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Exercício físico, ingestão de frutose e marcadores da síndrome metabólica em modelo experimental.

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Dedico este trabalho aos meus pais

José Archimedes e Enedir.

Resumo

A síndrome metabólica tornou-se grave problema médico-social tanto em países desenvolvidos quanto em desenvolvimento e o consumo excessivo de frutose na dieta parece ter papel importante nessa questão. Dessa forma, é de grande interesse o desenvolvimento de procedimentos mais efetivos para prevenção e tratamento desta doença. Um dos procedimentos que merece investigação é o exercício físico. O presente estudo visou avaliar os efeitos singulares de três protocolos de exercício físico na prevenção e tratamento de complicações metabólicas desencadeadas por uma dieta rica em frutose. Numa primeira série de experimentos, visou-se analisar os efeitos de uma dieta rica em frutose sobre biomarcadores hepáticos e da síndrome metabólica em ratos. Foram utilizados ratos da linhagem Wistar que a partir dos 120 dias de idade, foram separados em dois grupos, conforme a dieta: controle (ração comercial para roedores labina ®) e frutose (dieta semipurificada com 60% de frutose). Ao final foram analisados tolerância à glicose (teste de tolerância oral à glicose), sensibilidade periférica à insulina (teste de tolerância à insulina), marcadores de esteatose hepática não alcoólica (EHNA) (concentrações séricas de alanina aminotransferase - ALT, aspartato aminotransferase - AST). Foram, ainda, determinados no fígado: concentrações de lipídios totais e de triglicerídeos, taxa de lipogênese hepática, biomarcadores do sistema de defesa antioxidante (atividade das enzimas catalase - CAT e superóxido dismutase - SOD) e de peroxidação lipídica (substâncias que reagem ao ácido tiobarbitúrico – TBARs) e histologia hematoxilina-eosina. Os animais alimentados com dieta rica em frutose apresentaram uma redução na tolerância à glicose, hiperinsulinemia, resistência à insulina, aumento na glicose e nos triglicerídeos circulantes, aumento na relação AST/ALT, acúmulo de gordura no fígado e no tecido adiposo mesentérico, redução na atividade do sistema antioxidante no fígado e aumento das TBARs no mesmo. Numa segunda série de experimentos, foi avaliado o efeito de três diferentes protocolos de treinamento físico em biomarcadores hepáticos, marcadores da síndrome metabólica, peroxidação lipídica e atividade das enzimas antioxidantes de animais adultos da linhagem Wistar (120 dias). Os animais foram divididos em quatro grupos aleatoriamente: Controle: sedentários durante todo o experimento, grupo Aeróbio (A): realizou exercício de natação 1 hora por dia, cinco dias por semana na intensidade de 80% do limiar de lactato; grupo Força (S): treinados no treinamento de força realizando quatro séries de

10 saltos, cinco dias por semana; grupo Concorrente (AS): treinados três vezes por semana no protocolo aeróbio e duas vezes no protocolo de força. Os grupos A e S apresentaram redução no peso corporal se comparados ao grupo C. Além disso, todos os animais treinados apresentaram redução nas concentrações de triglicerídeos no tecido adiposo e fígado. Os animais apresentaram também uma redução da concentração dos marcadores de peroxidação lipídica (TBARs) e aumento da atividade enzimática da superóxido dismutase no soro. Ainda, os animais do grupo A, apresentaram uma elevação na atividade da catalase e superóxido dismutase no fígado. Concluindo, todos os protocolos de atividade física melhoraram o sistema antioxidante dos animais e reduziram os estoques de triglicerídeos nos tecidos analisados. A terceira fase experimental, avaliou os efeitos de três diferentes protocolos de exercício físico em animais portadores de desarranjos metabólicos induzidos por uma dieta rica em frutose. Para este experimento, utilizamos 40 animais Wistar (120 dias de idade) em cinco grupos distintos (n=8 animais por grupo): C (dieta controle e sedentários), F (dieta rica em frutose e sedentários), FA (dieta rica em frutose e exercitado no protocolo aeróbio), FAS (dieta rica em frutose e exercitado no protocolo concorrente) e FS (dieta rica em frutose e exercitados no protocolo de força). Ao final do experimento foram avaliados a regulação glicêmica (oGTT e ITT), bioquímica sanguínea, estresse oxidativo e inflamação, além de conteúdo de triglicerídeos em diferentes tecidos. Resultados: O exercício físico de força exerceu maiores efeitos na regulação glicêmica em comparação aos demais protocolos. Por outro lado, os três protocolos de exercício geraram notáveis respostas nos marcadores de inflamação, conteúdo de triglicerídeos teciduais e redução no estresse oxidativo (FA e FS). Concluindo, os protocolos de exercício físico geraram importantes repostas sistêmicas nos animais submetidos a dieta rica em frutose.

Palavras chave: Ratos Wistar, frutose, síndrome metabólica, esteatose hepática, estresse oxidativo.

Abstract

Metabolic Syndrome has become serious medical-social problem in developed and development country and the excessive fructose consumption in the occidental diet has a major role in this question. This way, it has been a great interest the development of procedures more effectives to prevent and treat this disease. The non-alcoholic fatty liver disease (NAFLD) emerges as a syndrome component and can be related to the development of insulin resistance. Once human studies have many limitations it is necessary the use of animal models adequate to this disease study. This research aims to investigate the potential effects of the physical exercise in the pathophysiology of fructose-induced metabolic syndrome, oxidative stress and inflammation.

The first step aimed to analyses the effects of fructose-rich diet in metabolic syndrome markers. We used Wistar rats (120 days), separated in two groups, according to the diet: control (balanced Labina ® dieta) and fructose-rich diet (60% of caloric content provided by fructose). The animals fed on a fructose rich diet for 60 days (120-180 days). At the end of experiment, they were euthanized to determine in order: the glucose tolerance, insulin sensitivity, insulin levels, biomarkers of nonalcoholic fatty liver disease, biomarkers of oxidative stress and liver histology. The animals fed on fructose rich-diet showed lower glucose tolerance, insulin sensitivity, hiperinsulinemia, raises on serum glucose and triglycerides, higher AST/ALT ratio, fat accumulation in the liver and in the mesenteric adipose tissue, a reduction in the anti-oxidant system activities and higher levels of TBARs.

The second step consisted to evaluate the effects of three exercise protocols in Wistar rats (120 days) on metabolic syndrome markers. Quarter of the animals were kept sedentary (C) during all the experiment (120-180 days); a quarter animals trained in the aerobic protocol (A) (80% of minimal lactate) 1h day, 5 days week, for 60 days; a quarter trained in strength exercise (S), consisted of 4 series of 10 jumps with 1 minute interval , 5 days a week, for 60 days; and the last quarter trained in the concurrent protocol 3 days in the aerobic protocol and 2 days in the strength protocol. At the end of experiment, we performed: oral glucose tolerance test (oGTT), peripheral insulin test (ITT), NAFLD markers- aspartate aminotransferase and alanine aminotransferase, lipids concentration in the liver, lipogenesis rate, biomarkers of antioxidant system (Catalase and Superoxide dismutase) and lipid peroxidation markers (TBARs). Groups A and S showed

reduction on body weight compared to C group. Moreover, all exercised animals revealed a reduction on the liver and adipose tissues concentrations and a reduction in the lipid peroxidation markers (TBARS) and higher activity of SOD in the serum. The A group animals, presented an elevation of the liver SOD and CATALASE activities. Concluding, all exercise protocols improved the anti-oxidant system and reduced the triglycerides storages in the animals at the end of experiment.

The third and last step, evaluated three different exercise protocols in animals with metabolic disturbs induced by a fructose rich diet. For this experiment, we used 40 Wistar rats (120d old) to produce five different groups (n=8 animals per group): C (control), F (fructose rich diet fed and sedentary), FA (Fructose rich diet fed and exercised on aerobic protocol), FAS (Fructose rich diet fed and exercised on concurrent protocol) and FS (Fructose rich diet fed and exercised on strength protocol). At the end of experiment, we evaluated the glucose regulation (trough oGTT and ITT), blood biochemistry, oxidative stress, inflammation and triglycerides content in different tissues. The strength protocol provided stronger effects in the glucose regulation compared to all other protocols. All exercise protocols provided remarkable responses like reduced inflammation stress, reduced triglycerides content in different tissues and reduced oxidative damage (FA and FS). Concluding, the physical exercise protocols provided truly and important systemic responses in animals fed on a fructose rich diet.

Key words: Wistar rats, fructose, metabolic syndrome, nonalcoholic fatty liver disease, oxidative stress.

Sumário

1. Introdução

Clinicamente, a síndrome metabólica, também conhecida como síndrome X ou síndrome de resistência à insulina, compreende um espectro de distúrbios, com a intolerância à glicose representando uma das mais importantes. Essas alterações incluem resistência à insulina, com ou sem diabetes mellitus tipo dois e hipertensão.

Estima-se que a prevalência da síndrome metabólica seja de 24% da população adulta e entre 50-60% na população acima de 60 anos nos Estados Unido, tornando esta síndrome grave problema um médico-social (SAMAD et al, 1999). Um aspecto que merece destaque é que há evidências clínicas e epidemiológicas da associação entre o consumo progressivo de frutose, largamente empregada como adoçante em refrigerantes e outros alimentos, e o desenvolvimento da síndrome metabólica (ELLIOT, et al, 2002; BRAY et al., 2004; NAKAGAWA et al., 2005; DHINGRA et al., 2007).

Na identificação da síndrome metabólica, existem controvérsias, pois não há um único critério internacional com a descrição definitiva. Reaven (1988) sugeriu a forte associação

existente entre indivíduos com os mesmos fatores de risco e designou de síndrome "X". Seu denominador comum era representado pela resistência à insulina. Na ocasião, propôs cinco consequências, todas com grande risco de doença cardiovascular: intolerância à glicose, hiperinsulinemia, aumento de triglicerídeos, diminuição do colesterol HDL e hipertensão arterial. A obesidade e a inatividade física aumentam a resistência à insulina e, portanto, agravam a síndrome. Entretanto, essa síndrome pode ser encontrada em indivíduos sadios, com peso e tolerância à glicose normais (ZAVARONI et al., 1989). De acordo com Duncan & Schimidt (2001), a síndrome metabólica caracteriza-se por um agrupamento, em populações, de fatores de risco para doenças cardiovasculares e diabetes, geralmente ligados à resistência à insulina e à obesidade central.

Recentemente, a Associação Europeia para o Estudo do Diabetes (EASD), em conjunto com a Associação Americana de Diabetes publicou posicionamentos a respeito da síndrome metabólica. De acordo com essas associações, a síndrome metabólica, que é considerada como preditor de doenças cardiovasculares, é definida inadequadamente, usada de maneira inconsistente, sendo necessárias mais pesquisas para ajudar na compreensão da maneira pela qual deve ser tratada. Ainda segundo essas associações, a síndrome metabólica é geralmente definida pela presença de três ou mais das seguintes características: alta circunferência da cintura, concentrações sanguíneas elevadas de triglicerídeos, pressão alta, colesterol HDL circulante reduzido e altas concentrações de glicose sanguínea. Tomadas individualmente, cada uma das condições citadas é considerada como fator de risco para doença cardiovascular e deveria ser tratada como tal, afirmam os autores (KAHN et al, 2005; GALE, 2005).

A Organização Mundial de Saúde (OMS) oferece uma definição um pouco diferente, incluindo qualquer um que tenha diabetes ou resistência à insulina e duas das seguintes

características: elevada razão cintura/quadril, concentrações elevadas de triglicerídeos ou quão baixa a concentração de colesterol HDL, pressão alta e alta excreção urinária de albumina (OMS, 1998). O conjunto dessas observações e divergências denota a urgência na ampliação dos estudos referentes aos mais pronunciáveis marcadores dessa síndrome.

1.1.Esteatose hepática não alcoólica e síndrome metabólica

A Esteatose hepática não alcoólica (EHNA) está emergindo como componente da síndrome metabólica (HANLEY et al., 2005). Essa é a desordem hepática mais frequente nos Estados Unidos, acometendo cerca de 30 milhões de pessoas (ÂNGULO, 1999; CLARK; BRANCATI; DIEHL 2003). Desenvolve-se de forma mais acentuada em adultos, porém, o número de crianças acometidas por essa doença vem crescendo acentuadamente (BROWNING; HORTON, 2004).

Suas causas mais comuns incluem obesidade, diabetes mellitus, desnutrição, ingestão de álcool e toxicidade em consequência de drogas. Sugeriu-se também que comprometimento do metabolismo lipídico, especialmente hipertrigliceridemia, cause esteatose hepática (RODEN 2006). A esteatose hepática parece acometer pessoas com idade mais avançada, enquanto 2,6% da população de crianças são portadores da desordem, 26% dos adultos entre 40 e 59 anos possuem a EHNA (GUPTE et al, 2004). Além disso, o seu aparecimento está correlacionada a presença de diabetes tipo 2 (50%), obesidade (76%) ou obesos mórbidos com diabetes tipo 2 (95%) (DEL GAUDIO et al, 2002).

Esteatose hepática não alcoólica (EHNA) refere-se a um espectro de alterações, variando desde esteatose hepática simples até manifestações mais severas, incluindo esteato-hepatite, que pode evoluir para fibrose, cirrose e falência hepática (MULHALL et al, 2002). EHNA é

diagnosticada na clínica através de biópsia hepática, técnica que dificulta sua aplicação em estudos epidemiológicos. Contudo, indivíduos portadores de EHNA tipicamente apresentam concentrações séricas elevadas de marcadores de lesão hepática, incluindo aspartato aminotransferase (AST) e alanina aminotransferase (ALT) (MUKAI et al., 2002) e sugeriu-se que EHNA seja a causa mais comum da elevação crônica das concentrações séricas das transaminases hepáticas (CLARK et al., 2003). Essas observações indicam que marcadores de lesão hepática podem ser também marcadores confiáveis de EHNA em estudos epidemiológicos (MUKAI et al., 2002).

Foi relatado que portadores de EHNA apresentam elevada prevalência de síndrome metabólica (MARCHESINI et al., 2001). Além disso, marcadores hepáticos mostraram-se correlacionados à síndrome metabólica em grandes amostras representativas da população em geral (CLARK et al., 2003 b) e existem evidências de que ALT e a razão AST/ALT predizem, de maneira significativa, a incidência da síndrome metabólica (HANLEY et al., 2005)

Ratos diabéticos por administração de estreptozotocina (DOMINGO et al., 1992; WAKAME, 1999) ou aloxana (KIM et al., 2006) apresentam aumento na atividade sérica das enzimas AST e ALT. Por outro lado, a alimentação de ratos com dieta rica em sacarose não alterou a atividade dessas enzimas no fígado (YAMINI et al., 1991)

O interesse na compreensão das causas e consequências da infiltração lipídica do fígado foi dinamizado em anos recentes pela constatação de que existe associação entre o acúmulo de triglicerídeos em células diferentes de adipócitos e o desenvolvimento de um estado de resistência à insulina (SALTIEL; KAHN 2001). Estudos em camundongos desprovidos de gordura demonstraram que acúmulo de gordura no fígado e nos músculos esqueléticos está associado com defeitos na sinalização insulina e resistência à esse hormônio (KIM et al., 2000, REUE et al.,

2000). Estudos em seres humanos também indicaram que o teor hepático de gordura está intimamente associado com as causas da resistência à insulina, tais como a obesidade (MARCEAU et al., 1999), e com defeitos na ação da insulina na supressão da produção de glicose, independente da obesidade (SEPPALA-LINDROOS et al., 2002). Estes dados fornecem apoio para o conceito emergente de que acumulo de gordura em tecidos insulino-sensíveis é deletério para a ação do hormônio. Por conseguinte, a redução do acúmulo hepático de gordura passou a ser considerado um novo alvo terapêutico no problema global da resistência à insulina.

1.2. Frutose e síndrome metabólica

O consumo excessivo de frutose está entre os fatores que podem conduzir ao acúmulo hepático de gorduras. Nas últimas décadas seu emprego se disseminou em refrigerantes, bolos, massas, entre outros alimentos. Isso gerou um aumento no consumo de frutose de aproximadamente 500% entres as décadas de 70 e 90 (RUMESSEN, 1992). Existem diversas fontes desse nutriente, entre as mais usadas pela indústria estão a sacarose, obtida da cana de açúcar no Brasil e o HCFS (high corn fructose syrup) obtido a partir do milho principalmente nos EUA.

Na década de 70 o consumo de frutose foi estimulado embasado em diversos estudos que apontavam que esse nutriente não induzia a produção e secreção da insulina, o que em tese seria altamente recomendável para diabéticos do tipo 2, intolerantes à glicose ou com a sensibilidade à insulina alterada. Com o avanço das pesquisas, o papel da frutose como alimento para diabéticos foi desmistificado, uma vez que diversos estudos apontaram que o consumo continuado de altas doses do nutriente acarretava aumento dos triglicerídeos hepáticos (Figura 1). Isso implica na alteração da sensibilidade à insulina, além de gerar aumento das concentrações de ácido úrico circulantes, hipercolesterolemia e dislipidemias. (BASCIANO et al, 2005).

Absorvida no intestino, a frutose então é lançada a corrente sanguínea, onde é transportada ao sistema porta hepático. Já no fígado, o processo de captação da frutose pelo hepatócito se deve aos transportadores de glicose-5 (GLUT-5). Dentro da célula, é transformada em frutose 1-fosfato (FRUTOSE 1-P), reação essa modulada pela frutoquinase. A frutose 1-P por sua vez pode ser convertida em gliceraldeido ou diidroxicetonafosfato. O gliceraldeido é então transformado em gliceraldeido 3-fosfato (GLICERALDEIDO 3-P), que por sua vez irá se transformar em piruvato. Este entrará na cadeia de respiração celular ou então produzir acetil-Coa, que fornecerá esqueletos de carbono para a síntese acil-Coa (ACILCOENZIMA A). Já a diidroxicetonafosfato será convertida em glicerol 3-P, que se ligará às moléculas de acil-Coa, convertendo-se em acil-glicerol que será utilizado na síntese de triglicerídeos ou, pela adição de moléculas de ApoB, na produção do VLDL “very low density cholesterol” (BASCIANO et al., 2005). Esta molécula de VLDL tem a função de carrear estes triglicerídeos a outras partes do organismo. Após a liberação de grandes quantidades de triglicerídeos o VLDL se transforma em LDL “low density cholesterol” que pode se acumular nas ranhuras dos vasos sanguíneos contribuindo para o processo aterosclerótico ou retornar ao fígado através do sistema circulatório e ser utilizado na produção dos sais biliares.

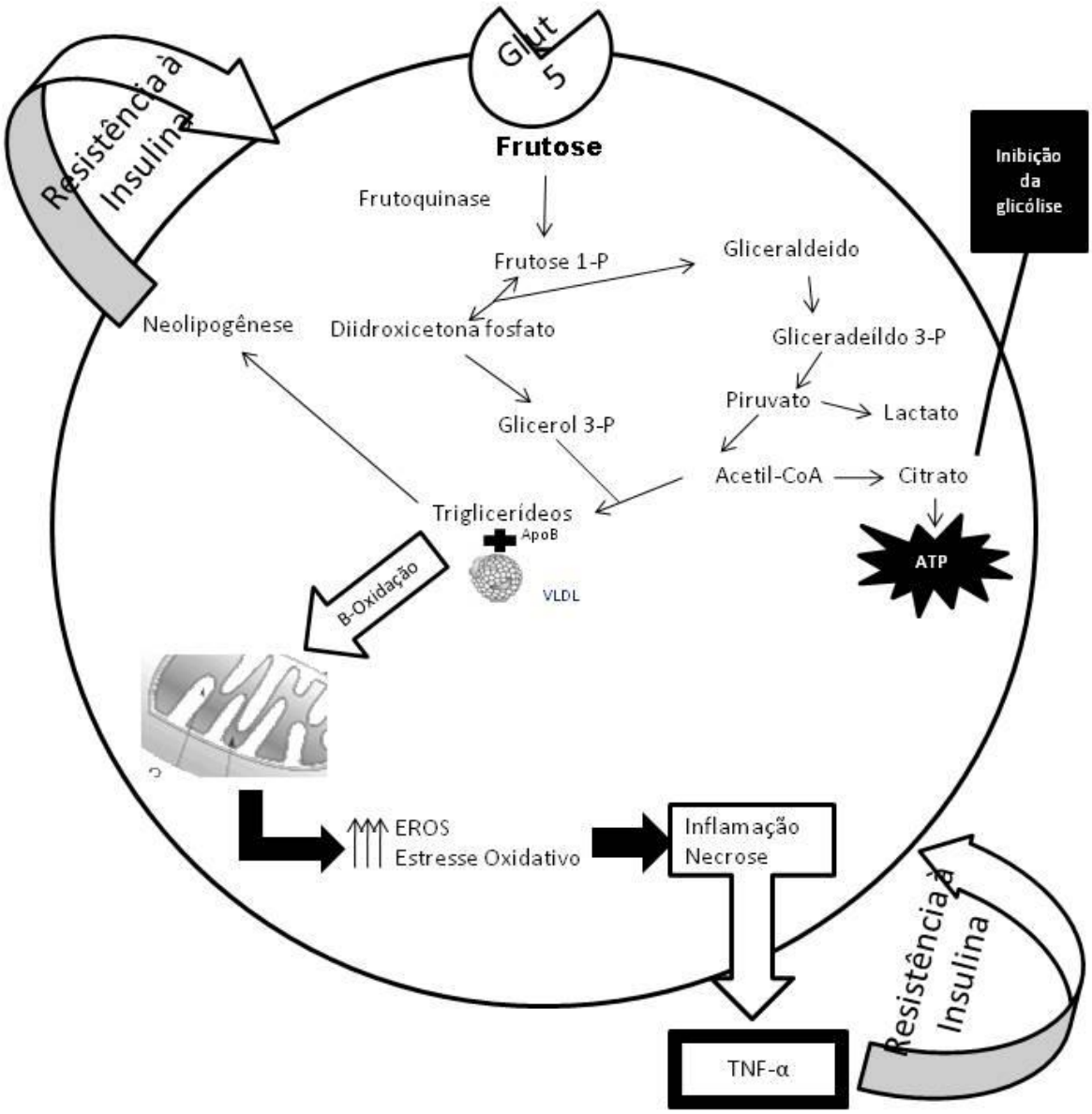

Figura1. Mecanismo da resistência à insulina em consequência do excesso de consumo de frutose (Botezelli et al., 2010 motriz).

O consumo excessivo de frutose leva a um aumento da produção de triglicerídeos, e consequente hipertrigliceridemia. Esse aumento nas concentrações de triglicerídeos no fígado passa agora a ser regulado pelo fígado, que tenta captar o excesso deste nutriente na corrente sanguínea. O aumento de reservas triglicerídeas no hepatócito leva a resistência à insulina,

formação de espécies reativas de oxigênio (EROS) e liberação de citocinas inflamatórias (BROWNING; HORTON, 2004; DANDONA et al., 2004). O aumento do estresse oxidativo e o processo inflamatório podem culminar, em longo prazo, na apoptose do hepatócito e necrose hepática. Além disso, importantes citocinas inflamatórias como o fator de necrose tumoral alfa (TNF-α) e interleucina 6 (IL-6) são liberados na corrente sanguínea. Estas citocinas são potentes inibidores da ação da insulina bem como potentes inibidores da produção e da ação da adiponectina, hormônio muito importante para a sensibilidade à insulina). A redução da sensibilidade à insulina e as altas concentrações de triglicerídeos séricos levam a aumentos nas concentrações de glicose, LDL-colesterol, colesterol total, insulina e redução no HDL-colesterol (BASCIANO et al, 2005. Todas estas alterações são componentes da síndrome metabólica e devem ser modificadas por mudança dietética, tratamentos medicamentosos ou através do aumento do dispêndio calórico gerado pelo exercício físico.

1.3. Exercício físico e síndrome metabólica.

Diversos estudos apontam o efeito benéfico do exercício sobre os distúrbios metabólicos das últimas décadas (MOTA et al, 2008; HICKMAN et al., 2004). Como apontado anteriormente, a OMS define a síndrome metabólica como a presença de diabetes tipo 2 ou resistência à insulina mais dois dos seguintes fatores: elevada razão cintura/quadril, concentrações elevadas de triglicerídeos ou baixa concentração de colesterol HDL, pressão alta e alta excreção urinária de albumina (OMS, 1991).

