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SANDRA DA SILVA SILVEIRA

IMPACT OF HIGH FRUCTOSE DIET AND MODERATE INTENSITY EXERCISE DURING ADOLESCENCE ON ADULT METABOLIC SYNDROME ONSET

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Orientador: Dr. Paulo Cezar de Freitas Mathias

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BIOGRAFIA

Sandra da Silva Silveira nasceu em Ilhéus/BA em 20/08/1988. Possui graduação em Biomedicina pela Universidade Estadual de Santa Cruz (2012) e Mestrado em Ciências Biológicas pela Universidade Estadual de Maringá (2014). Atualmente é Professora Curso docurso de Biomedicina da Faculdade São Paulo em Rolim de Moura/RO. Tem experiência na área de Biologia Celular e Bioquímica, atuando principalmente nos seguintes temas: I. Identificação e caracterização dos principais fungos de interesse médico; II. efeitos do stress oxidativo na proteína sérica albumina, utilizando o modelo de artrite induzida por adjuvante e III. insultos dietéticos e exercícios físicos de moderada intensidade como agente protetor em fases precoces e tardias do desenvolvimento, utilizando o conceito de programação metabólica, postulado pelo DOHaD (Developmental Origins of Health and Disease).

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APRESENTAÇÃO

Esta tese é composta de uma revisão e de dois artigos científicos. Inicia revisando o papel da frutose no desenvolvimento da síndrome metabólica em adolescentes no artigo "Metabolic Syndrome By Fructose In Adolecence: A Review". Tem continuidade com o artigo "Metabolic Programming Effects Of Fructose Diet And Moderate Intensity Exercise In Adolescent Rats" que avalia o papel da frutose na concentração de 10% durante a adolescência no desenvolvimento da síndrome metabólica na vida adulta e o papel do exercício físio de moderada intensidadade na proteção desses distúrbios metabólicos. Encerra com o artigo "Impact Of High Fructose Diet And Moderate Intensity Exercise During Adolescence On Adult Offspring Metabolism" que mostra a programação metabólica para obesidade através de uma alta dose de frutose (20%) no organismo de ratos adolescentes e o papel do exercicio físico de moderada intensidade na perda de peso e proteção do ornanismo. Em consonância com as regras do Programa de Pós-graduação em Ciências Biológicas, os artigos foram redigidos de acordo com as revistas European Journal of Nutrition, Metabolism e MolecularMechanism.

Artigo de revisão:
SILVEIRA, Sandra da Silva and MATHIAS, Paulo Cezar de Freitas. **Metabolic Syndrome By Fructose In Adolecence: A Review**. A ser submetido a revista *European journal of Nutrition*.

Artigos originais:
SILVEIRA, Sandra da Silva, PERES, Maria Natália Chimirri, FRANCISCO, Flávio Andrade, MARCON, Monique Suellen da Silva, SAAVEDRA, Lucas Paulo Jacinto, TOFOLO, Laize Peron, PRATES, Kelly Valério, BATAGLINI, Luiz Augusto, MOREIRA, Veridiana Mota, RIBEIRO,Tatiane Aparecida, FRANCO, Claudinéia Conationi da Silva, MATHIAS, Paulo Cezar de Freitas. **Adolescent rich fructose intake induced metabolic malprogramming in adult rats and early moderate exercise is able to atenuate it.** A ser submetido a revista *Diabetes.*

SILVEIRA, Sandra da Silva, PERES, Maria Natália Chimirri, MARCON, Monique Suellen da Silva, SAAVEDRA, Lucas Paulo Jacinto, PRATES, Kelly Valério, BATAGLINI, Luiz Augusto, FRANCISCO, Flávio Andrade, MOREIRA, Veridiana Mota, RIBEIRO,Tatiane Aparecida, MATHIAS, Paulo Cezar de Freitas**. Impact Of High Fructose Diet And Moderate Intensity Exercise During Adolescence On Adult Metabolism.** A ser submetido a revista *Molecular Mechanisms.*

RESUMO GERAL

INTRODUÇÃO. Distúrbios metabólicos como resistência à insulina, hiperglicemia, hipertensão, dislipidemia e obesidade abdominal são um conjunto de alterações clínicas que compõem a Síndrome Metabólica (MetS). O consumo de bebidas e alimentos adoçados aumentou em todas as faixas etárias, tendo os adolescentes como seus principais consumidores. O alto consumo de frutose tem sido associado ao desenvolvimento de obesidade, resistência à insulina, hiperlipidemia, diabetes tipo II e alterações no tecido adiposo. Os promissores efeitos do exercício físico na prevenção e no tratamento de distúrbios metabólicos têm sido evidenciados por diversos estudos que apontam sua importância na perda de peso, redução de gordura e na melhora da sensibilidade à insulina. O exercício de intensidade moderada tem mostrado ser protetor contra o desenvolvimento da MetS. Este modelo experimental de síndrome metabólica induzida pr frutose é relatado na literatura como o indutor de resistência à insulina com um perfil metabólico análogo à MetS observado em humanos. No entanto, o efeito duradouro da frutose sobre o metabolismo permanece inexplorado e também o efeito do exercício físico sobre a MetS é uma novidade e merece ser elucidado. Assim, a hipótese do primeiro artigo foi: (I) a ingestão de frutose durante a adolescência programa o metabolismo para MetS na idade adulta e (II) o exercício de moderada intensidade realizado na adolescência, concomitante à frutose, poderia proteger o metabolismo contra o desenvolvimento da MetS na vida adulta. A hipótese do segundo artigo foi: (I) verificar os efeitos de uma alta dose de frutose no desenvolvimento da obesidade e síndrome metabólica e (II) o exercício de moderada intensidade realizado na adolescência, concomitante à frutose, poderia proteger o metabolismo contra o desenvolvimento da obesidade e MetS na vidaadulta.

MÉTODOS. Após cinco dias de adaptação, aos 30 dias de idade, os animais foram divididos em quatro grupos: Controle sedentário (C-SED; n = 20), que recebeu água e ração padrão para ratos *ad libitum* durante todo o período; Frutose sedentário (F-SED; n = 20), que recebeu Frutose 10 % na água de beber dos 30 a 60 dias de vida e ração padrão para ratos ad libitum; Controle exercitado (C-EXE; n = 20), que foi treinado a partir dos 30 a 60 dias de vida e recebeu água e ração padrão para ratos *ad libitum* e Frutose exercitado (F-EXE; n = 20), que recebeu Frutose 10 % na água de beber e realizou exercício físico ao mesmo tempo dos 30 a 60 dias de vida. Para avaliar o efeito do tratamento (frutose e exercício) sobre o metabolismo imediatamente após o período de tratamento, os dados foram coletados em ratos com 60 dias de idade. Para avaliar o papel potencial da frutose na programação, os dados foram coletados em ratos com 120 dias de idade. Para realização do estudo com frutose 20%, os ratos receberam Frutose 20% na água, ao invés de 10%. Para a preparação de 10% de frutose, 10 g de frutose foram diluídos em 100 ml de água filtrada e as

garrafas cobertas com papel alumínio para evitar a fermentação induzida pela luz. Para a preparação de 20% de frutose, 20 g de frutose foram diluídos em 100 ml de água filtrada e realizado procedimento já descrito acima. Todos os ratos realizaram um teste de aptidão física para determinar seu consumo máximo de oxigênio individual (VO_2max) e velocidade máxima de corrida (MRS). O protocolo de treinamento em esteira foi realizado por 44 minutos por dia (9h às 10h), 3 dias por semana em um macrociclo de 4 semanas. Os protocolos de treinamento foram completados em 60 dias. Ingestão alimentar, consumo de bebida e ingestão calórica foram medidos a cada dois dias, dos 30 a 120 dias de vida. A ingestão calórica foi calculada com base na quantidade de alimentos e líquidos ingeridos e nas correspondentes constantes. Os animais foram pesados uma vez por semana durante o período experimental. Ao final da fase de tratamento (aos 60 dias de idade) e fase experimental (aos 120 dias de idade), os animais de todos os grupos foram pesados e decapitados, e amostras de sangue foram coletadas e centrifugadas. A insulina foi medida por radioimunoensaio (RIA) através de um contador de emissão de partículas gama, utilizando insulina padrão de rato, insulina anti-rato e insulina humana recombinante (I125). Asconcentrações de colesterol total e triglicérides foram medidas pelo método colorimétrico enzimático com kit disponível comercialmente. O teste intraperitoneal de tolerância à glicose (ipGTT) foi realizado no final da fase de tratamento (aos 60 dias de idade) e fase experimental (aos 120 dias de idade). Aos 60 dias e aos 120 dias de idade, todos os grupos foram eutanasiados e os depósitos de gordura (retroperitoneal, periepididimal e mesentérica) foram removidos e pesados para avaliar o estado de obesidade. O fígado foi dissecado e pesado, e a saída do sinal neural foi adquirida por 12 min, utilizando a interface Insight, a partir da qual 20 quadros gravados de 5 s de cada animal foram escolhidos aleatoriamente para contagem depulsos.

RESULTADOS E DISCUSSÃO. No primeiro artigo, os resultados aos 120 dias mostraram aumento do estoque de gordura, embora o ganho de peso tenha permanecido inalterado. Os níveis de colesterol total e triglicérides estavam elevados em F-SED em comparação com C-SED. Diversos trabalhos na literatura já mostraram o potencial da frutose em estimular a lipogênese no fígado. Esse alto rendimento leva a um depósito ectópico de lipídios no fígado e nos tecidos musculares, o que promove a resistência à insulina central e periférica no organismo, o que explica a resistência à insulina encontrada em nosso estudo. Relacionado à ANS (Autonomic Nervous System), descobrimos que a dieta de frutose aumenta a atividade elétrica vagal. Uma alta atividade do sistema nervoso parassimpático está relacionada a distúrbios metabólicos, como hiperinsulinemia e resistência à insulina, o que está de acordo com nossos resultados. O exercício físico também não alterou os estoques de gordura dos ratos suplementados com frutose. Isso pode ser explicado, possivelmente, pelo destreinamento ocorrido de 60 a 120 dias de vida.Nossos

resultados mostraram uma redução significativa nos níveis glicêmico e insulinêmico, bem como no índice HOMA IR e TYG, em ratos suplementados com frutose e submetidos ao protocolo de treinamento físico, comparado ao grupo sedentário (F-SED) nos 60 e 120 dias de vida. O exercício físico na ANS diminuiu a atividade do nervo parassimpático. Nosso laboratório já mostra que este protocolo de exercício iniciado antes do desmame é benéfico para a atividade do nervo vago em animais alimentados com HFD. Os resultados do segundo artigo mostram que a dose de 20% de frutose aumentou o peso corporal ao final dos 30 dias de tratamento e as reservas de gordura retroperitoneal, periepididimal e mesentérica do grupo sedentário (F-SED), em comparação ao controle de C-SED no final dos 120 dias de vida. Estudos recentes demonstraram que uma alta suplementação de frutose estimula a proliferação de adipócitos como os principais estoques de gordura, contribuindo para o desenvolvimento da obesidade. O exercício físico de intensidade moderada reduziu o peso corporal e os estoques das três gorduras do grupo treinado (F-EXE) comparado ao F-SED. Dados recentes publicados pelo nosso grupo mostraram que esse protocolo de exercício físico foi capaz de reduzir em 80% as reservas de gordura de ratos tratados com dieta hiperlipídica.

CONCLUSÕES. Os resultados mostraram que a suplementação com frutose durante a adolescência programa o metabolismo para MetS na idade adulta e o exercício físico de moderada intensidade atenua a programação metabólica contra disfunções metabólicas na idade adulta programada pela frutose em ratos adolescentes.

GENERAL ABSTRACT

INTRODUCTION. Metabolic disorders such as insulin resistance, hyperglycemia, hypertension, dyslipidemia and abdominal obesity are a set of clinical changes that make up Metabolic Syndrome (MetS). Consumption of beverages and sweetened foods increased in all age groups, with adolescents as their main consumers. High fructose consumption has been associated with development of obesity, insulin resistance, hyperlipidemia, type II diabetes and changes in adipose tissue. The promising effects of physical exercise on prevention and treatment of metabolic disorders have been evidenced by several studies that point out its importance in weight loss, fat production and in the improvement of insulin sensitivity. Moderate intensity exercise has been shown to be protective against the development of MetS. This experimental model of metabolic syndrome is reported in the literature as inducer of insulin resistance with a metabolic profile analogous to MetS observed in humans. However, the long-lasting effect of fructose on metabolism remains unexplored and also the effect of physical exercise on MetS is novel and deserves to be elucidated. Thus, the hypothesis of the first article was: (I) the ingestion of fructose during adolescence programs metabolism for MetS in adulthood and (II) the moderate intensity exercise carried out in adolescence concomitant with fructose could protect metabolism against the development of MetS in adult life. The hypothesis of the second article was: (I) to verify the effects of a high dose of fructose on the development of obesity and metabolic syndrome; and (II) the moderate intensity exercise performed in adolescence concomitant with fructose could protect the metabolism against development of obesity and MetS in adult life.

METHODS. After five days of adaptation, at 30 days of age, the animals were divided into four groups: Sedentary control (C-SED; n = 20), which received water and standard chow ad libitum throughout the period; Sedentary fructose (F-SED; n = 20), which received 10% Fructose indrinking water from 30 to 60 days of life and standard chow ad libitum; Exercised control (C-EXE; n = 20), who was trained from 30 to 60 days of age and received water and standard chow ad libitum, and Exercised fructose (F-EXE; n = 20). Wich received fructose 10% in drinking water and performed physical exercise at the same time from 30 to 60 days of life. To evaluate the effect of treatment (fructose and exercise) on metabolism immediately after the treatment period, data were collected in rats at 60 days of age. To evaluate the potential role of fructose in scheduling, data were collected from mice at 120 days of age. To perform the study with 20% fructose, rats received 20% fructose in water, instead of 10%. For the preparation of 10% fructose, 10 g of fructose were diluted in 100 ml of filtered water and the bottles covered with aluminum foil to avoid light-induced fermentation. For the preparation of 20% fructose, 20 g of fructose were diluted in 100 ml of filtered water and the preparation described above was performed.All rats

underwent a physical fitness test to determine their individual maximum oxygen consumption (VO_2max) and maximum running speed (MRS). The treadmill training protocol was performed for 44 minutes per day (9 am to 10 am), 3 days per week on a 4 week macrocycle. The training protocols were completed in 60 days. Food intake, beverage consumption and caloric intake were measured every two days, from 30 to 120 days of life. The caloric intake was calculated based on the amount of food and liquids ingested and the corresponding constants. The animals were weighed once a week during the trial period. At the end of the treatment phase (at 60 days of age) and experimental phase (at 120 days of age), animals of all groups were weighed and decapitated, and blood samples were collected and centrifuged. Insulin was measured by radioimmunoassay (RIA) with a gamma counter through a gamma particle emission counter, using standard rat insulin, anti-rat insulin and recombinant human insulin (I125). The concentrations of total cholesterol and triglycerides were measured by the enzymatic colorimetric method with commercially available kit. The intraperitoneal glucose tolerance test (ipGTT) was performed at theendofthetreatmentphase(at60daysofage)andexperimentalphase(at120daysofage).At
60 days and 120 days of age, all groups were euthanized and fat deposits (retroperitoneal, periepididimal and mesenteric) were removed and weighed to assess the obesity status. The liver was dissected and weighed, and the neural signal output was acquired for 12 min using the Insight interface, from which 20 engraved 5-s frames of each animal were randomly chosen for pulse counting.

