícil tratá-la como um todo. Basicamente, são realizadas diferentes estratégias de prevenção primária levando em consideração os principais fatores de risco conhecidos, que incluem perfil lipídico e aumento de gordura abdominal/visceral (Kohl et al., 2007; Oliveiros et al., 2014). Portanto, estratégias terapêuticas de natureza multifatorial podem ser benéficas no tratamento da SM. Em consonância com essa visão, o exercício físico possui um caráter multifatorial devido ao potencial de atuar em diferentes

vias em praticamente todos os tecidos corporais (Warbunton et al., 2006) O treinamento físico contribui de forma aguda e crônica na promoção da saúde auxiliando no tratamento e prevenção de diversas doenças, como distúrbios do SNC (Fagard & Cornelissen, 2007), doenças cardiovasculares crônicas (Slentz et al., 2005), distúrbios metabólicos como obesidade (Kraus et al., 2002), diabetes (Singal et al., 2007) e hipertrigliceridemia (Di Marzo & Silvestri, 2019) De fato, existe uma vasta literatura que demonstra o papel benéfico do exercício físico no tratamento dos fatores de risco associados à SM (Sökmen et al., 2008). No entanto, pouco se sabe sobre o efeito do exercício físico nas consequências neuroquímicas e cognitivas da SM no cérebro. A cafeína (1,3,7-trimetilxantina) pertence à família das xantinas e é amplamente consumida em todo o mundo, por meio de bebidas como café e chá (Schmidt, 2012). Tanto o consumo agudo quanto crônico de cafeína promove uma série de efeitos, desde que ingeridos em doses adequadas (Graham et al., 2008). Possui rápida absorção no sistema gastrointestinal e devido à sua característica química, tem rápida ação no SNC, antagonizando os receptores adenosinérgicos (A1, A2 e A3) (Fredhlom et al., 1999). Os receptores de adenosina são distribuídos em vários tecidos corporais além do SNC, como músculo cardíaco, vasos sanguíneos, rins, pulmões, trato gastrointestinal e tecido adiposo (Graham *et al*., 2001; (Guarino et al., 2013). Devido à ampla distribuição de seus receptores, diversos estudos demonstraram efeitos terapêuticos da cafeína em uma série de fatores de risco associados à SM, como controle da hipertensão (Yu *et al*., 2016), diabetes (Quan et al., 2013), obesidade (Panchal et al., 2012), redução da glicemia em ratos submetidos a dieta rica em sacarose (Barcelos et al., 2020) e redução da massa corporal total e massa adiposa em ratos submetidos à dieta hiperlipídica (Conde et al., 2012). Sabe-se também que a cafeína, quando associada ao exercício aeróbio, tem efeito ergogênico ao aumentar os níveis de epinefrina, reduzir o uso de glicogênio muscular, estimular a quebra de triglicerídeos, acelerando assim o uso de ácidos graxos como fonte de energia durante o esforço (Costill et al., 1978; Ivy et al., 1979). Considerando que tanto o exercício físico quanto a cafeína são terapias de baixo custo e facilmente acessíveis, além da possibilidade de ter um potencial efeito sinérgico no tratamento dos fatores de risco relacionados à

SM o presente estudo teve como objetivo investigar o efeito do treinamento aeróbio de natação combinado com a suplementação de cafeína nas alterações neuroquímicas e cognitivas, bem como nos fatores de risco associados à SM em ratos

# 2 REVISÃO DE LITERATURA

## 2.1 Síndrome Metabólica

A primeira descrição da Síndrome Metabólica (SM) foi feita por Reaven (Alberti et al., 2009; Hales et al., 1991), denominada na época por síndrome X, por apresentar relação no desenvolvimento de doenças metabólicas para a prevalência de doenças cardiovasculares. Atualmente a síndrome é caracterizada por apresentar associação de uma ou mais disfunções metabólicas inter-relacionadas, tais como, obesidade, resistência à insulina, intolerância à glicose, entre outras. (Wilson et al., 2005; Wiernsperger et al., 2001). No entanto, o primeiro acordo unificado sobre a definição da SM foi feito somente em 2005, durante um encontro organizado pela Federação Internacional de Diabetes (Eckel et al., 2005; McCracken et al., 2018; C. K. Roberts et al., 2013).

Quando procura-se entender sobre a fisiopatologia desta síndrome são encontrados vários mecanismos hipotéticos, mas o mais amplamente aceito é a resistência à insulina com o aumento de fluxo de ácidos graxos. Porém, outros mecanismos potenciais discutem sobre a inflamação crônica de baixo grau e estresse oxidativo. (Saltiel & Kahn, 2001).

### 2.1.1 Resistência à insulina

O hormônio polipeptídio conhecido como insulina é secretado pelas células beta da ilhota pancreática de Langerhans e atua através de receptores de glicoproteína encontrada nos tecidos-alvo do fígado, músculo esquelético e adipócitos. A insulina se liga à subunidade alfa do receptor tirosina quinase, transmitindo um sinal através da membrana plasmática e ativa o domínio da subunidade beta, que resulta em uma reação de autofosforilação intercelular dos resíduos de tirosina. Sendo assim, quando ativada a PI3-quinase é desencadeada uma sequência de outras reações de fosforilação para a indução da migração da proteína transportadora de glicose GLUT4 para a membrana

celular promovendo então a entrada da glicose na célula. (Lizcano & Alessi, 2002). A glicose é então fosforilada para ser armazenada como glicogênio ou metabolizada para produzir trifosfato de adenosina (ATP) (McCracken et al., 2018). Através desta cascata metabólica a insulina inibe a gliconeogênese e a glicogenólise, além de promover o armazenamento de glicose, estimular a transcrição genética de enzimas envolvidas nas vias sintéticas glicolíticas e de ácidos graxos. É desta forma que ocorre o metabolismo da glicose em mamíferos, variando de acordo com a necessidade energética, com a sua captação e principalmente com a ingestão de glicose pelo indivíduo.

Como mencionado anteriormente, a resistência à insulina é um dos principais mecanismos para o desenvolvimento da SM. Neste processo as células beta secretam quantidades maiores de insulina como mecanismo compensatório para manter os níveis glicêmicos no sangue. (Eckel et al., 2005; Savage et al., 2007). Eventualmente ocorrerá uma descompensação, sendo o precursor para o desenvolvimento da diabetes tipo II. Uma das principais manifestações da resistência à insulina será a redução na síntese de glicogênio e no transporte de glicose.

Segundo Savage et al., a redução de fosforilação da tirosina, inibindo a ativação subsequente do PI3-quinase, pode ser o precursor do acúmulo de lipídios no músculo esquelético. Além disso, níveis elevados de acetil-CoA podem reduzir a ativação da Akt/PKB e neste caso, o acúmulo de gordura pode ser devido a aumento de entrega de ácidos graxos aos tecidos, onde a ingestão de energia ultrapassa a capacidade de armazenamento (Randle et al., 1988; Unger, 1995).

Os ácidos graxos livres são derivados principalmente de reservas de triglicerídeos no tecido adiposo, liberadas por ação do AMP cíclico durante a lipólise, esse processo é inibido pela insulina através de um mecanismo proposto para reduzir a atividade do AMPc. No cenário de resistência à insulina, onde os efeitos da insulina são reduzidos, a taxa de lipólise aumentará, resultando em aumento da produção de ácidos graxos. Sendo assim, o acúmulo de lipídios no músculo esquelético e no fígado pode resultar no aumento da entrega / síntese de ácidos graxos nesses tecidos (Galassetti, 2012).

O desenvolvimento da SM ainda não é totalmente compreendido, mas sabe-se que a obesidade central e a resistência à insulina são fatores primários para desencadear esta síndrome.

### 2.1.2 Modelo experimental de Síndrome metabólica

A SM pode ser induzida por frutose experimentalmente em ratos pela alimentação com alta concentração de frutose na dieta (60%) (Hwang et al., 1987; Nakagawa et al., 2006) ou adicionando a frutose na água de beber (10 – 20%) (Dai; Mcneill, 1995). Diferentes vias de administração podem induzir um consumo variável de frutose e, por sua vez, resultar em manifestações variáveis de componentes da SM, no entanto, ambas as administrações são capazes de induzir hipertensão sistêmica, hiperuricemia e hipertrigliceridemia, sendo modelos muito úteis na pesquisa dos mecanismos da SM e potenciais farmacológicos (Sánchez-lozada et al., 2007).

A utilização da frutose em humanos e animais ocorre principalmente no fígado, rins e intestino delgado (Berghe, 1986). Ao contrário da glicose, a frutose pode entrar nas células musculares e adipócitos na ausência de insulina, usando GLUT facilitador. No entanto, a glicose pode entrar no músculo e no tecido adiposo na ausência de insulina, embora em quantidades muito pequenas. A frutose é transportada através da membrana basolateral pelo GLUT2 (Douard et al, 2008). A predominância do fígado, rim e intestino delgado no metabolismo da frutose é baseada na presença de três enzimas - frutocinase, aldolase tipo B e trioquinase, que convertem a frutose em intermediários da via glicolítico-gluconeogênica (Boesiger et al, 1994) . Tanto a frutose quanto a glicose podem ser degradadas em triose-fosfato e lactato e produzir intermediários glicolíticos. Suas duas etapas metabólicas iniciais são diferentes: a frutose em concentração fisiológica não é prontamente fosforilada pela hexoquinase (a enzima que catalisa a síntese de glicose-6-fosfato a partir da glicose em todas as células do organismo) é primeiro fosforilado em frutose-1-fosfato por uma enzima específica, frutoquinase, e então convertido em triose-fosfato por uma segunda enzima, aldolase B (Rippe, 2010). Essas variações metabólicas são pequenas,

no entanto, têm consequências metabólicas profundas que ao longo do tempo podem desencadear disfunções metabólicas levando á alguma patologia.

### 2.1.3 Processos inflamatórios e estresse oxidativo

Em geral, a SM já é caracterizada por um status pró-oxidante/pró-inflamatório, sendo o tecido adiposo o centro do desenvolvimento da fisiopatologia, podendo predispor pacientes a maiores eventos cardiovasculares (Furukawa et al., 2004).