Em um estudo longitudinal, Lee e colaboradores (2011) avaliaram os potenciais efeitos do exercício físico na redução da mortalidade em milhares de indivíduos com grande potencial de desenvolvimento de doença do aparelho circulatório. Neste estudo, cada incremento de 1-MET

(equivalente metabólico) gerava uma resposta de redução de 15-19% no risco de morte por distúrbios vasculares.

Diferentes estudos têm demonstrado relação direta entre nível de atividade física e sensibilidade à insulina (LAKKA et al., 2003; SCHNEIDER et al., 1995). Evidenciou-se menor concentração circulante de insulina e maior sensibilidade ao hormônio em atletas, quando comparados a indivíduos sedentários (EBELING et al., 1993; NUUTILA et al, 1994). Atletas másters apresentam maior proteção contra o comprometimento da tolerância à glicose associada ao envelhecimento (ROGERS et al., 1989).

Em trabalhos publicados por Ericsson (1997), Khan (1990) e Miller (1994), demonstrou-se que uma única sessão de exercício físico aumenta a captação de glicose mediada pela insulina em sujeitos normais, indivíduos com resistência à insulina com histórico familiar de diabetes tipo 2, obesos com resistência à insulina, bem como em portadores de diabetes tipo 2. O treinamento físico melhora a tolerância à glicose e a sensibilidade à insulina em indivíduos saudáveis, em obesos não-diabéticos e em portadores de diabetes tipo 1 e 2. (ERICSSON et al., 1997; KHAN et al., 1990; MILLER et al., 1994). O efeito do exercício físico sobre a tolerância à glicose e a sensibilidade à insulina tem sido constatado de 12 à 48 horas após a sessão, porém volta aos níveis basais de repouso de três a cinco dias após a última sessão de exercício físico (ERICSSON et al., 1997). Além disso, Verifica-se efeito benéfico sobre a sensibilidade à insulina tanto com exercícios aeróbios quanto com exercícios de força (PERSGHIN et al., 1996; POLLOCK et al., 2000; IVY et al., 1997; CIOLAC et al., 2002).

Shiva e colaboradores (1999), obtiveram sucesso em induzir obesidade em animais através da frutose. Como é um nutriente altamente lipogênico, a frutose pode induzir uma alteração na composição corporal. Por outro lado, estudos apontam o papel benéfico do exercício na redução

ponderal de peso, diminuição da adiposidade corporal, diminuição da razão cintura/quadril. (CIOLAC et al., 2004; HICKMAN et al. 2004). McArdle & Katch (1998), apontam que o exercício fisco é responsável por 20% do gasto energético em indivíduos sedentários e pode ultrapassar os 40% em indivíduos regularmente treinados. Uma maior massa muscular gera um aumento no metabolismo basal. Além disso, o músculo esquelético pode aumentar seu gasto energético em até 100 vezes durante o exercício físico.

A alteração nas concentrações de triglicerídeos circulantes é o primeiro e talvez principal mecanismo de indução da esteatose hepática tanto em modelo experimental como em humanos. A infiltração lipídica, em longo prazo, pode acarretar problemas adversos para o fígado, assim como a manutenção de elevados níveis de triglicerídeos circulantes pode causar leve resistência à insulina que posteriormente poderá evoluir para diabetes tipo 2, dislipidemias, aterosclerose, obesidade central entre outras alterações (QIN, et al., 2007; RECTOR, et al., 2008; MOTA, et al., 2007; HICKMAN, et al., 2003; UENO, et al., 1997; HUANG, et al., 2006) descritos na Tabela 1.

Tabela 1. Efeitos do exercício físico e da dieta de restrição calórica sobre marcadores da esteatose hepática não alcoólica e da síndrome metabólica.

Espécie	**Tratamento**	**Duração**	**Efeitos do exercício físico e da dieta de restrição calórica sobre marcadores de EHNA e síndrome metabólica**	Referência
Ratos	Exercício Físico Voluntário	2 semanas	Melhora na sensibilidade à insulina e tolerância à glicose	**Qin et al. (2007)**
Ratos	Exercício Físico Voluntário	16 semanas	Menor peso corporal, maior sensibilidade à insulina, maior tolerância à glicose, menores níveis de AGL e TG circulantes.	**Rector et al. (2008)**
Ratos	Exercício Físico	12 semanas	Prevenção da intolerância à glicose em ratos submetidos à administração de aloxana.	**Mota et al. (2008)**
Humanos	Dieta/ Exercício	15 meses	Diminuição do peso corporal e melhora no quadro de esteatose hepática	**Hickman et al. (2003)**
Humanos	Dieta/ Exercício	3 meses	Diminuição das concentrações de ALT, melhora no quadro de esteatose hepática.	**Ueno et al. (1997)**
Humanos	Dieta/ Exercício	16 semanas	Melhora no oGTT, diminuição do peso corporal.	**Rice et al. (1999)**
Humanos	Dieta/ Exercício	12 meses	Nenhuma melhora nos níveis de ALT.	**Huang et al. (2006)**

Botezelli et al. Consumo de frutose e exercício físico, impacto na síndrome metabólica. Motriz, 2004.

Estudos de Bezerra e colaboradores (2001) apontam que o exercício físico é um potente regulador das dislipidemias induzidas por uma dieta rica em frutose e/ou sedentarismo. A concentração de triglicerídeos circulantes pode ser reduzida pela atividade física, porém, esse processo se revela a partir da manutenção do exercício físico por um tempo relativamente grande. Essa redução se dá principalmente pelo aumento do gasto energético e diminuição da adiposidade corporal.

2.Objetivo

O presente estudo visou avaliar os efeitos singulares de três protocolos de exercício físico na prevenção e tratamento de complicações metabólicas desencadeadas por uma dieta rica em frutose.

Os resultados dessa pesquisa fornecem informações para aprofundar o entendimento do papel do exercício físico na prevenção e tratamento dos marcadores da síndrome metabólica, estresse oxidativo e inflamação.

2.1 Objetivos específicos

Para atingir o objetivo, este estudo foi dividido em três fases distintas.

Na primeira, avaliou-se os impactos deletérios da dieta rica em frutose em ratos Wistar (*Rattus Norvegicus)* alimentados com dieta rica em frutose (semi-urificada-60%frutose).

Para tal, determinamos a transição metabólica aeróbia/anaeróbia individual de ratos Wistar adultos (120 dias de idade) eutróficos, durante o exercício físico de natação pelo protocolo do Lactato Mínimo (LacMin), a tolerância à glicose e insulinemia (teste de tolerância à glicose oral-oGTT), sensibilidade periférica à insulina (teste de tolerância à insulina-ITT), marcadores da síndrome metabólica, marcadores da EHNA através da dosagem da razão da AST e ALT, status antioxidante (através da atividade das enzimas Catalase – CAT e Superóxido Dismutase - SOD) , biomarcadores de peroxidação lipídica (pelas substâncias que reagem ao ácido tiobarbitúrico – TBARs), lipogênese hepática e concentrações de triglicerídeos no fígado e tecido adiposo de animais alimentados com dieta rica em frutose dos 120 aos 180 dias;

Na segunda etapa, foram avaliados três diferentes protocolos de exercício físico (Aeróbio, Concorrente e de Força) em marcadores da síndrome metabólica e fatores associados em ratos alimentados com dieta balanceada.

Nesta etapa, foi determinada a transição metabólica através do LacMin e os efeitos do treinamento por natação em intensidade equivalente a 80% do LacMin (protocolo aeróbio), por exercício de salto suportando carga de 50% do peso corporal (protocolo força) e por exercício aeróbio associado ao exercício força (protocolo concorrente) sobre: tolerância à glicose (oGTT), sensibilidade periférica à insulina (ITT), marcadores da síndrome metabólica e concentrações de triglicerídeos no fígado e tecido adiposo dos animais treinados;

Por último, avaliamos os efeitos dos três protocolos de exercício físico no tratamento e prevenção de distúrbios metabólicos induzidos pela dieta rica em frutose.

Nesta etapa, foi determinada a transição metabólica individual de cada animal para posteriormente, verificar os efeitos dos três protocolos de exercício físico (Aeróbio, Concorrente e de Força) no peso corporal, na tolerância à glicose e na insulinemia (teste de tolerância à glicose oral-oGTT); sensibilidade periférica à insulina (teste de tolerância à insulina-ITT); nos marcadores bioquímicos sanguíneos (glicose, colesterol e triglicerídeos); nos marcadores da EHNA através da dosagem de concentrações de aspartato aminotransferase (AST) alanina aminotransferase (ALT) e histologia do fígado; no status antioxidante (através da atividade das enzimas Catalase – CAT e Superóxido Dismutase - SOD); nos biomarcadores de peroxidação lipídica (pelas substâncias que reagem ao ácido tiobarbitúrico – TBARs); na lipogênese hepática e nas concentrações de triglicerídeos no fígado e tecido adiposo e nos marcadores de inflamação (IL-1β, IL4, IL-6, IL-10, Interferon e TNF-α) em animais alimentados com dieta rica em frutose;

3.Materiais e Método

3.1 Animais e seu tratamento

Ratos da linhagem Wistar, adultos (120 dias), que foram mantidos em gaiolas coletivas, à temperatura de 25 ± 1º C e em ciclo claro/escuro de 12/12 horas, com livre acesso a água e ao alimento. Todos os animais tiveram peso corporal, ingestão alimentar e hídrica registrados uma vez por semana durante o experimento.

3.2 Aspectos Éticos

Todos os procedimentos realizados nos animais foram submetidos à apreciação e aprovados pela Comissão de Ética no uso de Animal em pesquisa (CEUA), Instituto de Biociências da UNESP, Campus Rio Claro, decisão n 005/2010. As carcaças remanescentes foram coletadas por uma empresa vinculada à Prefeitura Municipal de Rio Claro, para adequada disposição.

3.3 Dietas

Durante o experimento, o alimento consistiu de dietas semipurificadas, isocalóricas, balanceada ou rica em frutose. Foi utilizada a ração comercial Labina® como dieta controle. Para a dieta rica em frutose, realizamos uma adaptação da dieta padronizada por Bezerra e colaboradores (2004) composta por (em g/kg) 202 de caseína, 625,5 de frutose, 2 de l-cisteína, 50 de óleo de soja, 35 de mistura de sais minerais (AIN-93MX) (REEVES et al, 1993), 10 de mistura de vitaminas (AIN-93MX) (REEVES et al, 1993), 50 de fibra e 2,5 de cloridrato de colina.

3.4 Adaptação ao Meio Líquido

Todos os animais foram primeiramente adaptados ao meio aquático. A adaptação foi executada durante dez dias ininterruptos no mesmo tanque em que o treinamento foi realizado, com temperatura da água mantida em 31 ± 1°C (GOBATTO et al., 2001). O propósito da adaptação

foi de reduzir o estresse causado aos animais e impedir possíveis adaptações fisiológicas que poderiam aprimorar a capacidade física dos animais.

3.4.1 Adaptação ao Protocolo de Treinamento Aeróbio

Os ratos foram inseridos em água rasa por três dias durante 10 minutos. Posteriormente, o nível da água foi aumentado, bem como o tempo de duração do esforço e a carga (1% do peso corporal na forma de lastros de chumbo, inseridos em bolsa de velcro®, acoplada ao tórax) sustentada pelo animal. Assim, no quarto dia, os ratos nadaram em água profunda por cinco minutos, com acréscimo de dez minutos a cada dia até o décimo dia de adaptação (GOBATTO et al., 2001).

Tabela 2. Adaptação ao protocolo de treinamento aeróbio:

Dias	Água	Tempo	Carga (%PC)
1	10%	10	0
2	10%	10	0
3	10%	10	0
4	50%	10	0
5	100%	10	0
6	100%	10	0
7	100%	15	1
8	100%	20	1
9	100%	25	1
10	100%	30	1

Fig2. Adaptação ao protocolo aeróbio.

3.4.2. Adaptação ao Protocolo de Treinamento de Força

Os ratos foram alocados em tanques com agua rasa (31 ± 1°C) por 10 minutos nos dois primeiros dias. No terceiro, quarto e quinto dia, o nível da água foi aumentado e os animais foram mantidos nos tanques durante 5, 10 e 15 minutos consecutivamente. No sexto e sétimo dia foram acoplados ao tórax dos animais uma "mochila" de Velcro® com sobrecarga de 30% do peso

corporal e os animais foram mantidos no tanque com água rasa. Nos três últimos dias, os animais realizaram dez saltos com 30% de sobrecarga acoplados ao tórax em níveis progressivos de água (25, 50 e 100% da capacidade máxima do tanque) (ROGATTO; LUCIANO, 2001).

Tabela 3. Adaptação ao protocolo de treinamento de força:

Dias	Água	Tempo	Carga (%PC)
1	10%	10	0
2	10%	10	0
3	30%	5	0
4	50%	10	0
5	100%	15	0
6	10%	10	30%
7	10%	10	30%
8	25%	10 saltos	30%
9	50%	10 saltos	30%
10	100%	10 saltos	30%

Fig3. Adaptação ao protocolo aeróbio.

3.4.3. Adaptação ao Protocolo de Treinamento Concorrente

Os animais treinados no grupo concorrente passaram por ambas as adaptações, sendo estas realizadas em períodos diferentes, com oito horas de intervalo entre cada um.

3.5 Teste do Lactato Mínimo

Durante o exercício, existe uma zona de transição metabólica a partir da qual ocorre a mudança da predominância aeróbia para a anaeróbia, sendo essa zona extremamente importante para o condicionamento físico, treinamento e rendimento desportivo. Por essa razão, diversas investigações a cerca dessa zona de transição vêm sendo realizadas nas últimas décadas, resultando em diferentes protocolos de avaliação. Grande parte desses protocolos de avaliação utiliza a

resposta do lactato sanguíneo devido à fidedignidade dessa variável sanguínea na mensuração da intensidade de transição metabólica bem como excelente resposta ao treinamento físico, permitindo além da caracterização do esforço, acompanhamento da eficiência do treinamento.

No presente estudo, a transição metabólica aeróbia/anaeróbia durante a natação bem como a avaliação do condicionamento aeróbio dos animais foi efetuada pelo protocolo de lactato mínimo (LacMin). Esse teste baseia-se no princípio que durante teste de exercício com cargas progressivas realizados imediatamente após uma sessão de exercício máximo, que induz hiperlactacidemia, o lactato sanguíneo inicialmente declina para um valor mínimo para em seguida, aumentar novamente. Esse valor mínimo de lactato sanguíneo (LacMin) indica o limiar anaeróbio (TEGTBUR et al., 1993).

Para a realização do teste, inicialmente, os animais foram colocados individualmente em tanques (100 X 80 X 80cm) contendo água a 31 ± 1°C, suportando sobrecarga de 13% do peso corporal (sobrecarga utilizada para provocar hiperlactacidemia) e exercitaram-se durante 30 segundos. Após 30 segundos de repouso, foram submetidos à natação com carga de 13% até a exaustão. Decorridos 9 minutos de repouso, foi coletada amostra de sangue em capilares (25 µl) através de corte na extremidade distal da cauda, para a determinação da concentração de lactato e os animais iniciaram exercício com cargas progressivamente maiores (ARAUJO et al., 2007). A carga inicial foi de 2,0% do peso corporal, sendo acrescida de 0,5% a cada 5 minutos, até a exaustão. A cada troca de carga foi coletada amostra de sangue (25µl) para dosagem de lactato. LacMin foi determinado a partir de uma curva polinomial de segunda ordem ajustada à curva lactato sanguíneo por carga de trabalho. As concentrações de lactato sanguíneo foram determinadas em analisador de lactato (modelo YSI 1500 Sport, YellowSprings, OH, EUA). A menor concentração de lactato na curva representa, teoricamente, a máxima intensidade do

exercício onde sua produção se dá na mesma proporção que sua remoção (VOLTARELLI et al., 2005)**.**

3.6 Treinamento Físico

3.6.1 Treinamento Aeróbio

Consistiu de exercício de natação, em tanques coletivos, contendo água a 31±1°C, uma hora por dia, cinco dias por semana, com sobrecargas (chumbos inseridos em bolsas fechadas com Velcro® e atadas ao tórax com auxílio de um elástico), equivalentes à 80% da transição metabólica aeróbia/anaeróbia individual, identificada pelo protocolo de lactato mínimo.

3.6.2 Treinamento de Força

Consistiu de exercício de saltos na água a 31±1°C, com sobrecargas (chumbos inseridos em bolsas fechadas com Velcro® e atadas ao tórax com auxílio de um elástico) equivalentes a 50% do peso corporal de cada animal. A sessão de treinamento foi composta por quatro séries de dez saltos na água com um minuto de intervalo entre as séries e acontecerá uma vez por dia e cinco dias por semana.

3.6.3 Treinamento Concorrente

Os animais a serem treinados pelo protocolo concorrente realizaram exercício aeróbio três vezes por semana, intercalado com exercício força duas vezes por semana.

3.7 Delineamento dos grupos experimentais

Este estudo está sendo conduzido em três séries de experimentos. A figura 2 apresenta o desenho experimental ilustrado.

Na primeira série de experimentos visou verificar, em animais alimentados com dieta rica em frutose dos 120 aos 180 dias de idade: tolerância à glicose (teste de tolerância à glicose); sensibilidade periférica à insulina (teste de tolerância à insulina); marcadores de EHNA (concentrações séricas de aspartato aminotransferase-AST e alanina aminotransferase-ALT); bem como determinar, no fígado, as concentrações de triglicerídeos, a taxa de lipogênese, a histologia (hematoxilina-eosina) e a atividade de enzimas antioxidantes (catalase – CAT e superóxido dismutase - SOD) e biomarcadores de peroxidação lipídica (substâncias que reagem ao ácido tiobarbitúrico – TBARs). Nessa etapa serão formados dois grupos de animais dos 120 aos 180 dias de idade:

- Controle (C): alimentados com a dieta balanceada (AIN-93);
- Frutose (F), alimentados com a dieta rica em frutose.

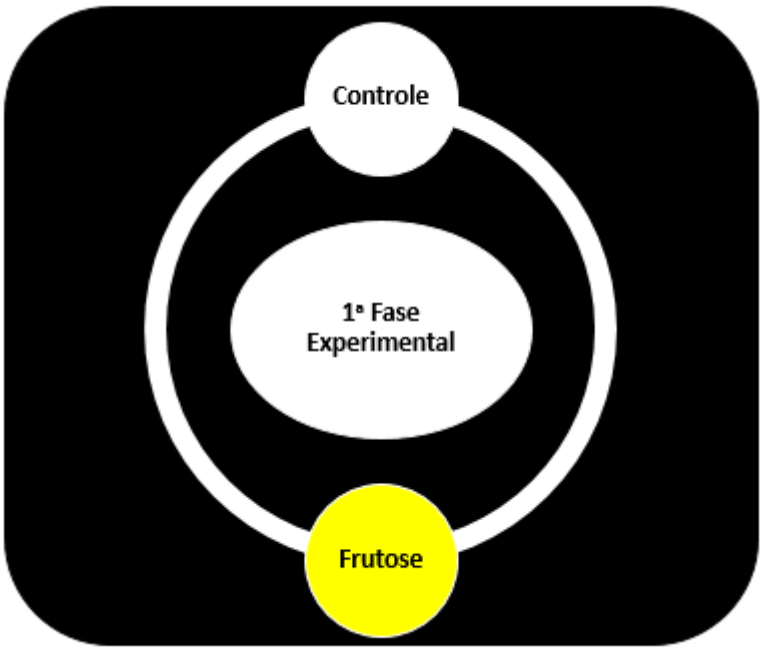

Figura2. Delineamento Experimental da 1ª Fase.

A segunda teve por objetivos determinar a transição metabólica aeróbia/anaeróbia individual de ratos eutróficos durante exercício de natação pelo protocolo de LacMin e analisar os efeitos do treinamento por natação na intensidade equivalente a 80% do LacMin (protocolo aeróbio); do treinamento por exercício de salto suportando 50% do peso corporal (protocolo força); e do protocolo combinado de exercício aeróbio e força (protocolo concorrente) sobre: tolerância à glicose (teste de tolerância à glicose oral), sensibilidade periférica à insulina de insulina (teste de tolerância à insulina), capacidade aeróbia (LacMin), perfil lipídico circulante (triglicerídeos, colesterol total, colesterol HDL e colesterol LDL) e concentração hepática de triglicerídeos após o treinamento desses animais.

Nessa etapa, formaram-se 4 grupos experimentais:

- Controle (C);
- Treinando protocolo Aeróbio (A);
- Treinado protocolo Força (S);
- Treinado protocolo Concorrente (AS);

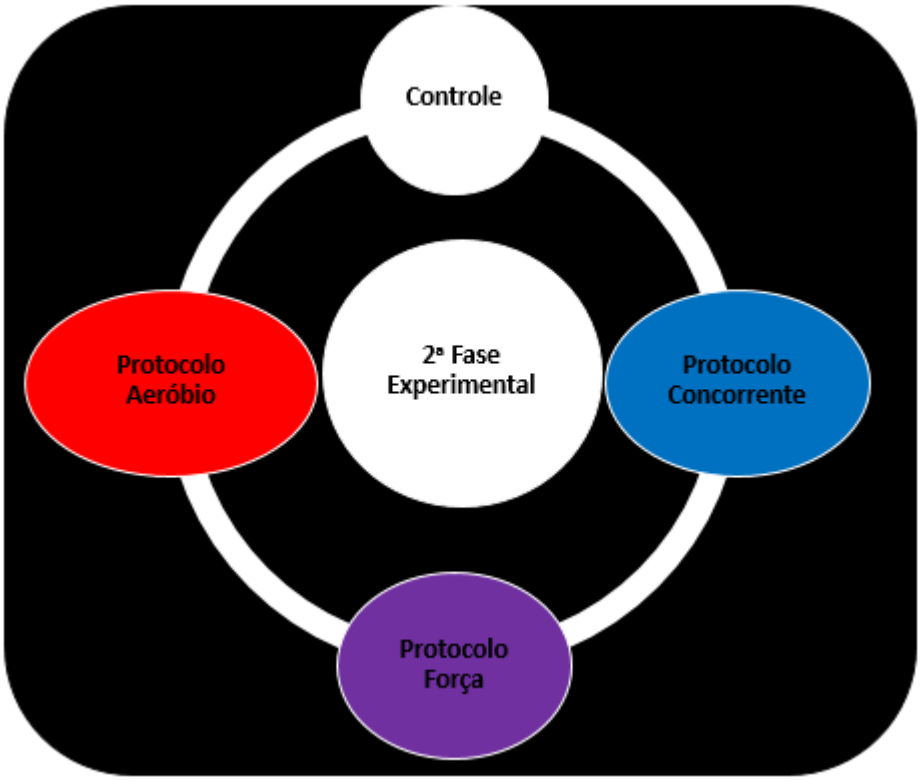

Figura 3. Delineamento experimental da 2ªFase.