RESULTS AND DISCUSSION. In the first article, the results at 120 days showed increased fat stores, although weight gain remained unchanged. Total cholesterol and triglyceride levels were elevated in F-SED compared to C-SED. Several works in literature have already shown the potential of fructose in stimulating lipogenesis in the liver. This high yield leads to an ectopic deposit of lipids in the liver and muscle tissues, which promotes resistance to central and peripheral insulin in the body, which explains the insulin resistance found in our study. Related to ANS, we found that the fructose diet increases vagal electrical activity. A high activity of the parasympathetic nervous system is related to metabolic disorders, such as hyperinsulinemia and insulin resistance, which is in agreement with our results. Physical exercise also did not alter the fat stores of rats supplemented with fructose. This can be explained, possibly, by the detraining occurring from 60 to 120 days of life. Our results showed a significant reduction in the glycemic and insulinemic levels, as well as the HOMA index IR and TYG, in rats supplemented with fructose and submitted to the physical training protocol, compared to the sedentary group (F-SED) at 60 and 120 days of life . Physical exercise in ANS decreased the activity of the parasympathetic nerve. Our laboratory already shows that this protocol of exercise started before weaningis

beneficial for the activity of vagus nerve in animals fed with HFD. The results of the second article show that 20% fructose dose increased body weight at the end of the 30 days of treatment and the stores of retroperitoneal, periepididimal and mesenteric fat of the sedentary group (F-SED), compared to the C-SED control at the end of the 120 days of life. Recent studies have shown that high fructose supplementation stimulates the proliferation of adipocytes as the main fat stores, contributing to the development of obesity. Moderate-intensity physical exercise reduced body weight and stocks of the three fats of trained group (F-EXE), compared to F-SED. Recent data published by our group showed that this protocol of physical exercise was able to reduce in 80% fat reserves of rats treated with hyperlipidic diet.

CONCLUSIONS. Fructose supplementation during adolescence program the metabolism to MetS in adulthood and moderate intensity exercise protects against metabolic dysfunction programmed by fructose in adolescent rats.

METABOLIC SYNDROME BY FRUCTOSE IN ADOLESCENCE: A REVIEW

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Keywords: Fructose, Adolescence, Metabolic syndrome, Metabolic programming.

Abstract

Metabolic Syndrome (MetS) is characterized as a set of metabolic disorders such as abdominal obesity, glucose intolerance, hyperinsulinemia, dyslipidemia and hypertension. The factors that contribute to development of metabolic syndrome are the sedentary lifestyle, high consumption of diets high in fats and carbohydrates, such as fructose. The literature has shown the fructose consumption by adolescents is increasing. The main cause is the consumption of sucrose and High Fructose Corn Syrup (HFCS) that produce glucose and fructose at high doses (55% fructose and 42% glucose). The liver is the main organ of fructose metabolism. In this organ, fructose induces lipogenic precursor production, such as acetyl-coA, leading to a high triglycerides and fat synthesis. This process causes fat deposition in the liver, developing hepatic steatosis and also central and peripheral insulin resistance both in adults and adolescents. On intestine, fructose is not immediately absorbed, interacting with the intestinal microbiota and changing it. In high levels of glucose conditions, non-insulin-dependent tissues such as nerve tissue, lense and erythrocytes can activate the polyol pathway and induce endogenous fructose production. That observation was obtained during adolescence and adult life. On adipose tissue, the metabolism of fructose in adipocyte results in generation of precursors of fatty acid synthesis, expression of GLUT-5 transporters in adipocyte plasma membrane and greater expression of enzyme responsible that contribute to glucocorticoids production, 11-hydroxysteroid dehydrogenase type 1 (11-HSD1). On pancreas, high fructose diet showed an increase in markers of stress in endoplasmic reticulum both in pancreas and in liver at the level of mRNAs and enzymes. On brain, fructose alters some functions related to hippocampal plasticity. This review focus on the effect of diet enriched by fructose in adolescence, that causes metabolic dysfunction in different tissues, characterizing the early metabolicsyndrome.

Key-words: Fructose, Adolescence, Metabolic syndrome.

Introduction

MetabolicSyndrome(MetS)ischaracterizedasasetofmetabolicdisorderssuchasabdominal obesity, glucose intolerance, hyperinsulinemia, dyslipidemia and hypertension (Nacagawa et al., 2006). It is estimated 20 to 25% of the adult population is diagnosedwiththissyndrome. The clinical characteristics of MetS depend on lifestyle, environmentalfactor,gender, ethnicity, and others (Grundy et al., 2005; Kwon et al., 2017). Many studies havesuggesteda combination of abdominal adipose tissue and MetS (Okosun et al., 2000). Thefactorsthat contribute to development of metabolic syndrome are sedentary lifestyle, high consumptionofdietsrich in fats and carbohydrates, such as fructose (Jean-Luc Gradidge et al., 2016; Kwon et al.,2017).

Fructose is a simple monosaccharide widely used as a sweetener in foods and beverages. Its form of consumption is as a free monosaccharide or as part of the disaccharide sucrose (Janssens et al., 2017). Fructose metabolism occurs mainly in liver, which increased production of lipid precursors, inducing a large production of triglycerides, total cholesterol and fractions, leading to a central and peripheral insulin resistance and hepatic steatosis. This evidence was found in adult rodents (Castro t al., 2015; Bezerra et al., 2000), however, same metabolic pathway was observed in adolescent rats (Legeza et al., 2017). In humans, fructose intake was associated with high fasting glucose, total trigicerides and high levels of apolipoproteins in adolescents (Egli et al., 2013) and increased triglicerides in adult men (Bantle et al., 2000) (Table2).

High ingestion of fructose, especially in the form of sweetened beverages, has been identified as a serious risk factor for the development of metabolic diseases in humans. Studies using experimental adolescent rats have shown that chronic consumption of fructose-rich diet induces systemic metabolic disorders that mimic aspects of the metabolic syndrome in humans, including hypertension, hypertriglyceridemia and hyperinsulinemia (Sadowska et al., 2017; White et al.,2008).

Diets high in fructose are directly related to excess calorie consumption. The high palatability favors the high consumption of sweetened foods and beverages mainly with HFCS (High Fructose Corn Syrup). It is a bad economic reasons, HFCS is a constituent of a industrial food and beverage why HFCS is really cheap and to have high economical profits. The capitalists don’t care about health population (Fernández-Novell et al., 2014; Harrell et al.,2015).

The literature has shown the fructose consumption by adolescents is increasing. The main cause is the consumption of sucrose and HFCS wich provide high doses of glucose and fructose (55% fructose and 42% glucose) (Whitton et al., 2011; White et al. 2008, Campos et al., 2016). It was suggested that fructose leads to long-term dysregulation in HPA (Hypothalamic-Pituitary-

Adrenal Axis), compromising adult life health (Harrel et al., 2015).

Adolescence is a transition phase between childhood and adulthood, marked by brain changes responsible specifically for behavior, mainly sexual ones. These alterations are modulated by hormones characteristic of this phase, such as testosterone to male individuals (Wierenga et al., 2018). According to the literature, hyperinsulinemia and obesity are related to a decline in testicular function and consequently changes in testosterone levels (Hart et al., 2018). In a study, high fructose diet caused testicular degeneration and low testicular concentration of testosterone in adolescent rats (Yildirim et al., 2018). Meydanli et al., 2017 found destruction of the germinal epithelium, a decrease in seminiferous tubules diameters and an increase in apoptotic index in adult rats. Lower testicular weight was also found in periadolescet rats (Shibata & Fukuwatari,2013).

High ingestion of fructose, especially in the form of sweetened beverages, has been identified as a serious risk factor for the development of metabolic diseases in adults, as well as in adolescents. Sadowska et al., 2017, in their study showed that the effects of beverages sweetened with HFCS were important in significantly reducing plasma HDL (High Density Lipoproteins) levels. The currently study consists in a literature review that aims to show that early metabolic syndrome found in adolescent who consumed high rich fructose diet; exploring malfunctions in diferent tissues.

Fructose And Sugar Consumption Among Adolescents

The consumption of fructose is currently a global problem and is strongly associated with the metabolic syndrome. Adolescents are the largest consumers of this sugar, consuming the equivalent of 78.2 g / day of fructose in the USA. In this country, a daily caloric intake of 15% is estimated, only from fructose (Vos et al., 2008). The high consumption of this sugar is related to the high rates of diabetes mellitus among the youngest (Harrel et al., 2015, de Oliveira et al., 2013). Fructose-sweetened foods are known to provide high palatability and are cheaper for the food industry. Its wide consumption is associated with the high consumption of fast foods as fast and tasty food alternative. Hypercaloric food intake is also associated with a reduction in healthy foods consumption. Since adolescence is a sensitive period of development of HPA axis, high fructose consumption can lead to development of metabolic alterations, which constitute the metabolic syndrome, such as diabetes mellitus, dyslipidemia, obesity, impaired glucose tolerance, among others (Basciano et al. al.,2005).

The lifestyle of many people in the world today favors the high consumption of unhealthy foods, such as those high in fructose. Added to this nutrient-poor diet is a sedentary lifestyle where children stay in front of the TV, computer or video game for most of the day (Harrel et al., 2015).

Several studies have shown that fructose has a broad spectrum of action in the human body, from the moment it is ingested, interfering in the metabolism of many organs such as liver (main metabolizer), pancreas, adipose tissue, intestine, brain, among others, causing its undesirable effects on the body, which characterize MetS (Basciano et al., 2005; Felice et al., 2017). The table 2 shows a selection of published articles where the authors treated adolescent rats with varying concentrations of fructose in water and observed a range of effects on various organs andtissues.

Metodology

This work is a bibliographic review research carried out on the following research platforms: Pubmed, Medline and Lilacs. The articles selected were those published between 2000 and 2018 and that worked with animals in late childhood, adolescence and early adulthood. The keywords used were 'fructose' and'adolescence'.

In total, 126 articles were found using the keywords. However, after analyzing each of these articles, it was noticed that most did not meet the criteria of this study, with only 39 articles being used (Table 1).

Table 1. Results of articles found and used in bibliographic research.

BASE DE DADOS	ARTIGOS	
	FOUNDED	USED
PUBMED	105	27
MEDLINE	20	13
LILACS	1	1
TOTAL	**129**	**42**

Results and Discussion

According to literature, the high fructose consumption causes changes in many organs and tissues. Table 2 lists the articles selected for this review, with lineage of used animals, as well as the age, the concentration of fructose, treatment period and its effects on theorganism.

In selected studies, the animals treatment age corresponds to adolescence and early adulthood. These results can be extrapolated to humans since the metabolism of rats reliably mimics humanmetabolism.

Due its wide spectrum of action in animal and human organism, it is necessary to study separately the performance of fructose in each organ and tissue.

Table 2. Experimental studies showing different consequences of fructose treatment from periadolescence to early adulthood, varying the concentrations of fructose, treatment period and animal strains.

AUTHOR	LINEAGE	AGE (or body weight)	FRUCTOSE CONCENTRATION	TREATMENT TIME	TREATMENT CONSEQUENCES
Orlandi et al., (2015)	Wistar	7 weeks	10%	5 weeks	**Plasma:** Hyperglycemia, increased triglycerides, total cholesterol and fractions. **Adipose Tissue:** Increased retroperitoneal and periepididimal fats.
Vasiljevic´ et al., (2014)	Wistar	21 days	60%	9 weeks	**Liver:** Increased levels of 11b-hydroxysteroid dehydrogenase type 1 (11bHSD1), increased pro-inflammatory status, through activation of NFκB and increased TNF. **Plasma:** Hypertriglyceridemia **Adipose tissue:** Increased visceral fat.
Svendsen et al., (2017)	Sprague-Dawley	120-140 g	70%	12 weeks	**Liver:** Rapid progression to steatosis, followed by inflammation, hepaticballooning (hepatocyte death), and fibrosis.
Román et al., (2014)	Wistar	180-200g	10%	3 weeks	**Plasma:** Increased levels of triacylglycerol, insulin and lipid peroxidation, associated with a state of insulin resistance and impaired glucose tolerance. **Pancreas**: Lower number of islets/area and lower density of beta cell.
Maiztegui et al., (2009)	Wistar	180-200g	10%	3 weeks	**Plasma:** Impaired glucose tolerance, hypertriglyceridemia, hyperinsulinemia with increased HOMAindex. **Pancreas:** A 33% decrease in beta cell mass and a 44% increase in apoptotic cells percentage.
Koo et al., (2008)	Sprague-Dawley	5 weeks	63%	2 weeks	**Liver:** Increased hepatic glycogen, indicating a rapid fructose conversion to hepatic glycogen, through gluconeogenesis, induction of genes that favor new lipogenesis and increased triglycerides levels.
Jiménez-Maldonado et al., (2017)	Sprague-Dawley	8 weeks	8 e 15%	7 days	**Brain:** Decreased levels of neural proteins (NeuN, Myelin Basic Protein and Axonal Growth Associated Protein 43), Decreased levels of Cytochrome C oxidase (indicative of mitochondrial dysfunction) and increased levels of GLUT 5 fructose transporter in hippocampus.
Felice et al., (2017)	Wistar	8 weeks	10%	2 weeks	**Plasma:** Hyperglycemia, hyperinsulinemia with increase in HOMA index, hypertriglyceridemia. **Bone tissue:** Decreased expression

					of TRAP and osteocyte density in trabecular bone and decreased osteogenic potential of mesenchymal cells.
Dupas et al., (2017**)**	Wistar	3 weeks	20-25%	21 weeks	**Kidney:** Hypertension without renalfailure. **Plasma**: Fasting hyperglycemia, insulin resistance with elevated HOMA index, high levels of triglycerides. **Liver:** Increased liver density (malfunction index).
Noble et al., (2016)	Sprague-Dawley	26 days	35%, 50% e 65%	6 weeks	**Intestine:** Change in intestinal microbiota.
Ibrahim et al., (2017)	Sprague-Dawley	21 days	20%	30 days	**Plasma:** Increased triglyceride levels. **Liver:** Increased hepatic lipid and glycogen stores due to conversion of fructose to glycogen by gluconeogenesis.
Meydanli et al., (2017)	Wistar	220-250 g	30%	8 weeks	**Testis:** Destruction of the germinal epithelium, decrease in seminiferous tubules diametersand increase in apoptotic index.
Egli et al., 2013	Adolescent humans	68 kg	30%	50 days	**Plasma:** High fasting glucose, total trigicerides and high levels of apolipoproteins.
Bantleet al.,2000	Adult humans	BMI > 39	17%	6 week	**Plasma:** High triglyceride levels.

Fructose Effects On Liver

Liver is the main organ of fructose metabolism. Unlike glucose is captured by GLUT2 transporter, fructose absorption is carried out by GLUT5 transporter, whose expression is positively regulated in response to fructose availability. Glucose metabolism is regulated by phosphofructokinase I (PFK I), an important glycolysis regulator. However, fructose enters the glycolytic pathway at the trioses level, bypassing the regulation by PFK I. This difference alters the metabolism of carbohydrates, as gluconeogenesis, increasing glycogen stores. Fructose also induces lipogenic precursors production, such as acetyl-coA, leading to a high triglycerides and fats production. This process causes fat deposition in liver, developing hepatic steatosis and also central and peripheral insulin resistance. These changes induce alterations in expressed hepatic genes involved in expression of enzymes involved in lipogenic, glycogenic and gluconeogenic activities (Basciano et al.,2005).

Vasiljevic *et al*., 2014 in your study with adolescent rats suggests the lipids increase in liver, caused by the high fructose intake, occurs by increased production and, consequently, increased glucocorticoid levels. Although MetS isn't characterized by increases in circulating glucocorticoid levels, there are specific tissue changes in glucocorticoid pre-receptor in cellular membrane. In this same work high levels of 11-hydroxysteroid dehydrogenase type 1protein

(11bHSD1) were found in liver microsomes, although gene expression wasn't affected.

Energy production via fructose increases citrate concentration in mitochondria. Citrate is a potent phosphofructokinase blocker. Phosphofructokinase is a glycolysis allosteric regulator. Therefore, less glucose will be directed to energy production and fructose will be metabolized into fats. This process contributes to hepatic resistance to insulin action. Resistance to insulin action impairs glucose uptake by skeletal muscle and adipose tissue and induces neoglycogenesis in the liver (Botezelli et al., 2012).