O estresse oxidativo apresenta correlação positiva como o acúmulo de gordura, estudos em humanos mostram que o aumento da expressão de NADPH oxidase é concomitante com a diminuição de enzimas antioxidante (Srikanthan et al., 2016). Além disso, camundongos obesos tratados com inibidor da NADPH oxidase apresentaram produção reduzida de espécies reativas de oxigênio (EROS) com melhora no fenótipo de diabetes (Cinti et al., 2005; Halberg et al., 2008).

Os adipócitos sofrem hipertrofia e hiperplasia em resposta ao excesso nutricional que pode levar as células a exceder seu suprimento sanguíneo com indução de um estado hipóxico (Lau et al., 2005). A hipóxia pode levar à necrose celular com infiltração de macrófagos e à produção de adipocitocinas, que incluem os mediadores pró-inflamatórios interleucina-6 (IL-6) e o fator de necrose tumoral alfa (TNF-α) (Bao et al., 2015; Bernberg et al., 2012).

Os níveis elevados de IL-6 foram medidos no tecido adiposo de pacientes com diabetes mellitus e obesidade, e também notavelmente em pacientes com características de SM. Estudos epidemiológicos demonstraram aumento das concentrações de IL-6 em associação com hipertensão, aterosclerose e eventos cardiovasculares (Azzawi, 1999). O TNF-α, denominada citocina pró-inflamatória, após sua atividade antitumoral, é um mediador significativo de inúmeras patologias cardiovasculares, incluindo aterosclerose e insuficiência cardíaca. (Duarte, 2015; Frisardi et al., 2010).

### 2.1.4 Síndrome metabólica e alterações comportamentais

A síndrome metabólica e a diabetes parecem demonstrar alterações cerebrais, aumentando o risco de comprometimento cognitivo, demência vascular e doença de Alzheimer (Convit et al., 2003; Duarte et al., 2009; Girault et al., 2019; S. M. Gold et al., 2007). Estudos mostram que estas condições de déficits cognitivos estão relacionadas com atrofia, degeneração sináptica e alteração do metabolismo do hipocampo, causada pela diabetes (Dai & McNeill, 1995).

Desta forma, a SM experimental em animais pode ser induzida pelo consumo de altas concentrações de frutose na dieta ou na água de beber (Agrawal & Gomez-Pinilla, 2012). Além disso, o alto consumo de frutose está relacionado com a sinalização da insulina no cérebro, indicando que a frutose também afeta a função neuronal. (Cournot et al., 2006). De forma que o alto consumo de frutose pode predispor danos cognitivos de longo prazo no cérebro e desordens neurológicas (Agrawal et al., 2016).

Neste sentido, a obesidade também tem sido relacionada a problemas cognitivos (Ward et al., 2005) e atrofia cerebral (Stefan M. Gold et al., 2005). Da mesma maneira, a hipertensão tem sido associada à atrofia cerebral e à disfunção cognitiva (Heyward et al., 2012).

Sabendo que essa síndrome é multifatorial, a obesidade, que é uma consequência ou um fator que leva a desenvolver esse distúrbio, pode levar prejuízo na memória e aprendizado. Estudos em animais, demonstraram que os ratos que tiveram uma dieta rica em gordura e sacarose tiveram prejuízo na memória e aprendizado em testes de reconhecimento de objetos (Vogel-Ciernia & Wood, 2014; Volk et al., 2013). O principal fator para esses déficits são as anormalidades na morfologia do hipocampo, déficits na plasticidade sináptica e neurogênese (Vogel-Ciernia & Wood, 2014; Volk et al., 2013)

A obesidade também está relacionada ao estresse, indivíduos obesos podem sofrer aumento de estresse e por sua vez, promover depressão. A influência do estresse na obesidade é exercida por mecanismo psicológico e fisiológico (Wardle et al., 2011). Um mecanismo fisiológico, pelo qual o estresse pode impactar tanto a depressão quanto a obesidade é através de sua ação no

eixo hipotálamo-hipófise-adrenal (HHA), com ativação em todos os níveis do eixo (Chrousos & Gold, 1992). Níveis elevados de cortisol, indicando a ativação do HHA, não são incomuns entre pessoas obesas e isso leva a origem da chamada obesidade abdominal - gordura principalmente na parede abdominal. A ativação do eixo HHA na depressão é responsável pelas pequenas associações, estatisticamente significativas, entre depressão e gordura corporal/abdominal (Bjorntorp e Rosmond, 2000). Segundo, Rosmond e Bjorntorp (1998), identificaram um grupo de indivíduos a quem denominavam "ansioso-depressivo", que pontuaram alto nas medidas de distúrbio psicológico. Esses autores descobriram que a resposta negativa ao Teste de Supressão da Dexametasona, a atividade do eixo HHA elevado, foi significativamente associada ao IMC e razão cintura / quadril.

Outro transtorno relacionado a SM é a ansiedade que demonstra semelhança quanto ao comportamento de depressão, estes transtornos estão associados aos marcadores de peroxidação lipídica, que por sua vez tem relação com os distúrbios relacionados com dietas hipercalóricas. Implicado como um fator inter-relacionado com os sinais endócrino e inflamatório que se pensa estar envolvido na mediação dessa conexão (Molteni et al., 2002).

Desta forma, considerando a SM como uma doença multifatorial, que apresenta diversos mecanismos da sua patogênese e fisiopatologia é difícil encontrar um tratamento específico para a síndrome. Sendo assim, busca-se alternativas terapêuticas que tenham potencial de reverter os distúrbios metabólicos, marcadores oxidativos e inflamatórios.

## 2.2 Cafeína

A cafeína é uma substância alcalóide de fórmula química 1,3,7 – trimetilxantina, que pertence à família das xantinas. É o psicoestimulante mais consumido mundialmente, sendo encontrado principalmente em bebidas como café, chás e refrigerantes, assim como em alguns alimentos como o cacau (Gibert et al., 1976; Bernstein et al., 2002).

Historicamente a cafeína só foi descoberta no ano de 1819, por Friedrich Runge, pois percebeu-se que alguns efeitos do café poderiam ser devido à algum princípio ativo da planta e o mesmo já era muito consumido mundialmente o que levou Runge a investigar e identificar a substância que conhecemos hoje como cafeína (Fredholm, 2011).

No entanto, a fórmula química da cafeína foi esclarecida pelo Hermann Emil Fischer em 1821, ele descobriu que a cafeína possuía um esqueleto heterocíclico similar ao ácido úrico (Fredholm, 2011).

Como já foi observado, na história mais antiga do uso de metilxantina, já eram observados efeitos medicinais importantes (Ukers, 1922; Mair e Hoh, 2009). Por exemplo, na Índia, onde o chá é nativo, o chá era usado há muito tempo para fins medicinais. Assim como havia alegações exageradas sobre os efeitos benéficos das bebidas feitas com café, chá ou cacau, havia alegações sobre as consequências negativas para a saúde. (Fredholm, 2011).

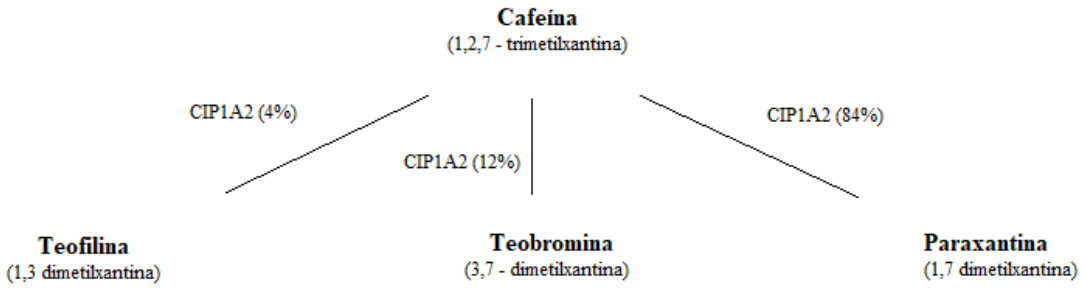
Somente em meados do século XIX os relatos de efeitos sobre a saúde obtêm uma base suficientemente científica para serem levados a sério. Como foi observado o relato da utilidade do café na asma (Salter, 1860), também houve os primeiros relatos sobre os efeitos negativos de altas doses de cafeína (Cole,1833).

### 2.2.1 Metabolismo

A cafeína é uma substância lipossolúvel, essas propriedades hidrofóbicas permitem sua passagem por todas as membranas biológicas sendo rapidamente absorvida pelo trato gastrointestinal, atingindo o seu pico no plasma entre 30 e 90 min após a ingestão oral (Fredholm et al., 1999; Tarnopolsky, 2010).

Desta forma a cafeína distribui-se por todos os tecidos podendo ser encontrada nos fluídos corporais, tais como: plasma sanguíneo, saliva, sêmen, leite materno, entre outros (Arnaud, 1976) ela atravessa rapidamente a barreira hematoencefálica, tanto por difusão como por um sistema de transporte saturável (Nehlinh et al., 1992). Sendo assim, tendo o seu principal mecanismo de ação no Sistema Nervoso Central (SNC).

A metabolização acontece no fígado principalmente, sendo o sistema enzimático do citocromo P-450 (CYP1A2) o responsável, sendo degradada em dimetilxantinas, como a paraxantina (83,9%), teobromina (12,2%) e teofilina (3,7%) como pode ser visto na figura abaixo. Cada um destes metabólitos têm suas funções no organismo, sendo excretados na urina após metabolizados. (Mclean e Graham, 2002; Arnaud, 1987).




Metabolismo cafeína

### 2.2.2 Mecanismo de ação

O principal mecanismo de ação da cafeína já conhecido é o antagonismo dos receptores de adenosina ($A_1$, $A_{2A}$, $A_{2B}$ e $A_3$). Os receptores $A_1$, $A_{2A}$ são ativados nas baixas concentrações basais de adenosina, sendo assim esses receptores são provavelmente os principais alvos da cafeína e da teofilina (Fredhlom, 1999). Estes são ambos acoplados à proteína G, porém o receptor $A_1$ é acoplado à proteína Gi causando a inibição da adenilato ciclase (AMPc) e de vários tipos de canais de Cálcio, enquanto os receptores $A_{2A}$ associam-se a proteína Gs, neste sentido à ativação desses receptores ativa a AMPc e os demais canais de cálcio. (Fredholm, 1994ª; Olah e Stiles, 1995).