A terceira série de experimentos encontra-se em andamento e objetiva avaliar os efeitos dos diferentes protocolos de treinamento físico sobre aspectos metabólicos e marcadores de EHNA e de estresse oxidativo no fígado em ratos submetidos à dieta rica em frutose dos 120 aos 180 dias de idade. Como controles, serão empregados ratos alimentados com dieta balanceada AIN 93. Parte dos animais será submetida aos diferentes protocolos de exercício dos 120 aos 180 dias e outra parte permanecerá sedentária. Dessa forma, serão compostos cinco subgrupos experimentais, na dependência da atividade física e da dieta administradas:

- Controle (C);
- Frutose (F);
- Frutose Aeróbio (FA);
- Frutose Força (FS);
- Frutose Concorrente (FAS);

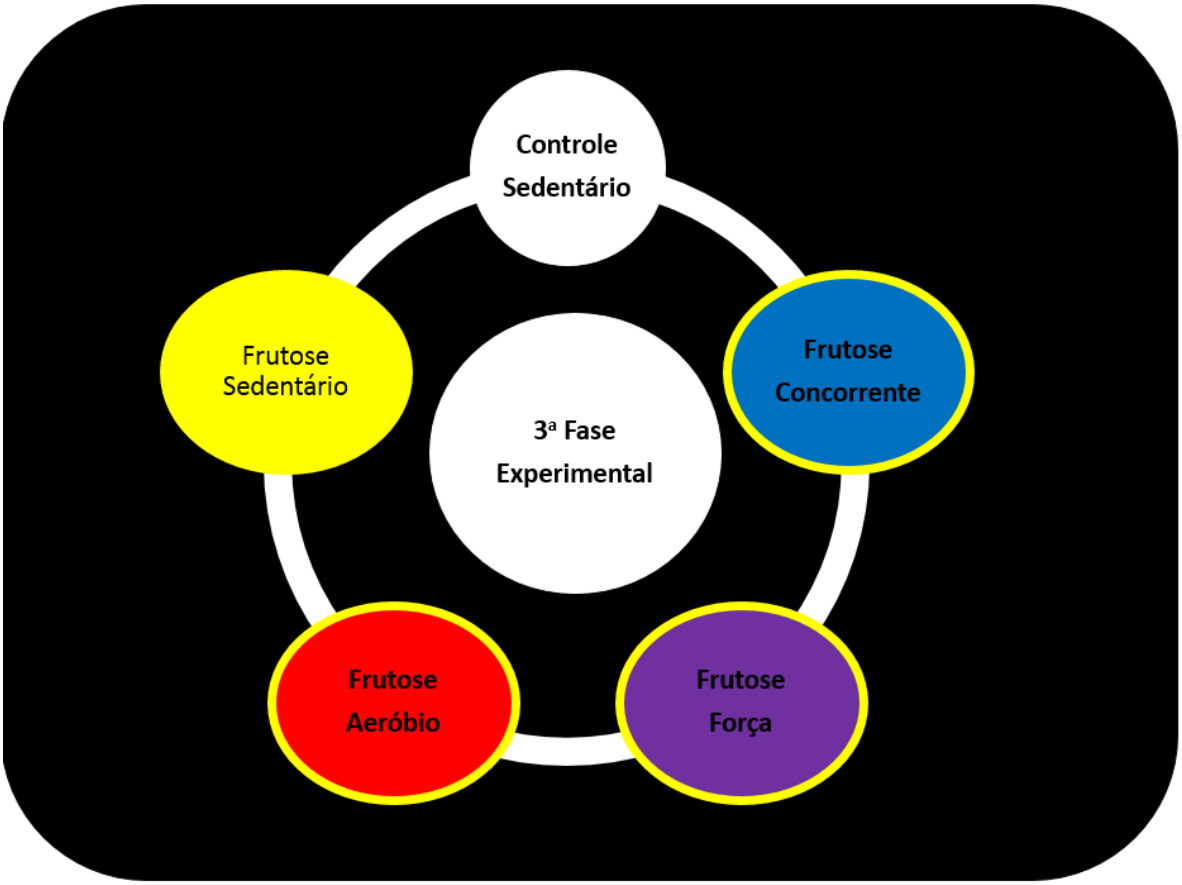

Figura 4. Delineamento da 3ª fase experimental

3.8 Teste de Tolerância Oral à Glicose (oGTT)

O oGTT foi realizado com os animais após 12 horas de jejum. Uma primeira coleta de sangue, feita através de corte na extremidade da cauda do animal (tempo 0). Em seguida, uma solução de glicose a 20% (2 g/Kg de peso) foi administrada aos ratos através de sonda gástrica de

polietileno. Amostras de sangue foram coletadas após 30, 60 e 120 minutos com capilares heparinizados e calibrados para 25µL, visando determinar as concentrações glicose e insulina. As concentrações de glicose sanguínea foram determinadas pelo método glicose-oxidase e as de insulina, pelo radioimunoensaio. Os resultados foram analisados através da determinação das áreas sob as curvas de glicose e de insulina séricas durante o teste pelo método trapezoidal (Mathews et al., 1990), utilizando-se o software Excel 2007 (2007).

3.9 Teste de Tolerância à Insulina

A sensibilidade à insulina foi avaliada através do teste subcutâneo de tolerância à insulina. O teste consistiu da administração subcutânea de insulina (300 mU/kg de peso corporal), seguida por coletas de sangue nos tempos 0, 30, 60 e 120 minutos. A taxa de remoção de glicose sanguínea (Kitt) é expressa em %.minuto^{-1} foi calculada pela fórmula (0, 0693/t/2) X100. A glicose sanguínea t/2 foi calculada pela curva de análise dos mínimos quadrados dos teores de glicose sérica enquanto houver decréscimo linear dos mesmos após a administração de insulina (LUNDBAEK, 1962).

3.10. Obtenção do Material Biológico

Quarenta e oito horas após a última avaliação *in vivo,* os animais foram anestesiados com tiopental sódico (40 mg/kg de peso corporal, intraperitoneal) e posteriormente mantidos em câmara de CO_2 até a completa paralisia respiratória. O sangue foi então coletado a partir da veia hepática e o soro separado para a dosagem das transaminases hepáticas AST e ALT, da fosfatase alcalina e de triglicerídeos (kits comerciais). O fígado foi extirpado e uma alíquota foi utilizada para a determinação da concentração de triglicerídeos (Nogueira et al., 1990). Outra alíquota foi empregada para avaliação de biomarcadores de estresse oxidativo, uma terceira, para determinação da taxa de lipogênese (FOLCH et al., 1956) e a última para a histologia HE.

3.11 Marcadores do Status Oxidante

3.11.1 Biomarcadores do Sistema de Defesa Antioxidante

Catalase

Para a determinação da atividade da Catalase, as amostras do tecido hepático (100-150 mg) foram colocadas em tubos tipo eppendorf contendo 1 ml de tampão fosfato gelado, submetidas à sonicação e centrifugadas 10.000 rpm por 5 minutos. O sobrenadante foi separado e armazenado a – 20 ºC para posterior analise. Os ensaios para dosagem da atividade foram conduzidos adicionando-se as amostras a tampão fosfato 50 mM e peróxido de hidrogênio (H_20_2) 10mM, (AEBI, 1984). O cálculo da atividade da catalase sanguínea foi feito pela seguinte equação: $(2,3/\Delta t)$. (a/b). (log A_1/A_2), onde a é o volume de hemolisado na cubeta e b é o volume total da cubeta, A_1 o valor da absorbância em t=0 e A_2 é o valor da absorbância no tempo final, que em nosso caso se dá aos 15 segundos após o início da reação (AEBI, 1984).

Superóxido Dismutase

Para a determinação da atividade da superóxido dismutase, as amostras do tecido hepático foram submetidas à lavagem em PSB pH 7,4 contendo heparina 0,16 mg/ml para remoção de células de sangue. Logo após o tecido foi então, homogeneizado (em gelo) em 1 ml de tampão HEPES 20 mM, pH 7,2 contendo (em mM): EGTA 1, Manitol 210 e sacarose 70. Em seguida, centrifugado a 10.000 x g por 15 minutos a 4ºC e o sobrenadante foi armazenado a – 20 ºC para dosagem de SOD Total (citoplasmático e mitocondrial) através de kit comercial Cayman®.

3.11.2. Biomarcadores de Peroxidação Lipídica

TBARS

Foram analisadas a quantidade de produtos finais da peroxidação lipídica (peróxidos lipídicos, malondialdeído, e outros aldeídos de baixo peso molecular) que, ao reagirem com o ácido 2-tiobarbitúrico (TBA), formam bases de Schiff. Esses complexos foram coloridos e sua concentração determinada espectrofotometricamente a 5nm, ou por fluorescência a 515nm de excitação e 555nm de emissão (OKAWA et al., 1979). Para a determinação de TBARs, as amostras (100-150 mg) foram colocadas em tubo plástico (tipo RIA) contendo1, 5 ml de tampão fosfato 0,05N frio e homogeneizado no Polytron e centrifugadas por 5 minutos a 10.000 rpm. Separado o sobrenadante, as amostras foram armazenadas a – 20 ºC para posterior análise.

3.12 Taxa de Lipogênese no fígado

A taxa de lipogênese no fígado foi mensurada a partir do uso de água tritiada. O trítio é incorporado em pontes C-H durante a síntese de ácidos graxos. Após 60 minutos de administração da dose, os animais foram mortos e amostras do tecido hepático foram isoladas. Os lipídeos então foram extraídos através do método de Folch et al., (1956) e a radiação emitida foi quantificada através de espectroscópio de cintilação (TI-CARB 2100TR). A síntese de ácidos graxos foi calculada de acordo com Windmuller e Spaeth (1967).

3.13 Histologia Hematoxilina-Eosina

Amostras do fígado foram coletadas e fixadas em Bouin. Os tecidos foram incluídos em historesina Leica e cortados em Micrótomo Leica RM2145 na espessura de 6 a 7 micrômetros. Os cortes foram, então, submetidos à técnica de coloração por Hematoxilina-Eosina. Para isso, os cortes serão hidratados, corados com hematoxilina (10 min), lavados e corados com e lavados e

corados com eosina (5 min). Em seguida, os mesmos foram lavados e montados em bálsamo do Canadá (Adaptado de JUNQUEIRA & JUNQUEIRA, 1983).

3.14 Resíduos

O descarte dos resíduos não-tóxicos ocorreu no sistema de esgoto comum. Os demais resíduos serão encaminhados para o Programa de Gerenciamento de Resíduos da UNESP para procedimento correto de sua eliminação.

4. Estatística

Os resultados foram analisados estatisticamente através do teste t-student ou pela Análise de Variância (ANOVA), onde apropriado. Quando necessário, utilizou-se o teste Post Hoc de Bonferroni, com nível de significância pré-estabelecido de 5%.

5. Resultados

Os resultados obtidos, referentes às três séries de experimentos, acham-se descritos na forma de dois artigos publicados e um submetido à publicação.

5.1 Resultados da Primeira Fase Experimental

Fructose-rich diet leads to reduced aerobic capacity and to liver injury in rats

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Abstract

The main purpose of this research was to investigate the alterations in the aerobic capacity and appearance of metabolic alterations in Wistar rats fed on fructose-rich diet. We separated twenty-eight rats into two groups according to diet: a control group (C) (balanced diet) and a fructose-rich diet group (F). The animals were fed these diets for 60 d (d 120 to 180). We performed insulin, glucose and fructose tolerance tests as well as a minimum lactate test, at d 120 and 180. At the end of the experiment, sixteen animals were euthanized, and the following main variables were analysed: aerobic capacity, the serum aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio, serum and liver triglyceride concentrations, serum and liver thiobarbituric acid reactive substance (TBARS) concentrations, serum and liver catalase and superoxide dismutase (SOD) activity and haematoxylin-eosin histology (HE) in hepatocytes. The remaining twelve animals were submitted to an analysis of their hepatic lipogenic rate. The animals fed a fructose-rich diet exhibited a reduction in aerobic capacity, glucose tolerance and insulin sensitivity and increased concentrations of triglycerides and TBARS in the liver. Catalase and SOD activities were reduced in the livers of the fructose-fed animals. In addition, the serum AST/ALT ratio was higher than that of the C group, which indicates hepatic damage, and the damage was confirmed by histology. In conclusion, the fructose-rich diet caused significant liver

damage and a reduction in insulin sensitivity in the animals, which could lead to deleterious metabolic effects.

Background

Nonalcoholic fatty liver disease presents like a new marker of metabolic syndrome [1]. This hepatic disease is most frequently diagnosed in the United States, affecting almost thirty million people [2, 3]. The aggressive form of this disease is most commonly found in adults, but the number of children affected is growing rapidly [4]. Interest in the causes and consequences of lipid infiltration in the liver has risen in recent years because of the association between triglyceride accumulation in different tissues and the development of insulin resistance [5].

The influx of triglycerides into hepatocytes leads to an overproduction of reactive oxygen species by beta-oxidation, which causes an anti-oxidant/oxidant imbalance [6]. The elevation of pro-oxidant species causes membrane and DNA damage and the inactivation of some regulatory proteins, which causes tissue inflammation and induces insulin resistance, apoptosis, cellular mutations and other effects.

Rats fed a triglyceride-rich diet have been used as an experimental model of human metabolic syndrome. Previous studies have had some success with inducing nonalcoholic fatty liver disease by feeding animals different concentrations of fructose [7, 8].

Moreover, physical activity is an important tool for the prevention of metabolic syndrome [8]. It has been shown that physical activity improves glucose tolerance and reduces insulin

resistance [8, 9]. Aerobic capacity can be a good indicator for physical conditioning and can be used after training or diet interventions to show alterations on physical conditioning parameters.

Objective

The main purpose of this research was to investigate the alterations in the aerobic capacity and appearance of metabolic alterations in Wistar rats fed on fructose rich diet. This study focused on the appearance of NAFLD and the subsequent alterations as systemic oxidative stress, liver damage, insulin resistance and hyperinsulinemic state.

Methods

Animals and treatments

Twenty-eight adult (aged 120 d) Wistar rats were used. The animals were kept in collective cages (four animals per group) at a controlled temperature of 25 ± 1 ºC and under a light/dark cycle of 12/12 h with free access to water and food. The experiment was performed at the Nutrition, Metabolism and Exercise Laboratory at São Paulo State University, Rio Claro, Brazil. The weights of the animals were recorded weekly during the experimental phase, and the area under the curve (AUC) was calculated with Microsoft Excel 2007 software using the trapezoidal method [10]. All experiments were analysed and approved by the Biosciences Institute Animal Ethics Committee, Rio Claro Campus (case number: 005/2010).

Experimental groups

At 120 d of age, the animals were separated randomly into two groups: the Control Group (C) was fed a commercial balanced diet from 120 to 180 d of age, and the Fructose Group (F) was fed a semi-purified, fructose-rich diet from 120 to 180 d of age.

Diet composition

We used commercial chow (Labina, Purina®) as a control diet (57,3% carbohydrate, 41,2% of corn-starch). For the fructose-rich diet, we used an adapted diet standardised by Bezerra and colleagues (2004) composed of (in g/kg) 202 of casein, 625.5 of fructose, 2 of l-cysteine, 70 of soy oil, 35 of mineral salt mix (AIN-93MX) [11], 10 of a vitamin mix (AIN-93MX)[11], 50 of fibre and 2.5 of choline chloridrate.

In vivo assays

Oral glucose tolerance test (oGTT)

The oGTT were performed at 8th week of experiment after the animals had fasted for 12 h. The first blood sample was collected by a small tail cut to determine the basal glucose and insulin concentrations (t0). Next, a 20% glucose solution (2 g/kg of animal weight) was administered via an intragastric catheter, and samples for glucose and insulin were collected 30, 60 and 120 min later into four heparinised capillaries calibrated to 25 µl each. Glucose levels were analysed by the glucose-oxidase method, and insulin levels were assessed using the radioimmunoassay method [12]. The results were analysed by calculating the glucose and insulin AUC using the trapezoidal method [10] in the Excel 2007 software.

Insulin sensitivity (ITT)

Insulin sensitivity was evaluated using the insulin tolerance test at 8th week of experiment. The test consisted of a bolus injection of insulin (300 mU/kg body weight) followed by blood sample collections (to measure glucose concentrations) from a cut at the tip of the tail before and 30, 60 and 120 min after the insulin injection. The serum glucose disappearance rate (Kitt) was calculated using the formula $0{\cdot}693/t_{1/2}$, wherein $t_{1/2}$ is the half-life of the process. The serum glucose half-life was calculated from the slope of a least-square analysis of serum glucose concentrations 0-60 min after the subcutaneous injection of insulin; during this time, the glucose level reduces linearly [13].

Minimum lactate test (ML)

Aerobic capacity was analyzed at the 8th week of experiment via the lactate threshold during swimming and calculated by determining the "minimum lactate test" [14], adapted to rats [15]. For this test, the animals were initially placed individually in tanks (100 cm X 80 cm X 80 cm) containing water at 31 ± 1°C. Animals carried an overload that was 13% of their body weight to induce hyperlactatemia and were then exercised for 30 sec. After resting for 30 sec, they swam carrying the 13% load until exhaustion. After a 9 min rest, a blood sample was collected by means of a cut in the distal end of the tail to determine lactate concentration. Animals then performed

exercise with progressively heavier loads [15]. The initial load was 2% of the body weight of the animal; the load was increased 0.5% every 5 min until exhaustion. After each load change, a blood sample was collected to measure lactate. The lactate minimum swimming workload (ML) was determined using a second-order polynomial curve adjusted to the blood lactate vs. workload curve. The blood lactate concentration was measured by spectrophotometry [16]. The lowest lactate concentration on the curve (minimum lactate) theoretically represents the maximum exercise intensity, where lactate production and removal occur in the same proportions [17].

In Vitro assays

Biological samples

Forty-eight h after the last "in vivo" evaluation (180 days old), sixteen animals (eight per group) were killed under anaesthesia (sodium thiopental, 40 mg/kg of body weight, intraperitoneally) for the collection of two blood samples from the hepatic vein. The first sample was used to obtain serum to evaluate glucose, triglyceride, HDL cholesterol, LDL cholesterol and total cholesterol concentrations as well as the activities of the hepatic transaminases aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes using colorimetric methods (commercial kits LABORLAB®) according to the methods of Nogueira [18]. The second sample was used to determine the TBARS concentration and catalase (CAT) and superoxide dismutase (SOD, commercial kit Cayman ®) activities. The liver was removed, and a sample was taken to determine the triglycerides concentration [19]; another sample was used to evaluate biomarkers of oxidative stress (TBARS, catalase and SOD). An additional sample was used for histological analysis using the haematoxylin/eosin method. The twelve remaining animals were used to determine the rate of liver lipogenesis (six per group) [19], also, a sample of the visceral adipose

tissue (mesenteric and retroperitonial) was removed to determine triglycerides concentration (calculated as mean of both regions) [18].

Biomarkers of the antioxidant defense system

Catalase (CAT)

To determine catalase activity, liver samples (100-150 mg) were placed in Eppendorf tubes® containing 1 ml of cooled phosphate buffer, subjected to agitation and centrifuged at 10,000 rpm for 5 min. Catalase activity dosage assays were performed, using phosphate buffer (50 mM) and oxygen peroxide (H_2O_2, 10 mM) [20]. The linear reduction in the H_2O_2 absorbance values was assessed by spectrophotometry (240 nm) according to the reaction below.

We calculated catalase activity using the formula $(2 \cdot 3/\Delta t)*(a/b)*(\log A_1/A_2)$, in which a is the haemolysed volume in the sample cell, b is the total volume of the sample cell, A_1 is the absorbance value at t = 0 s, and A_2 is the absorbance 15 s later (t = 15 s) [20].

Superoxide dismutase (SOD)

Superoxide dismutase activity was determined after washing the samples with PBS (pH 7·4) containing heparin 0·16 mg/L to remove blood cells. Then, the tissue was homogenised (on ice) using 1 ml of HEPES buffer (20 mM, pH 7·2) containing 1 mM EGTA, 210 mM manitol and 70 mM sucrose. Following centrifugation at 10,000 rpm for 15 min at 4º C, the samples were maintained at -20 ºC until the level of activity of total SOD (cytoplasmic and mitochondrial) was determined. For this procedure, we used a commercial kit (Cayman®), and a tetrazolium salt was used to detect superoxide radicals produced by xanthine-oxidase and hypoxanthine. In this reaction, one unit of

SOD is defined as the quantity of enzyme necessary to affect 50% of superoxide radical dismutation [21].

Biomarkers of Lipid-c Peroxidation

TBARs Concentration

We detected substances that react to thiobarbituric acid (TBARS). This method involves analysing the final products produced by lipid-c peroxidation (lipid peroxides, malonaldehyde and other low-weight aldehydes), which react with 2-thiobarbituric acid to produce Schiff bases. Blood samples were collected, and serum proteins were isolated using phosphotungstic acid. The supernatant was used in a colorimetric reaction with thiobarbituric acid, which uses MDA (the main product of lipid peroxidation), as the standard, and was analysed by spectrophotometry (5 nm). The liver samples (100-150 mg) were placed in plastic tubes containing phosphate buffer and were analysed by spectrophotometry (5 nm) [22].

Lipogenic rate in the liver

The lipogenic rate in the liver was measured using tritiated water as a radioisotope marker. Tritium is incorporated into stable C-H bonds during fatty acid synthesis by the exchange of ^{3}H with pyrimidine-reduced nucleotides (NADPH), which are produced via pentose or malic enzymes. The tritiated water was administered via an intraperitoneal injection. After 60 min, the animals were killed, and a liver samples was isolated and the lipids were extracted using the

method of Folch *et al*. [19] and were quantified using liquid scintillation spectroscopy (TRI-CARB 2100TR). The rate of fatty acid synthesis was calculated according to Windmuller and Spaeth [23]:

$$\text{Fatty acid } \mu\text{mol/2 h} = \frac{\text{dpm of } {}^{3}\text{H incorporated into liver FA x 109 x 1}}{\text{dpm of } {}^{3}\text{H /atom-g of H x 13·3 x t2-t1}}$$

Liver haematoxylin-eosin (HE) histology

Liver samples were collected and fixed in Bouin's fixative. The tissue was mounted in HitoResina (Leica) and sliced in a microtome (Leica RM2145) to a thickness of 6 μm. The slices were subjected to the haematoxylin-eosin staining method. The slices were hydrated and stained with haematoxylin (10 min) and were then washed and stained with eosin (5 min). Finally, they were washed and preserved in Canadian balsam [24].

Statistics

The Shapiro-Wilk W test was used to verify the normality of the sample. The results were analysed statistically using a Student's t-test.

Results

The minimal lactate example for one animal of each group is observed in the Figure 1.

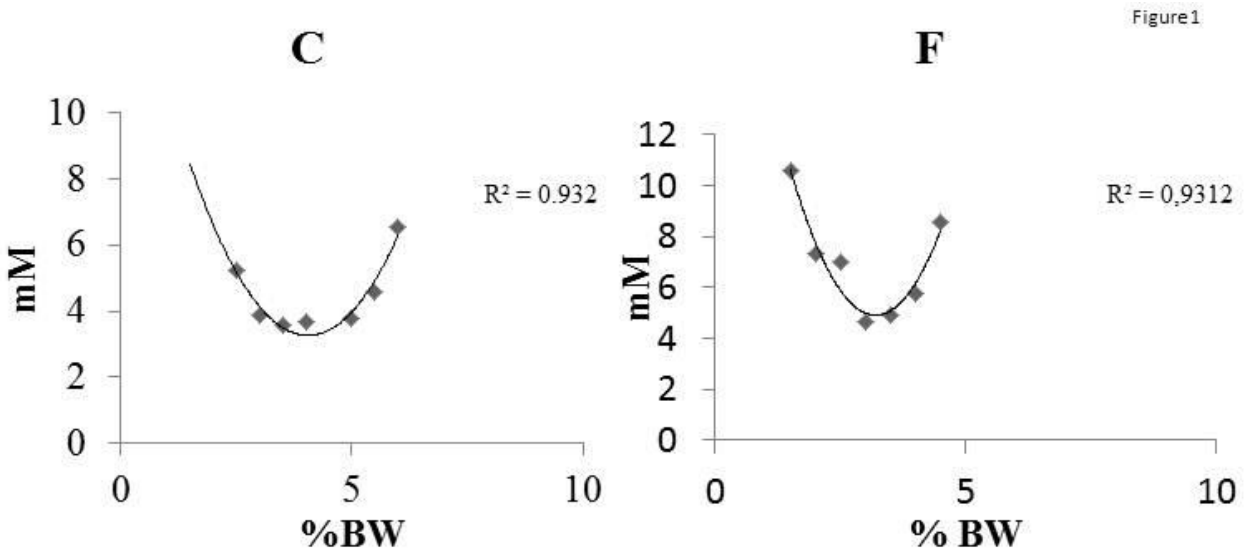

Figure 1. Minimum lactate test of one animal of each group, as an example, In this particular case, the estimated ML was 4.21% of body weight, while the interpolated blood lactate concentration was 3.96mM for the C animal and 2.53% of body weight and 3.94 mM blood lactate interpolated concentration.

The change in body weight, the AUC of the body weight measurements and of the animals during the 8 weeks of the experiment were not different between the groups (Figure 2).