Fructose Effects On Intestine

Fructose enters the intestinal cell in its apical portion, preferably by the GLUT-5 transporter, which is not regulated in this process and much of the fructose absorbed by enterocytes is directed into the circulation through enterocytes basolateral membrane via GLUT-2 transporter. Fructose, which is not immediately absorbed, interacts with the intestinal microbiota. Noble et al.(2016) argue that diet quality interferes with adolescent gut microbiota. Different fructose concentrations concomitant with glucose were tested in rat drink and he found altered amounts of Prevotella, *Lachnospiraceae incertae sedis*, Bacteroides, Alistipes, Lactobacillus, *Clostridium sensu*, Bifidobacteriaceae and Parasutterella. A study using adult rats (DiLucia 2015) showed an increase in genera Sutterella, Coprococcus and Ruminococcus and decrease in the bacteria of the genus Firmicutes. Changes in the intestinal microbiota caused by fructose affect the adolescent metabolism, wich metabolic desordersdevelopment.

Literature has shown that intestinal cells contain the highest amount of enzymes necessary for the metabolism of fructose, such as lipogenic and gluconeogenic enzymes (Campos 2016). There are few studies that confirm if fructose is mainly delivered to liver, thus leaving the supposition that enterocyte perhaps has a very significant contribution in fructose metabolism.

Fructose is a potent precursor to new lipogenesis, since this monosaccharide only requires a phosphorylation step via fructose kinase to form fructose-1-phosphate prior to conversion to the 3-carbon precursors (glyceraldehyde and dihydroxyacetone phosphate). Glucose, in addition to being controlled by insulin, is also regulated by ATP and citrate, which inhibit phosphofructokinase (Ibrahim et al., 2017).

Increased trioses phosphate production may lead to production of methylglyoxal (MG), which is a precursor to formation of advanced glycation products (AGEs). Methylglyoxal is known in literature as causing damage to proteins through glycation (Gugliuti et al., 2017). Studies using adolescent rats that ingested only fructose found greater production of AGEs in fructose-treated

rats, compared with glucose (Mastrocola et al., 2013).

ATP depletion promotes uric acid formation. An accelerated fructose metabolism into fructose-1-phosphate, through fructose kinase, consumes ATP forming ADP. This ADP, if not converted again to ATP through Adenylate-Cyclase, will be transformed into AMP, which will be converted to uric acid (Jhonson et al., 2013). Elevated levels of uric acid have been associated with a number of pathological conditions in adolescent rats such as insulin resistance, obesity, type II diabetes and chronic kidney disease and have been proposed as a risk for myocardial infarction and neurological disorders such as stroke (Legeza et al. al.,2017).

Fructose metabolism is known in literature for destroying many ATP molecules. This intense destruction causes oxidative stress and inflammatory response by disrupting organs and tissues functioning, resulting in an abnormal insulin production, inflammatory cytokines, adiponectin, leptin and and toxins, further aggravating the metabolic syndrome (Zhang et al., 2017).

Fructose Effects on Non-Insulin-Dependent Tissues

In high levels of glucose conditions (hyperglycemia), non-insulin-dependent tissues such as nerve tissue, lenses and erythrocytes can activate the polyol pathway and induce endogenous fructose production. This new fructose produced can cross the membrane passively or be transported, wich cause increase of fructose blood circulation adding to exogenous fructose intake. It was found that increased polyol pathway causes diabetic and vascular complications in adolescent rats (Gugliuti et al.,2017).

Fructose Effects On AdiposeTissue

According to Legeza et al., (2017) there is an interrelation between fructose concentration in adolescent body and glucocorticoids production in adipose tissue. Glucocorticoid production plays a key role in adipocytes differentiation and proliferation. The literature shows the metabolism of fructose in adipocyte results in generation of precursors of fatty acid synthesis, expression of GLUT-5 transporters in adipocyte plasma membrane and greater expression of glucocorticoids production enzyme responsible, 11-hydroxysteroid dehydrogenase type 1 (11-HSD1) (Vasiljevic´ et al.,(2014).

Orlandi et al., 2015 found retroperitoneal, periepididimal, hypertriglyceridemia and HDL increased after a treatment with 10% fructose for 5 weeks (Table 2). It can be explained by the fructose mechanism that form forming fructose-1-phosphate and is rapidly cleaved into tri-

phosphate, rising triglyceride levels.

Studies that tested adult human consumption of fructose and glucose separately in water showed the fructose consumption increased new lipogenesis, promoting a large lipid deposition in visceral adipose tissue, dyslipidemia, altered lipoprotein remodeling, decreased insulin sensitivity in overweight or obese individuals (Stanhope et al., 2009). Another theory proposes that fructose contributes to development of obesity by stimulating the insulin-independent Steroid Receptor 1-c Binding Protein Receptor (SREBP-1c), which activates genes involved in new lipogenesis, generating fatty acids for production of triglycerides in liver. However, this increase in lipids on liver is associated with an increase in synthesis and secretion of very low density lipoprotein VLDL. This systemic elevation of fatty acids and VLDL leads to an increase in lipid uptake by the peripheral organs, such as adipose tissue and skeletal muscle, contributing to a systemic insulin resistance. In addition, fructose is also linked to leptin resistance, worsening obesity and insulin resistance (Shapiro et al.,2008).

Studies in adolescents have shown that excessive fructose consumption exceeds the liver capacity to metabolize it and a small fraction remains in circulation, being captured by peripheral tissues. Although adipocytes have GLUT-5 receptor and are perfectly capable to capturing fructose, the mechanism of GLUT-5 hasn't been fully elucidated, although GLUT-5 is indicated in some studies as a regulator of adipocyte differentiation (Du et al., 2012; Legeza et al., 2017). In adipocyte, fructose is converted to fructose-6-phosphate by hexokinase enzyme, and converted to glucose-6-phosphate, which can promote glucocorticoids production by stimulating 11-HSD1 (Figure 1). Varma et al., Found that increasing fructose concentration enhances the conversion of pyruvate to acetyl-CoA via pyruvate dehydrogenase reaction, leading to glutamate. The pyruvate dehydrogenase flux enters in TCA cycle and result in an expanded acetyl-coA/citrate cycle in the synthesis of fatty acids and release of free palmitate. Therefore, the presence of fructose in adipocytes drives this alternative pathway, resulting in increased energy and CO2 production, which can be used in induced fructose lipogenesis and fat storage inadipocytes.

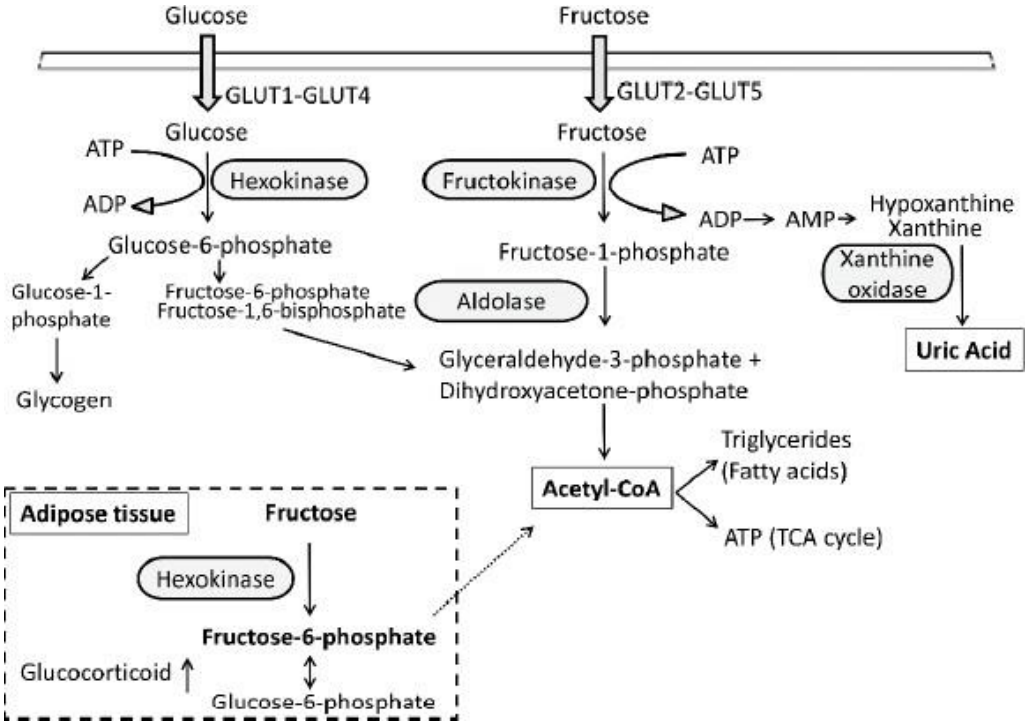

Figure 1. Differences in fructose metabolismon liver and adipose tissue. SOURCE: Legeza *et al.*, 2017.

Fructose Effects On Pancreas

Little is known about the specific action of fructose on insulin resistance and pancreatic beta cell function. Some articles have already linked a high consumption of fructose-rich diet to impaired glucose tolerance and insulin resistance, regardless of changes in body weight. Among several mechanisms studied to understand insulin resistance and beta cell functioning, oriented studies of the endoplasmic reticulum stress are an important molecular mechanism behind such pathologies.

Young adult rats undergoing a high fructose diet showed an increase in markers of stress in endoplasmic reticulum both in pancreas and in liver at the level of mRNAs and enzymes. The study showed a decrease in the mRNAs of the INSIG1 (regulates cholesterol metabolism, lipogenesis and glucose homeostasis) and PDX1 (transcription factor necessary for pancreatic development, including beta cell maturation) proteins, and increased mRNA levels of the regulatory proteins binding to sterol (SREBP1c), a regulator of cholesterol synthesis to fatty acids. The convergence of chronic ER stress for apoptosis in the pancreas/liver was also indicated by increased CHOP mRNA levels and increased JNK and Caspase-3 activity (Balakumar et al., 2016). Other work has shown that fructose-induced beta cell dysfunctions cause deregulation of leptin signaling and activation of protein kinase B in adolescent rat islets (Li et al., 2013). The Akt/FoxO1 pathway binds leptin signaling to Pdx1 uptake, related to the function and growth of pancreatic β cells. Akt/Fox01 and

INS-1 β-cells activation contributes to increased beta cell mass and insulin secretion. Under physiological conditions, leptin suppresses insulin secretion. Leptin resistance in the beta cell is related to hyperinsulinemia, beta cell failure, and glucose intolerance in obese rats (Morioka et al., 2007). The absence of leptin increases the phosphorylation of Akt and FoxO1, resulting in an increase in size and mass of the beta cell.

Pancreatic beta-cell failure in response to increased insulin demand willresult inimpaired glucose tolerance and type 2 diabetes development. According to Maiztegui et al.,adolescentratstreated with fructose presented reduced number of islets, endocrine total area, betacellarea, individual islet area and number of beta-cells/islet with a concomitant increase of 44%ofapoptotic cells. This apoptosis is related to stress mechanism of endoplasmic reticulumandglycolipotoxicity. Román et al. showed that adolescent rats fructosetreated had a decrease in number ofislets(50.3%) and beta-cell (36.8%), with an increasein plasma oxidative stress levels (elevatedplasma ThiobarbituricAcidReactiveSubstances-TBARSlevels).Theseisletsshowedhighlevels of O_2, and high NADPH oxidase activity, demonstrating the presence of local oxidative stress,which depends in part on the high enzymes activity in this complex.

Fructose Effects On Brain

Jimenez-Maldonado et al.(2011) showed one week of fructose supplementation in adolescent rats was able to alter some brain functions related to hippocampal plasticity, such as reduction of NeuN (Neuronal Nuclear Protein), Myelin Basic Protein, and Protein 43 associated with axonal growth concomitant with a reduction in hippocampal weight (table 2). There was also a reduction in the coactivator of gamma-receptor peroxisome-1 proliferator and cytochrome c oxidase II subunit as indicative of mitochondrial dysfunction and elevation of GLUT5 levels, indicating that fructose treatment is able to modulate positively the expression of its transporter. Agrawal et al. (2016) found that fructose consumption by young adult rats disrupts energy balance of hippocampus, decreasing the mitochondria functional bioenergetics (oxygen consumption rate and cytochrome C oxidase) and a worsening in effects of traumatic brain injury in molecular systems involved in cellular energy (sirtuin 1, gamma-1 peroxisome proliferator-activated receptor co-activator) and synapticplasticity.

Concluding Remarks

The high fructose consumption among adolescents is related to development of metabolic syndrome. This spectrum of changes is widespread and affects homeostasis of various organs and

tissues, such as liver, adipose tissue, intestine, pancreas, brain, among others. The current review

highlighted that food and beverages enriched by high fructose put in high risk the teenage health and open possibilities to also compromise their health adulthood. However, more care with adolescent, including deep decrease of fructose content in food and beverage to have impact in adolescent and adult health.

Declaration Of Interest

There are no conflicts of interest.

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Adolescent rich fructose intake induced metabolic malprogramming in adult rats and early moderate exercise is able to atenuate it

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Keywords: Fructose, Moderate intensity exercise, Metabolic programming.

ABSTRACT

Evidences in literature have shown that consumption of fructose increases metabolic syndrome (MetS) prevalence worldwide. Among the factors that predispose to MetS emergence, inadequate diet and lack of physical exercise do important role. The aim of this study was: (1) evaluate whether fructose would programme adolescents rats to MetS and (2) whether moderate intensity exercise training during adolescence protects adult rat to metabolic dysfunctions programmed by fructose supplementation during adolescence. Four-week-old male Wistar rats were randomly divided into 4 groups: control-sedentary (C-SED), fructose-sedentary (F-EXE), control-exercised (C-EXE) and fructose-exercised (F-EXE), with a fructose enriched drink (10% w/v fructose in water) and a moderate intensity exercise for 4 weeks. Food intake and body weight were measured weekly and the drink intake was measured each two days. After 120 days of life, analyses were performed. Data were analyzed with two-way ANOVA and the Tukey post-test. Rats F-SED presented hyperglycemia, hyperinsulinemia, high levels of total cholesterol and triglycerides when compared to the C-SED group. However, the F-EXE group showed reduced levels of these parameters, compared to F-SED group. Regarding fat stores, the F-SED group showed increased stocks of retroperitoneal and periepididymal fat pad and the exercise were not able to protect the F-EXE group from increase in stores of these fats. The F-SED group also show a significantly decrease in Sympathetic activity compared to C-SED group. Our results showed that 10% fructose supplementation during adolescence may program the metabolism to MetS in adulthood with development of insulin resistance, increased fat stores, dyslipidemia and alterations in ANS and the short-term moderate exercise may protect the metabolism from development of MetS through the increase insulin sensitivity, improving lipid metabolism and regulating the ANS (Autonomic NervousSystem).

Key-words: Fructose, Moderate Intensity Exercise, Metabolic Programming.

INTRODUCTION

Metabolic disorders such as insulin resistance, hyperglycemia, hypertension, dyslipidemia and abdominal obesity are a set of clinical changes that make up the Metabolic Syndrome (MetS)(1). The high consumption of carbohydrates, mainly fructose, has contributed to an epidemic incidence of MetS in the last decades(2), and now is a global problem(3). Consumption of beverages and sweetened foods has increased in all age groups, with adolescents as their main consumers(4; 5; 6). Several studies have already shown that stress factors, mainly dietary factors, in the early stages of life (gestation, lactation, adolescence) can trigger factors that lead to MetS in adult life(7; 8). This hypothesis is part of the DOHaD (Developmental Origins of Health and Disease) concept which postulates that insults in early life can be programmed for metabolic disorders in adult life. Previous studies already shows that adolescence is a dietary susceptible period that lead to metabolic programming(6), that can be induced by de high ingestion of sweetened drinks andfoods.

Although present in fruits in small amounts, the large quantity of fructose in the diet comes from industrialized products, largely sweetened beverages as soft drinks(3). The consumption of High Fructose Corn Syrup (HFCS) is what more provides fructose in the diet(5). Ingestion of fructose has been shown to cause obesity, insulin resistance(9), cardiovascular disorders(10) and type 2 diabetes(11). Fructose is first captured and metabolized by the liver, where it will act in the formation of lipogenic precursors, such as DHAP (dihydroxyacetone phosphate) and Acetyl-CoA(12), leading to hepatic fat deposition (steatosis), dyslipidemia and in hepatic insulin resistance(13).