Os receptores de adenosina são encontrados em diversos tecidos, como cérebro, músculo cardíaco, músculo esquelético e adipócitos, no entanto os receptores tipo $A_1$ são vistos em maior número no hipocampo, córtex, tálamo e em algumas terminações nervosas e os receptores do tipo $A_{2A}$ encontram-se em

regiões do cérebro ricas em dopamina. (Goodman e Snyder, 1982; Fastbom et al., 1987; Graham, 2008).

Outro mecanismo de ação já descrito é a cafeína como poupador de glicogênio, que sugere que essa substância aumenta a liberação de catecolaminas (especificamente a adrenalina), o que aumenta a liberação de ácidos graxos livres e preserva o glicogênio muscular durante o exercício. Esse mecanismo pode ser particularmente relevante para pessoas com diabetes, que costumam ter menos reservas de glicogênio no músculo e no fígado (Bischof et al., 2001; Shulman et al., 1990).

Além disso, a cafeína está associada com a preservação da memória e o menor risco de desenvolver doenças neurodegenerativas como o Alzheimer (Espinosa et al., 2013). Pois o tratamento crônico com cafeína demonstrou ser eficaz na prevenção na produção de β-amilóide e déficits de memória em modelos animais. (Cunha & Agostinho, 2010; Dall'Igna et al., 2007).

## 2.3 Exercício físico

O exercício físico, ao longo dos anos, tem se mostrado uma terapia eficaz para prevenção e tratamento de doenças crônicas, melhora no estilo de vida e saúde em geral (Powell e Paffernbarger, 1985; Garber et al., 2011). Já existem evidências que a prática de exercício físico, tanto em homens como mulheres pode aumentar a expectativa de vida. (Blair et al., 1989).

Os efeitos do exercício físico são bem descritos na literatura, principalmente, benefícios para a saúde cardiovascular (Fagard & Cornelissen, 2007), controle e prevenção de distúrbios metabólicos (diabetes, resistência à insulina, obesidade, síndrome metabólica) (Singal et al., 2007; Slentz et al., 2005), doenças musculares, articulares e em ossos (artrite, artrose, fibromialgia) (Metsiose et al., 2011), e também para a saúde mental, trazendo melhora na qualidade de vida de pessoas com depressão, ansiedade e outros transtornos psicológicos (Angevaren et al., 2008; Colcombe et al., 2006)

Neste sentido, nas últimas décadas pesquisadores têm estudado os efeitos neuroprotetores vindos da prática de exercício físico, acarretando

benefícios para o Sistema Nervoso Central (SNC). Diversos foram os estudos que mostraram melhora no quadro de pessoas com doenças degenerativas do SNC, como Esclerose Múltipla, Parkinson, Alzheimer e Epilepsia (Arida et al., 1999; Benedetti et al., 2009; Kohl et al., 2007; Laurin et al., 2001; Tillerson et al., 2003).

Além de seu efeito neuroprotetor, o exercício está relacionado positivamente com a regulação da plasticidade cerebral e os benefícios à memória, pois estudos mostram que após o treinamento as proteínas chaves, que são elas: fator neurotrófico derivado do cérebro (BNDF), fator de crescimento derivado da insulina (IGF-1) e fator de crescimento endotelial vascular (VEGF) (Carro et al., 2001; Fabel et al., 2003; Neeper et al., 1995), que são responsáveis por esta regulação, apresentam um aumento significativo, que podem promover a proliferação celular, crescimento e desenvolvimento neuronal

Em contrapartida, o exercício físico agudo de alta intensidade pode causar danos ao tecido muscular, aumentando a produção de espécie reativa de oxigênio (EROs) e aumento nos processos inflamatórios (Aoi et al., 2004; Fisher-Wellman & Bloomer, 2009). No entanto, a prática de exercício de forma crônica evidenciou que existe um processo de adaptação, no qual, as células tornam-se menos susceptíveis aos danos associados à atividade aguda do estresse oxidativo (Niess, 2007). Esta adaptação se dá em função ao aumento da biogênese mitocondrial, redução na produção de EROs e aumento das enzimas antioxidantes (Packer & Cadenas, 2007; Sachdev & Davies, 2008).

### 2.3.1 Exercício Físico e Síndrome Metabólica

Com a atual evolução da tecnologia, o estilo de vida sedentário tem aumentado progressivamente e é frequentemente acompanhado por um aumento no consumo de alimentos e bebidas processados, ricos em calorias e frutose, devido à fácil preparação, disponibilidade rápida e boa palatabilidade (Choi et al., 2005; Dale et al., 2014). Entre os fatores determinantes para o desenvolvimento da síndrome, principalmente entre os adolescentes, destaca-

se os altos níveis de inatividade física moderada e vigorosa, e baixos níveis de aptidão cardiorespiratória (Steele et al., 2008)

Foi demonstrado que a SM é considerada uma doença inflamatória de baixo grau, elevando consideravelmente as citocinas e processos inflamatórios em geral (Rogowski et al., 2008). Sendo assim, o exercício físico já foi considerado uma ferramenta não farmacológica em potencial para o tratamento desta síndrome, pois os estudos apontam os efeitos anti-inflamatórios e antioxidantes do exercício físico (Farinha et al., 2015; Karolkiewicz et al., 2009).

O exercício regular ajuda a reduzir o peso, reduzir a pressão arterial e melhorar os distúrbios lipídicos, incluindo o aumento do HDL e a redução dos triglicerídeos (Myers, 2014; Pucci et al., 2017). Além disso, outro fator importante é que os autores observaram um efeito favorável da atividade física regular na elasticidade arterial (Joo et al., 2017) e sabe-se das também do impacto favorável do exercício físico nas respostas fisiológicas da resistência à insulina (Henriksen, 2002; C. K. Roberts et al., 2013). Tendo um importante papel no tratamento da diabetes, pois no momento de repouso a glicose é sensível à insulina, no momento do exercício, as contrações musculares aumentam a captação de glicose da circulação, independente de insulina. De fato, o GLUT4 responde tanto à insulina quanto à contração muscular de forma independente.

Em situações onde o metabolismo aumenta, como por exemplo, treinamento físico de alta intensidade (Korivi et al., 2012; Liu et al., 2000) e doenças metabólicas (Brandt e Peterson, 2010), O corpo está sujeito ao aumento de espécies reativas de oxigênio durante e após a prática de exercício físico de forma aguda. No entanto, o estresse oxidativo como o aumento da peroxidação lipídica e carbonilação de proteínas e juntamente com a diminuição das defesas antioxidantes (Liu et al., 2015) no treinamento físico crônico representa um estresse físico transitório que altera o metabolismo e a homeostase (Mastorakos et al., 2005), que causa uma adaptação benéfica e tem um papel preventivo e terapêutico nas principais doenças associadas ao estresse oxidativo.

Desta forma, o exercício físico atua de forma benéfica em diversos tecidos do corpo e em diversas funções fisiológicas, sendo uma ferramenta importante e completa para a prevenção e tratamento da Síndrome Metabólica.

### 2.3.2 Exercício Físico e cafeína

A cafeína é popularmente conhecida como uma substância ergogênica, amplamente utilizada por atletas de todos os níveis. Os efeitos vindos da cafeína durante a prática de exercício físico é estudado durante anos, desde então busca-se conhecer os efeitos produzidos para a melhora do desempenho físico.

O mecanismo atualmente considerado responsável pelos efeitos ergogênicos da cafeína é a ativação do SNC através dos receptores A1 e A2 de adenosina (Davis et al., 2003; Zheng et al., 2014), e não poupando a utilização de glicogênio muscular (Costill et al.,1978). O bloqueio dos receptores A2 de adenosina com cafeína, por exemplo, promove uma potencialização excitatória direta dos receptores de dopamina D2 e aumenta a atividade psicomotora em animais (Ferré, 2016). A resposta ergogênica da cafeína durante o exercício de resistência é afetada por vários fatores, como a dose (Desbrow et al., 2009; Graham & Spriet, 1995), status de treinamento (O'Rourke et al., 2008), tempo de ingestão (Conway et al., 2003; Cox et al., 2002), efeitos de abstinência (Irwin et al., 2011; Van Soeren & Graham, 1998) e fonte de cafeína (Graham et al., 1998; Hodgson et al., 2013). Embora os efeitos do exercício agudo no receptor de adenosina e na sensibilidade à cafeína não sejam conhecidos, relatou-se que uma única sessão de exercício de 1 hora alterou a resposta mediada pelo receptor de adenosina à insulina no músculo sóleo de ratos (Langfort et al., 1993).

A cafeína melhorou as habilidades motoras necessárias para ter sucesso durante jogos simulados de taekwondo, tênis, rúgbi e futebol (Hornery et al., 2007; Lara et al., 2014; S. P. Roberts et al., 2010)(, e melhorou a agilidade, decisão e tempo de reação antes e depois de quatro circuitos de 20 min que replicavam os padrões de movimento e demandas de exercício em esportes

coletivos (Duvnjak-Zaknich et al., 2011). Devem ser feitos mais estudos, aprofundados, sobre o efeito da cafeína no SNC que reduzem a sensação de dor ao esforço, pois existem relatos sobre este efeito que não devem ser descartados (Plaskett & Cafarelli, 2001; S. P. Roberts et al., 2010).

A cafeína tem efeitos positivos sobre o desempenho cognitivo e físico quando estudados separadamente, estudos mostram que doses moderadas melhoram muitos aspectos da função cognitiva, como atenção, memória de reconhecimento e velocidade psicomotora complexa, após o exercício (Hogervorst et al., 1999).

# 3. OBJETIVOS

## 3.1 Objetivo Geral

Investigar o efeito do treinamento aeróbico de natação combinado com suplementação de cafeína em ratos previamente tratados com frutose nas alterações neuroquímicas e cognitivas.