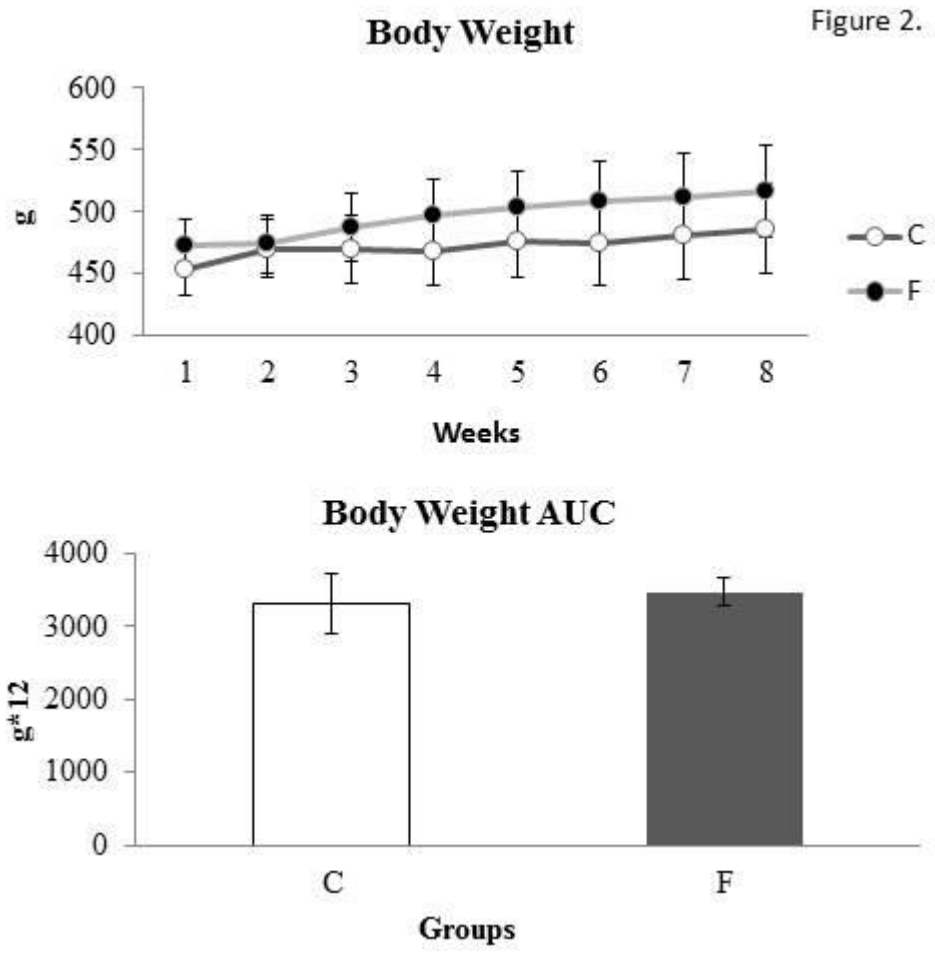

Figure 2. Body weight change, the area under the curve (AUC) for body weight and the weight gain of the animals during the 8 weeks of the experiment.
C: Control; F: Fructose. n = 14 animals per group. *Significantly different from the control group ($p \leq 0{\cdot}05$).

Similarly, no difference was observed between the two groups, both in weekly food intake and the AUC of food intake (Figure 3).

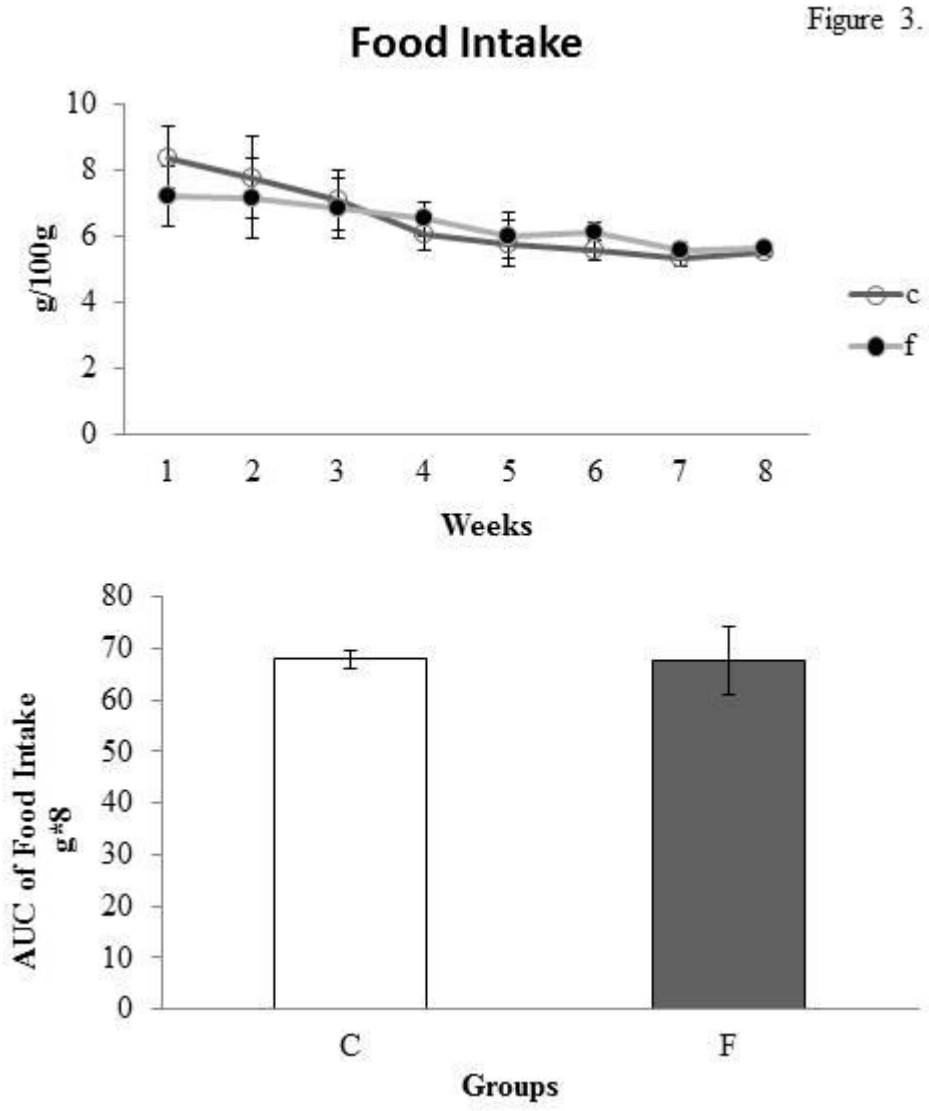

Figure 3. Weekly food intake (g/100 g) and area under the curve for food intake (g/100 g*8).
C: Control; F: Fructose. n = 14 animals per group. *Significantly different from the control group ($p \leq 0{\cdot}05$).

Table 1 shows the blood lactate concentrations and the workloads corresponding to the minimum lactate for both groups. Fructose feeding reduced the ML without alteration in blood lactate concentrations.

Table 1. Blood lactate concentrations (mM) and the workloads (%BW) corresponding to the minimum lactate for both groups.

Groups	Rest	Minimum Lactate	
	mM	mM	%BW
C	**1.3±0.31**	**3.2±1.2**	**3.9±0.5**
F	**1.5±0.2**	**4.3±0.7**	**2.5±0.3***

C: Control; F: Fructose. n = 8 animals per group.
*Significantly different from the control group ($p \leq 0{\cdot}05$).

Table 2 shows the serum glucose kinetics of the animals during the insulin tolerance test and the Kitt values (glucose disappearance rate) at the end of experiment. The F animals exhibited lower Kitt scores compared with C animals, which indicates insulin resistance.

Table 2. Glucose kinetics (mg/dl) and glucose removal rate (%/min, Kitt) during the insulin tolerance test (ITT).

Groups	Serum Glucose and KITT levels in the ITT				Kitt
	Time				
	0	30	60	120	
C	85.8± 4.5	80.6± 5.7	80.5 ± 5.4	91.3± 9.3	1.04± 0.3
F	87.5± 7.4	86.9± 5.2	88.3 ± 14.8	95.2± 9.0	0.54± 0.16*

C: Control; F: Fructose. n = 8 animals per group.

*Significantly different from the control group ($p \leq 0{\cdot}05$).

The serum glucose and serum insulin levels as well as the area under the curve scores for serum glucose and insulin during the oral glucose tolerance test at the end of the experiment are shown in Table 3. The animals fed the fructose-rich diet exhibited higher serum glucose AUC values compared to C. The serum concentrations of insulin and the insulin AUC values were higher in group F compared when to group C.

Table 3. Serum glucose kinetics (mg/dl), serum insulin (ng/dl), area under the curve for serum glucose (mg*120 min/dl, AUC) and serum insulin (ng*120 min/dl, AUC) during the oral glucose tolerance test (oGTT).

Groups	Serum glucose and insulin in the oGTT				
		Time			Area Under the Curve
	Variable	0	30	60	120	
C	Glucose (mg/dl)	69.6 ± 2.2	80.8± 6.7	81.8± 4.2	73.4± 1.3	9353.7 ± 456.6
	Insulin (ng/ml)	2.1± 0.3	2.4± 0.1	2.3± 0.4	2.1± 0.3	277.4± 32.6
F	Glucose (mg/dl)	75.2± 4.8	90.6± 7.3	85.8± 3.8	83.7± 2.3	10219.9± 530.2*
	Insulin (ng/ml)	2.5 ± 0.3*	2.5± 0.3	3.6± 0.5*	3.4± 0.4*	381.2± 49.5*

C: Control; F: Fructose. n = 8 animals per group.

*Significantly different from the control group ($p \leq 0{\cdot}05$).

The concentrations of serum glucose, triglycerides, total cholesterol, HDL cholesterol and LDL cholesterol and measurements of liver injury markers (AST/ALT ratio), catalase and SOD activities and lipid peroxidation markers (TBARS) at the end of the experiment are shown in Table 4. The animals fed the fructose-rich diet exhibited higher levels of glucose and triglycerides, an increased AST/ALT ratio, TBARS levels and catalase activity.

Table 4. Glucose, triglycerides, total cholesterol, HDL cholesterol and LDL cholesterol concentrations, AST/ALT ratio, SOD activity, catalase activity and TBARS concentrations in animal sera at the end of the experiment.

Variable	C	F
Glucose (mg/dl)	83.6±4.5	98.5±10.5*
Triglycerides (mg/dl)	123.4±49.0	268.3± 35.1*
Total cholesterol (mg/dl)	91.7± 9.6	96.3± 15.5
HDL cholesterol (mg/dl)	42.7± 5.3	43.5± 6.9
LDL cholesterol (mg/dl)	62.8± 3.9	65.4± 1.3
AST/ALT ratio	1.08± 0.4	2.8± 0.8*
SOD (U/ml)	1.7± 0.4	1.5± 0.6
Catalase (U/ml)	42.2± 10.4	68.4± 6.8*
TBARS (µM)	16.5± 2.0	22.2± 4.4*

C, Control; F, Fructose. n = 8 animals per group.
*Significantly different from the control group ($p \leq 0{\cdot}05$).

As shown in Table 5, fructose-fed group exhibited higher concentrations of triglycerides and TBARS in the liver than the control group. Conversely, fructose-fed animals showed a reduction in liver catalase activity.

Table 5. Triglyceride concentrations, SOD activity, catalase activity and TBARS concentrations in animal livers at the end of the experiment.

Variable	C	F
Triglycerides (mmol/mg)	6.4± 1.9	72.8± 25.7*
SOD (umol/min.mg protein)	4.69± 1.6	2.6±1.4
Catalase (umol/min.mg protein)	5.7± 1.8	2.2±1.1*
TBARS (umol/mg)	0.30±0.03	0.35±0.02*

C, Control; F, Fructose. n = 8 animals per group.
*Significantly different from the control group ($p \leq 0{\cdot}05$).

The HE histology is presented in Figure 4. The image shows a substantial increase in fatty accumulation in hepatocytes (diffuse macrovesicular steatosis) in the animals fed the fructose-rich diet.

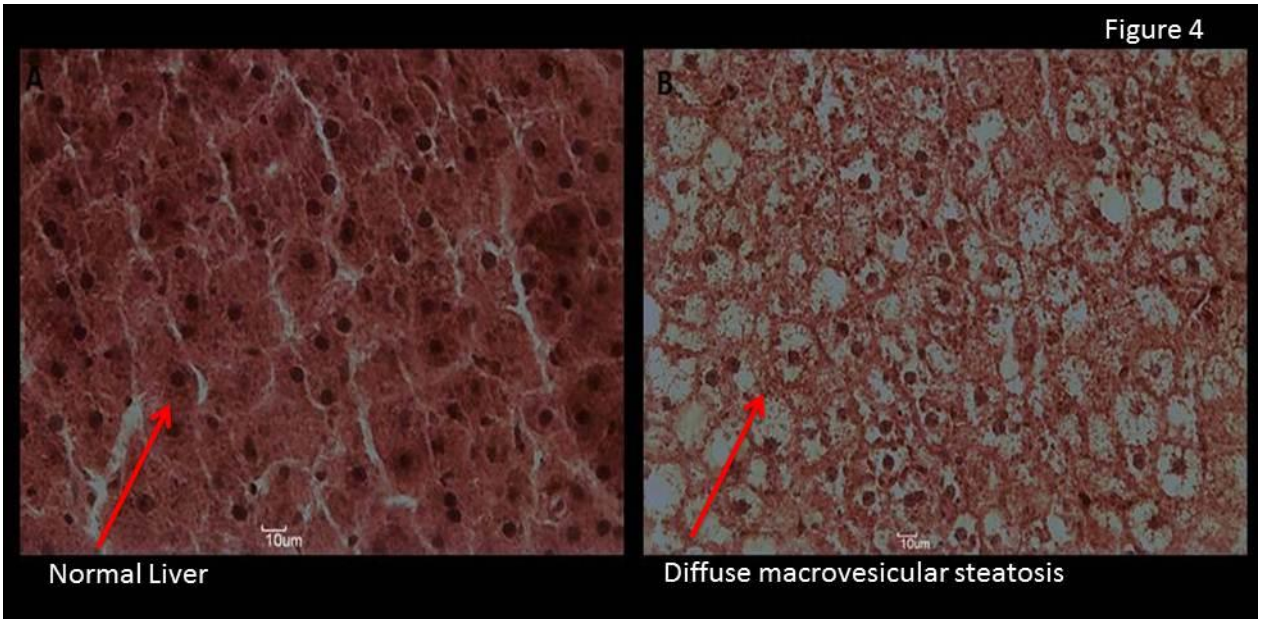

Figure 4. Liver HE histology of one rat at the end of the experiment as a representative example. A: Control Group; B: Fructose Group.

Figure 5 shows the values of lipogenic rates. The animals fed the fructose rich diet revealed an increase in lipogenic rate.

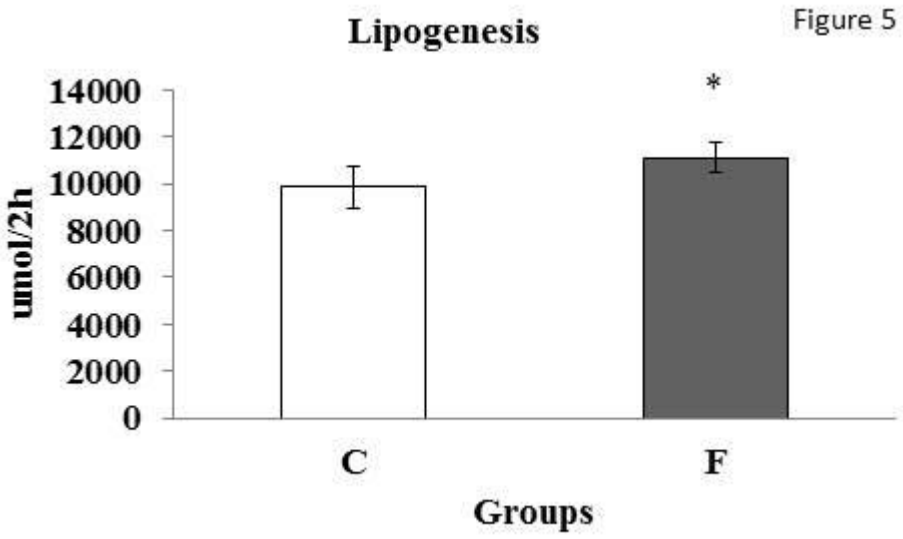

Figure 5. Lipogenesis rate (µmol/2 h) of six rats at the end of the experiment.
C: Control; F: Fructose. n = 6 animals per group. *Significantly different from the control group ($p \leq 0{\cdot}05$).

Figure 6 shows the triglycerides concentration in the visceral adipose tissue (retroperitoneal and mesenteric region) of the animals at the final of experiment. F showed high concentrations of triglycerides compared to C group.

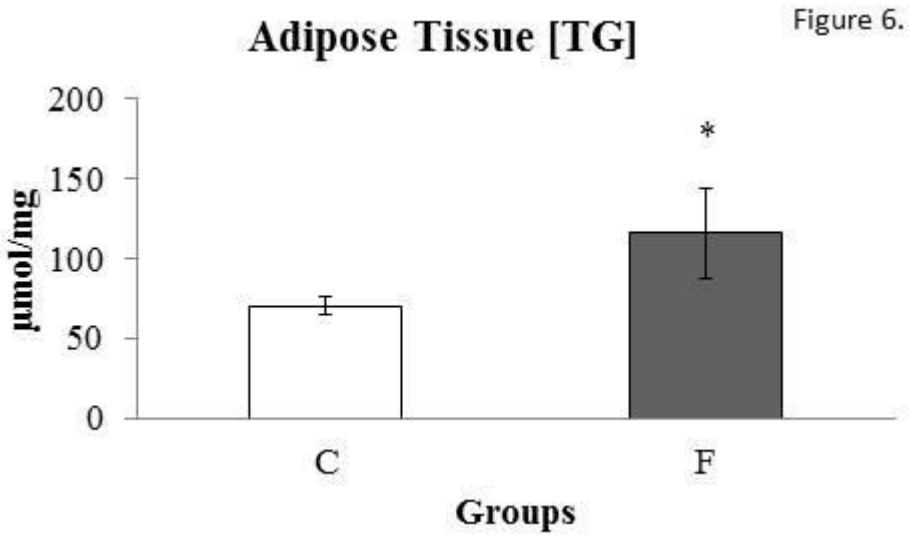

Figure 6. Triglycerides concentration (µmol.mg) in the visceral adipose tissue of eight rats at the end of the experiment.

C: Control; F: Fructose. n = 8 animals per group. *Significantly different from the control group ($p \leq 0{\cdot}05$).

Discussion

Previous studies have shown that an increase in metabolic syndrome incidence is highly correlated with changes in alimentary behaviour. Western diets, which are rich in sugars and saturated fats, have been implicated as the main cause of the global pandemic in obesity, glucose intolerance, type-2 diabetes mellitus, hypertension and other conditions [25-26]. Once our main purpose is to investigate changes in the aerobic capacity and the main metabolic alterations caused

by the fructose-rich diet administration, we performed an eight weeks experiment starting 120 d of age.

At d 180, the animals in this group had similar weights to those fed a normal diet. This finding corroborates other studies that showed that animals fed on fructose diet for a short term did not present excessive weight [8, 27, 28].

The exercise is an important and powerful weapon against the metabolic syndrome markers. The minimal lactate zone is situated in a transition domain between intense and severe exercise. In this zone, both glycolytic and oxidative pathways contribute to the energy production, and a reduction on the efficiency in one of them can be responsible for lower performance. In this sense, we performed this test to evaluate the aerobic capacity of the animals. The lactate response allowed us to determine the minimal lactate intensity in both groups and revealed that the aerobic capacity of F group was reduced if compared to C after 60 days of fructose-rich diet consumption. Associated with the reduced aerobic capacity, the F group presented insulin resistance.

Several studies have reported an association of changes in aerobic capacity, insulin resistance and obesity [29, 30, 31], but the cause of these alterations was not completely elucidated. Some studies suggest that the insulin can modulate the production and activity of glycolytic and oxidative enzymes [32, 33]; thus an insulin resistance could reduce the mitochondrial efficiency and, at least in part, can be responsible for the reduced workloads at the same lactate concentrations showed in the F group. In a previous study our group showed that, rats fed on fructose-rich diet had higher lactate concentrations in a same overload during the maximal lactate steady state test [34].

This insulin resistance occurrence in F group leads to both overproduction and secretion of insulin by the beta cells, then triggering the hyperinsulinemic state, as found during the oral

glucose tolerance test. Over 60 d, the F group exhibited a rise in the serum glucose AUC value and a pronounced elevation in both serum insulin concentrations and serum insulin AUC in this test. This hyperinsulinemic state can strongly increase the insulin resistance in peripheral tissues and different organs [35], which reduce, again, the glucose uptake and, consequently, lead to a considerable increase in glycaemia, as was observed in F group, characterizing a vicious cycle. The impairment on carbohydrates metabolism is compensated with higher contributions of lipids as the mainly source of energy.

The fructose metabolism occurs in the liver, which has a great capacity to uptake and phosphorylates this nutrient. This nutrient can be transformed in glucose and glycogen, but this pathway is very "inefficient". So, the liver choice is to produce pyruvate, which is transferred to mitochondria and is transformed in fatty acids. These fatty acids are used as the mainly liver energy source, stored as triglycerides depots or released in the blood stream as VLDL and NEFA. This characteristic makes fructose a highly lipogenic nutrient [36]. In the present study, the F animals showed higher concentrations of triglycerides in the serum, liver and visceral adipose tissue; in addition, higher liver lipogenic rate was found. This result corroborates several studies that demonstrated the lipogenic capacity of fructose [36, 37]. The excessive production and uptake of triglycerides can be viewed in the Figure 3. The animals fed on fructose rich diet presented bigger triglycerides content. High concentrations of liver triglycerides lead to an increase of the activity of fatty-acyl-coA oxidase activity and also stimulate the liver to produce energy trough beta-oxidation. The fructose-rich diet used in our study induced an elevation in liver TBARS concentrations and a reduction in hepatic catalase activity. The higher levels of blood TBARS concentrations in the F group can be correlated with an overutilization of non-esterified fatty acid (NEFA) as energy source. Also, NEFA can induce insulin secretion by pancreatic islets, leading

to a hypersulimenic state, as presented by the F group. High insulin levels can downregulate the Malonil-CoA activity, reducing the mitochondrial NEFA transport via CPT-1, leading to an excessive NEFA oxidation at peroxisomes and endoplasmic reticulum [38-41]. Fatty oxidation in these cytoplasmic organelles areas releases high amounts of reactive oxygen species, which can damage mitochondrial membranes (rich in polyunsaturated fat), producing lipid peroxidation metabolites. These metabolites are extremely toxic to the cells and are released into the bloodstream, as observed in the F group [42]. These events could cause an acute systemic response that leads to changes in the antioxidant system, denoted by the higher levels of serum catalase enzyme observed in the present study, which represent a protective mechanism in order to reduce the cellular damage in the F animals [43]. Interestingly, Kakkar *et al*. [44] demonstrated that Sprague-Dawley rats showed an increase in anti-oxidant activity for several weeks followed by a dramatic reduction in the anti-oxidant response, which consequently increased the level of cellular damage. The chronic imbalance of reactive oxygen species production can impair the ability of the anti-oxidant system to reduce the levels of these radicals, which attenuates its protective function [43].

The imbalance between the production and the removal of free radicals is intimately linked to a structural damage and cellular injury; this was confirmed by the increase in the AST (aspartate aminotransferase)/ALT (alanine aminotransferase) ratio (markers of liver damage) in the F animals. Alterations in the levels of serum AST and ALT are considered important markers of hepatic injury and liver fibrosis [45].

Stored hepatic triglycerides are related to acute local insulin resistance. This mechanism can be resulted of a local pro-inflammatory response which alters the circulating AST/ALT ratio [8]. This response releases high amounts of pro-inflammatory mediators such TNF-α and IL-6,

reducing the hepatic insulin sensitivity. These substances impair the ability of insulin receptor substrate 1 (IRS-1) and insulin receptor substrate (IRS-2) to be phosphorylated in tyrosine as well as stop the insulin signal and all processes related to glucose uptake [46-49]. Moreover, high levels of serum NEFA leads to insulin overproduction and secretion by the pancreatic beta-cell.

This sequence of events results in a massive influx of triglycerides into beta cells and increases the energy production through the beta-oxidation, resulting in this way, in a cellular lipotoxicity, which can generate structural damage and deficient beta-cell regulation [49]. The animals' glucose disappearance rates after exogenous insulin administration (Kitt) values were reduced drastically in the F animals, indicating reduction in the insulin sensitivity [35]. This reduction could be responsible for the reduced aerobic capacity, increase on fatty triglycerides, overproduction of EROS; the last may induce liver and islet apoptosis in a long term [25, 48, 50, 51, 52]. Moreover, these findings are closely connected to the appearance of type-2 diabetes mellitus in NAFLD and hypertriglyceridemia patients [1].

Conclusion

In summary, the fructose-rich diet administration protocol impaired aerobic capacity, induced fatty liver disease and trigger the hypertriglyceridemia, hyperglycaemia, insulin resistance and glucose intolerance, all markers of metabolic syndrome. Furthermore, the fructose reduced the antioxidant activity, enhanced the EROS production and damaged the liver of the animals. These findings may provide the foundation for future studies to elucidate the role of fructose in metabolic syndrome and to develop new treatment procedures for related disorders.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

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Authors' contributions

JDB was responsible for the experimental design, data collection, statistical analysis and preparation of the manuscript. LTC, ACG and RAD were responsible for the data collection and the preparation of the manuscript. FAV was responsible for the preparation of the manuscript. MARM was responsible for the experimental design, financial support, and coordination of the research and preparation of the manuscript.