Studies have shown that a diet rich in fructose for three weeks, during adolescence, increased insulin levels, as well as triglycerides, oxidative stress, insulin resistance(13; 14; 15) and high fasting glycemia(16). Other work showed that animals supplemented with fructose for two weeks exhibited hepatomegaly, moderate steatosis, disorganized liver histoarquitecture, altered TAG (triacylglycerols) synthesis and lipid deposition out of adipose tissue(lipotoxicity)(17).

The promising effects of physical exercise on the prevention and treatment of metabolic disorders have been evidenced by many studies that point out it's importance in weight loss, fat reduction and in the improvement of insulin sensitivity(18; 19). The moderate intensity exercise has been showed to be protective against development of the MetS(21). Physical exercise during young age in rats showed better results than in adult rats due to increased neuroplasticity processes, such as neurogenesis and angiogenesis(22). Our laboratory recently demonstrated that themoderate

intensity physical exercise when applied during adolescence in rats is able to decrease the size of walker tumor and ameliorate metastasis(23).

This experimental model of metabolic syndrome is reported on literature as insulin resistance inducer with a metabolic profile analogue to MetS observed in humans(9). However, the long-lasting effect of fructose on metabolism remains unexplored and also the effect of physical exercise on metabolic programming is a novelty and deserves to be elucidated. So, our hypothesis was: (I) the fructose intake during adolescence program metabolism to MetS in adulthood and (II) the short-term moderate exercise at low frequency performed at adolescence, concomitant with fructose, could protect the metabolism against MetS development in adult life.

MATERIALS AND METHODS

Ethical Approval

The handling of animals and experimental procedures were in accordance to the rules of National Council of Animal Experiments Control (CONCEA) and the Brazilian Society of Science in Laboratory Animals (SBCAL) and approved by the Ethics Committee on Animal Use of Universidade Estadual de Maringa – CEUA/UEM (protocol number 5669210917).

Animals and Experimental Design

Male Wistar rats were obtained at 25 days of age. They were kept in appropriate cages (5 rats per cage) under controlled temperature conditions (22±2°C), and a light/dark cycle of 12 h (07:00 a.m. to 07:00 p.m.), with *ad libitum* access to water and a standard diet (Nuvital®, Curitiba, PR, Brazil). After five days of adaptation, at 30 days of age, the animals was divided into four groups: Control sedentary (C-SED; n = 20), that received water and standard rat chow *ad libitum* during all the period; Fructose sedentary (F-SED; n = 20), that received Fructose 10% in the drinking water from the 30 to 60 days of life and standard rat chow *ad libitum*; Control exercised (C-EXE; n = 20), that was trained from the 30 to 60 day of life and received water and standard rat chow *ad libitum*, and Fructose exercised (F-EXE; n = 20), that received Fructose 10% in the drinking water and performed physical exercise at the same time from the 30 to 60 days of life. In order to evaluate the effect of the treatment (fructose and exercise) on metabolism immediately after the treatment period, data was collected in 60 day-old rats. To evaluate the potential role of fructose in programming, data was collected in 120 day-oldrats.

Preparation of Fructose Drinking Water

The fructose used in this protocol was D-Fructose >99% (Labsynth®, São Paulo/SP, Brazil).The fructose drink was prepared each two days and based on the formula weight/volume (w/v). For the preparation of 10% of fructose, 10 g of fructose was diluted in 100 ml of filtered water and the bottles covered with aluminium foil to prevent fermentation induced by light(5).

Training Protocol

All rats performed a physical fitness test to determine their individual maximal oxygen uptake (VO_2max) and maximal running speed (MRS). The test utilized a gas analyzer coupled to a treadmill for rodents (Panlab, Harvard Apparatus®, LE405 76- 0195 O2/CO2, Cornellà, Barcelona, Spain). The test began with a warm up (5 min, 10 cm/s, 0◦ of inclination), after which the velocity was increased by 9 cm/s every 3 min until exhaustion of the animal to obtain VO_2max and MRS, using Metabolism software, version 2.2.02. The decision to use 3 min at each stage was previously described(24), who reported that oxygen consumption stabilized after approximately 3 min at each stage of exercise after a change in workload. At the end of the treadmill line, a stainless steel grid emitted electrical stimuli (0.2 mA in < 1 s) to keep the animal in motion, as previously reported(25). The animal's inability to maintain the pace was considered to be a sign of exhaustion(26). A physical fitness test was performed before (initial: 30 days old), in the final of treatment and aerobic exercise (middle: 60 days old), and in the final of the experimental period (final: 120 days old). Incremental tests were performed every 15 days to adjust the training load. Exercise training was performed with running on a treadmill (Panlab, Harvard Apparatus®, LE8710R 76-0308, rat 5-lanes). Previous adaptation was performed in 5 sessions with durations of 10, 12, 14, 16 and 18 minutes and an intensity of 16 cm/s. Two days of rest were established to apply the ET. The prescription was based on effort test (ET) and was performed by the individual value of the final workload (FWL) corresponding to 55% and 65% of VO_2max to optimize the fat metabolism zone(27). The treadmill training protocol was performed for 44 minutes a day (9 am to 10 am), 3 days a week in a 4-week macrocycle. The sessions were distributed with 2 minutes of warming up and cooling down at 20 cm/s and 40 minutes of continuous running at a moderate intensity (~ 55% to 65% FWL of the ET). Rats that reached the same speed or FWL training at the same time. The training protocols were completed at 60days.

Caloric intake and body weight gain

Food intake, drink intake and caloric intake was measured each two days from 30 to 120 days of life. The food intake was calculated as the difference between the amount of food remaining and the total provided, which was divided by the number of days and the number of rats in the box(28). The caloric intake was calculated based on the amount of food and fluid intake and the corresponding constants(29).The animals were weighed once a week during the experimental period.

Biochemical Analysis

At the end of the treatment phase (at 60 days of age) and experimental phase (at 120 days of age) animals from all groups were weighed and decapitated, and blood samples were collected and centrifuged (10,000 rpm for 5 min) to obtain plasma for further biochemical analysis. The plasma was used to measure glucose by the enzymatic method using a commercial colorimetric kit (Gold Analisa R, Belo Horizonte, Brazil) and quantified by spectrophotometry (BIO200FL, Bio Plus R, São Paulo, Brazil). Insulin was measured by radioimmunoassay (RIA)(30) with a gamma counter through a gamma particle emission counter (Wizard Automatic Gamma Counter, TM-2470, PerkinElmer R, Shelton, CT, United States), using standard rat insulin, anti-rat insulin (Sigma-Aldrich R , St Louis, MO, United States) and recombinant human insulin ([125I]-Insulin (h)] (PerkinElmer R , Shelton, CT, United States). Insulin resistance markers were evaluated (HOMA - IR and Triglyceride and glucose (TyG) index). Total cholesterol and triglyceride concentrations were measured using the enzymatic colorimetric method with a commercially available kit (Gold Analisa; Belo Horizonte, MG, Brazil).

Intraperitoneal Glucose Tolerance Test (ipGTT)

The Intraperitoneal Glucose Tolerance Test (ipGTT) was performed at the end of the treatment phase (at 60 days of age) and experimental phase (at 120 days of age)(31). Food was withdrawn 8 - 12 h before the test, and free access to water was allowed. The rats received an intraperitoneal injection of glucose (2g/kg of BW). Blood samples were obtained through tail venesection,) at 0 (prior to glucose injection), 15, 30, 60 and 120 minutes after injection and centrifuged (13.000 rpm for 5 min). The glucose was measured by the enzymatic method using a commercial colorimetric kit (Gold Analisa R, Belo Horizonte, Brazil) and quantified by

spectrophotometry (BIO200FL, Bio Plus® , São Paulo,Brazil).

Fat Pad Stores Measurements and tissueextractions

At 60 days and 120 days of age, all the groups were euthanized and their fat pad stores (retroperitoneal, periepididymal and mesenteric) were removed and weighted to assess the state of obesity. The liver were dissected and weighed. Each of the fat pad stores values and liver values were correlated with the bw of each rat and were calculated as g/100kg of bw(7).

Parasympathetic and sympathetic activity

To verify de ANS (Autonomic Nervous System) activity, at 120 days of age a longitudinal surgical incision was made on the anterior cervical region of the animals. The left vagus superior branch was isolated and placed over a silver electrode inside a faraday cage, as previously described Peron *et al*., 2014(32). After 12 min of vagus nerve electrical recordings, a laparotomy was performed to isolate the branch of the sympathetic nerve that is located in the splanchnic region; this branch originates in the lumbar plexus at the level of L2 and extends to the retroperitoneal adipose tissue. The neural signal output was acquired using the Insight interface (Insight®, RiberãoPreto, SP, Brazil) for 12 min, from which 20 recorded frames of 5 s from each animal were randomly chosen for spike counting. Spikes >0 mV were considered. The average number of spikes was used as the nerve firingrate.

Statistical Analysis

Data were expressed as the mean ± SEM. GraphPad Prism version 6.01 for Windows (GraphPadSoftware, La Jolla, CA, USA) was used for statistical analyses and developing graphs. Two-way analysis of variance (ANOVA) followed by the Tukey multiple comparisons test was used for 60 and 120-day-old animals. A p value <0.05 was considered significant when considering the main effect of fructose (F), exercise (E), their interaction (I; fructose vs exercise) and the differences between groups.

RESULTS

Caloric intake and Body Weight

During the period of treatment, from 30 to 60 days, the food intake of F-SED group was significantly less than C-SED group (Figure 1A). Yet, immediately after the nutritional transition, from day 60, the F-SED group experienced a catch-up in the chow consumption, recovering the normality in the following week. The exercise reduced food intake of fructose supplemented group during the treatment period. But, after this period, F-EXE had a catch-up, maintaining this pattern until the end of experimental period.

The total caloric intake during the treatment period was not significantly different among both C-SED and F-SED, but after this period, the caloric intake of F-SED group was significantly higher than C-SED (Figure 1C). The energy intake of C-EXE group was less than the C-SED group during the 30 days of treatment, and after this was not significantly. The F-EXE group ingested significantly more calories than C-EXE during the treatment period and, after this, no difference between these groups was found. However, F-EXE ingested significantly more calories than F-SED from 60 to 120 days of life.

Wasn't found significantly differences in weight gain in all the groups (Figure 1B).

Fat Pad Stores and Liver Weight

At 60 day of treatment, was not found significantly differences in periepididymal, retroperitoneal and mesenteric fat pad in F-SED, compared to C-SED group (Table 1). The exercise decreased the stores of retroperitoneal and mesenteric fat stores only in the C-EXE compared to C-SED group. The reduction in F-EXE compared to F-SED in periepididymal and retroperitoneal was only a trend. Yet, at 120 days of life, was found significantly increased stores of periepididymal and retroperitoneal fat in F-SED compared to C-SED group and the exercise did not decreased this fat stores (Table 2). Contrariwise, the exercise increased significantly the stores of periepididymal fat in C-EXE compared to C-SED group. Wasn't found significantly differences in all groups in mesenteric fat (data notshown).

At 60 days of life wasn't found any differences between the groups on liver weight (Table 1), but at 120 days of life, a significantly decrease on liver weigh was found in F-SED compared to C-SED and the exercise didn't improved this alteration (Table2).

Glycaemia and insulinaemia

At 60 days of life, wasn't found differences among all groups in fast glycaemia (Table 1),

but during the ipGTT the F-SED group showed a significantly increase in blood glucose when compared to C-SED group (Figure 2A). The exercise was able to significantly decrease the blood glucose of F-EXE compared to F-SED. At, 120 days of life, was found a significantly increase in fast glycaemia in F-SED compared to C-SED group (Table 2). The physical exercise did not reduced this alteration, but during the ipGTT the exercise reduced significantly the blood glucose of F-EXE compared to F-SED group (Figure 2B).

At 120 days of life, the fast insulinaemia of F-SED was significantly increased compared to C-SED and the exercise decreased de insulinaemia of the F-EXE compared to F-SED significantly (Table 2). During the ipGTT, the F-SED group showed an increased insulinaemia compared to C-SED and the exercise reduced significantly this insulinaemia in F-EXE compared to F-SED group (Figure 3).

The HOMA-IR (Homeostasis Model Assessment-index) showed a significantly increase in F-SED compared to C-SED group and the exercise significantly reduced this parameter in F-EXE compared to F-SED group (Table 2). The Triglyceride and glucose (TyG) index also showed a significantly increase in F-SED compared to C-SED group and the exercise reduced significantly this parameter (Table2).

LipidProfile

At 60 days of life, wasn't found differences in total cholesterol and triglycerides and the exercise didn't change anything (Table 1).

At 120 days of life, the F-SED increase the total cholesterol and triglycerides significantly compared to C-SED group and the exercise decreased significantly these parameters in F-EXE compared to F-SED group (Table 2).

Parasympathetic and Sympathetic Activity

At 120 days of life, wasn't found significantly difference in parasympathetic activity between C-SED and F-SED groups, but the F-SED showed a trend to increase and the exercise reduced significantly this increase in F-EXE (Figure 4). However, we found significantly decrease in Sympathetic activity in F-SED compared to C-SED group and the exercise only showed a trend toincrease.

DISCUSSION

For the first time, this work showed that fructose supplementation in adolescent rats program metabolism to metabolic syndrome in adulthood and moderate physical exercise mitigates the metabolic schedule caused by fructose supplementation in adolescence. We obtained consistent data that fructose already changes glycemic homeostasis in rats with 60-days old rats. After 30 days receiving sugar suplementation, young rats present glucose intolerance. Interesting that the animals when they turn to adult life, 120-day-olds ones, still presenting glucose intolerance and plus showed fast hyperglicemia, hyperinsulinemia, tissue insulin resistance, elevated fat pad stores, dyslipidemia. Those adult rats also presents changes in ANS activity umbalance, high Parasympathetic Nervous System (PNS) and low Sympathetic Nervous system (SNS). Our results showed that the concomitant exercise with fructose-rich diet was evident and beneficial with respect to glycemic metabolism, both at 60 and 120 days of life. However, at 120 days-old, short-term exercise atenuated dyslipidemia and ANSumbalance.

Many studies using rich fructose intake after weaning and during adolescence found insulin resistance, increased fat stores and dyslipidemia at the end of treatment(32). Fructose consumption in 5 month-old rats increasing lipid content of the liver, leading to hepatic steatosis, insulin resistance(13) cardiovascular diseases (CVD), type 2 diabetes in adult humans (33; 34; 35) and 8-18 years-old boys is also pointed out as responsible for non-alcoholic fatty liver (NAFLD) development(36). Our results obtained with 60 days-old rats show a decrease in food intake with no changes in total caloric intake and glucose intolerance, without fast hyperglicemia. These results suggest although food intake is lower, fructose supplementation provides a high amount of calories, sufficient to disturb glucose metabolism at end of the 30 days of treatment, but insufficient to increase fat stores at the sametime.

The early exposure to bad environmental factors is described on literature to be responsible for many health outcomes in adult life(37). Interestingly, our studies suggest that the ingestion of fructose in an early stage of life may result in a long-last programming composed for an onset of disorders that compound metabolic syndrome in adult life. Rats that were exposed to 10% of fructose supplementation during adolescence showed at the end of the 120 days of treatment disorders that constitute the metabolic syndrome, such as hyperglycemia, hyperinsulinemia with a insulin tissue resistance, showed by high HOMA and TYG index. Also, dyslipidemia, high levels of fat and alterations in the ANS umbalaced were observed. Those dysfunction can be considered as a metabolicsyndrome(40).

At 120 days, our data shows increased fat stores, although weight gain remained unchanged.Thesedatashowthat,althoughfoodintakeduringthetreatmentperiodislower,the

potencial of fructose to promote lipogenesis is high, which justifies the increase in the main fat stores. The study by Harrel *et al*(38) showed that the consumption of fructose by adolescents increased fat stores without body weight gain. Studies in literature that used 10% fructose in rats showed no increase in body weight(1; 2). However, in studies that used fructose 20%, there is a weight gain compared to controls(5). So, we can infer, therefore, that the absence of weight gain shown in the fructose-treated rats in this work was due to low concentration of fructose administered.