## 3.2 Objetivos Específicos

Avaliar se o tratamento aeróbico com frutose induz alterações nos parâmetros bioquímicos e cognitivos;

Analisar se o treinamento físico e/ou a suplementação com cafeína altera os parâmetros bioquímicos e cognitivos;

Analisar o efeito do treinamento aeróbico combinado com a suplementação com cafeína em animais tratados com frutos nos parâmetros bioquímicos e cognitivos

## 4 . RESULTADOS

Manuscrito científico

# Effect of physical training and caffeine supplementation on neurochemical and cognitive parameters in fructose pretreated rats

Adson de Souza Pereira[1], Mariele da Silva Hernandez[1], Jozyê Milena da Silva Guerra[1], Bruno Henrique Nieswald[1], Matheus Chimelo Bianchini[1], Douglas Buchmann Godinho[2], Leandro Severo[2], Robson Luiz Puntel[1], Luiz Fernando Freire Royes[2], Leonardo Magno Rambo[1]*

# Effect of physical training and caffeine supplementation on neurochemical and cognitive parameters in fructose pretreated rats

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## Abstract:

Metabolic syndrome is a disorder that has been growing in the world population. Changes in life style and the in dietary habits is believed to have an impact on the increased prevalence of this disease. Therefore, the search for non-medication and low-cost therapies for the treatment of this disorder is of great interest. In this sense, the objective of this study is to investigate the effect of aerobic exercise on the neurochemical and cognitive parameters of rats submitted to a model of fructose-induced metabolic syndrome . To assess the effect of exercise training and caffeine supplementation, animals were assigned to 8 different groups . Animals received either fructose or vehicle for 10 weeks. The swimming training started in the fourth week after the start of fructose administration. During the period of exercise animals were also administered with caffeine. Our results show that fructose consumption increased visceral fat, however there did not increase the body weight and neither chaged the weight of the soleus muscle. Animals that were exposed to physical exercise showed an increase in muscle weight and a decrease in total body weight as well as visceral fat. Caffeine supplementation was able to prevent against the gain of visceral fat induced by the fructose treatment. Furthermore, both the caffeine treatment and the physical exercise were also able to decrease the glucose clearance over time. Physical exercise also lowered the total cholesterol, HDL and triglycerides, but not LDL levels induced by the fructose treatment. Statistical analysis showed that the administration of fructose for 10 weeks impaired

short-term memory and localization, but not long-term memory, however physical training improved short-term, long-term and localization memory and reverted the effect fructose in short-term memory. Physical training increased the levels of NRF2 compared to sedentary animals. Thus, it was possible to conclude that the high consumption of fructose caused animals to develop metabolic disorders such as: the increase of visceral fat, increase in lipid levels, increase in the clearance of glucose and that, in addition, generates cognitive damages impairing memory and causing anxiety, on the other hand, physical training and caffeine reverted a some of the damages caused by fructose, therefore, one can consider physical training and caffeine intake as an alternative and non-medication therapy to help reduce the risk factors associated with metabolic syndrome.

Key-words: fructose; caffeine; physical exercise; metabolic syndrome.

## INTRODUCTION

The modern lifestyle brought mainly by technology evolution makes the human being progressively sedentary and it is often accompanied by increased consumption of processed, calorie- and fructose-rich food and beverage, due to easy preparation, quick availability and good palatability (CHOI, et al, 2005; DALE et al, 2014). It has been shown that physical inactivity associated with high fructose consumption are related with several metabolic dysfunctions such as obesity (BLÜHER, 2019), glucose intolerance, increased blood pressure, hypertriglyceridemia, low levels of high density lipoprotein (HDL) and atherosclerosis (BIDWELL, 2017). When the same individual accumulates at least three of the aforementioned metabolic disorders, it is considered a metabolic syndrome (MS) (SWARUP et al, 2019).

Nowadays, MS is a public health problem, with difficult clinical management, presenting a low-grade systemic inflammatory condition as well as increased oxidative stress (ROGOWSKI et al, 2008; FARINHA et al, 2015; DEVRIES et al, 2008). More than a systemic disease, MS has several

consequences at the central nervous system (CNS) level. In this sense, it has been shown that individuals with MS present reduced cerebral blood flow and that it may be related to cognitive decline, memory deficits and dementia (YATES et al, 2012; BIRDSILL et al, 2013; LIVINGSTON et al, 2020). In fact, lower cerebral blood flow may alter brain energetic metabolism, as well as increase reactive oxygen species production, oxidative stress and neuroinflammation (CONVIT et al., 2003; GOLD et al., 2007). Taking into account that MS has a multifactorial pathogenesis (ZAFAR et al, 2018), it is difficult to treat the syndrome as a whole. Basically, different strategies for primary prevention are carried out taking into account the main known risk factors, which includes lipid profile and increased abdominal/visceral fat (OLIVEIROS, et al 2014; Scaglione, et al, 2010). Therefore, therapeutic strategies with a multifactorial nature could be beneficial in MS treatment. In line with this view, physical exercise has a multifactorial character due to the potential to act in different pathways in virtually every body tissue (WARBURTON, et al 2006). The physical training contributes both in acute and chronic ways in health promotion assisting in the treatment and prevention of several diseases, such as disorders of the CNS (KOHL, et al 2007) chronic cardiovascular diseases (FAGARD and CORNELISSEN, 2007), metabolic disorders as obesity (SLENTZ, et al, 2005), diabetes (SINGAL, et al, 2007) and hypertriglyceridemia (KRAUS, et al, 2002). In fact, there is a vast literature demonstrating the beneficial role of physical exercise in the treatment of risk factors associated with MS (DI MARZO and SILVESTRI, 2019). However, little is known about the effect of physical exercise on the neurochemical and cognitive consequences of MS in the brain. Caffeine (1,3,7-trimethylxanthine) belongs to

the xanthine family and is widely consumed worldwide, through drinks such as coffee and tea (SHIMIT, 2002). Both acute and chronic caffeine consumption promotes a series of effects, provided they are ingested in adequate doses (GRAHAM, et al, 2008). It has a rapid absorption in the gastrointestinal system and due to its chemical characteristic, it has a rapid action in the CNS, antagonizing adenosinergic receptors (A1, A2 and A3) (FREDHLOM, et al, 1999). Adenosine receptors are distributed in several body tissues in addition to the CNS, such as cardiac muscle, blood vessels, kidneys, lungs, gastrointestinal tract and adipose tissue (GRAHAM, et al, 2001; SöKMEN, et al, 2008). Due to the wide distribution of its receptors, several studies have shown therapeutic effects of caffeine on a series of risk factors associated with MS, such as hypertension control (YU et al, 2016), diabetes (GUARINO, et al, 2013), obesity (QUAN, et al, 2013), reduction in blood glucose in rats submitted to a rich-sucrose diet (PANCHAL et al, 2012) and reduction in total body mass and adipose mass in rats submitted to a high-fat diet (CONDE et al., 2012). It is also known that caffeine, when associated with aerobic exercise, has an ergogenic effect by increasing epinephrine levels, reducing muscle glycogen usage, stimulating triglycerides breakdown, thus accelerating fatty acids usage as an energy source during effort (COSTILL et al 1978, IVY et al 1979; BARCELOS et al, 2020). Considering that both physical exercise and caffeine are low cost and easily accessible therapies, in addition to the possibility of having a potential synergistic effect in the treatment of risk factors related to MS, and that to the best of our knowledge, they have never been tested in combination on risk factors associated with MS, nor even on cognitive consequences and neurochemical

changes in the brain, the present study aimed to investigate the effect of aerobic swimming training combined with caffeine supplementation in rats previously treated with fructose on neurochemical and cognitive changes, as well as risk factors associated with MS.

## MATERIAL AND METHODS

### Animals and Reagents

Adult male Wistar rats approximately two months old (200-250 g; n=64) were purchased from the central bioterium of Federal University of Santa Maria. Rats were transported and maintained in the Animal Bioterium of the Federal University of Pampa (UNIPAMPA) in appropriate cages (4 rats per cage) in a controlled environment (12:12 h light-dark cycle, 24±1 °C, 55% relative humidity), with free access to water (when they were not under fructose treatment) and food (standard rat chow) during all the experimental procedures. Animals body weight, food intake, water (or fructose) intake were measured daily throughout the study. Animal utilization protocols followed the Official Government Ethics guidelines and were approved by the University Ethics Committee of Federal University of Pampa (#026/2018). All reagents were purchased from Sigma (St. Louis, MO, USA) or from local suppliers.

### Fructose administration protocol and experimental design

After a 10-days acclimatization period, animals were randomly divided into the control (n= 32; drinking water) or fructose (n=32; 15% w/v) group. Fructose

treatment and lasted 10 weeks, as shown in timeline (Figure 1). Four weeks after fructose treatment in drinking water started, all animals were subjected to the Glucose Tolerance Test (GTT) and then randomly divided into the following eight groups (Table 1):

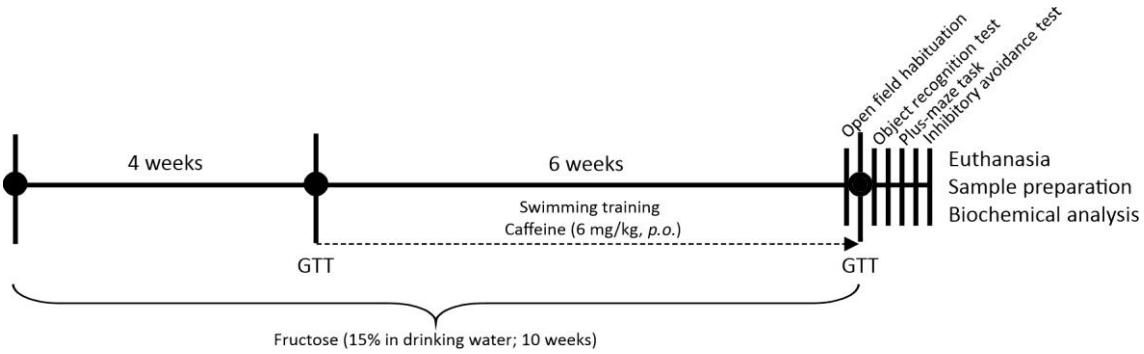



Figure 1 - Timeline showing the periods of fructose administration (10 weeks), caffeine supplementation (6 weeks, concomitantly with physical training and fructose administration) and swimming training (6 weeks, concomitantly with caffeine and fructose administration) as well as behavioral and biochemical assays timepoint.