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5.2 Resultados da Segunda Fase Experimental

Different exercise protocols improve metabolic syndrome markers, tissue triglycerides content and antioxidant status in rats.

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Abstract

Background: An increase in the prevalence of obesity entails great expenditure for governments. Physical exercise is a powerful tool in the combat against obesity and obesity-associated diseases. This study sought to determine the effect of three different exercise protocols on metabolic syndrome and lipid peroxidation markers and the activity of antioxidant enzymes in adult Wistar rats (120 days old).**Methods:** Animals were randomly divided into four groups: the control (C) group was kept sedentary throughout the study; the aerobic group (A) swam1 h per day, 5days per week, at 80% lactate threshold intensity; the strength group (S) performed strength training with four series of 10jumps, 5 days per week; and the Concurrent group (AS) was trained using the aerobic protocol three days per week and the strength protocol two days per week. **Results** Groups A and S exhibited a reduction in body weight compared to group C. All exercised animals showed a reduction in triglyceride concentrations in fatty tissues and the liver. Exercised animals also exhibited a reduction in lipid peroxidation markers (TBARS) and an increase in serum superoxide dismutase activity. Animals in group A had increased levels of liver catalase and superoxide dismutase activities. **Conclusions** We concluded that all physical activity protocols improved the antioxidant systems of the animals and decreased the storage of triglycerides in the investigated tissues.

Keywords: Physical exercise, liver damage, oxidative stress, rats

Background

The increase in the incidence of obesity in industrialized countries in recent years has been associated with a decrease in daily energy expenditure. Studies indicate that, for the last 20 years, daily caloric intake has decreased; however, energy expenditure by means of physical activity has decreased even more. Thus, it is believed that the ongoing obesity epidemic might be more related to a reduction in physical activity than to an increase in caloric intake [1].

Obesity is associated with the appearance of systemic metabolic disorders, such as glucose intolerance, hyperinsulinemia, increased triglyceridemia, HDL cholesterol reduction and arterial hypertension. These disorders are highly associated with cardiovascular disease. This association is known as metabolic syndrome [2]. It is estimated that the prevalence of metabolic syndrome is 34% among adults and 50-60% in the population over 60 years of age in the United States, which constitutes a serious medical-social and economic problem [3, 4].

Metabolic syndrome patients may also exhibit a state of chronic inflammation caused by an increased dependence on lipids as an energy source, which leads to the formation of oxygen reactive species and subsequent cell structure damage and protein structure disarray [5].

Physical activity is an important tool for the prevention of metabolic syndrome. It has been shown that aerobic training improves metabolic syndrome markers and tissue triglycerides content [6]. Also, the strength exercise can ameliorate the muscle loss and insulin sensitivity in insulin-resistant subjects [7, 8]. Also, both aerobic and strength exercise can improve glucose tolerance and insulin sensitivity [6-9]. For this reason, this study sought to determine the effects of an aerobic, a strength and a concurrent (aerobic plus strength) exercise protocols on metabolic syndrome markers, lipid peroxidation markers and antioxidant enzyme activity in Wistar rats.

Methods

Animals and handling

Thirty-two freshly weaned Wistar rats were used in this study. Animals were kept in shared cages (four animals per cage) at a controlled temperature of 25 ± 1ºC and a 12h sleep-awake cycle. Animals had free access to water and pelleted food Labina® (Purina®, São Paulo, Brazil). This study was performed at the Nutrition, Metabolism and Exercise Laboratory of São Paulo State University, São Paulo, Brazil. The weights of the animals were recorded weekly during the study, and the area under the curve (AUC) values were calculated using the trapezoidal rule [10] with Microsoft Excel 2007. This study was approved by the Animal Use Ethics Committee of the São Paulo State University, Biosciences Institute (CEUA), Rio Claro campus, and protocol n º 005/2010.

Experimental groups

The Control (C) group was kept sedentary from 120 to 180 days of age. The Aerobic Training (A) group performed aerobic training 5 days per week (at 80% lactate threshold intensity) for 1 h per day from 120 to 180 days of age. The Strength Training (S) group performed strength training exercises [11] 5 days per week from 120 to 180 days of age. The Concurrent Training (AS) group performed aerobic training (at 80% lactate threshold intensity) 2 days per week (Tuesdays and Thursdays) for 1 h per day and strength training [11] 3 days per week (Mondays, Wednesdays and Fridays) from 120 to 180 days of age.

Exercise protocol

Aerobic training adaptation

The sedentary (C) and aerobically trained (A and AS) groups were first adapted to the water environment. Adaptation was performedover10uninterrupted days in the same tank where the training was performed. The water temperature was kept at 31 ± 1°C [12]. The aim of adaptation was to reduce animal stress and to avoid possible physiological adaptations that might improve the physical capacity of the animals.

Rats were placed in shallow water for 10 min for three days. The water depth was then increased, as was the effort length and load (1% body weight in the form of lead ballasts placed in a Velcro® backpack attached to the thorax) carried by animals. By the fourth day, animals swam for 5 min in deep water. The length of time was increased by 10min each day until the 12^{th} day of adaptation [12].

Strength training adaptation

Rats were placed in shallow water tanks (31 ± 1°C) for 10 min the first two days. On the third, fourth and fifth days, the depth level was increased, and the animals were kept in the tanks for 5, 10 and 15 min, consecutively. On the sixth and seventh days, a 30% body weight overload in a Velcro® "backpack" was attached to the thorax of the animals, and they were swept into the tank with shallow water. In the last three days, the animals performed 10jumps with a 30% overload attached to the thorax, while the depth of water was progressively increased (25, 50 and 100% maximum tank capacity) [11].

Concurrent training adaptation

Rats were placed in shallow water (31 ± 1°C) for 10 min on the first two days. On the third, fourth and fifth days, the depth level of water was increased, and the animals were kept in the water for 5, 10 and 15 min, consecutively. On the fifth, sixth and seventh days, the animals were subjected to increased exposure times (5 min per day) in the water with a 1% body weight load attached by means of a Velcro® backpack. On the last three days, the animals were subjected to strength training adaptation: on the first day, they were kept in shallow water with a load attached to the thorax (30% body weight);during the following days, they performed 10jumps carrying this same load inside tanks filled to 50% and 100% of their maximum capacity.

Lactate threshold

The lactate threshold during swimming was calculated by determining the adapted "minimum lactate" test [13, 14]. For this test, the animals were initially placed individually in tanks (100 cm X 80 cm X 80 cm) containing water at 31 ± 1°C.Animals carried an overload that was 13% of their body weight to induce hyperlactacidemy and were then exercised for 30 sec. After resting for 30 sec, they swam carrying the 13% load until exhaustion. After a 9min rest, a blood sample was collected by means of a cut in the distal end of the tail to determine lactate concentration. Animals then performed exercise with progressively heavier loads [14]. The initial load was 2% of the body weight of the animal; the load was increased 0.5% every 5 min until exhaustion. After each load change, a blood sample was collected to measure lactate. The lactate minimum speed (LMS) was determined using a second-order polynomial curve adjusted to the blood lactate vs. workload curve. The blood lactate concentration was measured by

spectrophotometry [15]. The lowest lactate concentration on the curve (minimum lactate) theoretically represents the maximum exercise intensity, where lactate production and removal occur in the same proportions [16].

Physical training

Aerobic protocol

This protocol consisted of the animals swimming in individual tanks that contained water at 31 ± 1°C for 1 h per day, 5 days per week. Exercise was performed with the 80% individual minimum lactate intensity overload attached to the thorax of the animal.

Strength Protocol

Animals performed jumps in individual tanks with the water level standardized at 150% body length and a water temperature of 31 ± 1°C. Animals performed four 10-jump series with a 50% body weight overload attached to the thorax and a 1-min rest between series for5days per week.

Concurrent protocol

Animals were trained using the aerobic protocol three times a week (Mondays, Wednesdays and Fridays) and using the strength protocol twice a week (Tuesdays and Thursdays).

Metabolic syndrome markers

Body weights, oral glucose tolerances, insulin sensitivities, blood glucose levels, lipid profiles and liver and fatty tissue triglyceride concentrations from multiple areas of the bodies of the animals were used as metabolic syndrome markers.

Tests performed

Oral glucose tolerance test – oGTT

Oral GTT was performed in animals after a 12-h fast. First, a blood sample was collected from the tail end (fasting). Then, a 20% glucose solution (2g/kg body weight) was administered to rats by a polyethylene gastric tube. Blood samples were collected after 30, 60 and 120 min by heparinized capillary tubes calibrated for 25µL to establish the glucose and insulin concentrations. The blood glucose concentration was measured using the glucose oxidase method [17]. Results were analyzed by establishing the serum glucose AUG values by means of the trapezoidal rule [10] using Excel 2007.

Insulin tolerance test-ITT

Insulin sensitivity was assessed by means of a subcutaneous insulin tolerance test. It consisted of subcutaneous administration of Humalog® (Lilly®, São Paulo Brazil) (insulin, 300 mU/Kg body weight), followed by blood sampling at 0, 30, 60 and 120 min. The blood glucose removal rate (KITT), which was expressed as %/minute, was calculated using the formula (0.0693/t/2) x100. The t/2 blood glucose was calculated by the least-square analysis of the curve of serum glucose contents, as long as a linear decrease after insulin administration was evident [18].

Biological material

Forty-eight hours after the last *in vivo* test, animals were sacrificed by intraperitoneal anesthesia (sodium thiopental, 40mg/kg body weight).Two blood samples were collected via the liver portal vein. One sample was used to measure glucose, triglycerides, HDL cholesterol, LDL cholesterol and total cholesterol concentrations by means of a commercial kit (Laborlab®, São Paulo, Brazil) [19]. The other sample was used to assess TBARS concentration and to estimate catalase and superoxide dismutase (SOD) activities. Two liver samples were collected, one to determine the triglyceride concentration and the other to assess oxidant status biomarkers (TBARS concentration and catalase and SOD activities). Finally, fatty tissue was removed from the subcutaneous, retroperitoneal and mesenteric areas to assess the triglyceride concentrations.

Oxidant Status Markers in the Liver

Antioxidant System Biomarkers

Catalase

To assess catalase activity, liver tissue samples (100-150 mg) were placed in Eppendorf-like tubes (1.5mL)containing 1 mL of frozen phosphate buffer saline (PBS) and were subjected to sonication and centrifugation at 10,000 rpm for 5 min. The supernatant was separated and stored at -20ºC for later analysis. Activity assays were performed by adding 50mM phosphate buffer and 10mM hydrogen peroxide (H_2O_2) to samples [20]. The drop in absorbance values by H_2O_2 spectrophotometry was followed. The calculation of catalase activity was performed using the equation $(2.3/\Delta t).\ (a/b).(\log A_1/A_2)$, where a is the volume of hemolysate in the bucket, b is the total bucket volume, A_1 is the absorbance value at t=0 and A_2 is the absorbance value at the final time point (which, in our case, was 15 sec after the onset of the reaction) [20].

Superoxide dismutase (SOD)

To assess superoxide dismutase activity, samples of liver tissue were washed in PBS, pH 7.4, containing 0.16 mg/mL heparin, to remove blood cells. Then, tissue was homogenized (on ice) in 1 mL of 20 mM 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES) buffer, pH 7.2, containing 1 mM ethylene glycol tetra-acetic acid (EGTA), 210 mM manitol and 70 mM sucrose. Next, the tissue was centrifuged at 10,000 x g for 15 min at 4ºC, and the supernatant was stored at -20ºC to measure total SOD (both cytoplasmic and mitochondrial levels). Measurements were performed using a commercial kit Cayman® (Michigan, US) .A tetrazolium salt was used to detect superoxide radicals generated by xanthine-oxidase and hypoxanthine. In this procedure, one SOD unit is defined as the amount of enzyme needed to exhibit 50% dismutation of superoxide radicals. This assay measures all three SOD types (Cu/Zn-, Mn- and Fe-SOD) [21].

Lipid Peroxidation Biomarkers

Concentration of thiobarbituric acid reactive substances (TBARS)

The method for TBARS assessment consisted of analyzing the final products of lipid peroxidation (i.e., lipid peroxides, malonaldehyde and other low-molecular-weight aldehydes) that form Schiff bases upon reacting with 2-thiobarbituric acid (TBA). To assess TBARS, samples (100-150 mg) were placed in plastic tubes (RIA-type) containing 1.5 mL of cold 0.05 N phosphate buffer and were homogenized in a Polytron and centrifuged for 5 min at 10,000 rpm. The supernatants were separated, complexes were stained and their concentrations were determined by spectrophotometry at 5nm [22].

Statistics

Results were analyzed statistically using one-way analysis of variance (ANOVA). When indicated, a Bonferroni contrast test was applied at the 5% significance level.

Results

Figure 1 shows the body weight measurements during the study and the AUC values at the end of the study. At the end of the study, A and S groups exhibited reductions in the AUC values compared to the C group.

Figure 2 shows the blood lactate and the overload values at the lactate threshold during the minimum lactate test. The results for one animal are shown as an example. For this animal, the lactate threshold was obtained with a 2.6% body weight load and a 4.86mM lactacidemia value.

Table 1 shows the values of the areas under the serum glucose curves (AUG) during oral glucose tolerance test. No difference was found between the groups.

Table 2 shows the glucose removal rates during the insulin tolerance test. No differences were found between the groups.

Table 3 shows glucose, triglyceride, HDL cholesterol, LDL cholesterol and total cholesterol serum concentrations at the end of the study. Animals in groups A and S exhibited higher HDL cholesterol concentrations compared to group C.

Figure 3shows the catalase and superoxide dismutase activities and TBARS concentrations in the sera of animals at the end of the study. All exercised groups (A, AS and S) exhibited increases in SOD activities and decreases in TBARS concentrations. Moreover, group A exhibited a reduction in catalase activity compared to groups AS and C.

Figure 4 shows the catalase and SOD activities and TBARS concentrations in the livers of animals at the end of the study. Animals in group A exhibited increase sin catalase activity in the liver compared to the other groups. Animals in group A also exhibited increases in SOD activity compared to group C.

Table 4shows the triglyceride levels in the liver and in the mesenteric, retroperitoneal and subcutaneous fatty tissue at the end of the experiment. Exercised animals (groups A, AS and S) had reduced levels of triglycerides in the liver and fatty tissues in all three areas.

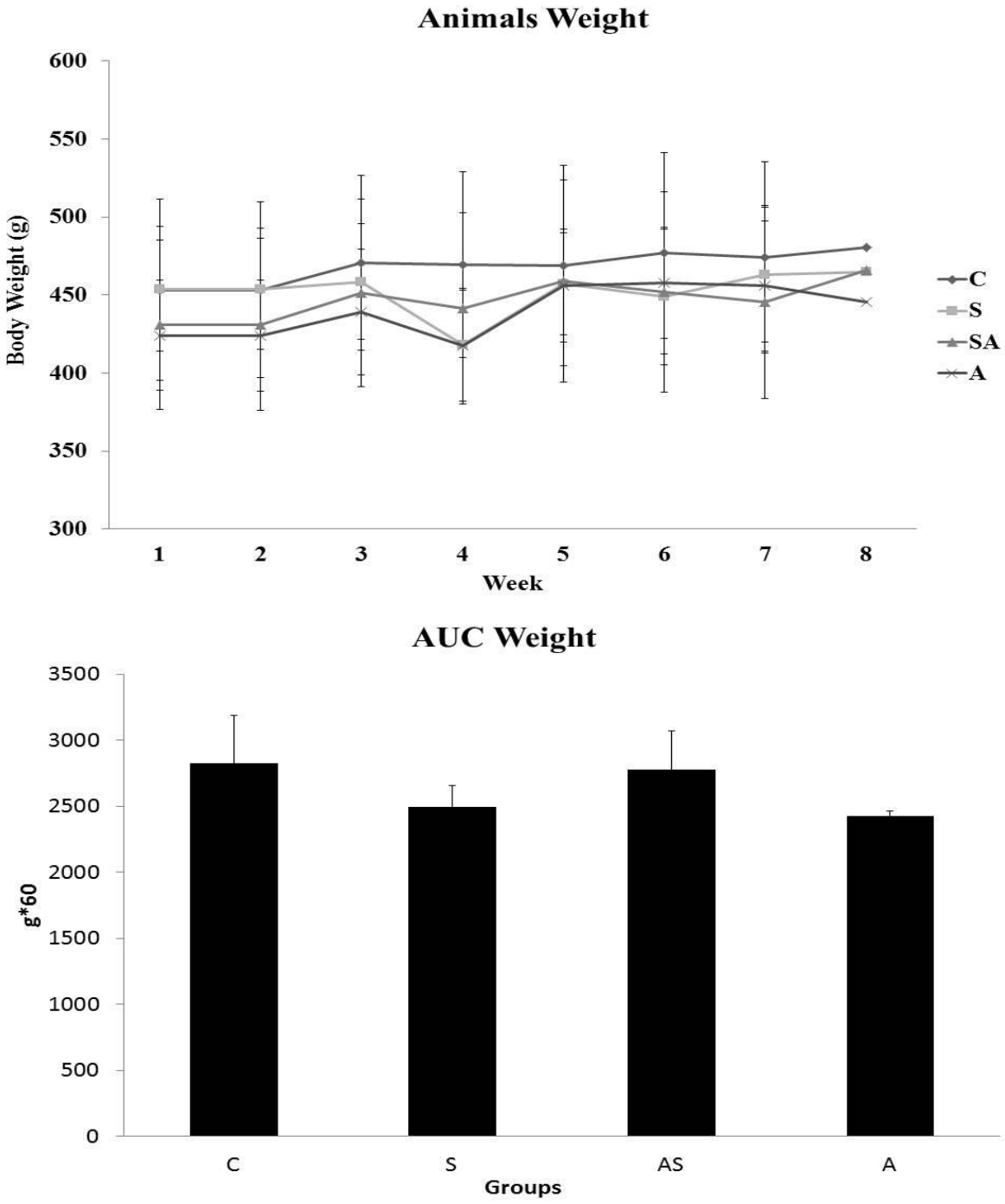


Figure 1. Body weights during the study and the body weight AUC values at the end of the study.
C=Control; S=Strength Training; AS=Concurrent Training; A=Aerobic Training. n=8 animals per group. Results are expressed as the means ±standard deviation. Different letters indicate the significant differences between groups ($p \leq 0.05$).

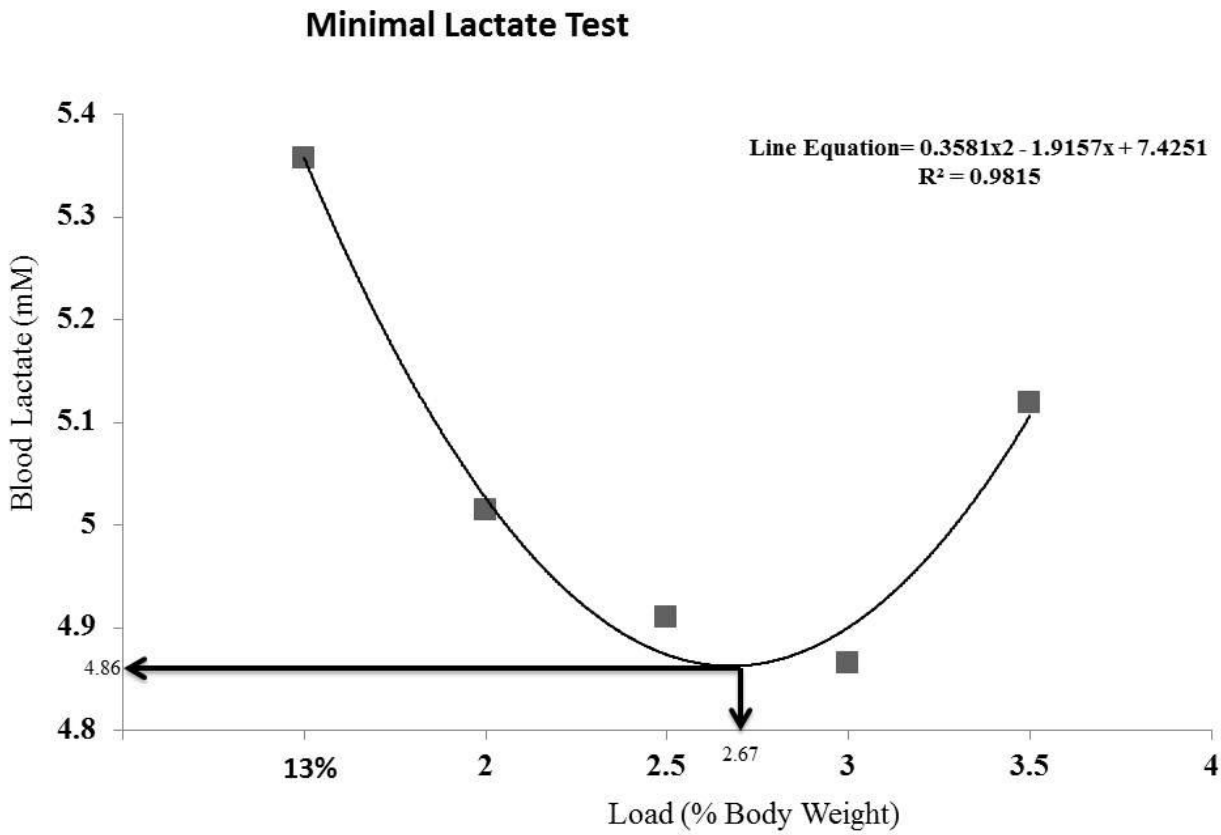


Figure 2. Lactacidemia and lactate threshold equivalent overload of one animal during the minimum lactate test.
C=Control; S=Strength Training; AS=Concurrent Training; A=Aerobic Training. n=8 animals per group. Results are expressed as the mean± standard deviation. Different letters indicate the significant differences between groups ($p \leq 0.05$).

Table 1.Blood glucose (mg/dl) and glucose areas under the curve (AUG) values during the oral glucose tolerance test (oGTT).

Serum Glucose and AUG					
Groups	**T0**	**T30**	**T60**	**T120**	**AUG**
C	80.2±6.0	105.8±13.8	102.1±6.7	79.2±8.8	11354±676
A	91.1±8.9	94.0±9.9	97.3±8.1	92.6±10.9	11343±1129
AS	93.8±8.1	95.9±6.4	94.7±7.5	94.1±6.5	11371±850
S	93.4±6.3	93.3±6.2	92.2±6.5	87.7±5.0	10981±727

C=Control; S=Strength training; AS=Concurrent Training; A=Aerobic Training. n=8 animals per group.

Table 2. Glucose removal rates (%.min^{-1}-KITT) after the insulin tolerance test (ITT).

Groups	KITT
C	1.04±0.31
S	0.9±0.3
AS	0.9±0.2
A	0.6±0.2

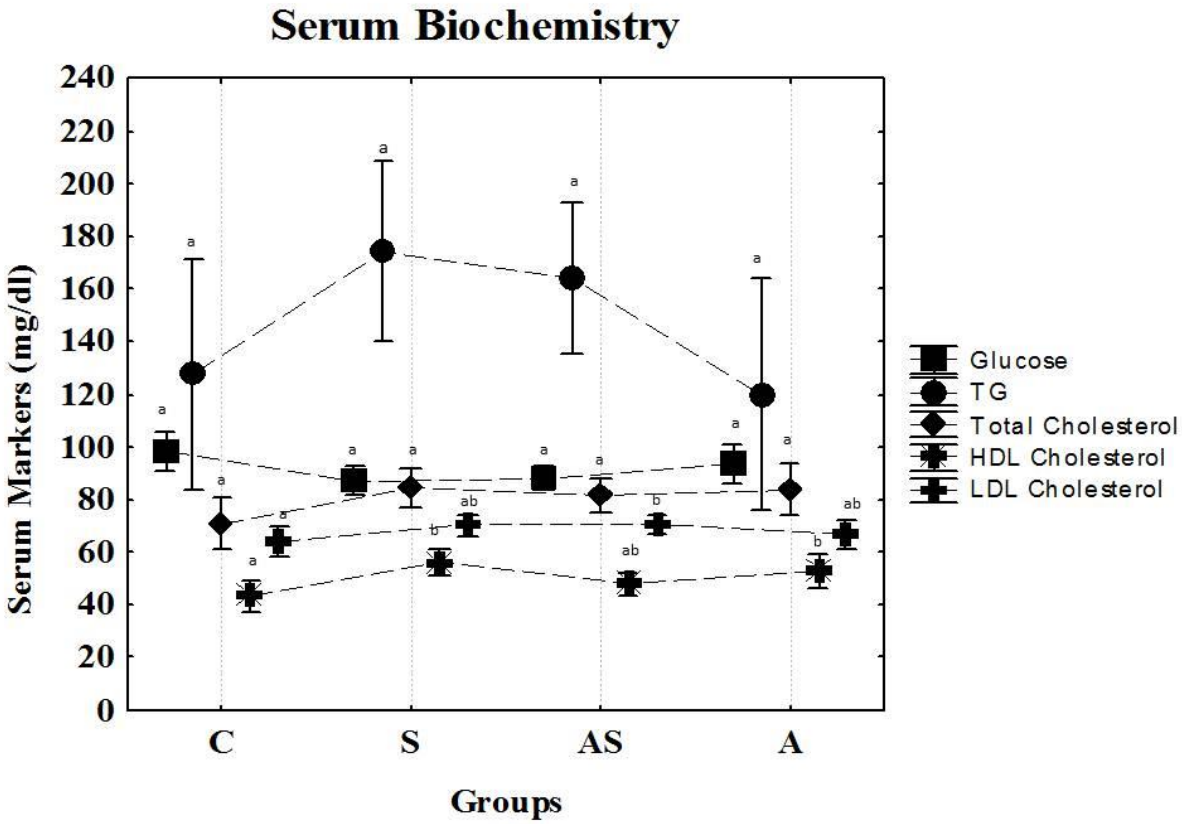
C=Control; S=Strength training; AS=Concurrent Training; A=Aerobic Training. n=8 animals per group. Different letters indicate significant differences between groups ($p \leq 0.05$).