All dysfunction were programmed by a fructose drinking from a fructose solution of 10%; which was based in volume drunk daily. Then the dose was 5 g of fructose consumed by day. This dose offer is high compared to control group that received no fructose supplementation. Considering that a teenage rats weighs an average of 90 g and consumes approximately 5 g of fructose per day and an American teenager weighs an average of 70 kg and consumes approximately 72.8 g / day of fructose(38), it is observed that the ingestion of fructose by the adolescent rats is about 50 times greater than the intake of fructose by the human adolescent. In contrast, the literature has shown that this concentration mimics fructose consumption by human adolescents (5), being the lowest dose found in the literature. However, the data is consistent to suggest that fructose intake was able to disrupt glucose homeostasis is adolescent rats and also remarkably programs adult offspring toMetS.

Our data showed that the administration of fructose induces a state of insulin resistance, according the HOMA IR and TYG index suggest. Maiztegui et al.,(47) showed similar results when treating adolescent wistar rats with fructose for 3 weeks. The TYG index has been indicated in the literature to reliably portray insulin resistance. Unlike the HOMA IR index based on glycemia and insulinemia baseline, the TYG index uses triglyceride values instead insulin, making a relation between glycemia and lipid profile, being strongly associated with diabetes, NAFLD and metabolicsyndrome(48).

The current study also showed that the treatment with fructose did not altered the lipid profile of adolescent rats. However, at 120 days of age, total cholesterol and triglyceride levels were elevated in F-SED compared to C-SED. Several works in the literature have already shown the potential of fructose in stimulating lipogenesis in liver. This high yield leads to an ectopic deposition of lipids in the liver and muscle tissues, which promotes central and peripheral insulin resistance on the body, which explains insulin resistance found in our study. The high lipogenesis in the liver also leads to a large production and elevation in triglyceride levels(12; 16; 35;36).

Related to ANS, we found that the fructose diet enhances the vagal electrical activity in adult life. Our laboratory already shown that the vagal electrical activity is increasedin an obesity

state(39). A high activity of the parasympathetic nervous system is related to metabolic disorders such as hyperinsulinemia and insulin resistance(7), which is in agreement with our results. We did not found statisticall increase of the vagal activity in adult rats, who was treated with fructose in adolescence, however, observing the sympathetic electrical activity we shown that fructose treatment programe a large decrease in sympathetic nervous system of adult rats. It has been shown that sympathetic nervous system activity is decreased in many animal model obesity in human beans (52). Our observation is in agreement of "MONA LISA" hypothesis (Most Obesities kNown Are Low In Sympathetic Activity), proposed by Bray, which says that the resistance to leptin, responsible for obese state, is related to a decreased local sympatheticactivity(53).

Our data also showed that the moderate intensity physical exercise plays an important role protecting the body against development of fructose induced MetS. In the prevention of risk factors for the metabolic syndrome, many studies have demonstrated an effectiveness in use of the physical exercise alone(40) and a direct correlation between physical exercise among young adults and improved insulin sensitivity(41; 42). Our laboratory has already demonstrated that the moderate intensity physical exercise when applied in adolescence protects the body from the metabolic syndrome caused by a high-fat diet in adolescence (HFD)(21). In our study, at 60 days of life, the physical exercise reduced the retroperitoneal and periepididimal fat stores of C-EXE compared to C-SED group. However, there was no significant change in fat stores in the exercised group supplemented with fructose, F-EXE, compared to F-SED. At 120 days of age, physical exercise also did not change fat stores of rats supplemented with fructose. It can be explained possibly by the detraining occurred from 60 to 120 day of life. Studies showed when exercise training was stopped, most of the benefits that was gained during the training was lost progressively during the time, but not alter the insulin sensitivity(20). Our data also showed no differences in soleus and gastrocnemius muscle weight (data not shown) at 120 days of life, reinforcing the hypothesis of detrainingperiod.

Consistent with previous findings on the protective ability of moderate-intensity physical exercise on glycemic and lipidic metabolism(21; 23), our results showed a significant reduction in glycemic and insulinemic levels, as well as in HOMA IR and TYG index, in rats supplemented with fructose and submitted to the physical training protocol during adolescence, compared to sedentary group (F-SED) at both 60 and 120 days of life. Exercise programs to insulin conserve good tissue insulin sensitivity. Increased insulin-mediated glucose utilization in insulin-resistant individuals with a family history of type 2 diabetes, as well as in type 2 diabetes patients was improved with only one physical exercise session (49; 43). Scomparin *et al*(22) demonstrated that physical exercise performed early in life protects against the low supply of catecholamines inthe

adrenal medulla in rats with obesity induced by monosodium glutamate (MSG). The total cholesterol was improved both at 60 and 120 days of life and the triglyceride was improved only at 120 days of life. Bezerra and contributors(44) found the physical exercise is a regulator of fructose or sedentary dislipidemia, reducing the plasma levels of triglycerides.

The physical exercise decreased the parasympathetic nerve activity and these results is similar to Jia *et al*(45) that found a decrease in parasympathetic activity in trained young adult human beins. Our laboratory already shows that this exercise protocol starting before weaning is beneficial to vagus nerve activity to animal fed with HFD(46). Although the exercise did not increased significantly the electrical sympathetic activity in adult who was fructose treated, it was evident that fructose rich intake decrease sympathetic activity. Those data are in agreement with many observations in obesity programmed experimental animal(53;54).

In conclusion, our results showed that the fructose supplementation during adolescence program to metabolic syndrome in adulthood, and the short-term moderate exercise syncronized with fructose intake atenuates metabolic dysfunctions onset in adultrats.

DECLARATION OF INTEREST

There are no conflicts of interest.

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FIGURE LEGENDS

Figure 1. (A) Food intake; (B) Weight gain and (C) Total caloric intake from 30 to 120 days of life (n = 20). C-SED sedentary rats subjected a normal diet, F-SED sedentary rats subjected a fructose diet, C-EXE exercised animals subjected to a normal diet and F-EXE exercised animals subjected to a fructose diet. F fructose factor, E exercise factor and I interaction between fructose and exercise factors. +++ $p < 0.001$, ++ $p < 0.01$ and + $p < 0.05$ for the probability based on a two-way analysis ofvariance.

Figure 2. (A) Glycemia at 60 days of life and (B) Glycemia at 120 days of life (n = 20). C-SED sedentary rats subjected a normal diet, F-SED sedentary rats subjected a fructose diet, C-EXE exercised animals subjected to a normal diet and F-EXE exercised animals subjected to a fructose diet. F fructose factor, E exercise factor and I interaction between fructose and exercise factors. +++ $p < 0.001$, ++ $p < 0.01$ and + $p < 0.05$ for the probability based on a two-way analysis of variance.

Figure 3. Insulinemia at 120 days of life (n = 20). C-SED sedentary rats subjected a normal diet, F-SED sedentary rats subjected a fructose diet, C-EXE exercised animals subjected to a normal diet and F-EXE exercised animals subjected to a fructose diet. F fructose factor, E exercise factor and I interaction between fructose and exercise factors. +++ $p < 0.001$, ++ $p < 0.01$ and + $p < 0.05$ for the probability based on a two-way analysis ofvariance.

Figure 4. Parasympathetic and sympathetic activity at 120 days of life (n = 20). C-SED sedentary rats subjected a normal diet, F-SED sedentary rats subjected a fructose diet, C-EXE exercised animals subjected to a normal diet and F-EXE exercised animals subjected to a fructose diet. F fructose factor, E exercise factor and I interaction between fructose and exercise factors. +++ $p < 0.001$, ++ $p < 0.01$ and + $p < 0.05$ for the probability based on a two-way analysis of variance.

TABLES

Parameters	C-SED	F-SED	C-EXE	F-EXE	P VALUE
Fast Glycemia (mg/dL)	68,4±3,72	70,75±3,54	70±1,52	64,8±2,15	>0.05
Periepydidimal fat (g/100g)	0,67±0,04	0,71±0,02	0,55±0,02	0,61±0,00	>0.05
Retroperitoneal fat (g/100g)	0,67±0,03	0,73±0,02	0,43±0,0481	0,58±0,03	>0.05
Liver weight (g/100g)	3,32±0,06	3,24±0,08	3,19±0,05	3,30±0,06	>0.05
Total cholesterol (mg/dL)	82,7±3,29	87±3,39#	73,78±2,49	67,71±3,20#	<0.05
Triglycerides (mg/dL)	81,17±4,7	74±3,65	79,67±1,82	77±3,033	>0.05

Table 1. Biometric parameters from wistar rats at 60 days of life. Values are reported as mean S.E.M and were statistically significant when P<0.05. *Represent difference between C-SED and F-SED; #Represent difference between F-SED and F-EXE by Two-way ANOVA, followed by Tukey's post hoc test.

Parameters	C-SED	F-SED	C-EXE	F-EXE	P VALUE
Fast Glycemia (mg/dL)	79,38±2,47*	92±3,68*	87,6±1,28	83,33±1,68	<0.05
Fast insulinemia (ng/mL)	0,22±0,01*	0,30±0,01*#	0,21±0,01	0,24±0,00#	<0.05
HOMA IR	0,98±0,05*	1,67±0,14*#	1,11±0,13	1,18±0,06#	<0.05
TyG index	7,71±0,03*	8,49±0,08*#	7,76±0,08	7,91±0,08#	<0.05
Periepydidimal fat (g/100g)	1,07±0,06*	1,55±0,06*	1,59±0,07	1,53±0,14	<0.05
Retroperitoneal fat (g/100g)	1,21±0,13*	1,66±0,12*	1,48±0,10	1,50±0,15	<0.05
Liver weight (g/100g)	3,02±0,20*	2,75±0,05*	3,01±0,06	2,94±0,05	<0.05
Total cholesterol (mg/dL)	61,84±2,62*	94,66±4,83*#	61,29±4,15	64,78±2,83#	<0.05
Triglycerides (mg/dL)	58±1,82*	104,7±6,81*#	54,8±4,49	67,57±4,47#	<0.05

Table 2. Biometric parameters from wistar rats at 120 days of life. Values are reported as mean S.E.M and were statistically significant when P<0.05. *Represent difference between C-SED and F-SED; # Represent difference between F-SED and F-EXE by Two-way ANOVA, followed by Tukey's post hoc test.

Figure 1

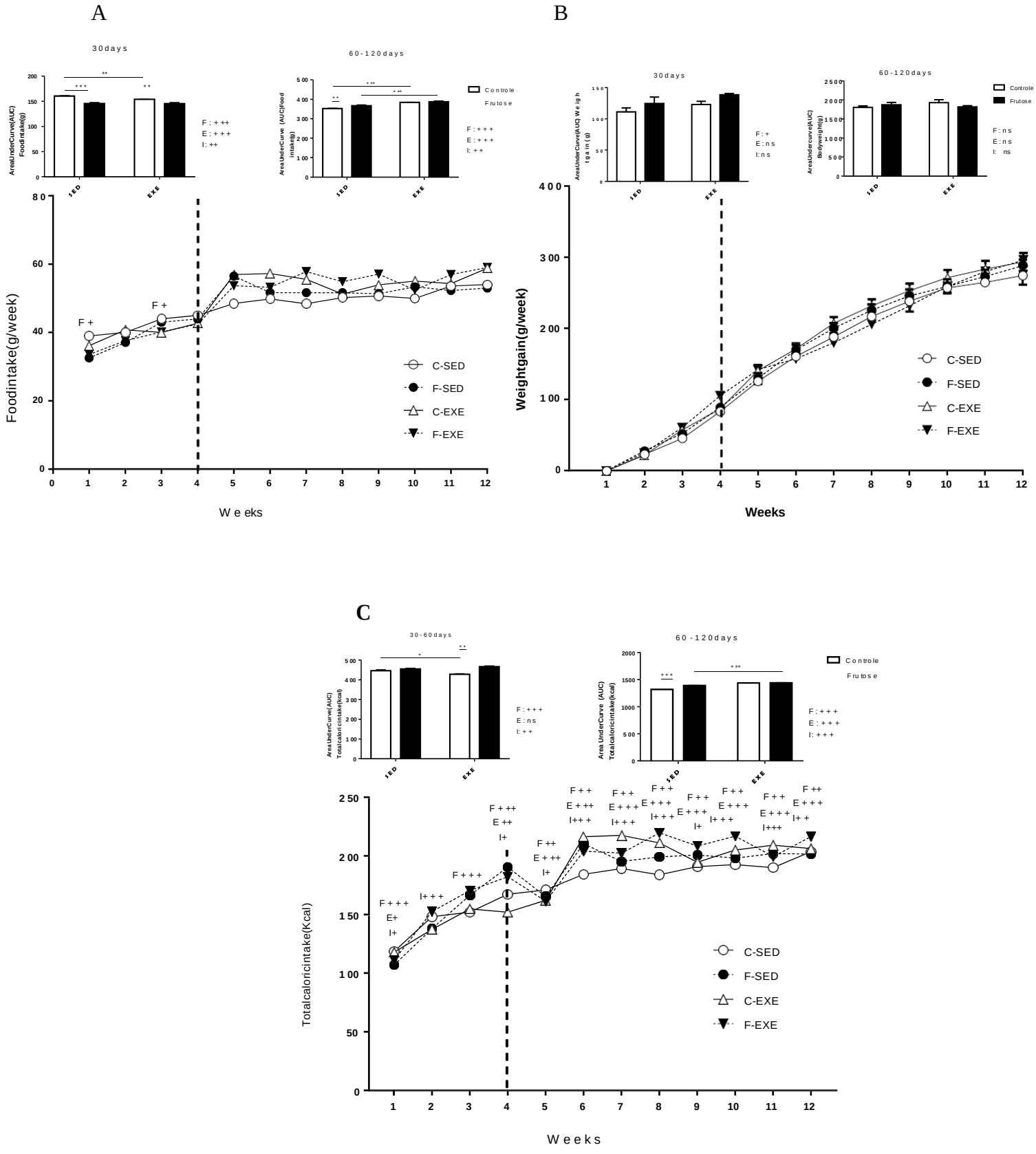
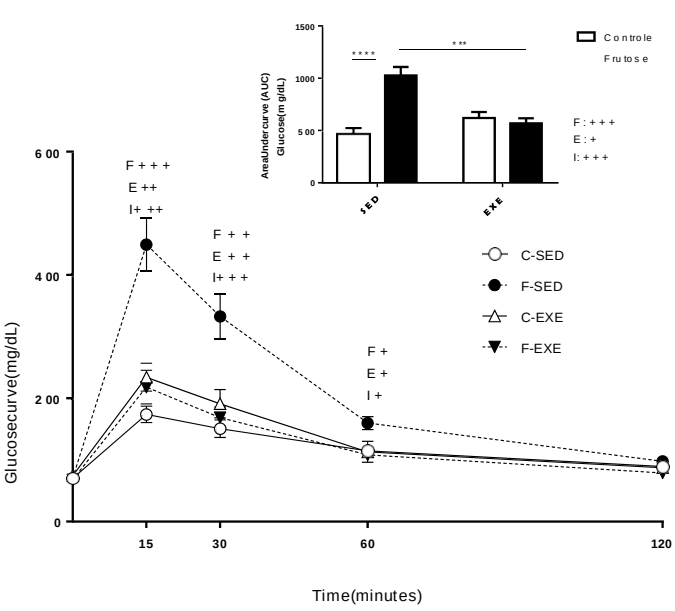

A
B
C
30days
60-120days
30-60days
Controle
Frutose
F : + + +
E : + + +
I: ++
F : +
E : ns
I: ns
F : ns
E : ns
I: ns
F : + + +
E : ns
I: + +
F : + + +
E : + + +
I: + + +
1ED
EXE
Foodintake(g/week)
Weightgain(g/week)
Totalcaloricintake(Kcal)
Weeks
C-SED
F-SED
C-EXE
F-EXE