Table 1 - experimental groups design

| **Experimental group** | **Fructose (15% w/v)** | **Swimming Training** | **Caffeine (6 mg/kg)** | ***n*** |
|---|---|---|---|---|
| **Group 1** | - | - | - | 8 |
| **Group 2** | + | - | - | 8 |
| **Group 3** | - | - | + | 8 |
| **Group 4** | + | - | + | 8 |
| **Group 5** | - | + | - | 8 |
| **Group 6** | + | + | - | 8 |
| **Group 7** | - | + | + | 8 |
| **Group 8** | + | + | + | 8 |

**Glucose Tolerance Test (GTT)**

GTT was performed in two moments: 1) four weeks after fructose treatment begins, before the physical training and caffeine supplementation starts and 2) at the end of the physical training period and supplementation with caffeine. Blood glucose level was measured using a glucose meter (AccuChek active® Roche Diagnostics, Mannheim, Germany). After 8-hours of overnight fasting it was collected a small drop of blood from the tail vein for fasting glucose level measuring. After first measurement (time 0 - fasting glucose level), rats were injected with 50% dextrose (2 g/kg body weight; i.p.) and a drop of blood was collected again at 15, 30, 60, 90 and 120 minutes after dextrose administration to measure glucose clearance (Agrawal et al., 2016).

**Swimming training protocol and caffeine supplementation**

Animals were subjected to the swimming training protocol by a period of 6 weeks, beginning 4 weeks after fructose treatment. The training tank used for this study was 80 cm in diameter and deep, with water temperature at 32 ± 1 °C. Swimming training was always performed between 9:00 and 11:00 a.m.. In the first week of the aerobic physical training, animals (groups 5, 6, 7 and 8) were subjected to the water adaptation during 5 days, by 20 minutes on the first day, 30 minutes on the second day and so on, until 1 hour on day 5, without body overload. The aerobic swimming training during the remaining 5 weeks consisted of swimming sections lasting 60 minutes a day, 5 times a week, with an overload of 5% of the body weight adjusted in backpacks in the dorsal region. The overload was adjusted once a week due to the natural weight gain of rats. Animals of the

sedentary groups (groups 1, 2, 3 and 4), which were not submitted to physical training, were placed in the tank with shallow water, at a height of 5 cm, at the same conditions, for the same time as the training groups, for reduce the stress caused by daily handling, without promoting adaptation to physical training (Souza et al., 2009). During all physical training period the animals were supplemented with caffeine (6 mg/kg) or water, by an orogastric probe 30 minutes before swimming sections (Fredholm, et al., 1999).

**Behavioral Analysis**

*Open field (exploratory and locomotor activity and habituation memory)*

On the penultimate day before the end of the physical training period, rats were placed in a wooden square box open field measuring 50 x 50 x 50 cm with the floor divided into 25 equal squares. Each rat was left 5 minutes into the arena in a room and the exploratory and locomotor behavior was recorded in a camera for further off-line analysis for the number of crossing and rearing. To evaluate the habituation memory to the open field, 24-hours after, animals were placed again in the center of the open field and the same parameters were recorded. The entire apparatus was cleaned with 30% ethanol before and after each animal be placed in the arena (Barros et al., 2006).

*Object Recognition Test*

The object recognition test was performed 24 hours after the last habituation session in the same arena as open field. The objects to be discriminated were figures of similar size and texture (8–10 cm high) fixed to the

floor with adhesive tape. To avoid olfactory stimuli, the objects were carefully cleaned and washed with 30% ethanol solution after each individual session. The previous open field task was considered as an habituation, in turn to reduce possible neophobic responses. In the first trial animals were placed in the open field with two distinct objects (A and B) for free exploration and the time spent exploring each object was recorded during 5 minutes. The short-term memory was tested three hours after the first trial and the animals were replaced in the same arena for 5 minutes with one of the objects changed by a new one (objects A and C). To assess the long-term memory, 24 hours after the first trial animals were replaced in the open field for 5 minutes with one different object (objects A and D). The cumulative time that each rat spent at each of the objects was manually recorded by an observer, who was not aware of treatments. Exploration of an object was defined as follows: directing the nose to the object or touching it with the nose or front paws; turning around or sitting on the object was not considered as exploratory behavior. The percentage of total time exploration that each animal spent investigating the novel object was the measure of recognition memory, defined at the Discrimination Index (DI). It was calculated using the following formula: (time spent investigating new object) − (time spent investigating known object)/time spent in investigating both objects*100 (Ferreira et al., 2014).

*Elevated Plus-maze Test*

The elevated plus-maze consisted of two horizontal wooden surfaces aligned at the ends (open arms), crossed at right angles with two other surfaces

of the same alignment (closed arms). All arms have the same size (50 x 10 cm), with the closed arms being surrounded by 40 cm high walls, except in the central part (10 x 10 cm), where the surfaces intersect. The entire apparatus is raised 50 cm from the floor. The test consisted of gently placing each animal in the central area of the apparatus, with the snout facing one of the closed arms, left alone in a room, and free exploration was allowed for five minutes. The number of entries and the time spent within each arm were measured. All the behavior was recorded in a camera for further off-line analysis. In the intervals between animals analysis the entire apparatus was cleaned with 30% ethanol. The elevated plus-maze test was performed 24 hours after the last object recognition session (Izquierdo et al., 2002).

*Inhibitory avoidance task*

Twenty four hours after elevated plus-maze test, all the animals were subjected to a single training session in a step-down inhibitory avoidance apparatus, consisting of a 25 x 25 x 35 cm acrylic box with a grid floor composed by parallel stainless steel bars (1.0 mm diameter) spaced 1.0 cm apart and the left portion covered by a wooden platform measuring 7 x 25 x 2.5 cm. Each individual rat was placed gently on the platform facing the rear left corner. Once the rat stepped down with all 4 paws on the grid, a 3 s, 0.3 mA shock was applied to the grid. After training session, animals were returned to their home cages and tested for retention 24 h later. A step-down latency test was taken as a measure of retention, with a cut-off time of 300 s (Guerra et al., 2012).

## Sample processing

*Blood, muscle and visceral adipose tissue*

Twenty four hours after the last behavioral test animals were euthanized by decapitation and their blood was collected from decapitated neck in heparinized test tubes for further biochemical assays. Immediately after collection, the blood was centrifuged at 3000 g for 15 min at 4 ºC to obtain serum samples. Serum was maintained in a -4 ºC freezer until use. Soleus muscle and visceral adipose tissue were removed and immediately weighted in a high precision balance.

*Brain*

At the same time that blood was being collected, an experimenter collected the hippocampus by quickly skull opening in the sagittal suture and parietal bones removal. Brain was gently watered with cold artificial cerebrospinal fluid (aCSF) and immediately removed from the skull to a petri dish, with ice underneath, cold aCSF and filter paper inside to facilitate tissue adherence and removal of the hippocampi. Hippocampi was collected and immediately frozen in liquid nitrogen and maintained in a -80 ºC freezer until use.

## Biochemical assays

Serum HDL, LDL, total cholesterol and triglycerides level were determined with standard commercial kits (Bioclin-Quibasa, MG, Brazil), according to the

manufacturer's protocol. Samples absorbances were read spectrophotometrically at a wavelength of 500 nm for triglycerides and total cholesterol, 550 nm for HDL and 546 nm for LDL.

**Western blot test**

Western blot analysis was performed according to De Zorzi et al. (2019) with some modifications. Hippocampal samples were lysed on ice in RIPA (radio-immunoprecipitation assay) and centrifuged for 20 min at 12,700 rpm at 4 °C. The protein concentration of each sample was determined by bicinchoninic acid protein assay (Thermo Fisher Scientific). Then, samples (30 µg protein) were subjected to a 10% SDS polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane using Trans-Blot® Turbo™ Transfer System. Equal protein loading was confirmed by Ponceau S staining (Sigma Aldrich - P7170). After specific blocking, the blots were incubated overnight at 4 °C with rabbit anti-Nrf2 (1:1000; sc-722, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and rabbit anti-Adenosine A1-Receptor (1:1000; sc-28995, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Mouse anti-β-Actin antibody (1:10.000; sc-69879, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was stained as additional control of protein loading. After primary antibody incubation, membranes were washed with TBS-T (TBS plus 0.1% Tween 20) twice at room temperature for 15 min and incubated with anti-rabbit (sc-2004, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-mouse (sc-2005, Santa Cruz Biotechnology, Santa Cruz, CA, USA) secondary antibodies conjugated with horseradish peroxidase (1:5000) for 2 h at room temperature. Bands were

visualized by enhanced chemiluminescence using ECL Western Blotting Substrate (Pierce ECL, BioRad) and the signals were captured with a ChemiDoc XRS+ (BioRad). The blots were stripped and probed again for mouse anti-β-Actin (43 kDa) antibody (sc-69879, Santa Cruz Biotechnology, Santa Cruz, CA, USA) to verify equal protein loading. The density of the specific bands was quantified with Image Lab software 6.0 (Bio-Rad). Values are expressed as a percentage of the control.

### Statistical analysis

Statistical analyses were carried out by t-test, one- or two or three-way analysis of variance (ANOVA). Values of F are only presented if $P<0.05$. Post hoc analyses were carried out, when appropriate, by the Tukey's test. All data are expressed as mean and S.E.M.

## RESULTS

### Dietetic analysis

Figure 1 shows the food (Fig. 1A), water (or fructose) (Fig. 1B) and total caloric intake (Fig. 1C) throughout the experimental period. Statistical analysis revealed that fructose-treated animals eat less food [$F(1,56)=2168$; $p<0.001$], drink more water [$F(1,56)=272$; $p<0.001$] and ingested more calories [$F(1,56)=626$; $p<0.001$] over time in comparison to animals on a regular diet.