Table 3. Glucose, triglycerides, HDL cholesterol, LDL cholesterol and total cholesterol (mg/dl) levels in the sera of the animals at the end of the study.

Parameter	C	S	SA	A
Glucose	98.8±9.9[a]	90.0±5.2[a]	88.7± 3.0[a]	93.0± 4.1[a]
Triglycerides	182.8±40.9[a]	174.0±46.3[a]	124.5±14.4[a]	163.8±31.7[a]
HDL-Cholesterol	42.7±5.3[a]	54.4±7.8[b[p<0.05]]	53.1±4.6[ab]	47.4±2.2[b[p<0.05]]
LDL-Cholesterol	62.9±3.9[a]	70.4±6.5[ab]	70.0±4.7[b[p<0.05]]	71.1± 7.5[ab]

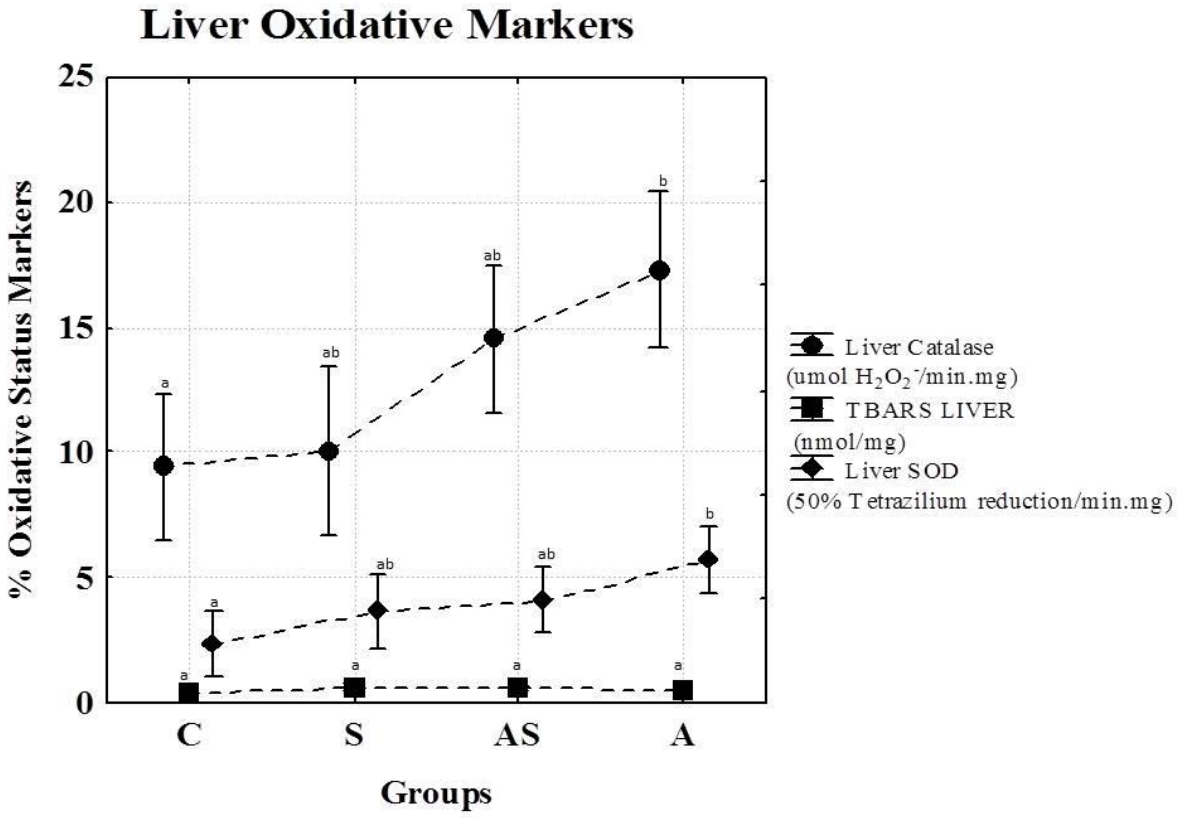
C=Control; S=Strength Training; AS=Concurrent Training; A=Aerobic Training. n=8 animals per group. Results are expressed as the means ± standard deviation. Different letters indicate the significant differences between groups ($p \leq 0.05$).

Figure 3. Catalase and SOD activities and TBARS concentrations in animal serum at the end of the study.



C=Control; S=Strength Training; AS=Concurrent Training; A=Aerobic Training. n=8 animals per group. Results are expressed as the means ±standard deviation. Different letters indicate the significant differences between groups ($p \leq 0.05$).

Figure 4. Catalase and SOD activities and TBARS concentrations in animal livers at the end of the study.



C=Control; S=Strength Training; AS=Concurrent Training; A=Aerobic Training. n=8 animals per group. Results are expressed as the means ±standard deviation. Different letters indicate the significant differences between groups ($p \leq 0.05$).

Table4. Concentrations of triglycerides in the liver and fatty tissue in the mesenteric, retroperitoneal and subcutaneous areas at the end of the study.

Triglycerides (mmol/mg)	C	S	AS	A
Liver	13.5 ± 3.5^{a}	$9.1\pm1.2^{b}[p<0.05]$	$9.0\pm 2.8^{b}[p<0.05]$	$9.9\pm 1.5^{b}[p<0.01]$
Mesenteric	78.6 ± 3.0^{a}	$60.1\pm6.0^{b}[p<0.01]$	$57.4\pm15.1^{b}[p<0.01]$	$51.2\pm12.0^{b}[p<0.001]$
Subcutaneous	140.4 ± 24.0^{a}	$82.2\pm6.3^{b}[p<0.01]$	$95.5\pm12.5^{b}[p<0.01]$	$85.2\pm10.0^{b}[p<0.05]$
Retroperitoneal	101.5 ± 27.0^{a}	$55.5\pm10.5^{b}[p<0.01]$	$42.0\pm3.5^{b}[p<0.01]$	$50.5\pm 2.0^{b}[p<0.01]$

C=Control; S=Strength training; AS=Concurrent Training; A=Aerobic Training. n=8 animals per group. Different letters indicate significant differences between groups ($p\leq0.05$).

Discussion

The reduction in daily energy expenditure is strongly correlated with the abrupt increase in the number of obese individuals [23]. Physical exercise is an important tool for controlling body weight [24]. In this study, animals in groups A and S exhibited reductions in the body weight AUC values compared to group C. During physical exercise, the skeletal muscle might increase its energy expenditure up to 100 times during physical exercise [25]. Minimum lactate (ML) intensity corresponds to approximately 77% of the maximum oxygen consumption (VO_2; VO_2max, for maximum value). In our study, animals in groups A and AS were subjected to training at 80% ML intensity, corresponding to approximately60% of VO_2max [26]. The energy expenditure during long duration exercise performed at moderate intensity is obtained mostly by lipid mobilization, followed by carbohydrates and finally by proteins. Triglyceride mobilization might explain the weight loss of endurance-trained animals at the end of the study. Animals from group S might

have responded to the increase in energy expenditure and muscular hypertrophy caused by this type of high-intensity training [27].

Insulin sensitivity and glucose tolerance are significant tests to determine the efficiency of carbohydrate metabolism. In the strength training exercises used in this study (group S), lactic glycolytic energy predominates. In this system, glucose captured by cells is degraded into two acetyl-CoA molecules that are transformed into lactate, supplying two ATP molecules [28]. This reaction is a powerful activator of carbohydrate metabolism and glucose capture by glucose transporters-4 (Glut-4). According to the results described in Table 2, there were no significant differences between the exercised groups and control in this regard. However, the glucose tolerance and insulin sensitivity values found in the exercised groups approach the values found in other studies [11, 29]. Moreover, no differences were seen in the blood glucose levels of animals at the end of the study, suggesting that no alteration of carbohydrate metabolism occurred.

The lipid metabolism levels of animals were altered. There were increases in blood HDL cholesterol in groups A and S compared to the control group. Moreover, exercised animals exhibited reductions in the concentrations of triglycerides in the liver and fatty tissue in all investigated areas compared to the control. An increase in energy expenditure might explain the reductions in the concentrations of these lipids. During exercise lasting 1 to 2 h, intramuscular triglycerides are consumed, and the lipolysis mechanism is activated in the fatty tissue that supplies carbon skeletons for physical exercise. Alternatively, triglycerides might be re-esterified and stored in the muscle tissue for future use [30]. During strength exercise, there might be an increase in energy expenditure and basal metabolism due to the muscular hypertrophy [28] developed by this type of training [11]. The reduction of liver lipids in group A might be related to an improvement in liver function and the subsequent increase in HDL cholesterol production. Several

studies suggest an improvement in the lipid profiles in endurance-trained rats [31-34]. The reductions of triglycerides were significant in all fatty tissue areas investigated in the trained animals. An increase in energy expenditure caused by physical training and possible metabolic alterations might be the major cause behind the improvements in the lipid profiles of rats. Similar to previous studies involving different exercise protocols [35-37], this finding shows that at different intensities, volume and energy predominance, physical exercise is an important tool in the fight against obesity.

Another beneficial effect of physical training that was observed is related to the concentrations of TBARS lipid peroxidation markers in the blood of exercised animals (groups A, AS and S). This finding is interesting because an increase in lipid oxidation is also seen in obesity. However, in this condition, lipid peroxidation is caused by the increased production of free radicals [38, 39]. In the long run, this imbalance in reactive oxygen species might make the antioxidant system unable to reduce free radicals, causing its failure, decreasing its activity and blocking its protective role [40]. Physical exercise might have improved the efficiency of lipid oxidation and the antioxidant status, which was diagnosed by means of superoxide dismutase activity in animal livers in groups A, AS and S and catalase activity in the animal livers in group A. These enzymatic antioxidants are present in all mammalian cells and are associated with the reduction of $O^-$and $H_2O_2^-$ in the respiratory chain [41].

Maintenance of the antioxidant mechanism and a decrease in the structural damage caused by reactive oxygen species are very important for obese individuals. A steady high-level reactive oxygen species state could lead to cell structural damage, which, in turn, increases the concentrations of inflammatory markers such as TNF-α, interleukin 6 and interferon-γ and decreases the concentrations of adiponectin and anti-inflammatory interleukins [42, 43].

Maintenance of inflammation leads to the damaging effects present in metabolic syndrome, such as endothelial dysfunction and insulin resistance [43]. Peroxidation of blood LDL cholesterol allows atheromatous plaques to accumulate on the endothelial walls, which might eventually detach and block blood flow in important small-caliber blood vessels, causing cerebrovascular accidents, myocardial ischemia and infarction [44].

Inflammatory markers decrease insulin sensitivity by causing dysfunction of insulin receptor substrate-1 (IRS-1) and insulin receptor substrate-2 (IRS-2), decreasing glucose capture and consequently increasing the dependence on lipid metabolism, thus creating a vicious circle [45]. Physical exercise might interrupt the full chain of events and make this unhealthy status regress [6].Physical exercise might also increase the efficiency of lipid oxidation and the activity of anti-oxidizing enzymes.

In conclusion, physical exercise was efficient with respect to the following: reducing the storage of triglycerides in animal livers and fatty tissue; decreasing the body weights of animals in groups A and S; decreasing the lipid profiles of animals in group A; significantly increasing the activities of the anti-oxidizing system; and reducing the concentrations of lipid peroxidation markers. Further studies are needed to identify other physical activity intensity zones that can achieve improvement in these markers.

Competing Interests

The authors declare that they have no competing interests.

Author's Contributions

JDB, was responsible for experimental design, data collection, statistical analysis and preparation of the manuscript. LTC, ACG, RAD, PPMS and CR were responsible for data collection. FAV was responsible for collecting data and preparing the manuscript. MARM was responsible for experimental design, coordination of research and preparing the manuscript. All authors read and approved the manuscript.

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5.3 Resultados da Terceira Fase Experimental

Different physical exercise protocols attenuate the pathophysiology of metabolic syndrome, inflammation and oxidative stress in rats fed on fructose rich diet.

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Abstract

Background: The association between the consumption of a fructose-rich diet and metabolic disorders has been established in the literature. On the other hand, exercise has proven a valuable tool preventing and treating some metabolic disorders both in humans and in experimental models. The aim of this study was to evaluate the effects of three different exercise protocols in animals presenting metabolic disorders induced by a fructose-rich diet. **Method**: For this experiment , we used 40 Wistar (120 days old) separated in five groups (n = 8 animals per group): C (control diet and sedentary), F (fed on a fructose rich-diet and sedentary), FA (fed on a fructose rich-diet and exercised in the aerobic protocol) , FAS (fed on a fructose rich-diet and exercised in the concurrent protocol) and FS (fed on a fructose rich-diet and exercised power in the protocol) . At the end of the experiment the follow analyses were performed: the glucose homeostasis (oGTT and ITT), blood biochemistry, oxidative stress and inflammation , and triglyceride content in different tissues.. Results: The Strength protocol exerted greater effects on glucose homeostasis compared to other protocols. All three exercise protocols provided remarkable responses in markers of inflammation, tissue triglycerides content and reduced oxidative stress (FS and FA) . In summary , the protocols of exercise generated important systemic responses in animals received a diet rich in fructose. However, the strength protocol generated sharper responses than aerobic and concurrent protocol in most of the metabolic syndrome and inflammation markers.

Key words : Fructose , Exercise , Metabolic syndrome , hyperinsulinemia , inflammation

Background

The metabolic syndrome (MS) (coexistence of metabolic disorders in the same individual) is a world-wide growing problem (1) and is associated with an excessive intake of sugar and fat (2) and also, with a decrease in daily energy expenditure (3). The average prevalence of the MS in the United States is 34% among adults and 50-60% in the population over 60 years of age, which constituting a harmful health and economic problem (4) (5). A state of chronic inflammation occurs with the MS installation and can be a cause-effect of an increased dependence on lipids as an energy source, insulin resistance, formation of oxygen reactive species that results in subsequent protein structure disarray, cell structure damage, mutations and apoptosis, leading to a vicious cycle (6) (7).

The physical exercise is a well-described treatment against obesity, cardiovascular diseases, diabetes and other metabolic disorders. The extra caloric expenditure and physiological adaptations can be responsible for reduction in the weight, triglycerides content, glycaemia, total-cholesterol, inflammatory markers, oxidative stress and improving in the sensitivity of several hormones, muscle mass, glucose tolerance, basal metabolism etc… (8), (9), (10), (11).

Objective

This research aims to investigate the potential effects of the physical exercise in the pathophysiology of fructose-induced metabolic syndrome, oxidative stress and inflammation.

In order to explore this research hypothesis, Wistar rats (*Rattus Norvegicus)* were fed on a standard diet (Labina®) and a fructose-rich diet (semi-purified, 60% of fructose) and treated with three different exercise protocols: Aerobic, Concurrent and Strength.

The outcomes of this study will provide a better understanding of the role of physical exercise in the prevention and treatment of metabolic pathophysiologies.

Material and Method:

Forty adult (120 days) Wistar rats (*Rattus Norvegicus)* rats were used. The animals were kept in collective cages (four animals per cage) at a controlled temperature of 25 ± 1 ºC and under a light/dark cycle of 12/12 h with free access to water and food. The experiment was performed at the Nutrition, Metabolism and Exercise Laboratory at São Paulo State University, Rio Claro, Brazil. All experiments were analyzed and approved by the Biosciences Institute Animal Ethics Committee, Rio Claro Campus (case number: 005/2010).

Diet composition

We used commercial chow (Labina, Purina®) as a control diet (57,3% carbohydrate, 41,2% of cornstarch), this diet was used in previous researches as a control diet and none improving in the animals was detected (12), (13).

For the fructose-rich diet, we used an adapted diet standardized by Bezerra and colleagues (14) composed of (in g/kg) 202 of casein, 625.5 of fructose, 2 of l-cysteine, 70 of soy oil, 35 of mineral salt mix (15) 10 of a vitamin mix (15), 50 of fiber and 2.5 of choline chloridrate.

Experimental groups

At 120 d of age, the animals were separated randomly into five groups:

Control Group (C) was fed on commercial balanced diet from 120 to 180 d of age;

Fructose Group (F) was fed on semi-purified fructose-rich diet from 120 to 180 d of age.

Fructose Aerobic Group (FA) was fed on semi-purified fructose rich diet from 120 to 180 d of age. Also these animals were trained in the same period in the Aerobic exercise protocol one hour per day, five days per week.

Fructose Concurrent Group (FAS) was fed on semi-purified fructose rich diet from 120 to 180d of age. These animals were trained during the same period in the Aerobic exercise protocol and Strength exercise protocol in alternate days, five days per week.

Fructose Strength Group (FS) was fed on semi-purified fructose-rich diet from 120 to 180d of age. These animals were trained in the Strength exercise protocol five days per week.

A baseline group is described in the supplementary file. This group was used just to show the initial state of the rats before the experiment. These data include: oGTT, ITT, serum biochemistry, triglycerides concentration in different tissues and oxidative status markers.

Physical training

Aerobic protocol

This protocol consisted of the animals swimming in individual tanks that contained water at 31 ± 1°C for 1 h per day, 5 days per week. Exercise was performed with the 80% individual minimum lactate intensity overload attached to the thorax of the animal.

Strength Protocol

Animals performed jumps in individual tanks with the water level standardized at 150% body length and a water temperature of 31 ± 1°C. Animals performed four 10-jump series with a

50% body weight overload attached to the thorax and a 1-min rest between series for 5 days per week.

Concurrent protocol

Animals were trained using the aerobic protocol three times a week (Mondays, Wednesdays and Fridays) and using the strength protocol twice a week (Tuesdays and Thursdays).

Body weight

The weights of the animals were recorded weekly during the experimental phase, and the area under the curve (AUC) was calculated with Microsoft Excel 2010 software using the trapezoidal method Mathews.

Food intake

The food intake was recorded once a week during the experimental phase. The AUC was calculated using the Microsoft Excel 2010 software through the trapezoidal method (16).

Oral glucose tolerance test – oGTT

Oral GTT was performed in animals after a 12-h fast. First, a blood sample was collected from the tail end (fasting). Then, a 20% glucose solution (2g/kg body weight) was administered to rats by a polyethylene gastric tube. Blood samples were collected after 30, 60 and 120 min by heparinized capillary tubes calibrated for 25µL to establish the glucose and insulin concentrations. The blood glucose concentration was measured using the glucose oxidase method (17) and the

serum insulin levels were measured using ultrasensitive mouse insulin ELISA kit (EZRMI-13K.EIA: Millipore, St. Charles, MI). Results were analyzed by establishing the serum glucose AUG values by means of the trapezoidal rule Mathews using Excel 2007 (16).

Insulin tolerance test-ITT

Insulin sensitivity was assessed by means of a subcutaneous insulin tolerance test. It consisted of subcutaneous administration of Humalog® (Lilly®, São Paulo Brazil) (insulin, 300 mU/Kg body weight), followed by blood sampling at 0, 30, 60 and 120 min. The blood glucose removal rate (KITT), which was expressed as %/minute, was calculated using the formula (0.0693/t/2) x100. The t/2 blood glucose was calculated by the least-square analysis of the curve of serum glucose contents, as long as a linear decrease after insulin administration was evident (18).

In Vitro Assays

Biological material

Forty-eight hours after the last *in vivo* test, animals were sacrificed in a CO_2 chamber. Three blood samples were collected via the liver portal vein. One sample was used to measure glucose, triglycerides, HDL cholesterol and total cholesterol concentrations by means of a commercial kit (Laborlab®, São Paulo, Brazil). The other sample was used to assess TBARS concentration and to estimate catalase and superoxide dismutase (SOD) activities. The third one was used to measure the IL-4, IL-1β, IL-6, IL-10, INF-γ and TNF-α. Two liver samples were collected, one to determine the triglyceride concentration and the other to assess oxidant status biomarkers (TBARS concentration and catalase and SOD activities). Finally, heart and fatty tissue

were removed from the subcutaneous, retroperitoneal and mesenteric areas to assess the triglycerides concentrations.

Liver haematoxylin-eosin (HE) histology

Liver samples were collected and fixed in Bouin's fixative. The tissue was mounted in HitoResina (Leica) and sliced in a microtome (Leica RM2145) to a thickness of 6 μm. The slices were subjected to the haematoxylin-eosin staining method. The slices were hydrated and stained with haematoxylin (10 min) and were then washed and stained with eosin (5 min). Finally, they were washed and preserved in Canadian balsam (19).

Statistics

For the statistical tests and graphs we used the software Statistica 7.0®.The Shapiro-Wilk's W test was used to verify the normality of the sample. The results were analysed statistically using an ANOVA One way test and when necessary a post hoc contrast test (Bonferroni) was performed.

RESULTS

Minimum Lactate Test

The figure 1 shows the minimum lactate test of one rat used as example. For this specific rat the minimum lactate was obtained in a workload of 2.67% and 4.87mM. The results provided by this test were used as standards for each during each session of physical exercise and adjusted weekly according to the changes in body weight.

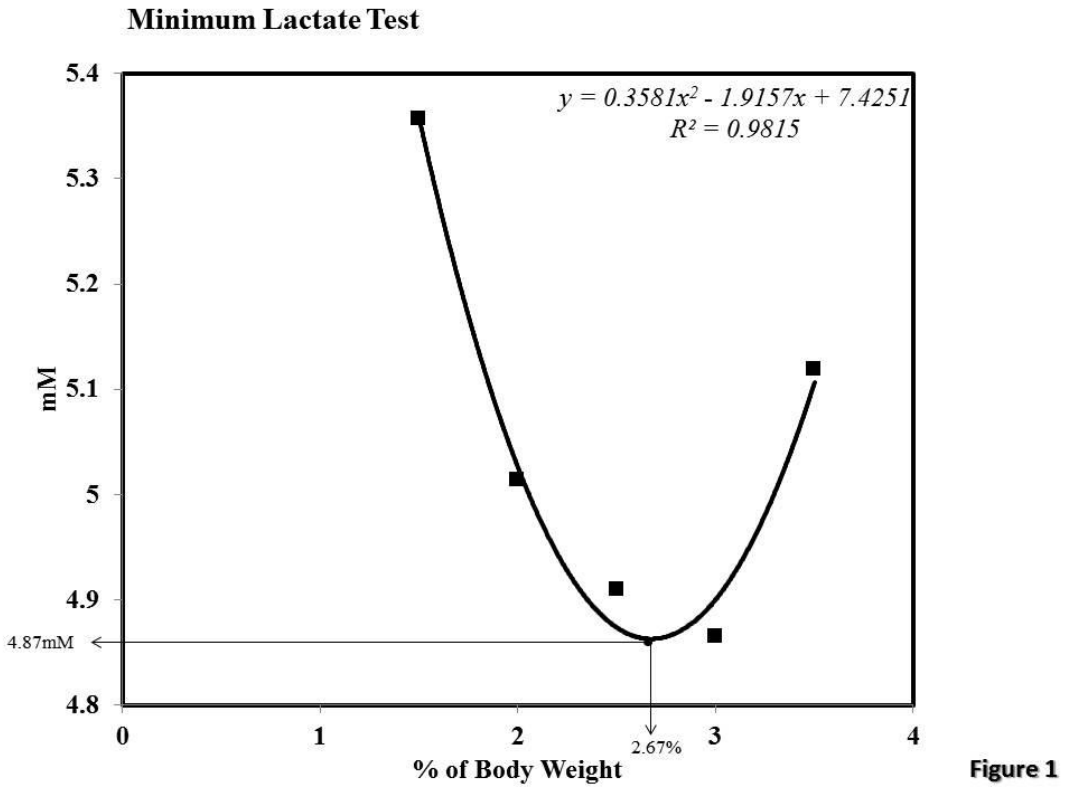

Figure 1

Figure 1: Minimum lactate test of one animal, as an example, In the case **A**, the estimated ML was 2.67% of body weight, while the interpolated blood lactate concentration was 4.87mM. In the case **B** the estimated ML was 4.37% of body weight, while the interpolated blood lactate concentration was 5.03mM. In the case

Aerobic exercise seems to reduce the food intake and body weight.