Figure 2

A


Glucosecurve(mg/dL)
6 00
4 00
2 00
0
15
30
60
120
Time(minutes)
F + + +
E ++
I+ ++
F + +
E + +
I+ + +
F +
E +
I +
C-SED
F-SED
C-EXE
F-EXE
AreaUndercurve (AUC)
Glucose(m g/dL)
1500
1000
500
0
****
**
SED
EXE
Controle
Frutose
F : + + +
E : +
I: + + +

B

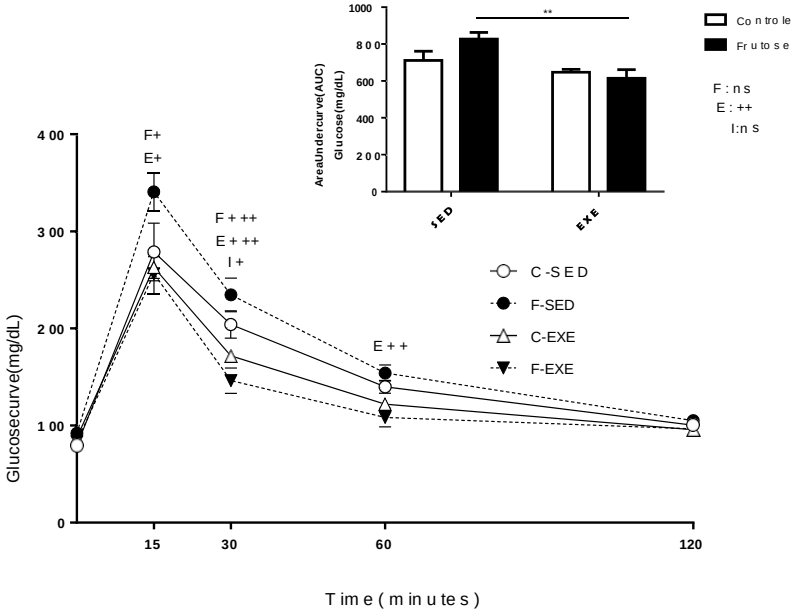
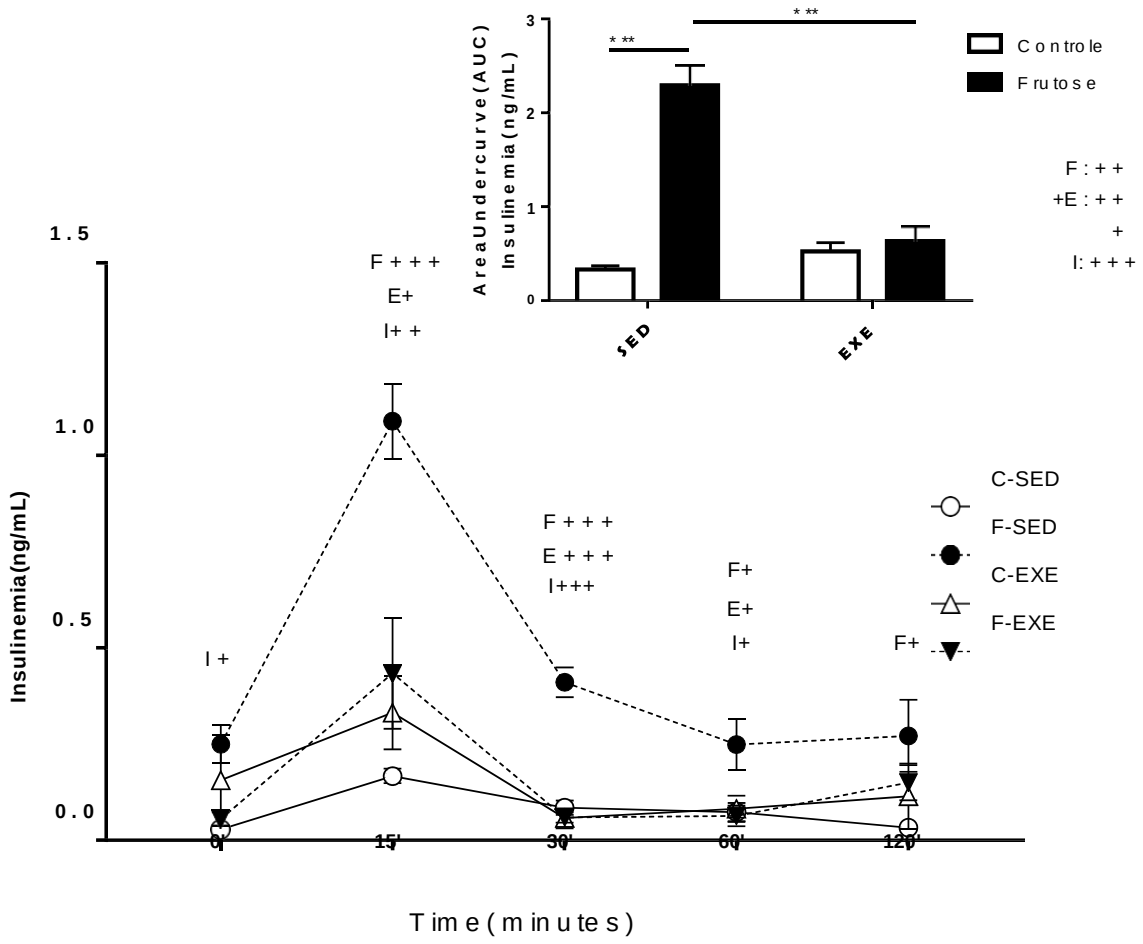

Glucosecurve(mg/dL)
4 00
3 00
2 00
1 00
0
15
30
60
120
Time (minutes)
F+
E+
F + ++
E + ++
I +
E + +
C -SED
F-SED
C-EXE
F-EXE
AreaUndercurve(AUC)
Glucose(mg/dL)
1000
8 00
600
400
2 00
0
**
SED
EXE
Controle
Frutose
F : ns
E : ++
I:n s

Figure 3


Area Under curve (AUC)
Insulinemia (ng/mL)
3
2
1
0
* **
* **
SED
EXE
Controle
Frutose
F : + +
+E : + +
+
I: + + +
1.5
1.0
0.5
0.0
Insulinemia(ng/mL)
F + + +
E+
I+ +
F + + +
E + + +
I+++
F+
E+
I+
F+
I +
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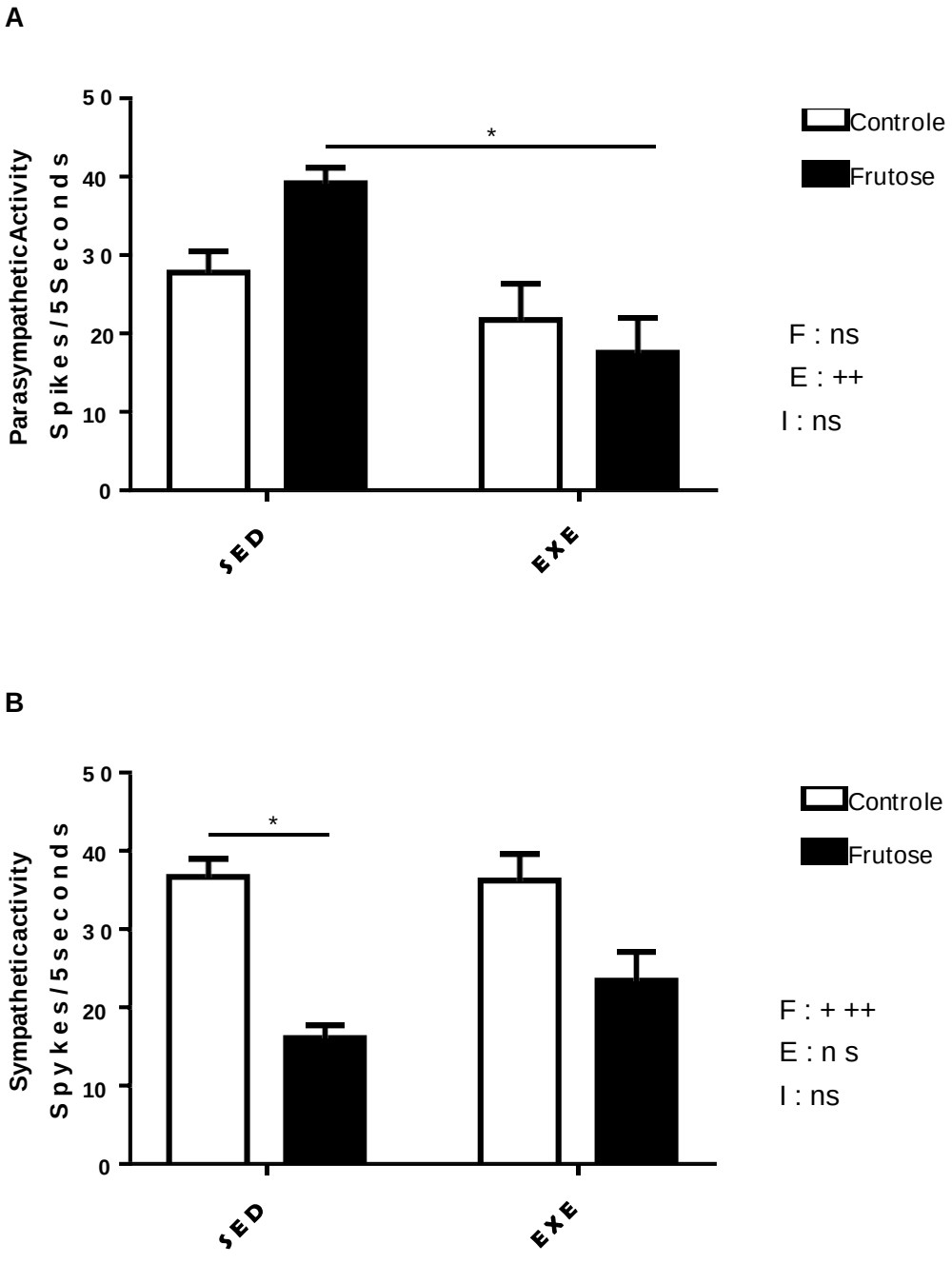
C-EXE
F-EXE
0'
15'
30'
60'
120'
Time (minutes)

Figure 4


A
ParasympatheticActivity
Spikes/5Seconds
50
40
30
20
10
0
*
SED
EXE
Controle
Frutose
F : ns
E : ++
I : ns
B
Sympatheticactivity
Spykes/5seconds
50
40
30
20
10
0
*
SED
EXE
Controle
Frutose
F : + ++
E : n s
I : ns

Impact Of High Fructose Diet And Moderate Intensity Exercise During Adolescence On Adult Metabolism

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Keywords: High Fructose Diet, Obesity, Moderate intensity exercise, Metabolic programming.

ABSTRACT

Ojective. Due to new western eating habits, obesity has become a public health problem in the modern world, being linked to other problems such as dislipidemia and diabetes mellitus, diseases that compound metabolic syndrome (MetS). This work aimed to verify the effects of a high dose of fructose on obesity and metabolic programming development and whether the moderate intensity physical exercise would be able to protect the body from development of metabolic abnormalities in adultlife.

Methods. Four-week-old male Wistar rats were randomly divided into 4 groups: Sedentary-control (C-SED), Sedentary-fructose (F-EXE), Exercised-control (C-EXE) and exercised-fructose (F-EXE), with a fructose enriched drink (20% w/v fructose in water) and a moderate intensity exercise for 4 weeks. Food intake and body weight were measured weekly and the drink intake was measured each two days. After 120 days of life, analyses were performed. Data were analyzed with two-way ANOVA and the Tukeypost-test.

Results. The 20% fructose dose increased body weight at the end of the 30 days of treatment and retroperitoneal, periepididimal and mesenteric fat stores of the sedentary group (F-SED), compared to C-SED control at the end of the 120 days of life and moderate-intensity physical exercise reduced body weight and stocks of the three trained group fats (F-EXE) at the same time as F-SED.

Conclusion. The results showed that a high fructose supplementation during adolescence program the metabolism to MetS in adulthood with development of obesity and high fat stores and the moderate intensity exercise performed at adolescence protects against fructose-programed metabolic dysfunctions in adultlife.

Key-words: High Fructose Diet, Obesity, Moderate intensity exercise, Metabolic programming.

INTRODUCTION

Although many other environment aggression, due new western eating habits, obesity has become a public health problem in the modern world, being linked to other health problems, such as dyslipidemia, hypertension and diabetes mellitus, diseases that compound metabolic syndrome (MetS). Western diets often include high fat and carbohydrate, such as fructose [1; 2], being adolescents the main group involved in high consumption of fructose [3], leaving them susceptible to metabolic dysfunction in adult life[4].

Fructose found in natural foods has beneficial effect since it supplies energetic need of the body. However, high fructose intake from sweetened foods with sucrose (fructose + glucose) or HFCS (High Fructose Corn Syrup) causes negative effects on body metabolism, provoking metabolic syndrome deleterious effects in any age [5; 6].

The high fructose consumption in early and adult live has been associated to development of obesity, insulin resistance, hyperlipidemia, type II diabetes and tissue fat accumulation [7]. Fructose ingestion are mainly metabolized in liver and intestine, but a residual part in circulation is absorbed by white adipose tissue (WAT), leading to physiological changes such as hypertrophy, insulin resistance and inflammation [8]. In liver, its entry is rapid and does not depend on mediators such as hexokinase enzyme and doesn't undergo insulin control, being transformed into lipogenic mediators such as dihydroxyacetone and glyceraldehyde-3-phosphate, which will increase triglycerides production[5].

The increase in the use of foods sweetened with fructose produced by food industry is accompanied by the increase in rates of obesity and metabolic syndrome among adolescents and young adults [9]. Recent studies have shown that adolescence is a period of neural plasticity and may be vulnerable to metabolic programming by dietary insults [4; 10].

The benefits of non-pharmacological and non-invasive therapies such as physical exercise have already been well recognized. Several studies have already shown the ability of physical exercise to alleviate the metabolic syndrome symptoms or even protect the body from its development [10; 11; 13]. The practice of regular physical exercise improves healthy living; by other hand sedentary life increases the risk of developing chronic diseases or premature deaths [12]. Recent studies have shown that the practice of physical exercise has positive effects on fat reduction, body weight and obesity prevention. Fiebig et al. (1997) [13] showed the antilipogenic effect of physical training down-regulating the liver lipogenic enzymes by reducing the amount of long chain fatty acids available for triglyceride synthesis. The protocol of moderate intensity exercise has been shown as a good modality for physical training, being well established by recent studies in our group. Tofolo et al., (2014) [10] showed that moderate-intensity physicalexercise

attenuated weight gain and fat stores of rats fed with high fat diet (HFD). In another study, moderate intensity exercise functioned as an adjuvant, improving post-vaccination immune system capacity[14].

Our group has already shown that supplementation with 10% fructose induces metabolic syndrome in adulthood without change body weight and the moderate intensity exercise concomitant with the high fructose insult protects body from the metabolic syndrome in adult life (unpublished data). The literature has shown that fructose supplementation in concentrations above 10% may be more efficient in inducing obesity in adolescents, but most articles use very high doses (over 50%), being unusual doses [15; 16; 17]. Several studies have shown that fructose at a concentration of 15 to 20% more accurately simulates the fructose intake of adolescents, but the effects of 20% fructose supplementation on metabolic programming and the protective effects of moderate intensity exercise protocol are still unknown. Thus, this work aimed to verify the effects of a high dose of fructose during adoslecence programming MetS when they turned to adult life and whether the moderate intensity physical exercise would be able to protect the youngest to metabolic abnormalities in adultlife.

MATERIALS AND METHODS

Ethical Approval

The handling of animals and experimental procedures were in accordance to the rules of National Council of Animal Experiments Control (CONCEA) and the Brazilian Society of Science in Laboratory Animals (SBCAL) and approved by the Ethics Committee on Animal Use of Universidade Estadual de Maringa – CEUA/UEM (protocol number 5669210917).

