Revista Contexto & Saúde
Editora Unijuí
Programa de Pós-Graduação em Atenção Integral à Saúde
ISSN 2176-7114 – v. 21, n. 43, jul./set. 2021

http://dx.doi.org/10.21527/2176-7114.2021.43.11623

COMO CITAR:
FERNANDES, Carlos Alexandre Molena *et al*. Effects of the ingestion of Flaxseed (Linum Usitatissimum l.) oil and physical exercise in animal model for metabolic syndrome. *Revista Contexto & Saúde*, v. 21, n. 43, p. 165-179, 2021.

Effects of the Ingestion of Flaxseed (*Linum Usitatissimum l.*) Oil and Physical Exercise in Animal Model for Metabolic Syndrome

Carlos Alexandre Molena Fernandes,[1] Karine Oltramari,[2] Nelson Nardo Junior,[3] Greice Westphal[3]

ABSTRACT

Introduction: Metabolic syndrome (MetSyn) is a disorder that involves a series of symptoms associated to obesity and metabolic modifications, and increases the risk of developing cardiovascular disease and type 2 Diabetes Mellitus. The study of new possibilities of non-pharmacological strategies is fundamental for the primary prevention of MetSyn. **Objective**: The aim of this work was to evaluate the responses to the ingestion of flaxseed oil associated or not associated with physical exercise in hypertensive rats (SHR) with lipid changes induced by the administration of dexamethasone (Dexa) regarding the body weight, and glycaemic and lipid profiles, in comparison with the drug treatment with metformin. **Methodology**: After 24 days of experiment (details at the material and methods session), the 20-week-old animals were submitted to the glucose tolerance test (GTT) and lipid profile evaluation. **Results**: The results showed that the administration of Dexa in SHR animals promoted a framework of hyperglycaemia, dyslipidaemia and attenuated the insulin resistance common in the SHR, thus producing an experimental model with several components of the human MetSyn. The effects on weight gain indicated an important preventive action of flaxseed oil and exercise on the weight evolution of SHR-Dexa animals. Furthermore, the ingestion of flaxseed oil associated or not with exercise promoted significant improvements on hyperglycaemia and glucose intolerance in these animals. Regarding the lipid profile, the treatments with flaxseed oil and exercise promoted reductions in triglyceride levels and HDL-c increase. **Conclusion**: However, the combination of these two non-pharmacological interventions was more effective than the isolated treatments.

Keywords: metabolic syndrome; natural product; physical exercise; glucose intolerance; hypertension.

EFEITOS DA INGESTÃO DE ÓLEO DE LINHAÇA (LINUM USITATISSIMUM L.) E EXERCÍCIO FÍSICO EM MODELO ANIMAL PARA SÍNDROME METABÓLICA

RESUMO

Introdução: A síndrome metabólica (SMet) é uma condição que envolve uma série de sintomas associados à obesidade e às modificações metabólicas, e aumenta o risco de desenvolver doenças cardiovasculares e Diabetes Mellitus tipo 2. O estudo de novas possibilidades de terapias não medicamentosas é fundamental para a prevenção primária da SM. **Objetivo**: O objetivo deste trabalho foi avaliar as respostas à ingestão de óleo de linhaça associada ou não ao exercício físico em ratos espontaneamente hipertensos (SHR) com alterações lipídicas induzidas pela administração de dexametasona (Dexa) em relação ao peso corporal, e perfis glicêmico e lipídicos, em comparação com o tratamento medicamentoso com metformina. **Metodologia**: Após 24 dias de experimento (detalhes na sessão material e métodos), os animais com 20 semanas de idade foram submetidos ao teste de tolerância à glicose (GTT) e avaliação do perfil lipídico. **Resultados:** Os resultados mostraram que a administração de Dexa em animais SHR promoveu um quadro de hiperglicemia, dislipidemia e atenuou a resistência à insulina comum em SHR, produzindo um modelo experimental com diversos componentes da SM humana. Os efeitos no ganho de peso indicaram uma importante ação preventiva do óleo de linhaça e do exercício na evolução ponderal de animais SHR-Dexa. Além disso, a ingestão de óleo de linhaça, associada ou não ao exercício físico, promoveu melhorias significativas na hiperglicemia e intolerância à glicose nesses animais. Em relação ao perfil lipídico, os tratamentos com óleo de linhaça e exercícios promoveram redução nos níveis de triglicerídeos e aumento de HDL-c. **Conclusão**: A combinação dessas duas intervenções não medicamentosas, no entanto, foi mais eficaz do que os tratamentos isolados.

Palavras-chave: síndrome metabólica; produtos naturais; exercício físico; intolerância à glicose; hipertensão.

RECEBIDO EM: 19/10/2020
MODIFICAÇÕES SOLICITADAS EM: 27/1/2021
ACEITO EM: 15/3/2021

[1] Universidade Estadual do Paraná – Unespar. Paranavaí/PR, Brasil.