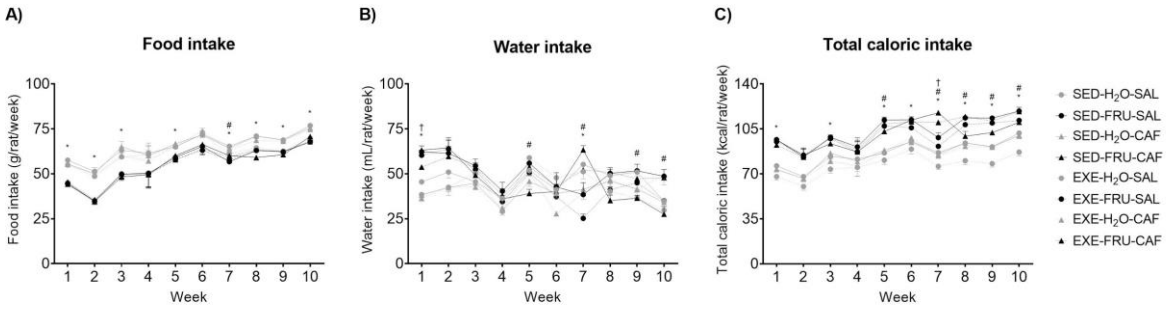



**Figure 1.** Effect of fructose (FRU), training (EXE) and caffeine (CAF) on food intake (g/rat/week), water intake (mL/rat/week) and total caloric intake (kcal/rat/week) during ten weeks of treatment. Data are expressed in mean ± SEM. * indicates significant difference between groups SED-FRU-SAL versus SED-$H_2O$-SAL; # between groups SED-FRU-CAF versus SED-FRU-SAL; † between groups EXE-FRU-CAF versus SED-FRU-SAL.

**Anthropometric assays**

Figure 2 shows the effect of caffeine and/or physical training on fructose-induced changes in total body weight (Fig. 2A), soleus weight (Fig. 2B) and visceral fat mass (Fig. 2C). Statistical analysis revealed that fructose treatment by 10-weeks increased visceral fat mass but interessantly didn't increase the average soleus weight nor the body weight. In opposition, physical training decreased visceral fat mass, increased soleus weight and decreased body weight *per se* compared with untrained rats. Caffeine supplementation had no *per se* effect on any of the parameters analyzed, but was able to decrease the fructose-induced visceral fat mass gain [$F(1,56)=5,236$; $p<0.05$]. Swimming training also decreased the effect of fructose on visceral fat mass [$F(1,56)=7,306$; $p<0.01$] and body weight [$F(1,56)=4,480$; $p<0.05$].

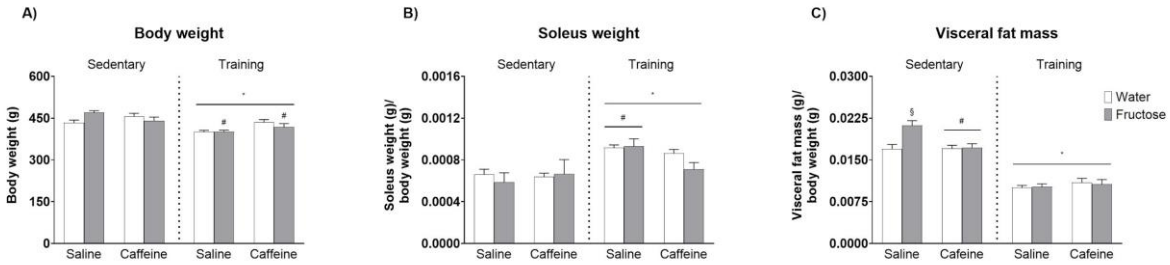



**Figure 2.** Effect of fructose, training and caffeine on body weight (g), soleus weight (g) and visceral fat mass (g) after ten weeks of treatment. Data are expressed in mean ± SEM. * indicates a significant difference of groups Sedentary; # of group Sedentary more Fructose and Saline; § different from all groups.

## GTT

Figure 3 shows the effect of fructose treatment by four weeks (Fig. 3A) and the effect of caffeine and/or physical training on fructose-induced changes on glucose clearance (Fig. 3B). Statistical analysis (paired t-test) showed that fructose treatment (15%) by four weeks altered glucose clearance after fasting dextrose injection [t(62)=3,463; $p<0.05$]. Individual comparison revealed that except for baseline, the fructose-treated group presented higher glucose levels in all other time points. These results confirmed that fructose treatment by four weeks was effective in promoting at least one metabolic syndrome-like effect. After this analysis, all animals were randomly divided into eight experimental groups. Animals receiving fructose continued under fructose regimen for six more weeks, concomitantly to physical training or caffeine treatment. The three-way ANOVA (fructose x caffeine x training) with repeated measures (different points of time) showed a significant interaction effect [F(5,51)=3,028; $p<0.05$). Post hoc analysis revealed that aerobic swimming training as well as caffeine treatment for

6 weeks were able to revert the effect of fructose on decreasing glucose clearance along time.

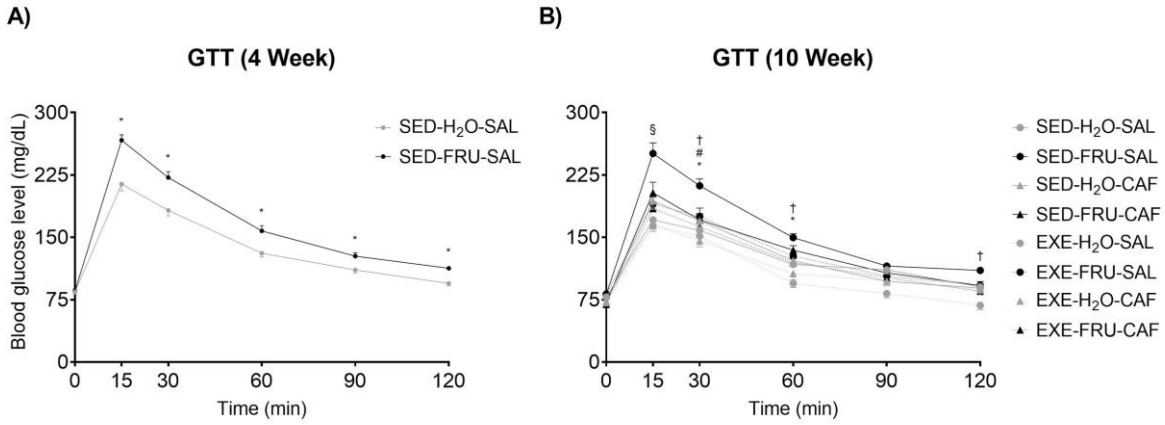



**Figure 3.** Effect of fructose (FRU), training (EXE) and caffeine (CAF) on glucose clearance (mg/dL) at six different points of times (0, 15, 30, 60, 90 and 120 min) after fasting dextrose injection. The treatment consisted of four weeks of fructose (A), followed for six weeks of physical training plus caffeine, concomitantly with fructose (B). Data are expressed in mean ± SEM. * indicates significant difference between groups SED-FRU-SAL versus SED-$H_2O$-SAL; # between groups SED-FRU-CAF versus SED-FRU-SAL; † between groups EXE-FRU-CAF versus SED-FRU-SAL; § SED-FRU-SAL group is different from all groups.

**Lipid Profile**

In order to analyze the effect of physical training and caffeine supplementation on fructose-induced lipid profile changes, we performed the analysis of total cholesterol, triglycerides, HDL and LDL. Statistical analysis revealed a significant effect of interaction for total cholesterol [$F(1,48)=7,130$; $p<0,05$] (Fig. 4A), triglycerides [$F(1,48)=17,180$; $p<0.0001$] (Fig. 4B), and HDL [$F(1,48)=14,650$; $p<0.001$] (Fig. 4C) but not for LDL (Fig. 4D). Statistical analysis also confirmed the hypothesis that fructose ingestion could increase total cholesterol and LDL as well as decrease HDL levels, but not altered triglycerides levels. In the same way, physical training factor was able to revert the effect of fructose on total

cholesterol, HDL and triglycerides but not on LDL levels. Caffeine supplementation was able to revert the effect of fructose on total cholesterol, triglycerides, HDL and LDL levels. Interessantly, post hoc analysis showed that physical training *per se* increased HDL levels. Caffeine *per se* increased HDL and decreased LDL levels.

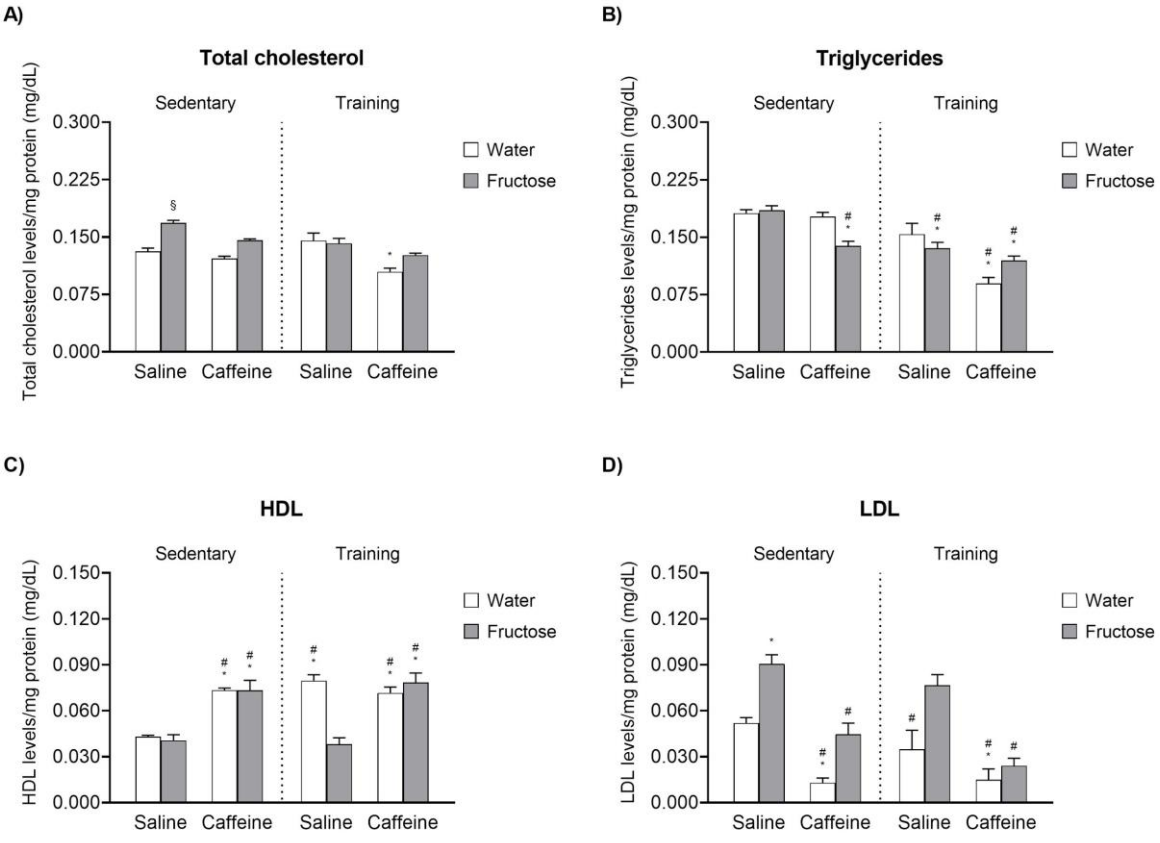



**Figure 4.** Effect of fructose, training and caffeine on levels of Total cholesterol (A), Triglycerides (B), HDL (C) and LDL (D) after ten weeks of treatment. Data are expressed in mean ± SEM. * indicates a significant difference of groups Sedentary; # of group Sedentary more Fructose and Saline; § different from all groups.