Animals and Experimental Design

Male Wistar rats were obtained at 25 days of age. They were kept in appropriate cages (5 rats per cage) under controlled temperature conditions (22±2°C), and a light/dark cycle of 12 h (07:00 a.m. to 07:00 p.m.), with *ad libitum* access to water and a standard diet (Nuvital®, Curitiba, PR, Brazil). After five days of adaptation, at 30 days of age, the animals was divided into four groups: Control sedentary (C-SED; n = 20), that received water and standard rat chow *ad libitum* during all the period; Fructose sedentary (F-SED; n = 20), that received Fructose 20% in the drinking water from the 30 to 60 days of life and standard rat chow *ad libitum*; Control exercised (C-EXE;n = 20), that was trained from the 30 to 60 day of life and received water and standard rat

chow *ad libitum*, and Fructose exercised (F-EXE; *n* = 20), that received Fructose 20% in the drinking water and performed physical exercise at the same time from the 30 to 60 days of life. In order to evaluate the effect of the treatment (fructose and exercise) on metabolism immediately after the treatment period, data was collected in 60 day-old rats. To evaluate the potential role of fructose in programming, data was collected in 120 day-oldrats.

Preparation of Fructose Drinking Water

The fructose used in this protocol was D-Fructose >99% (Labsynth®, São Paulo/SP, Brazil).The fructose drink was prepared each two days and based on the formula weight/volume (w/v). For the preparation of 20% of fructose, 20 g of fructose was diluted in 100 ml of filtered water and the bottles covered with aluminium foil to prevent fermentation induced by light [15].

Training Protocol

All rats performed a physical fitness test to determine their individual maximal oxygen uptake (VO_2max) and maximal running speed (MRS). The test utilized a gas analyzer coupled to a treadmill for rodents (Panlab, Harvard Apparatus®, LE405 76- 0195 O2/CO2, Cornellà, Barcelona, Spain). The test began with a warm up (5 min, 10 cm/s, 0◦ of inclination), after which the velocity was increased by 9 cm/s every 3 min until exhaustion of the animal to obtain VO_2max and MRS, using Metabolism software, version 2.2.02. The decision to use 3 min at each stage was previously described [18], who reported that oxygen consumption stabilized after approximately 3 min at each stage of exercise after a change in workload. At the end of the treadmill line, a stainless steel grid emitted electrical stimuli (0.2 Ma in < 1 s) to keep the animal in motion, as previously reported [19]. The animal's inability to maintain the pace was considered to be a sign of exhaustion [20]. A physical fitness test was performed before (initial: 30 days old), in the final of treatment and aerobic exercise (middle: 60 days old), and in the final of the experimental period (final: 120 days old). Incremental tests were performed every 15 days to adjust the training load. Exercise training was performed with running on a treadmill (Panlab, Harvard Apparatus®, LE8710R 76-0308, rat 5-lanes). Previous adaptation was performed in 5 sessions with durations of 10, 12, 14, 16 and 18 minutes and an intensity of 16 cm/s. Two days of rest were established to apply the ET. The prescription was based on effort test (ET) and was performed by the individual value of the final workload (FWL) corresponding to 55% and 65% of VO_2max to optimize the fat metabolism zone [21]. The treadmill training protocol was performed for 44 minutes a day (9 am to 10 am), 3 daysa

week in a 4-week macrocycle. The sessions were distributed with 2 minutes of warming up and cooling down at 20 cm/s and 40 minutes of continuous running at a moderate intensity (~ 55% to 65% FWL of the ET). Rats that reached the same speed or FWL training at the same time. The training protocols were completed at 60 days.

Caloric intake and body weight gain

Food intake, drink intake and caloric intake was measured each two days from 30 to 120 days of life. The food intake was calculated as the difference between the amount of food remaining and the total provided, which was divided by the number of days and the number of rats in the box [22]. The caloric intake was calculated based on the amount of food and fluid intake and the corresponding constants [23].The animals were weighed once a week during the experimental period.

Biochemical Analysis

At the end of the treatment phase (at 60 days of age) and experimental phase (at 120 days of age) animals from all groups were weighed and decapitated, and blood samples were collected and centrifuged (10,000 rpm for 5 min) to obtain plasma for further biochemical analysis. The plasma was used to measure glucose by the enzymatic method using a commercial colorimetric kit (Gold Analisa R, Belo Horizonte, Brazil) and quantified by spectrophotometry (BIO200FL, Bio Plus R, São Paulo, Brazil).

Intraperitoneal Glucose Tolerance Test (ipGTT)

The Intraperitoneal Glucose Tolerance Test (ipGTT) was performed at the end of the treatment phase (at 60 days of age) and experimental phase (at 120 days of age) [24]. Food was withdrawn 8 – 12 h before the test, and free access to water was allowed. The rats received an intraperitoneal injection of glucose (2g/kg of BW). Blood samples were obtained through tail venesection,) at 0 (prior to glucose injection), 15, 30, 60 and 120 minutes after injection and centrifuged (13.000 rpm for 5 min). The glucose was measured by the enzymatic method using a commercial colorimetric kit (Gold Analisa R, Belo Horizonte, Brazil) and quantified by spectrophotometry (BIO200FL, Bio Plus® , São Paulo, Brazil).

Fat Pad Stores Measurements and tissue extractions

At 60 days and 120 days of age, all the groups were euthanized and their fat pad stores (retroperitoneal, periepididymal and mesenteric) were removed and weighted to assess the state of obesity. The liver were dissected and weighed. Each of the fat pad stores values and liver values were correlated with the bw of each rat and were calculated as g/100kg of bw [4].

Statistical Analysis

Data were expressed as the mean ± SEM. GraphPad Prism version 6.01 for Windows (GraphPadSoftware, La Jolla, CA, USA) was used for statistical analyses and developing graphs. Two-way analysis of variance (ANOVA) followed by the Tukey multiple comparisons test was used for 60 and 120-day-old animals. A p value <0.05 was considered significant when considering the main effect of diet (D), exercise (E), their interaction (I; fructose vs exercise) and the differences between groups.

RESULTS

Food intake and Body Weight

During the treatment period, F-SED group presented significantly lower food intake (33,99 ± 2,5), compared to C-SED group (38,54 ± 2,6). Physical exercise further reduced F-EXE food intake (29,25 ± 1,1) compared to sedentary group (F-SED). After the treatment period, F-SED group significantly increased its consumption (54,91 ± 0,7) compared to C-SED (49,52 ± 0,8). The physical training was able to significantly reduce the food intake of the F-EXE group (48,73 ± 0,8) compared to sedentary F-SED (Figure 1).

Regarding body weight, from 30 to 60 days there was no difference in body weight of the experimental groups. From 60 days, the F-SED group presented a catch-up (400,1 ± 20,8), compared to sedentary control (C-SED = 309,5 ± 12,7). Physical exercise significantly reduced body weight of F-EXE group (275± 6,0) compared to F-SED (Figure 2).

Fat Pad Stores and Liver Weight

There was no increase in the fat stores of the main fats evaluated at 60 days of life in F-SED group compared to C-SED, and physical exercise did not change these parameters (Figure 3A). However, at 120 days of life, the F-SED group presented a significant increase in periepididimal,

retroperitoneal and mesenteric fats stores compared to C-SED group. The exercise protocol significantly reduced fat stores in both three tissues evaluated (Figure 3B).

At 60 days of age, the liver of the F-SED group presented a significant increase compared to the C-SED group and the exercise was not able to reduce this parameter. At 120 days of life, there was no significant difference between the sedentary groups (C-SED = 3,02 ± 0,2; F-SED = 3,688 ± 0,05), but exercise significantly reduced liver weight in the F-EXE group (3,132 ± 0,07), compared to F-SED (Figure4).

Glycaemia

Regarding the glycemic profile, there were no significant differences between groups in fasting glycemia and exercise did not change this parameter (data not shown). During the ipGTT, there was no significant difference between the sedentary groups, but the physical exercise significantly reduced glycemia of F-EXE group (84 ± 6,4), compared to F-SED group (149 ± 24,9).

DISCUSSION

This work for the first time showed that high rich fructose supplementation during adolescent programs young rats to metabolic syndrome in adult life, revealing well characterized: body weight gain, huge tissue fat accumulation, dyslipidemia, gross glucose intolerance and hyperglycemia. Surprising, even considering detraining time, youngest rats submitted to moderate exercise presented diminished clinical signals of MetS in adulthood. A high dose of fructose was used to mimic the modern Western diet of adolescents. Bad diets consist not only of foods high in cholesterol and fat but also beverages that are sweetened with HFCS that may lead to the development of metabolic syndrome later in life [16]. There is no data in the literature that shows the metabolic effects in adulthood of high fructose supplementation during adolescence, nor the possible protective effects of physical exercise against deleterious effects in metabolism; although, much is known about the health beneficial effects of physical exercise.

The fructose dose used (20%) is equivalent to a consumption of 10g of fructose per rats/ day. As a teenage rat weighs an average of 90 g and a human adolescent weighs on average 70 kg, it is then verified that this is a very high dose offered to rats, but the literature does not show doses lower than 10% that show some metabolic effect inrats.

Our group has already demonstrated that the lowest dose found in the literature (10%) already changes adolescent rats metabolism and program for metabolic syndrome emergence in adulthood, but without change body weight gain (unpublished data). In this work, we found that a 20% supplementation of fructose ingested during adolescence not only alters fat metabolism, but

also leads to body weight gain after the treatment period (Figure 2). This can be explained because the caloric intake of the fructose-supplemented rats is higher (data not shown) and because the fructose is highly lipogenic. After the treatment period, there is an increase in food intake of the F-SED group (Figure 1).

During the treatment period, from 30 to 60 days, the food intake of fructose-treated groups (F-SED and F-EXE) were lower, compared to controls (C-SED and C-EXE), might be due to high amount of ingested calories from drinking water. After the withdrawal of fructose supplementation, there was a reversal, and F-SED group increased food intake by the end of the 120 days. This inversion occurred because the rats were adapted to high calories ingested and the high palatability, and after the withdrawal of this source of calories, they compensated by ingesting more chow (F-SED group) but in the F-EXE group exercise prevented this increase. In the study by Gordish et al., 2017 [25], found that the addition of 20% fructose reduced 18% of food intake in treated group, compared to controls in adult rats.

Recent studies have shown that a high fructose supplementation stimulates the adipocytes proliferation, considered the main fat stores, contributing to obesity onset [7; 11]. Our result show that the absence of an increase in the main fat stores at 60 days of life is similar to our data obtained with 10% fructose supplementation (unpublished data). At 120 days, high fructose supplementation was shown to promote increase periepididimal, retroperitoneal and mesenteric fat stores, quite different results obtained with 10% fructose drinking water, that was not observed huge fat tissue stores (results not published). The ability of fructose to promote lipogenesis and to be captured by adipocytes and induce hypertrophy of these cells is already well reported in the literature [7; 6; 15]. Vasiljevic et al., 2014 [26] suggests that the increase lipids in liver, caused by the high fructose intake, occurs by increased production and, consequently, increased glucocorticoid levels. Although MetS isn't characterized by increases in circulating glucocorticoid levels, there are specific tissue changes in glucocorticoid pre-receptor metabolism. According to Legeza et al., (2017) [8] there is an interrelation between fructose concentration and glucocorticoids production in adipose tissue. Glucocorticoid production plays a key role in adipocytes differentiation and proliferation. Another theory proposes that fructose contributes to development of obesity by stimulating the insulin-independent steroid receptor 1-c binding protein receptor (SREBP-1c), which activates genes involved in new lipogenesis, generating extra fatty acids for production of triglycerides in liver. However, this increase in lipids on liver is associated with an increase in synthesis and secretion of very low density lipoprotein (VLDL). This systemic elevation of fatty acids and VLDL leads to an increase in lipid uptake by the peripheral organs, such as adipose tissue and skeletal muscle, contributing to a systemic insulinresistance. In

addition, fructose is also linked to leptin resistance, worsening obesity and insulin resistance [27]. Studies have shown that an excessive fructose consumption exceeds the liver capacity to metabolize it and a small fraction remains in circulation, being captured by peripheral tissues. Although adipocytes have GLUT-5 receptor and are perfectly capable to capturing fructose, the mechanism of GLUT-5 hasn't been fully elucidated, although GLUT-5 is indicated in some studies as a regulator of adipocyte differentiation [28;8].

Studies have shown that physical exercises decrease abdominal circumference and visceral fat, blood pressure, HDL cholesterol, plasma triglyceride levels; and especially increased GLUT-4 protein in the skeletal muscle membrane, improving the transport of blood glucose to the muscles [29; 30]. Our data showed that rats supplemented with fructose subjected to a moderate intensity exercise protocol had a reduction in food intake during the treatment period and kept the levels low post-treatment up to 120 days. Remarkable is that exercised youngest rats reduced fructose and chow intake. These data suggest ability of physical exercise to increase satiety, decreasing the leptin resistance [31] and decrease the taste for unhealthy foods. Our data also showed a reduction in body weight gain after the treatment period in F-EXE, compared to F-SED group. The current study and other studies suggest the long-lasting protective effect of exercise performed early in life and how it can efficiently protect the body from the development of obesity and metabolic syndrome [10].

According to Carrol et al.(2004) [30], the metabolic benefits of exercise are more accentuated when performed at least three times a week and lasting up to 60 minutes, at an intensity of approximately 40 to 60% of VO_2max. He also suggests that the metabolic benefits of moderate exercise are also linked to a concomitant body fat loss. Our data showed a reduction in all three body fat stocks analyzed in the group supplemented with fructose that was submitted to the physical training protocol. Recent data published by our group showed that this protocol of physical exercise was able to reduce 80% the fat stores of rats treated with high-fat diet [10]. Mostarda *et al* [32] showed that three weeks of detraining was not able to change the hemodynamic benefits (BP, heart rate, sympathetic and vagal tonus). In this current work, detraining is too long, eight week. The benefits of exercise still work and fat stores were maintained reduced in fructose treated rats. Beside the strick results regarden detraining, we do not have any data to find the responsible mechanisms. Maybe the early moderate exercise reduced expression and increase gene expression throughout epigeneticpathways.

Insulin resistance is a condition that impairs the uptake of glucose by tissues, causing hyperglycemia and diabetes mellitus. Our data also showed a reduction in glycemia of rats supplemented with fructose and submitted to the physical exercise protocol (F-EXE), showing that

physical exercise improves insulin sensitivity by increasing the concentration of glucosetransporter in the skeletal cell membrane[29].

Combining all results obtained in the current work it is possible to suggest that high enriched fructose supplementation during adolescence programs to metabolic syndrome in adult life; however, moderate physical exercise concomitant to fructose treatment is able to attenuate metabolic disruption onset. Although, using a high rich fructose supplementation, our data tried to emulate over fructose consumption human teenagers, with other environmental aggression may contributes to metabolic syndrome pandemic; however, reducing fructose into industrialized food and beverage could be a necessary maneuver to improve health adolescence and moderate exercise can be a strong tool to combat this malaise to youngest and theirfuture.

DECLARATION OF INTEREST

There are no conflicts of interest.

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FIGURE LEGENDS

Figure 1. Food intake from 30 to 120 days of life (n = 20). C-SED sedentary rats subjected a normal diet, F-SED sedentary rats subjected a fructose diet, C-EXE exercised animals subjected to a normal diet and F-EXE exercised animals subjected to a fructose diet. F fructose factor, E exercise factor and I interaction between fructose and exercise factors. +++ $p < 0.001$, ++ $p < 0.01$ and + $p < 0.05$ for the probability based on a two-way analysis ofvariance.