Figure 2 shows the body weight during the experiment, the AUC of body weight, weight gain and AUC of food intake. No differences were observed in the weight gain and AUC of weight at the end of experiment. On the other hand, the animals of FA group showed a reduction in the AUC of food intake when compared to F group ($p \leq 0.01$). The aerobic exercise seems to be a good control for the food intake when compared to other exercise protocols.

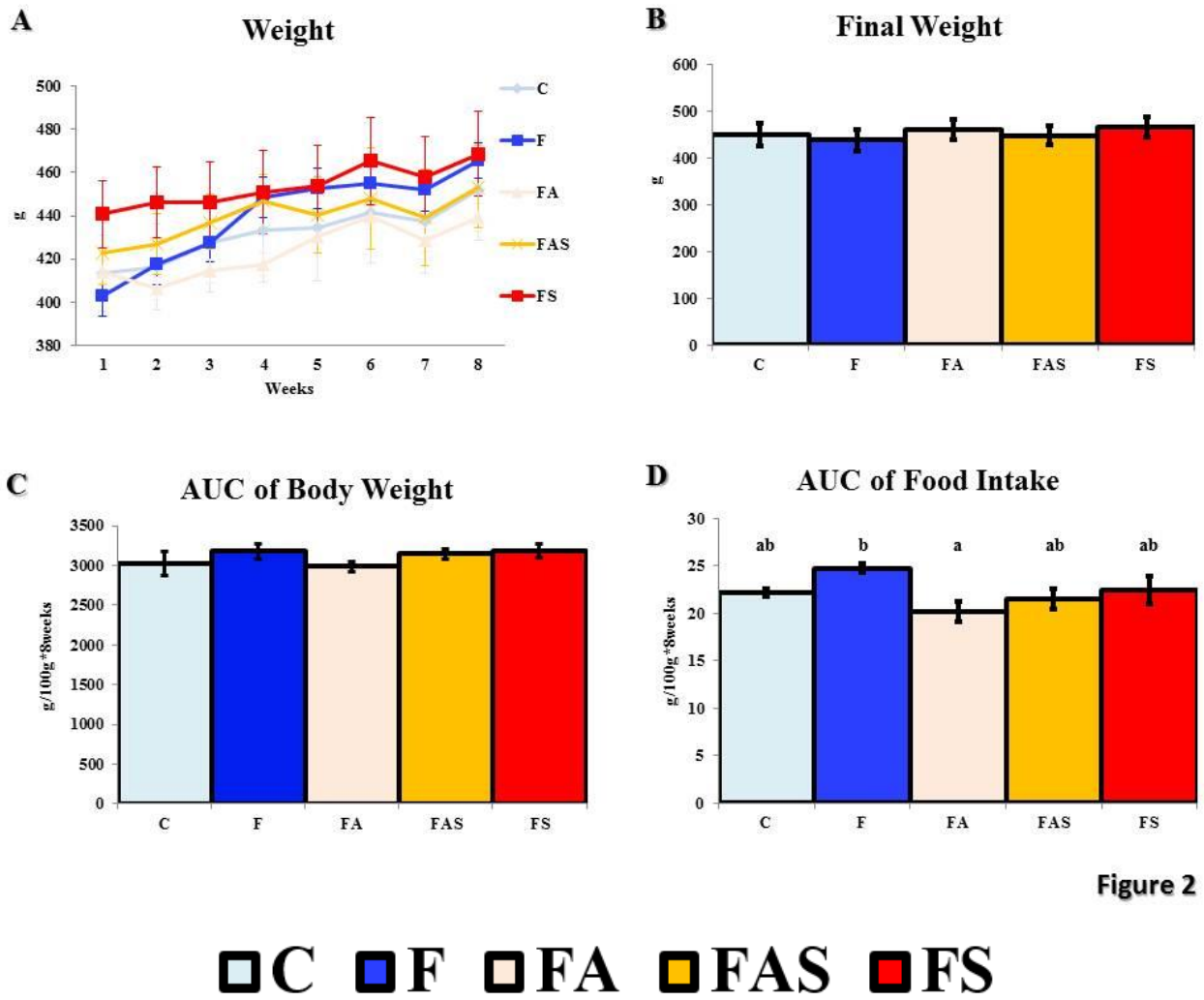

Figure 2. (A) Body weight change, **(B)** final weight and **(C)** weight gain did not change during and in the end of experiment. **(D)** F animals showed higher food intake (AUC) compared to FA group ($p \leq 0.01$).
C: Control; F: Fructose; FA: Fructose Aerobic; FAS: Fructose Concurrent; FS: Fructose Strength. n = 14 animals per group. Different letters means statistical difference

Hyperinsulinemia, glucose intolerance and insulin resistance are attenuated by aerobic physical exercise while strength training was able to restore all these markers to control levels.

There are no differences in the serum glucose during the oGTT. Otherwise, the animals of the FS group showed lower Areas Under Glucose curve during oGTT compared to the group F ($p \leq 0.05$) (Figure 3B).

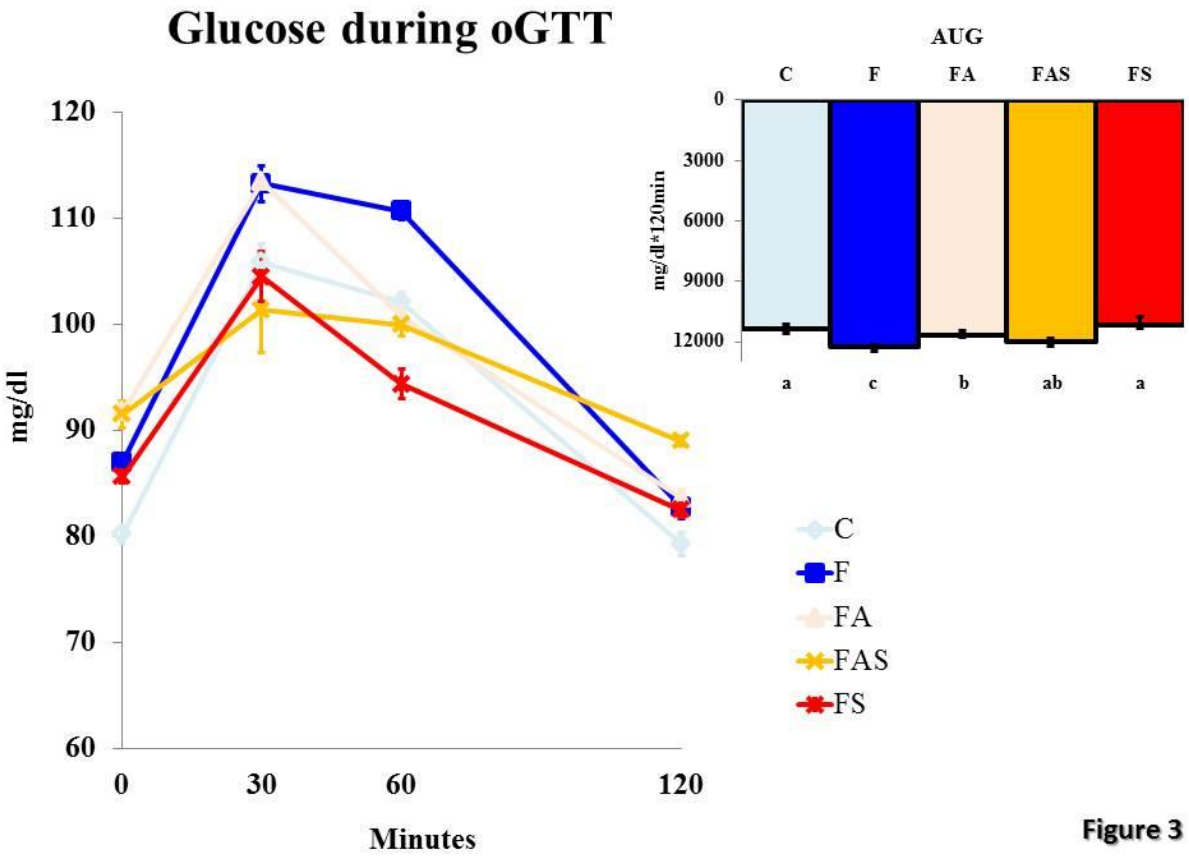

Figure 3. (A) Serum glucose kinetics (mg/dl), serum insulin kinetics (ng/dl), **(B)** area under the curve for serum glucose (mg*120 min/dl, AUC) and serum insulin (ng*120 min/dl, AUC) during the oral glucose tolerance test (oGTT). Animals of F and FA group showed higher AUC of glucose compared to C ($p \leq 0.01$) and FS ($p \leq 0.01$ *vs* F, and $p \leq 0.05$ *vs* FA).
C: Control; F: Fructose; FA: Fructose Aerobic; FAS: Fructose Concurrent; FS: Fructose Strength. n = 14 animals per group. Different letters means statistical difference.

The insulin levels were higher in the F groups compared to all other groups in the 30 minutes point ($p \leq 0.001$-0.005), the F group also showed higher concentrations of serum insulin in the 120 min point compared to the C group ($p \leq 0.01$) (Figure 4). The F group showed the highest levels of the insulin AUC among the groups ($p \leq 0.001$-0.005). Finally, the FA group showed higher insulin AUC values compared to the C and FS group ($p \leq 0.01$-0.05).

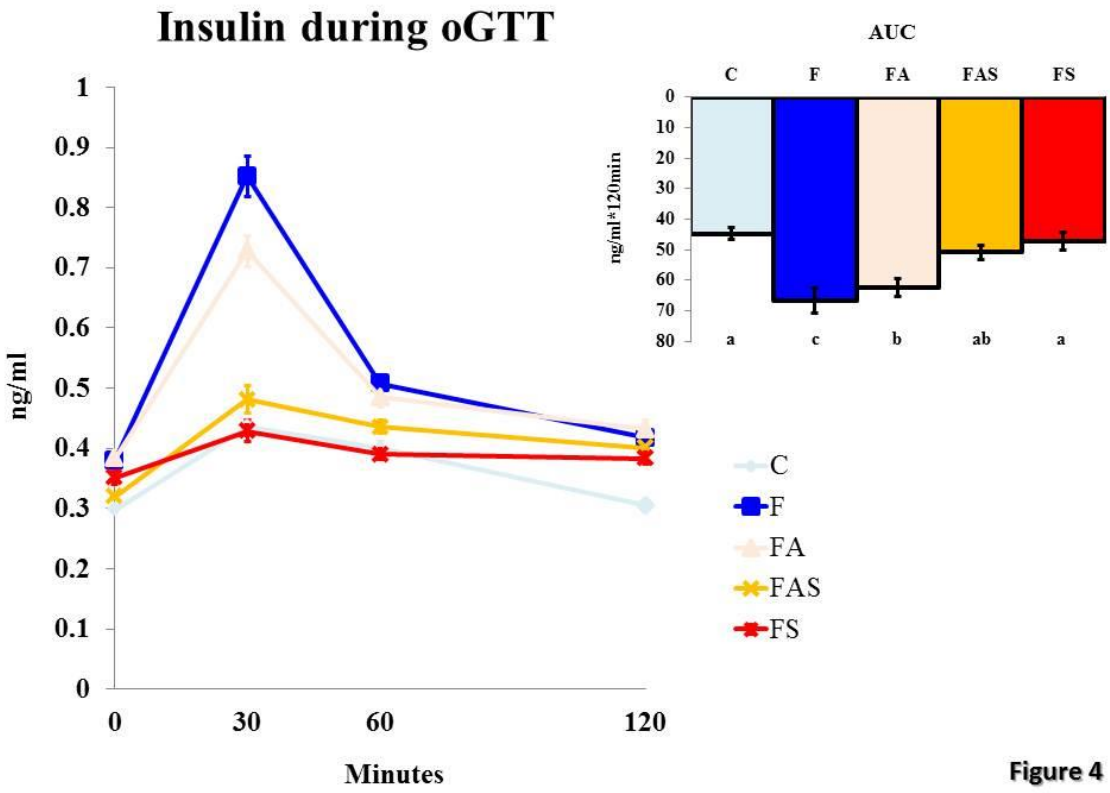

Figure 4. (A) Serum insulin kinetics (ng/dl), **(B)** and area under the curve for serum insulin (ng*120 min/dl, AUC) during the oral glucose tolerance test (oGTT). The animals of F group showed higher insulin values at 30 minutes peak; $p \leq p \leq 0.01$-0.005) compared to all other groups. Also, the animals of F group showed higher AUC of insulin ($p \leq 0.001$-0.005) compared to all other groups.
C: Control; F: Fructose; FA: Fructose Aerobic; FAS: Fructose Concurrent; FS: Fructose Strength. n = 14 animals per group. Different letters means statistical difference.

The basal insulin levels were measured using the first sample of the insulin tolerance test (Fig. 5A). All animals fed on fructose-rich diet (F, FA, FAS and FAS), showed higher insulin levels compared to the control animals (*C diet vs F diet*; $p \leq 0.0001$). In contrast, the animals trained in all exercise protocols presented lower insulin levels compared to F (*F vs FA, FAS and FS*; $p \leq$. 0.01-0.005). (**Fig. 5A**). Glucose kinetics (mg/dl), and area glucose removal rate (KITT in $\%/min^{-1}$) during the insulin tolerance test (ITT) is shown in Figure 5B and Figure 5C. There is a reduction in the insulin sensitivity of F and FA animals. Moreover, F group showed reduced insulin sensitivity (KITT) compared to all other groups ($p \leq p \leq 0.001$-0.0001) and FA group showed lower KITT levels compared to C ($p \leq 0.001$) and FS $p \leq$-0.005) (**Fig. 5C**). Taking the results, the strength training was successful to return the insulin sensitivity to normal control levels (vs C, $p \geq 0.05$).

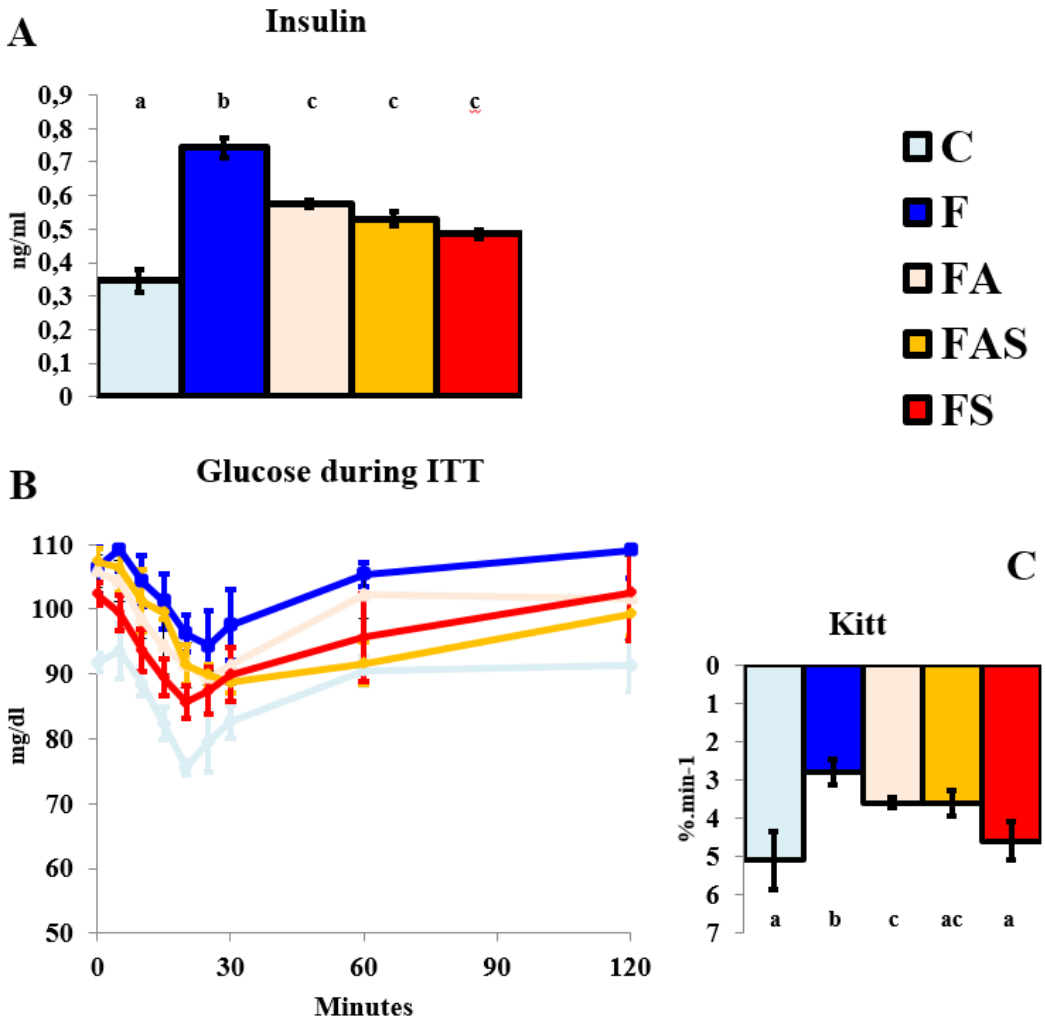

Figure 5. (A) The fructose-rich diet was able to induce an hyperinsulinemic (*F diet vs C diet*; p≤0.0001). On the other hand, all exercise protocols were successful to reduce the insulin levels (*Sedentary vs Exercised*; p≤0.01-0.005), but still, these levels are higher than the control animals (p≤0.05-0.01). **(B)** Glucose kinetics (mg/dl), and **(C)** glucose removal rate (KITT in %/min^{-1}) during the insulin tolerance test (ITT). F group showed reduced lower insulin sensitivity (KITT) compared to all other groups (p ≤ p≤0.001-0.0001). Also FA group showed lower insulin sensitivity compared to C (p≤0.001) and FS p≤-0.005). C: Control; F: Fructose; FA: Fructose Aerobic; FAS: Fructose Concurrent; FS: Fructose Strength. n = 14 animals per group. Different letters means statistical difference.

As we described, this hyperinsulinemic response to the fructose-rich diet administration was prevented by all exercise protocols. Also, the improvements in the glucose tolerance and insulin sensitivity seem to be more responsive to the strength exercise protocol compared to the aerobic protocol.

Physical exercise results in an almost complete restoration of the liver microscopic aspect to control animals with a reduction on the liver and muscle triglycerides.

A portion of the right lobe was extirpated to the H&E staining histology. The histology revealed that, fructose-fed animals have bigger storages of triglycerides characterizing diffuse macro vesicular steatosis (**Fig. 6**).

Figure 5

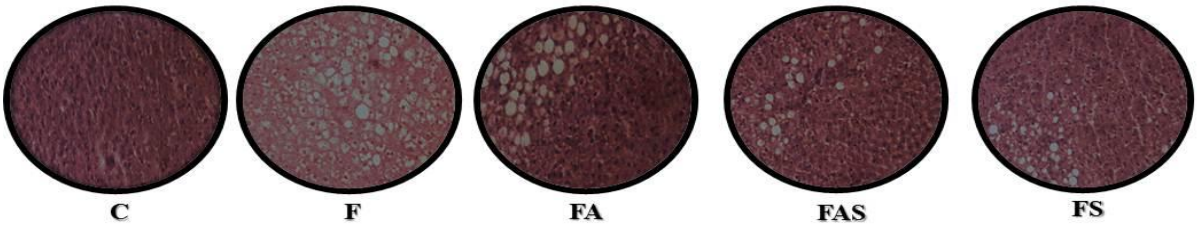

Figure 6. A portion of the right lobe was extirpated to the H&E staining histology. The histology revealed that, fructose-fed animals have bigger storages of triglycerides characterizing diffuse macro vesicular steatosis (Figure 5). C: Control; F: Fructose; FA: Fructose Aerobic; FAS: Fructose Concurrent; FS: Fructose Strength.

The triglycerides concentration in the liver, soleus muscle, heart and adipose tissue is showed in the table 1. The fructose-rich diet was successful to induce the triglycerides accumulation in the liver (ANOVA effect; *C diet vs F Diet;* ≤0.0001) and soleus muscle (ANOVA effect; *C diet vs F Diet;* ≤0.005). However the exercise was able to reduce this triglycerides infiltration to lower levels (ANOVA effect: *Sedentary vs Exercised;* Liver: p ≤0.005; Muscle: p ≤0.05). Taking individually, F and FA groups presented an increase in the concentrations of liver triglycerides compared to the other groups (p≤0.0001-0.05); both FAS and FS presented higher concentrations of triglycerides in the liver compared to the C group (p≥0.05). The F group showed also, an increase in the retroperitonial triglycerides content compared to the C and FS group (p≤0.001-0.01). All exercised animals showed higher concentration of muscle triglycerides

compared to C ($p \leq 0.05$) but when compared to F, lower concentrations ($p \leq 0.001$). No differences were found in the heart and the two other regions of the adipose tissue.

Table 1. Triglycerides concentrations(mg/dL) in the liver, heart and in the adipose tissue (Mesenteric, Retroperitonial and Subcutaneous regions) at the end of the experiment.

Groups	Liver (umol/mg)	Heart (umol/mg)	Soleous (umol/mg)	Mesenteric (umol/mg)	Retroperitoneal (umol/mg)	Subcutaneous (umol/mg)
C	2.7±0.8[a]	0.64±0.07	0.31±0.02[a]	46.1±1.83	70.1±1.94[a]	53.7±4.20
F	16.5±0.35[c]	0.71±0.03	1.34±0.11[b]	57.5±3.46	115.7±10.11[b]	50.3±3.21
FA	11.5±0.32[c]	0.56±0.04	0.45±0.03[c]	44.9±4.87	81.7±5.44[ab]	51.3±6.39
FAS	6.8±0.32[b]	0.53±0.03	0.59±0.06[c]	48.1±3.11	93.3±9.01[b]	56.5±7.91
FS	4.6±0.15[b]	0.55±0.07	0.67±0.04[c]	51.9±4.56	65.7±3.25[a]	37.6±2.68

F and FA groups presented an increase in the concentrations of liver triglycerides compared to the other groups ($p \leq 0.0001$-0.05); both FAS and FS presented higher concentrations of triglycerides in liver compared to the C group ($p \geq 0.05$). The F group also showed an increase in the retroperitonial triglycerides content compared to the C and FS group ($p \leq 0.001$-0.01). All exercised animals showed higher concentration of muscle triglycerides compared to C ($p \leq 0.05$) but when compared to F, lower concentrations ($p \leq 0.001$). No differences were found in the heart and the two other regions of the adipose tissue.
C: Control; F: Fructose; FA: Fructose Aerobic; FAS: Fructose Concurrent; FS: Fructose Strength. n = 8 animals per group. Different letters means statistical difference.

Hypertriglyceridemia is a main hallmark in rats fed on fructose-rich diet.

No differences were observed for the serum glucose and Total-Cholesterol concentrations at the end of experiment (Table 2). The fructose diet was able to induce hypertriglyceridemia in all F animals (ANOVA effect: *C diet vs F diet*; $p \leq 0.001$). On the other hand, FA and FAS group showed higher concentrations of HDL-Cholesterol compared to C and F group ($p \leq 0.05$). The fructose diet induces the overproduction of triglycerides in the liver and consequently the constant release of these fatty-acid chains in the bloodstream. At the consequence, a hypertriglyceridemia state was achieve and this condition contributes to an increase in the triglycerides uptake in the muscles (higher triglycerides concentrations in the soleus muscle) and production/reuptake in the

liver (liver droplets). However, the aerobic exercise showed an important role as tool to enhance the HDL-cholesterol levels.