## Object recognition

Aiming to analyze a putative effect of fructose on cognitive performance we design a series of experiments as follows, beginning in object recognition task.

Statistical analysis (three way ANOVA) showed that fructose administration for 10 weeks impaired short-term [F(1,56)=4,154; p<0.05] (Fig. 5A) and location [F(1,56)=6,931; p<0.05] (Fig. 5C), but not long-term memory [F(1,56)=0,025; p>0.05] (Fig. 5B) in object recognition task. On the other hand, physical training improved short-term [F(1,56)=18,250; p<0.0001], long-term [F(1,56)=5,083; p<0,05] and location [F(1,56)=10,810;p<0.01] memory and reverted the effect of fructose on short-term memory. Caffeine supplementation had no effect *per se* on object recognition task but was able to revert the effect of fructose on short-term memory.

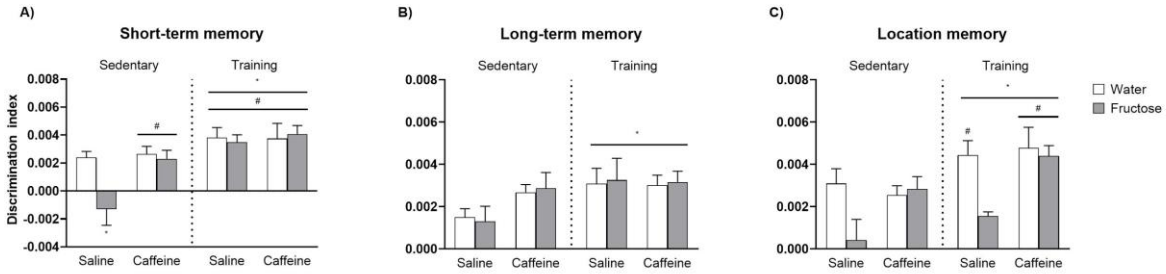



**Figure 5.** Effect of fructose, training and caffeine on discrimination index during the Short-term memory test (A), Long-term memory test (B) and Location memory test (C) after ten weeks of treatment. Data are expressed in mean ± SEM. * on the line indicates a significant difference of groups Sedentary; * only indicates a significant difference of groups Sedentary more Water and Saline; # of group Sedentary more Fructose and Saline.

**Elevated plus maze**

Statistical analysis (three way ANOVA) showed that fructose administration for 10 weeks induced rats to spend more time in closed arms [F(1,56)=10,160; p<0.01] (Fig. 6A) in elevated plus maze task. In addition, physical training decreased the time spent in closed arms [F(1,56)=7,549; p<0.01] (Fig. 6A) and reverted the effect of fructose on time spent in closed arms [F(1,56)=19,930;

p<0.001] (Fig. 6A). Caffeine supplementation had no effect *per se* on any task analyzed on elevated plus maze, but was able to revert the fructose-induced decrease on time spent in closed arms [F(1,56)=4,685; p<0.05] (Fig. 6A). None of the treatments changed the number of head dipping incidence in elevated plus maze task (Fig. 6B).

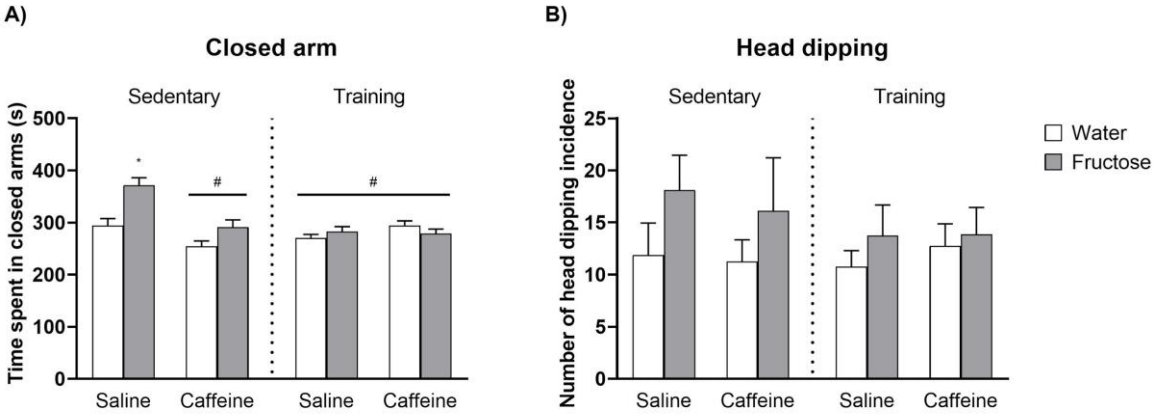
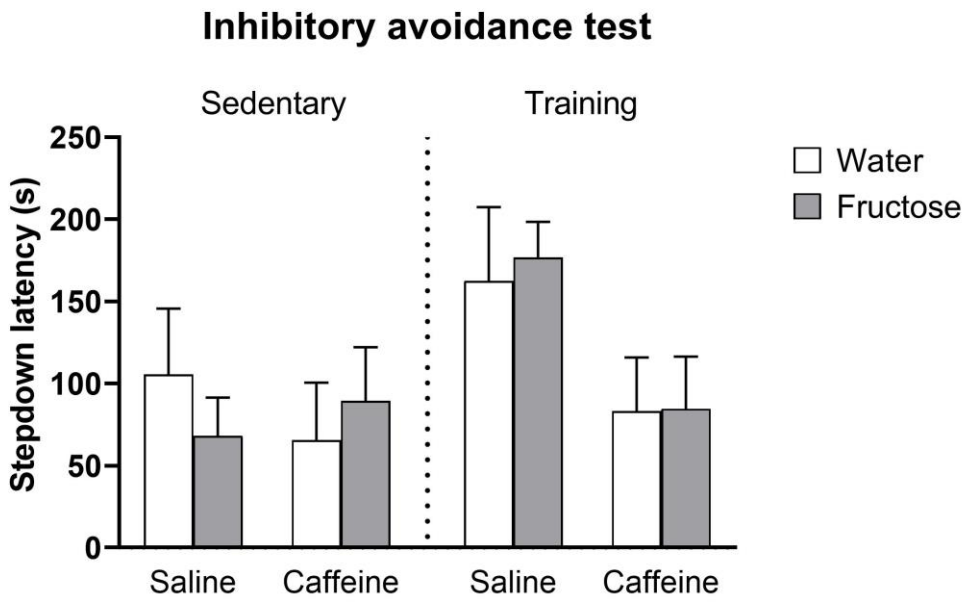



**Figure 6.** Effect of fructose, training and caffeine on time spent in closed arms (A) and the number of head dipping incidence (B) during the plus maze task, after ten weeks of treatment. Data are expressed in mean ± SEM. * indicates a significant difference of groups Sedentary more Saline and Water; # of group Sedentary more Fructose and Saline.

**Inhibitory avoidance**




Statistical analysis did not show any influence of the treatments on step down latency to the platform in the inhibitory avoidance test (Fig. 7).

**Figure 7.** Effect of fructose, training and caffeine on Inhibitory avoidance test (Stepdown latency) after ten weeks of treatment. Data are expressed in mean ± SEM. There was no significant difference between groups.

**Hippocampal NRF2 levels**

In order to understand a possible mechanism involved in the effects of caffeine and/or physical exercise on fructose-induced changes, we assessed NRF2 protein levels. Statistical analysis revealed that physical training increased NRF2 levels compared with sedentary animals [$F(1,56)=21,480$; $p<0.001$] (Fig. 8). Both fructose and caffeine did not alter NRF2 levels.

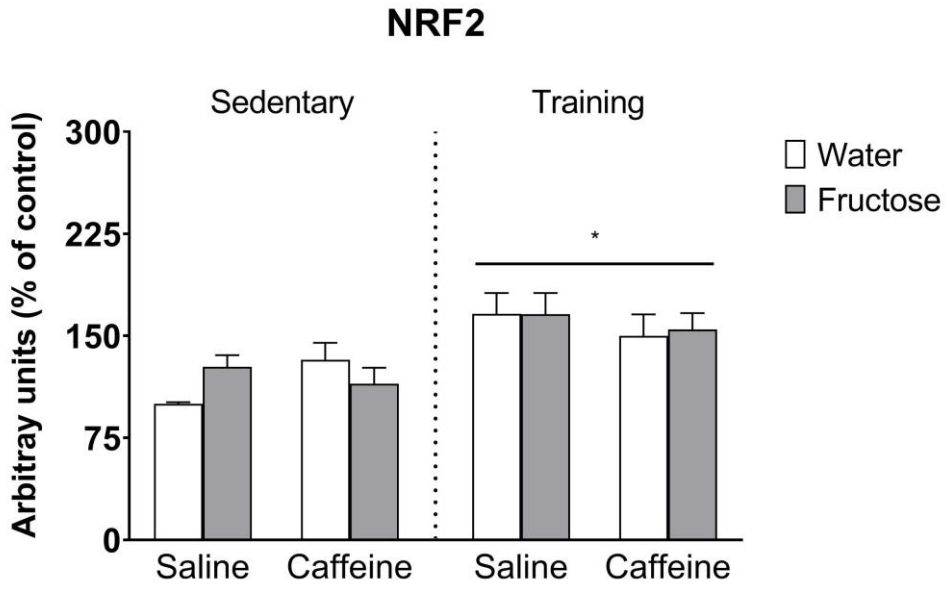



**Figure 8.** Effect of fructose, training and caffeine on NRF2 (Arbitray units) after ten weeks of treatment. Data are expressed in mean ± SEM. * indicates a significant difference of groups Sedentary.