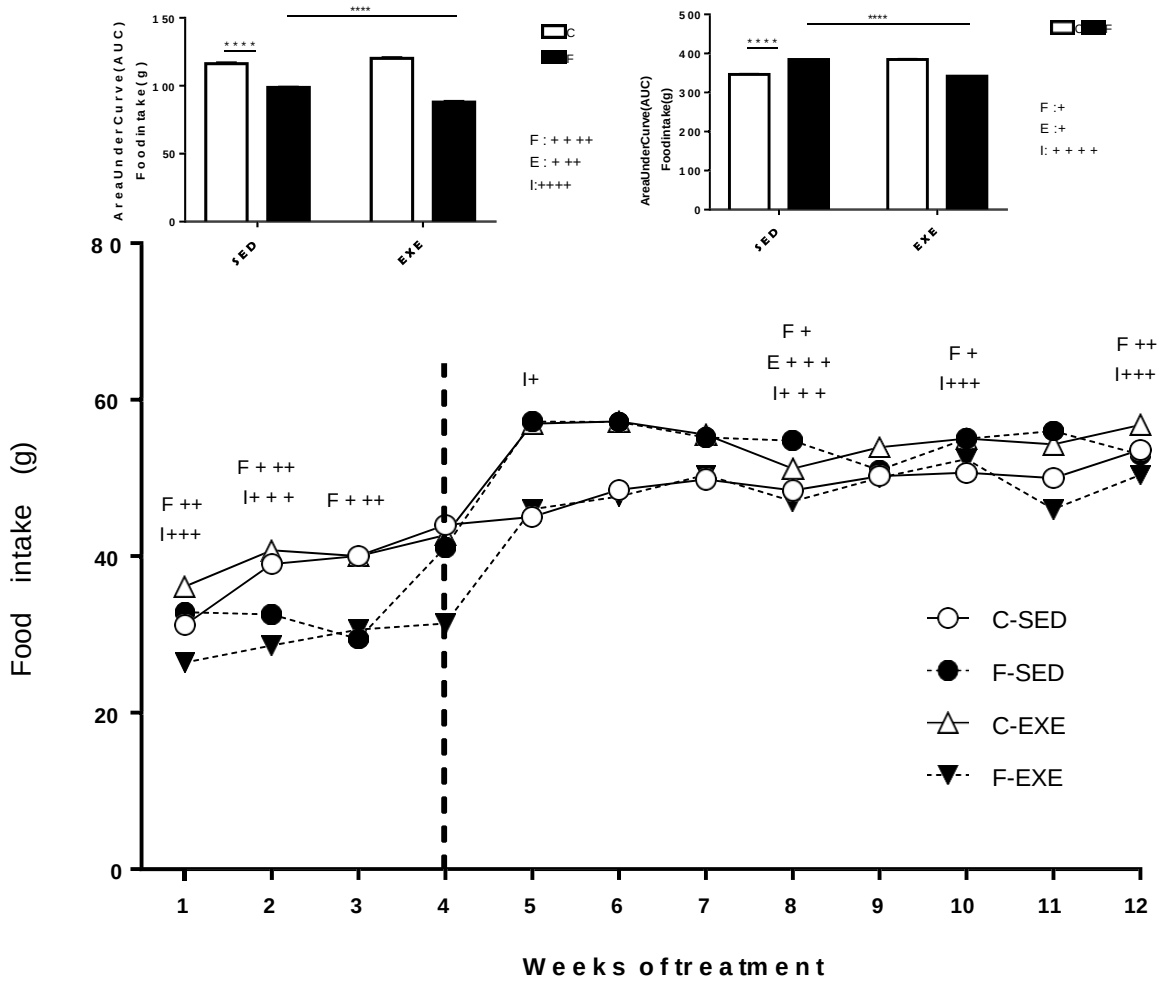
Figure 2. Body weight from 30 to 120 days of life (n = 20). C-SED sedentary rats subjected a normal diet, F-SED sedentary rats subjected a fructose diet, C-EXE exercised animals subjected to a normal diet and F-EXE exercised animals subjected to a fructose diet. F fructose factor, E exercise factor and I interaction between fructose and exercise factors. +++ $p < 0.001$, ++ $p < 0.01$ and + $p < 0.05$ for the probability based on a two-way analysis ofvariance.

Figure 3. (A) Fat pad stores at 60 days of life and (B) 120 days of life (n = 20). C-SED sedentary rats subjected a normal diet, F-SED sedentary rats subjected a fructose diet, C-EXE exercised animals subjected to a normal diet and F-EXE exercised animals subjected to a fructose diet.Ffructosefactor,EexercisefactorandIinteractionbetweenfructoseandexercisefactors.
+++ $p < 0.001$, ++ $p < 0.01$ and + $p < 0.05$ for the probability based on a two-way analysis of variance.

Figure 4. Liver weight at (A) 60 days of life and (B) 120 days of life (n = 20). C-SED sedentary rats subjected a normal diet, F-SED sedentary rats subjected a fructose diet, C-EXE exercised animals subjected to a normal diet and F-EXE exercised animals subjected to a fructose diet. F fructose factor, E exercise factor and I interaction between fructose and exercise factors. +++ $p < 0.001$, ++ $p < 0.01$ and + $p < 0.05$ for the probability based on a two-way analysis of variance.

Figure 5. Glicemia at 120 days of life (n = 20). C-SED sedentary rats subjected a normal diet, F-SED sedentary rats subjected a fructose diet, C-EXE exercised animals subjected to a normal diet and F-EXE exercised animals subjected to a fructose diet. F fructose factor, E exercise factor and I interaction between fructose and exercise factors. +++ $p < 0.001$, ++ $p < 0.01$ and + $p <0.05$ for the probability based on a two-way analysis ofvariance.

Figure 1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Weeks of treatment

Figure 2

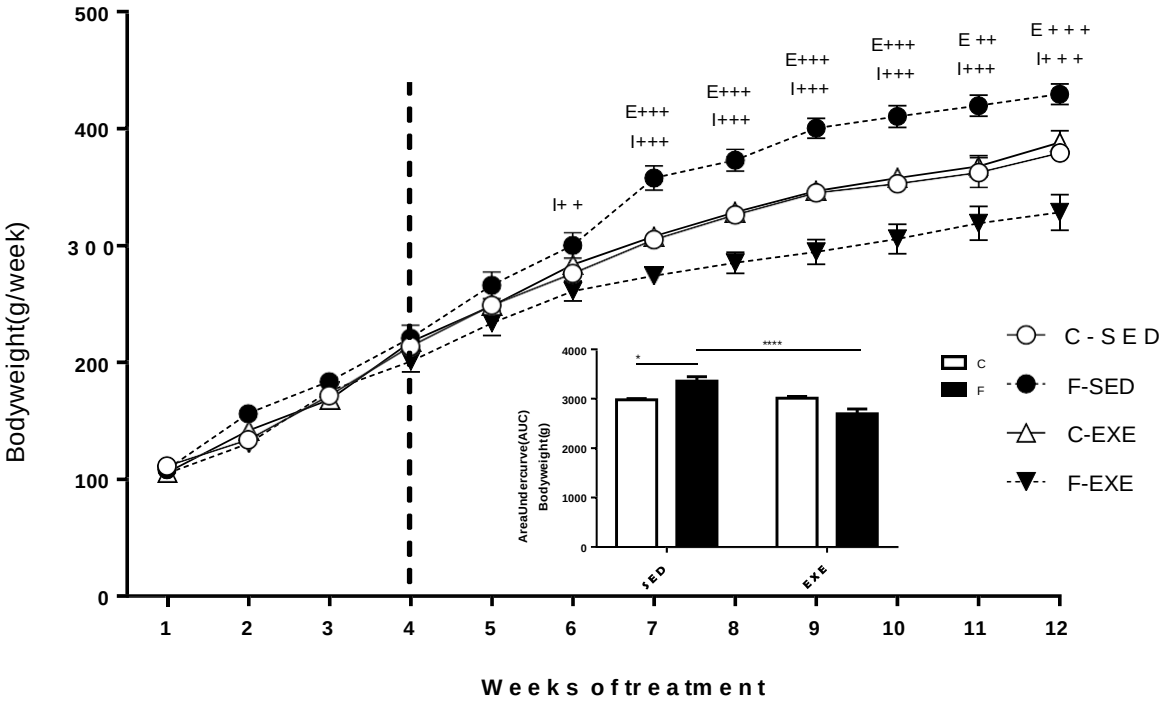
Bodyweight(g/week)
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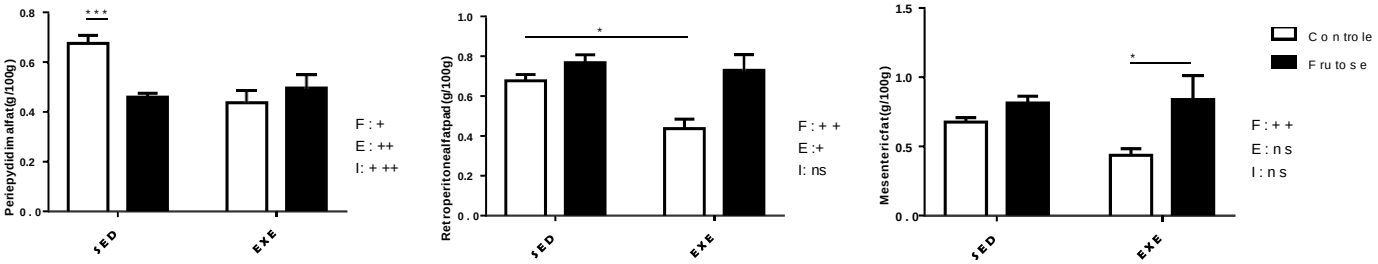
Figure 3

A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Frutose

B

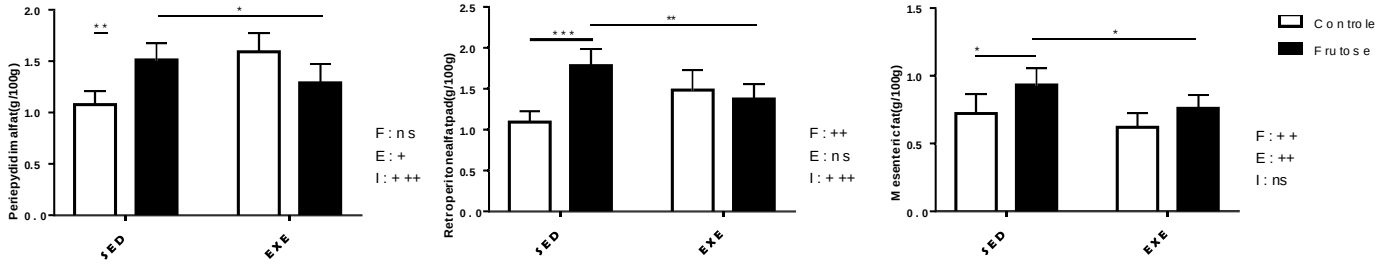

Periepydidimalfat(g/100g)
2.0
1.5
1.0
0.5
0.0
**
*
SED
EXE
F : n s
E : +
I : + ++
Retroperitonealfatpad(g/100g)
2.5
2.0
1.5
1.0
0.5
0.0
***
**
SED
EXE
F : ++
E : n s
I : + ++
Mesentericfat(g/100g)
1.5
1.0
0.5
0.0
*
*
SED
EXE
F : + +
E : ++
I : ns
Controle
Frutose

Figure 4

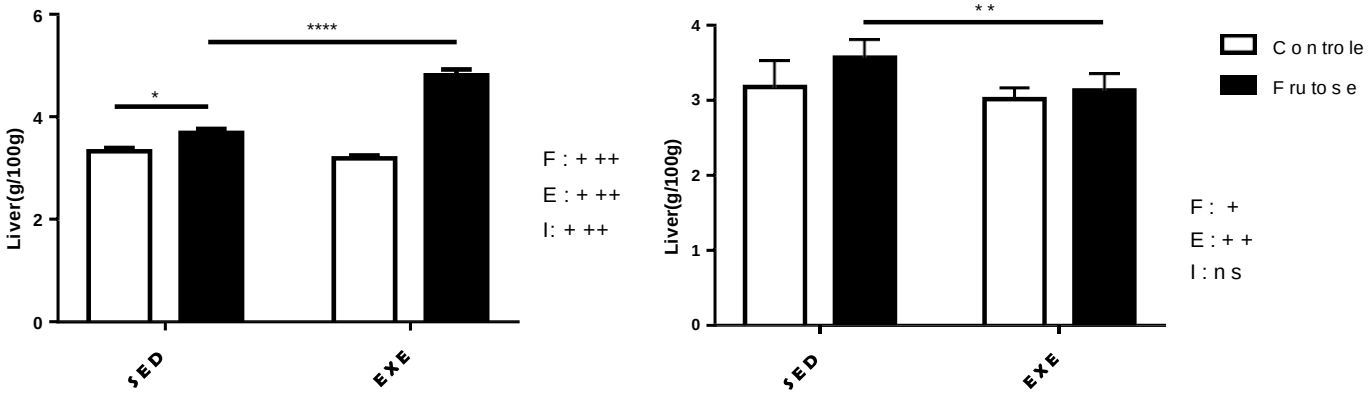
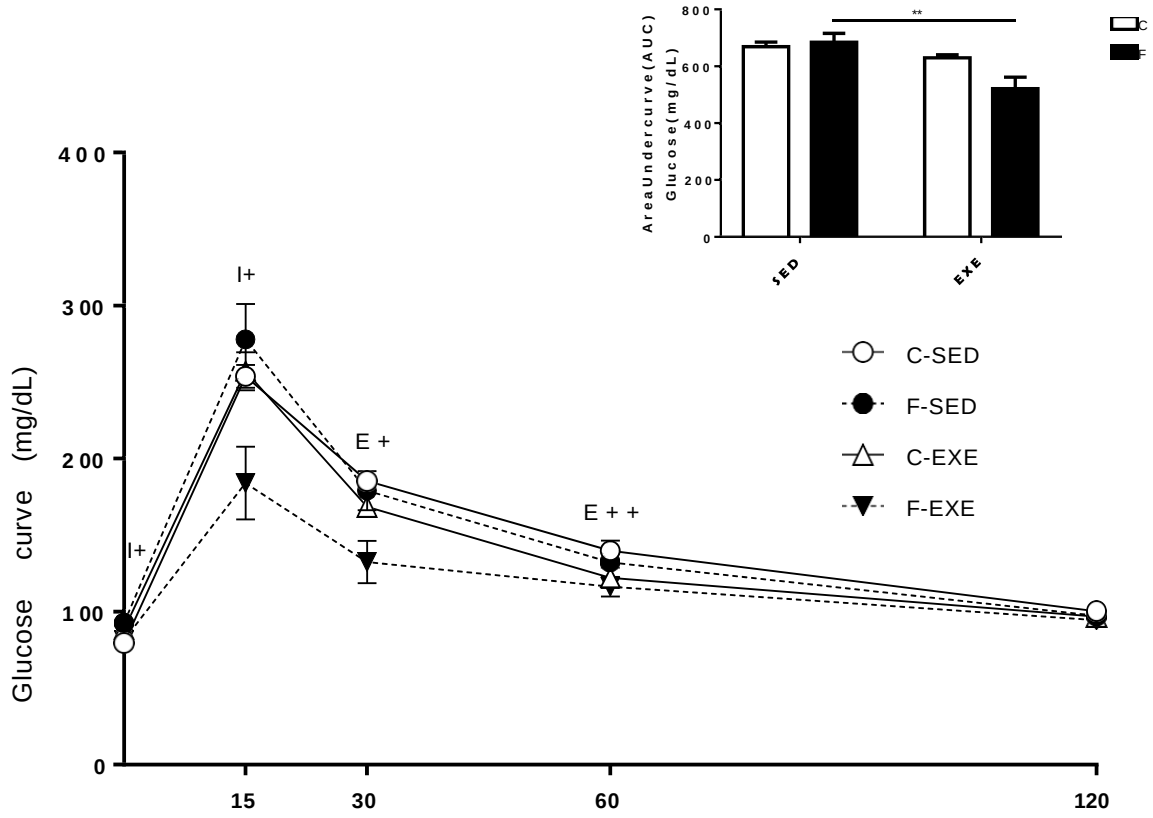

Liver(g/100g)
6
4
2
0
*
****
SED
EXE
F : + ++
E : + ++
I: + ++
Liver(g/100g)
4
3
2
1
0
* *
SED
EXE
Controle
Frutose
F : +
E : + +
I : n s

Figura 5


Glucose curve (mg/dL)
400
300
200
100
0
15
30
60
120
I+
I+
E +
E + +
C-SED
F-SED
C-EXE
F-EXE
Area Under curve (AUC) Glucose (mg/dL)
800
600
400
200
0
**
SED
EXE