Table 2. Glucose, triglycerides,, HDL cholesterol and Total-cholesterol concentrations, in animal serum at the end of the experiment.

Groups	Glucose (mg/dL)	Tryglicerides (mg/dL)	HDL-Cholesterol (mg/dL)	Total-Cholesterol (mg/dL)
C	98.8±3.50	172.2±15.02[a]	22.7±1.20[a]	78.9±5.58
F	110.9±5.02	261.4±4.49[b]	22.3±1.48[a]	89.1±5.44
FA	106.4±3.35	262.4±9.05[b]	31.3±1.13[b]	85.9±1.69
FAS	105.0±5.19	235.4±12.65[b]	35.2±2.51[b]	70.7±7.03
FS	104.5±4.98	242.0±7.8[b]	24.4±1.09[ab]	79.9±8.23

No differences were observed for the serum Glucose and Total-Cholesterol concentrations at the end of experiment. All fructose-fed animals (F, FA, FAS and FS) showed higher concentrations of serum triglycerides compared to C ($p \leq 0.0001$-0.01). Also, FA and FAS group showed higher concentrations of HDL-Cholesterol compared to C and F group ($p \leq 0.05$).
C: Control; F: Fructose; FA: Fructose Aerobic; FAS: Fructose Concurrent; FS: Fructose Strength. n = 8 animals per group. Different letters means statistical difference.

Liver and serum lipid peroxidation is reduced in animals exercised on aerobic protocol

The serum oxidant status was achieved through a puncture in the portal vein of the animals. No difference was observed in the levels of the antioxidants SOD. On the other hand, the Catalase activity has shown increased in the F compared to all other groups ($p \leq 0.01$). Also, the animals C and FA presented lower concentrations of lipid peroxidation markers compared to all other groups ($p \leq 0.01$-0.05) (Fig.7C).

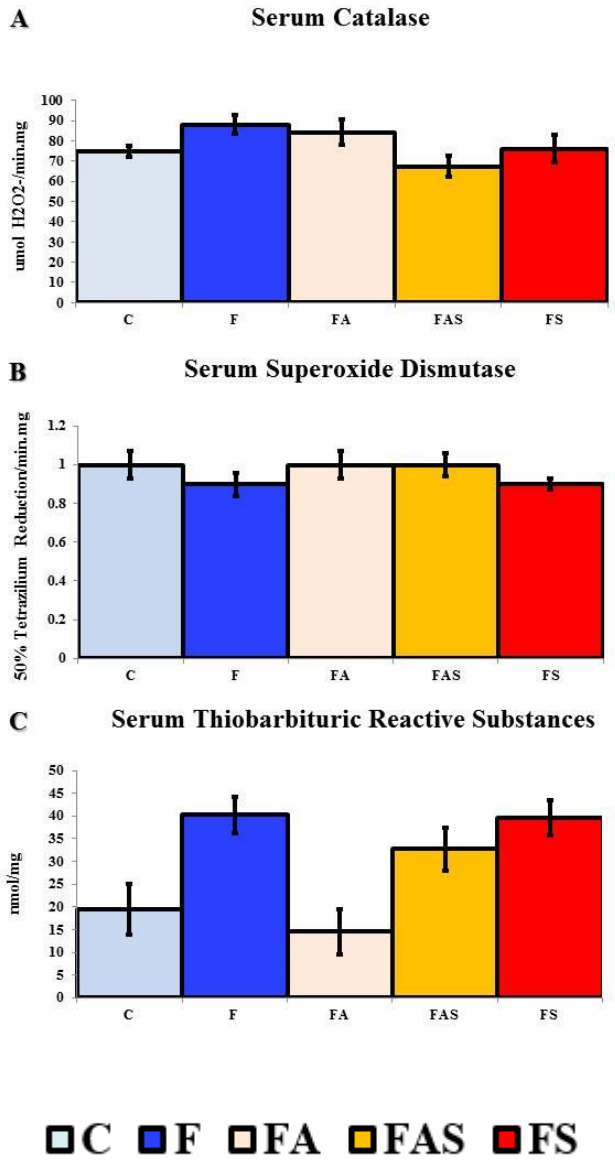

Figure 7: (A) Serum catalase, **(B)** SOD and **(C)** TBARs at the end of the experiment. No differences were observed in the antioxidants levels (SOD and catalase) neither in the lipid peroxidation products (TBARs). C: Control; F: Fructose; FA: Fructose Aerobic; FAS: Fructose Concurrent; FS: Fructose Strength. n= 8 animals per group.

A medial portion of the liver was extirpated to achieve the antioxidant status at the end of experiment (Fig.8). The F group showed a reduction on the Catalase levels compared to C ($p \leq 0.01$) while, the FA and FS showed an improvement in this enzyme activity compared to F ($p \leq 0.05$) (Fig. 8A). Finally, the F and FAS animals presented higher values of lipid peroxidation sub products (TBARs) compared to all other groups (Fig. 8C).

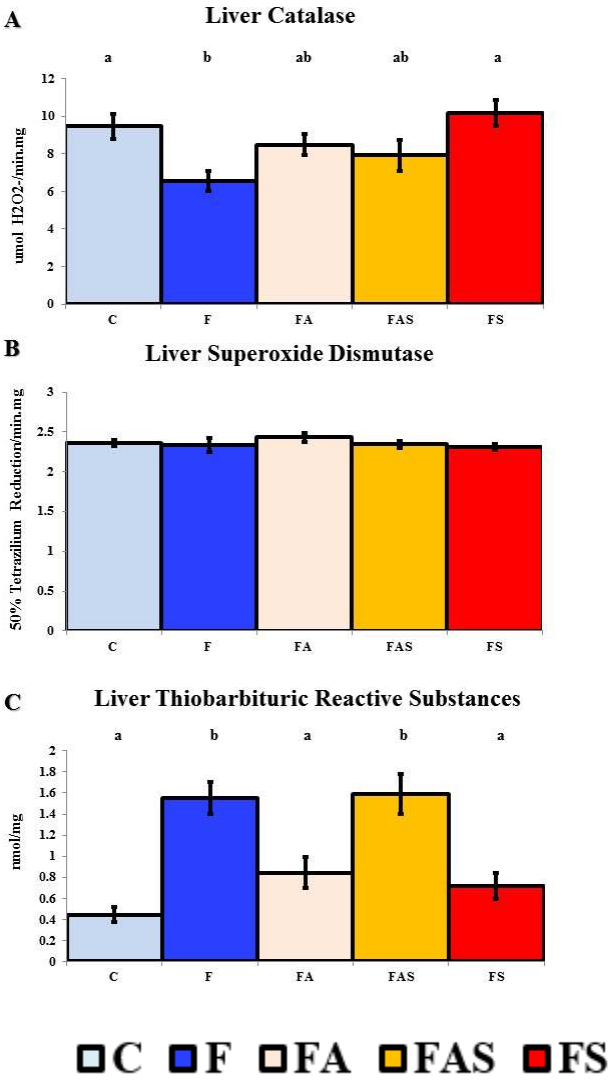

Figure 8: (A) Liver catalase, (B) SOD and (C) TBARs at the end of the experiment. The liver oxidant status was modified at the end of experiment. The F group showed a reduction on the Catalase levels compared to C ($p \leq 0.01$) while, the FA and FS showed an improving in this enzyme activity compared to F ($p \leq 0.05$). Finally, the F and FAS animals presented higher values of lipid peroxidation products (TBARs) compared to all other groups ($p \leq 0.001$-0.0001). C: Control; F: Fructose; FA: Fructose Aerobic; FAS: Fructose Concurrent; FS: Fructose Strength. n= 8 animals per group.

Physical exercise attenuate systemic inflammation.

The inflammatory response to the treatment showed in the Table 3 was achieved through the serum cytokines concentration. The F animals showed a high-level inflammatory state (**Fig.9B** IL-4, **Fig.9C**IL-6, **Fig.9E** Interferon-γ and **Fig.9F** TNF-α) compared to all other groups ($p \leq 0.01$-0.001). Serum IL-6 concentration (**Fig.9C**) was higher in the groups FAS when compared to the C and FA groups ($p \leq 0.0001$-0.0005). All exercises protocols were successful to prevent an increase in some inflammatory markers which are correlated with insulin resistance, obesity, NASH, atherosclerosis and other correlates diseases.

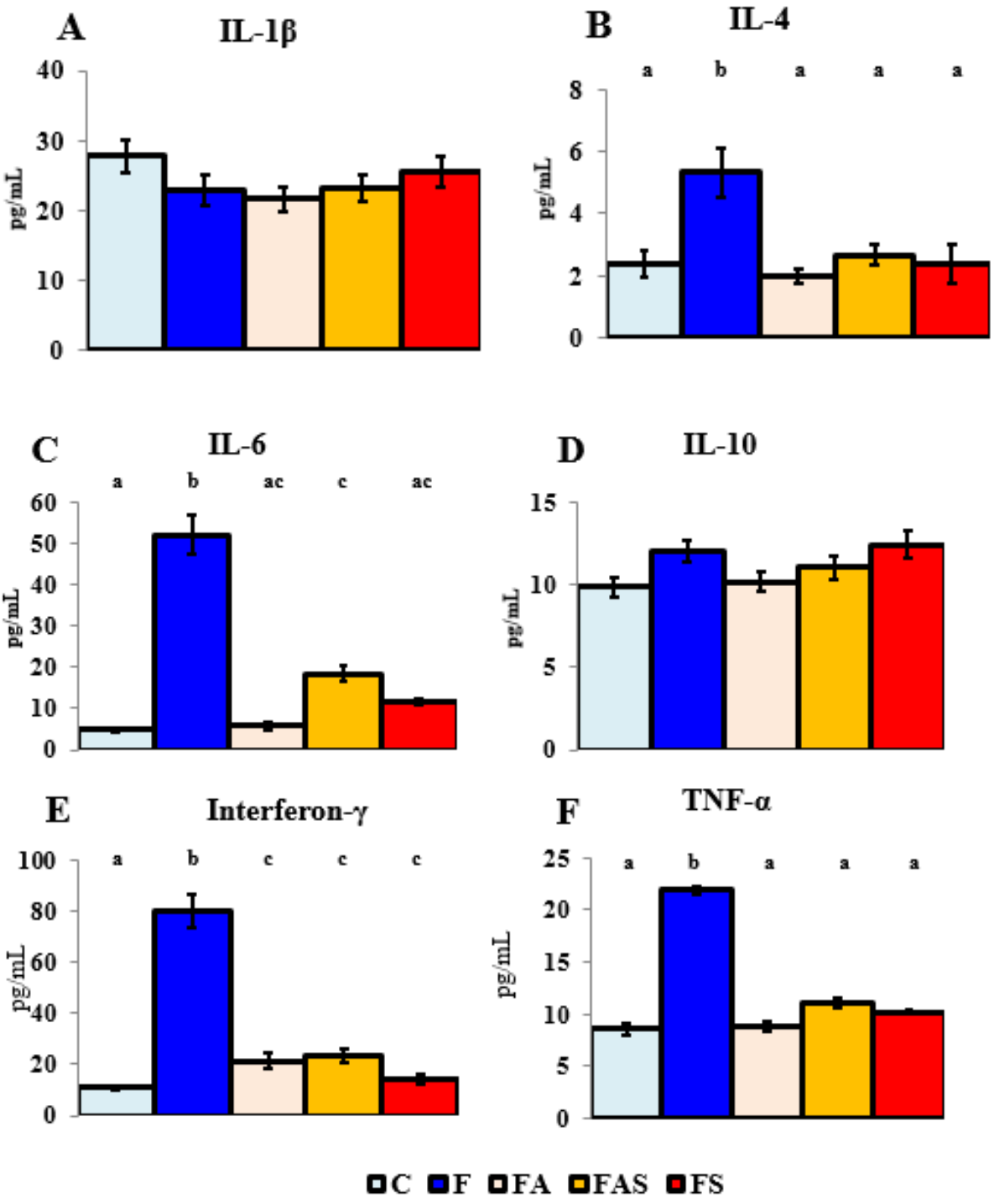

Figure 9: All exercises protocols were successful to prevent an increase in some inflammatory cytokines. The concentrations of IL-4, IL-6, Interferon-γ and TNF-α were higher in the group F compared to all other groups ($p \leq 0.01$-0.001). The serum IL-6 concentration was higher in the groups FAS when compared to the C and FA groups ($p \leq 0.0001$-0.0005).
C: Control; F: Fructose; FA: Fructose Aerobic; FAS: Fructose Concurrent; FS: Fructose Strength. n = 8 animals per group. Different letters means statistical difference

Discussion

There is already a well-established consensus that correlates the consumption of sugar-rich diets and physical inactivity global epidemics of obesity, diabetes, hypertension, chronic oxidative stress, systemic inflammation, cancer and other metabolic conditions associated (Eliot et al., 2002; Odegaard et al., 2012) which increase the risk of premature death .

Exercise is a powerful tool against obesity and related diseases (BOTEZELLI et al., 2011; BROOKS et al., 2007; PERSEGHIN et al., 1996; LEE et al., 2011; GHEZZI et al., 2012; Cambria et al., 2011). Our study allowed us to observe the peculiar settings of three different exercise protocols on the mainly metabolic syndrome markers, oxidative stress and inflammation in animals fed on a fructose-rich diet.

Aerobic exercise has been successful in reducing animal food intake receiving a fructose-rich diet. The association between high energy expenditure and decreased appetite and relative food intake has been described by research in humans (JACKSSON, 2012) but, there is still a controversy in animal studies (BOTEZELLI et al., 2011).

The particularities of the three different exercise protocols become more pronounceable when we take the oral glucose tolerance, insulin and insulin sensitivity (Figures 3, 4 and 5). The animals of group F showed significant reductions in glucose tolerance and insulin sensitivity to a concurrent increase in circulating insulin. These findings corroborate previous data from our group (BOTEZELLI et al., 2011; . CAMBR et al., 2011; . BOTEZELLI et al., 2011.) And other research groups (BASCIANO et al., 2005; . RUTLEDGE et al., 2007.) evaluating the role of fructose in the dysregulation of glucose homeostasis and insulin signaling. Moreover, the strength exercise seemed to have greater effects on the regulation of these parameters . Predominantly anaerobic (ATP - CP and glycolytic), the jump exercise preferentially utilizes ATP , creatine phosphate and

glycolysis as energy source during this exercise, which could generate better responses in the glucose uptake compared to the others. In the cited figures, we see a "progressive effect" where the earnings are lower in the aerobic group (FA) with intermediate response in the concurrent protocol (FAS) and the highest in the strength protocol (FS). These findings corroborate studies in humans (HOLTEN et al., 2004) but differ up to a previous study by our group that evaluated the effects of these exercise protocols in rats fed a balanced diet and found no improvement in the exercised animals (BOTEZELLI et al., 2011). It is worth mentioning that the values of the FS group are similar to those in the control group in most of these results.

Hyperinsulinemia is perhaps the most remarkable trigger for all sequential metabolic disorders (MEHRAN et al., 2012). The excess of this hormone appears to block the energy expenditure in animals and activate the "economic mode" in the body while increases the dependence of lipids resulting in an inflammatory processes in several tissues (SHANIK et al, 2008; PARTY et al., 2000; DANDONA et al., 2005). All exercise protocols were successful to reduce the concentrations of circulating insulin but. This unique finding leads us to believe that probably the caloric expenditure may be the major regulator of the concentration of circulating insulin, regardless of the kind of exercise performed as described in human studies (WEISS et al., 2006;. ALMIND; KHAN, 2004; KANG et al., 1996).

Other notable findings of this study are described in the Figure 6 and Table 1. Our research demonstrated how lipid accumulation in the liver and bloodstream becomes pronounceable in a fructose-rich diet. The exercise, on the other hand was able to reduce this hepatic lipid accumulation, beside now effects on the circulating triglycerides concentration. Given that, any disruption in the liver lipid content can be triggered by the fructose via neolipogênese (BASCIANO et al., 2005). While the glucose breakdown is modulated by phosphofructokinase

(high concentrations of citrate can inhibit the action of Phosphofructokinase), fructose continuously enters the glycolytic pathway providing carbon chain in the neolipogenesis. Providing a reduced rate of conversion to glycogen (irreversible reaction), fructose is predominantly converted into triglycerides, which literally "spill " into the bloodstream, where they can be stored in peripheral tissues, binds to a molecule of apolipoprotein B (ApoB) thus producing VLDL cholesterol (BROWNING , HORTON, 2004; . GRIFFIN et al., 1999), or head back to the liver where it can be recaptured and stored into a cellular droplet fat depots. The whole process generates high concentrations of triglycerides in the heart, liver and retroperitoneal adipose tissue of animals analyzed. The three exercise protocols were successful in reducing the concentrations of hepatic triglyceride corroborating previous studies in animals (RECTOR et al., 2008) and human (KRASNOFF et al., 2008; DEVRIES et al., 2008), but surprisingly the FS group had lower triglycerides concentrations in the liver tissue between all groups exercised. In Table 1, we can again observe a “progressive effect” among the four groups fed on a fructose-rich diet. Moreover is visually perceptible difference in the area of fat deposits in the liver tissue. This peculiar finding agrees with the studies of Mehran and colleges (2012) showing that a reduced concentration of circulating insulin in knockout animals triggers a protective effect against lipogenic diets. In the present study, the animals of the FS group showed lower concentrations of circulating insulin and the smaller reserves of hepatic triglycerides.

Taking the serum parameters (glucose, triglycerides, HDL - cholesterol and total cholesterol), exercise exerted only a pronounceable change. HDL-cholesterol in the Aerobic and Concurrent protocols showed a considerable increase (above the control group and other groups). The cholesterol pathway production was not evaluated in this study, but an improvement in liver function or increased consumption of triglycerides by aerobic exercise may have caused this

changes. The workload standardized in our study (80 % of Lacmin) corresponds to the transition between moderate and intense effort domain of exercise. At this particular point, both carbohydrates and lipid contribute virtually equally for the energy demand. This effect could be the responsible for any improvement in the lipid metabolism of animals, thus, improving the concentrations of HDL cholesterol corroborating previous studies in animals (BOTEZELLI et al., 2011 GHEZZI et al., 2012 CAMBRI et al., 2011) and human (KIENS et al., 1980) . On the other hand, a fructose-rich diet increased the serum triglycerides concentrations by near 65%. This accumulation may have been triggered by the neolipogenesis as described by Basciano (2005) and Loria (2008). The imbalance between the oxidation of lipids and carbohydrates associated to an excessive circulating triglycerides and hiperinsulinemia may have trigger oxidative stress and a subclinical inflammatory response (GRIFFIN et al., 1999).

Another beneficial result of aerobic exercise and strength was shown in Figure 8 (A and C). These animals showed reduced levels of lipid peroxidation markers (TBARS). This finding stands out because the increased dependence of lipids as energy source leads to an overproduction of reactive oxygen species resulting in oxidation of proteins or important cellular structures in the cell cycle regulation (GIRARD; MADANI; BOULORTT, 2006; SIMONEAU; KELLEY; 1997). In a long term, such an imbalance in the production of reactive oxygen species can cause an inability of the antioxidant system to reduce this radicals leading to system failure by decreased activity and fail to exert its protective role (KAKKAR et al., 1998). Besides aerobic group showed an increase in the activity of catalase assets. This antioxidant enzyme is present in all mammalian cells and plays a protective role in the reduction of hydrogen peroxide (H2O2 -) from the respiratory chain (LEHNINGER; NELSON and COX 1993). The chronic overproduction of

oxygen reactive species leads to new protein and structural damage which can impact the normal cellular cycle and trigger a systemic inflammatory process.

To reach this data, we also analyzed the levels of inflammatory markers (cytokines) in all groups studied. Exercise attenuated the concentrations in four of the six assessed cytokines. Interleukin-6 may play a pro-and anti-inflammatory. Released in large amounts after a workout or septic shock, this cytokine plays a key role in the rebuilding of muscle cells and fight infection through the activation of macrophages (Pedersen et al., 2005; BRUUNSGAARD ; BENTE 1995). However, in the absence of exercise, high levels of this cytokine can generate a “blind “body’s inflammatory response by interfering in several key processes in the regulation of glucose homeostasis. In the present study animals fed on a fructose-rich diet presented high circulating concentration of IL-6. Even we did not performed a tissue analyses, the adipose tissue must be responsible for this cytokine chronic release. Moreover, IL-4, TNF-α and interferon-y are exclusively inflammatory cytokines (MCGILLICUDDY et al., 2009; WADDA et al., 2011; SHIBA; Higashi ; NISHIMURA , 1998) and were found elevated in F group in this study. Besides that, the exercised animals have reduced concentrations of all these three cytokines, showing levels compared to the control group. These three molecules can affect the insulin-signaling cascade by preventing the normal functioning of the insulin receptor and all kinases involved in this reaction chain. In addition, they lead to an infiltration of leukocytes in various tissues and consequent inflammation site (HOTAMISLIGIL, 1999; BORST, 2004; KOIVISTO; PELKONEN; CANTEL , 1989). Infiltrated leukocytes increase oxidative stress in a "blind” generating a breakdown of proteins and molecules of neighboring cells (LEEUWENBURGH; HEINECKE, 2001).

In short, through a fructose-rich diet we generated a group of animals that manifested the classic metabolic syndrome symptoms, oxidative stress and inflammation. At the same time, part

of the animals were subjected to three different exercise protocols for assessing the individual metabolic responses. The strength exercise provided responses that are more pronounceable in glucose tolerance, insulin sensitivity and liver lipid infiltration. On the other hand, the greater responses triggered by the aerobic protocols led to better responses in the oxidative stress and HDL cholesterol. Finally, all protocols were successful to reduce the concentrations of circulating inflammatory cytokines. Future studies can evaluate individually the effect of physical exercise with a greater specificity.

Competing Interests

The authors declare that they have no competing interests.

Author's Contributions

JDB was responsible for experimental design, data collection, statistical analysis and preparation of the manuscript. PPMC, LTC and ACG were responsible for data collection.. MARM was responsible for experimental design, coordination of research and preparing the manuscript. All authors read and approved the manuscript.

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6. Considerações Finais

Na primeira série de experimentos, o presente estudo avaliou os efeitos de uma dieta rica em frutose sobre biomarcadores hepáticos da síndrome metabólica de ratos sedentários. Os animais alimentados com dieta rica em frutose apresentaram uma redução na tolerância à glicose, hiperinsulinemia, resistência à insulina, aumento na glicose e nos triglicerídeos circulantes, aumento na relação AST/ALT, acúmulo de gordura no fígado e no tecido adiposo mesentérico, além de uma redução na atividade do sistema antioxidante no fígado um aumento das TBARs no mesmo.

Na segunda série experimental, os animais foram submetidos a três diferentes protocolos de exercício e foram avaliados os marcadores da síndrome metabólica, peroxidação lipídica e atividade dos antioxidantes enzimáticos. Os grupos A e S apresentaram redução no peso corporal se comparados ao grupo C. Além disso, todos os animais treinados apresentaram redução nas concentrações de triglicerídeos no tecido adiposo e fígado. Os animais apresentaram também uma redução da concentração dos marcadores de peroxidação lipídica (TBARs) e aumento da atividade enzimática da superóxido dismutase no soro. Ainda, os animais do grupo A, apresentaram uma elevação na atividade da catalase e superóxido dismutase no fígado.

Na terceira série experimental lançamos mão de uma dieta rica em frutose para geramos um grupo de animais que manifestou os sintomas da síndrome metabólica, estresse oxidativo e inflamação. Em seguida os animais foram submetidos a diferentes protocolos de exercício físico para avaliar as repostas metabólicas de cada uma destas diferentes ferramentas. O exercício físico de força gerou respostas mais pronunciáveis na tolerância à glicose, sensibilidade à insulina e na

prevenção da esteatose hepática. Por outro lado, o exercício aeróbio desencadeou melhores respostas no balaço oxidativo e na circulação do colesterol HDL. Por fim, todos os protocolos tiveram sucesso em reduzir as concentrações de citocinas inflamatórias circulantes. Próximos estudos podem avaliar separadamente o efeito do exercício físico com maior especificidade.

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8.Apêndices

Apêndice 1:

BOTEZELLI, J. D.; MOURA, R. F.; ROSSI C.A.; DALIA, R. A.; CAMBRI, L. T.; MELLO, M. A. R. Consumo de frutose e exercício físico, impacto na síndrome metabólica. **Motriz : Revista de Educação Física (Online)**, v.16, p.231 - 239, 2010.

Apêndice 2:

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Apêndice 6:

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