## DISCUSSION

We showed here that fructose intake for four weeks decreased glucose clearance compared with regular dietary intake. We also noticed that rats already treated for four weeks with fructose who started a six-week physical training protocol or caffeine supplementation, or the combination of these two factors were able to reverse the deleterious effects of fructose on glucose clearance, even when the consumption of fructose remains the same. Several evidence indicates that increased fructose availability in diet, especially in sugar-sweetened beverages may be associated with an increased risk of cardiovascular diseases (Khan et al., 2019), type 2 diabetes and metabolic syndrome (Malik et al., 2010). In an opposite way, It has been shown over the last few years that physical exercise has the ability to improve glucose tolerance as well as insulin sensitivity (Sato et al., 2003; D. Yang et al., 2019). It is important

to note that in our study we didn't analyze the insulin sensibility, however it is plausible to purpose that our fructose regimen has impaired the sensibility to insulin, since we have demonstrated several other changes similar to those found in metabolic syndrome, including the decreased glucose clearance, that in turn is associated with insulin downstream (Petersen & Shulman, 2018). It has also been shown that long-term caffeine supplementation improves glucose metabolism in some metabolic disorders (Park et al., 2007; Urzúa et al., 2012). Our results are in accordance with literature data, since we found that both physical exercise and caffeine reverted the effect of fructose on glucose clearance decrease. We also found here an addictive effect of training and caffeine in all time points after fasting dextrose injection at the end of the experimental period (10 weeks of fructose; 6 weeks of caffeine and 6 weeks of physical training), except at the 90-minute point, where we found no difference between groups.

In the present study we showed that fructose regimen (10 weeks) did not increase the total body weight, nor changed the soleus muscle weight, but increased the visceral fat mass. This is an important fact about our fructose treatment. Studies have shown that increased visceral fat mass is a predictor for the development of cardiometabolic diseases and metabolic syndrome (Cho er al., 2017; Sasai et al., 2015; Sullivan et al., 2015). In fact, it has been shown that the increase of visceral fat mass may be associated with higher risk of metabolic syndrome development, even in subjects with normal body weight (Goodpaster et al., 2005). Both caffeine and physical exercise counteracted the effect of fructose on visceral fat mass. Furthermore, physical exercise decreased visceral fat mass *per se*. This finding was expected, since physical training was effective

in promoting physiological adaptations. It has been previously shown that physical training reduces abdominal visceral fat in different populations studied (Irvin et al., 2009; Dugan et al., 2010; Zaiac-Gawlak et al., 2017). In the same sense, caffeine may decrease fat accumulation due to inhibition of fatty acid synthesis, as well as increase in β-oxidation enzymes activity (Sugiura et al., 2012). In addition to individual effects, a recent meta-analysis showed that caffeine supplementation prior to an exercise session may effectively increase fat oxidation during aerobic exercise (Collado-Mateo et al., 2020). However, in our experimental model we did not find an additive effect of exercise and caffeine on reducing visceral fat mass. Our experimental data suggests that both interventions can be considered as therapy to treat or even prevent the development of metabolic syndrome or associated metabolic disorders, such as obesity or increased visceral fat mass.

One can question whether the training was indeed effective in promoting physiological adaptations. In fact, we did not perform any measurement of physiological or biochemical parameters (i.e. resting heart rate, citrate synthase enzyme activity, lactate levels in an increasing load test, etc.) to confirm that aerobic swimming training for six weeks promoted adaptations. However we can assume that the increase of soleus weight, decrease of total body weight and visceral fat mass may be considered as an adaptation, reflection of six weeks of aerobic swimming training and thus serve as a proof that physical training was effective in promoting physiological adaptations.

We avoided naming our protocol of fructose administration of a model of metabolic syndrome, but our data show several parameters similar to those found

in human metabolic syndrome. In this sense, another important fact that corroborates the view that our model can be considered similar to the metabolic syndrome are the findings on the lipid profile after 10 weeks of fructose intake. We found an increase in LDL and total cholesterol as well as a decrease in HDL levels. This body of evidence is in accordance with the dyslipidemia found in conditions of high-fructose consumption (Basciano et al., 2005; Stanhope e Havel, 2008; Park et al., 2020). It has been shown that a single session of aerobic physical exercise was able to alter the lipid pattern in women with dyslipidemia (Costa et al., 2020). In another study in a specific population authors found that physical exercise increased HDL levels (Hsu et al., 2019). Two reviews showed that aerobic exercise has the potential to increase HDL as well as decrease LDL and triglycerides level (Mann et al., 2014; Wang & Xu, 2017). Our results completely agree with literature data. Our findings on the effect of caffeine treatment on fructose-induced dyslipidemia did not agree with the findings in the literature if consumption of caffeinated drinks is considered. In fact, some studies have shown that the intake of caffeinated coffee is related to increased total cholesterol, LDL and triglycerides level, also depending on different ways of preparing coffee (Haffner et al., 1985) Lee et al., 2001; Cai et al., 2012). However, most studies present several biases, i.e. they do not consider whether beverages are ingested with sugar or even the other components of the beverages that may contribute, in some way, to the increase in blood lipids. On the other hand, other studies showed that acute caffeine (isolated) administration prior to exercise increased lipid metabolism (Collado-Mateo et al., 2020). Currently, even the consensus on the effects of caffeine on the improvement in physical performance

has been contested. A recent study lists the factors that may influence the discrepant results regarding the effects of caffeine supplementation. It is possible that these factors may also be responsible for the controversy regarding the effects on the lipid profile. To know, these factors are subdivided into the effects of caffeine, daily habits, physiological and genetic factors (Martins et al., 2020). In our study we treated animals with caffeine diluted in saline and we found an important reversal effect of caffeine on fructose-induced dyslipidemia.

Our protocol also revealed that the treatment with fructose for 10 weeks impaired the object recognition memory in the short-term and localization memory and induced treated animals to stay longer in the closed arm in the elevated plus-maze apparatus. Except for the localization memory, physical exercise and caffeine supplementation reverted the effect of fructose on cognitive and anxiety parameters. Interestingly, the combination of treatments (caffeine and physical training) did not present an additive effect. In fact, the deleterious effects of fructose on cognitive performance were expected, since increased visceral fat mass, as well as obesity and diabetes (isolated components of the metabolic syndrome) are associated with cognitive deficits (Kumari et al., 2000; Schwartz et al., 2013; Tanaka et al., 2020). In addition, several studies have shown the cognitive decline in individuals with metabolic syndrome (Yaffe et al., 2004; Yates et al., 2012). Several mechanisms may be involved in cognitive impairments, such as systemic inflammatory status, reduced brain circulation and oxygenation (Guicciardi et al., 2019; Mellendijk et al., 2015). It has been shown that caffeine supplementation has the potential to prevent weight gain in a high-fat diet, and therefore also reduce cognitive impairments (Moy & McNay, 2013). One putative

mechanism proposed is the caffeine-induced increase in BDNF levels. In the same way, physical exercise has the potential of improve cognitive functions *per se*, however the effects of physical exercise on syndrome metabolic-induced cognitive deficits are controversial, or at least needs more robust data to clarify if and how physical exercise improve cognition in this population (Mandolesi et al., 2018; Zhao et al., 2018; Lin et al., 2019). We found, in the present study, that physical training improved cognitive performance and also decreased the cognitive deficits induced by fructose. Additionally, we also found an effect of caffeine and physical exercise in reducing anxiety like-behavior induced by fructose. In fact, it has been shown that individuals presenting metabolic syndrome have an increased prevalence of anxiety and depression (Shinkov et al., 2018). There are few studies about anxiety and metabolic syndrome and even less focusing on how to treat or prevent anxiety and depression in this population. In our study we showed that both interventions have the potential to treat or prevent anxiety-like behavior as well as cognitive deficits induced by fructose.

One putative mechanism involved in the effects of physical exercise can be explained, at least in part, by Nrf2 increase. We found here that neither fructose nor caffeine altered Nrf2 levels, but physical training was able to increase hippocampal Nrf2 levels. It is important to note that Nrf2 deficient mice present exacerbated oxidative stress, neurovascular dysfunction, blood-brain barrier disruption and neuroinflammation (Tarantini et al., 2018). It was also shown that a decrease in Nrf2 levels may be associated with metabolic syndrome development (Zhang et al., 2014). Furthermore, therapies with the potential to activate the Nrf2 pathway has the potential to prevent or even treat metabolic

syndrome (Chartoumpekis & Kensler, 2013; Vasileva et al., 2020; M. Yang et al., 2018). Our study showed that physical exercise increased hippocampal Nrf2 levels and that can be responsible, at least for the effects of physical exercise on cognition and maybe anxiety.

## CONCLUSION

Thus, it is possible to conclude that the high fructose consumption develops metabolic disorders such as: the increase of visceral fat mass, increase in lipid levels, decrease in the glucose clearance and that, in addition, generates cognitive damages impairing memory and causing anxiety. On the other hand, physical training and caffeine can reverse, at least in part, the damages caused by fructose. Therefore, one can consider physical training and caffeine intake as an alternative and non-pharmacological therapy to help reduce the risk factors associated with the metabolic syndrome.

## 5. CONSIDERAÇÕES FINAIS

De acordo com os resultados apresentados nesta dissertação podemos concluir que:

- A frutose prejudica as funções metabólicas como: o aumenta da massa visceral, aumenta os níveis de colesterol total, LDL e diminui o HDL;

- O treinamento físico e a suplementação de cafeína puderam reverter o aumento no peso, nos níveis de colesterol total e triglicerídeos;

- Além disso, o treinamento físico foi capaz de melhorar a memória de curto prazo, longo prazo e localização e foi capaz de reverter o efeito da frutose na memória de curto prazo;

- O treinamento físico e a suplementação de cafeína pode ser um tratamento alternativo não medicamentoso na síndrome metabólica.

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