[2] Autora correspondente. Centro de Ciências da Saúde – Universidade Estadual de Maringá. Av. Colombo, 5790 – Zona 7. Maringá/PR, Brasil. CEP 87020-900 http://lattes.cnpq.br/5254277248724973. http://orcid.org/0000-0002-1383-1765. karineoltramari@gmail.com

[3] Universidade Estadual de Maringá – UEM. Maringá/PR, Brasil.

INTRODUCTION

One of the most important causes of worldwide morbidity and mortality is systemic arterial hypertension (SAH), which is responsible for the reduction of life expectancy and quality of life (GADAU *et al.*, 2018). Its association with diabetes mellitus (DM) is frequent, consistently increasing the risk of cardiovascular diseases (CVDs) at any stage of hypertension, which may lead to other comorbidities like dyslipidemia, thus configuring a condition named metabolic syndrome (MetSyn) (ROLIM *et al.*, 2018).

MetSyn can be defined as a disorder that involves a series of symptoms that are associated to obesity and metabolic modifications, and increases the risk of developing CVD and type 2 DM. The clinical manifestations of MetSyn include hyperglycemia, insulin resistance, hyperlipidemia, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis; being the persistent low-grade inflammation a crucial characteristic of the syndrome. MetSyn has become frequent due to the current lifestyle, marked by psychosocial stress, changes in eating habits, and a decrease in the practice of physical activity (SENAPHAN *et al.*, 2015). The study of new possibilities of non-drug therapies is fundamental for the development of effective and sustainable programs to public health services for the primary prevention of MetSyn (STRELKOVA; OVSYANNICOV; UTKINA, 2016).

In this sense, functional foods have been intensively investigated and exploited, since they constitute a food category that promotes biochemical and physiological benefits due to its bioactive compounds, in addition to the basic nutritional function (MILLAR *et al.*, 2018). Studies with flaxseed (*Linum usitatissimum* L.) and their derivatives have shown preventive and/or therapeutic benefits of their consumption, both in animals (KAUR; KISHORE; SINGH, 2017) and in humans (PARIKH; NETTICADAN; PIERCE, 2018). The practice of physical exercises has also been pointed out as essential in the prevention of CVD, being related to the decrease of adiposity and to the improvement of the lipid profile. Thus, physical exercises are recommended in light to moderate intensity to benefit individuals (OZEMEK *et al.*, 2018).

Animal models capable of mimicking human physiological responses may aid in the investigation of metabolic diseases. Nonetheless, MetSyn is difficult to study in experimental animals because of the lack of good animal models that simultaneously express all or almost all components of the syndrome (SENAPHAN *et al.*, 2015). Widely used in experimental studies, SHR (spontaneously hypertensive rat) is a model of genetic arterial hypertension, which resembles essential human hypertension, including the presence of glucose metabolism disorders commonly seen in essential hypertensives (EIBEL *et al.*, 2018). However, SHR rarely become obese, whereas the various animal models of diet-induced obesity or spontaneously obese unfrequently become hypertensive (VELLOSO, 2009).

Faced with the lack of a MetSyn experimental model that presents dyslipidemia, in this study we intended to set an animal model that combined hypertension, insulin resistance and lipid alterations through the administration of dexamethasone (Dexa) in adult SHR. The attempt to establish this model is based on the assumption that older SHR present important metabolic alterations, such

as changes in body weight and in the lipids profile, featuring a picture of dyslipidaemia. Therefore, the administration of Dexa associated with the advanced age of SHR allows in this experimental model two more alterations present in the MetSyn, in addition to the characteristic hypertension (GHEZZI *et al.*, 2012).

In view of the literature gap, the aim of this investigation was to develop a more complete experimental model for the study of MetSyn and to evaluate the effects of the dietary ingestion of flaxseed oil, associated or not to physical exercise practice, on body weight, glycaemic and lipid profiles, in comparison to the drug treatment with metformin.

MATERIAL AND METHODS

Adult male Wistar and SHR rats, at 20 weeks of age, were obtained from the bioterium of the Laboratory of Inflammation of the State University of Maringá (UEM). The animals received ration and water *ad libitum*, and were kept under a constant cycle of 12 hours of light and 12 hours of darkness, at a constant temperature of 22 °C. Throughout the experimental period, the animals were weighed at the 20th, 23th and 24th weeks. All of the procedures were performed in accordance with the Brazilian College of Animal Experimentation (Cobea), which comply with international laws, and were approved by the Ethics Committee on Animal Experimentation of the State University of Maringa (CEEA 033/2007).

Experimental groups:

The animals were divided into seven groups:

1. *Normotensive control group (NTR-C):* composed of 12 Wistar animals that received water by gavage and were not submitted to physical training.
2. *SHR control group (SHR-C):* composed of 12 SHR that received water by gavage and were not submitted to physical training.
3. *SHR dexamethasone sedentary group (SHR-Dexa-SED):* composed of 14 SHR with dexamethasone-induced glucose intolerance, which were not submitted to physical training.
4. *SHR dexamethasone trained group (SHR-Dexa-TR):* composed of 14 SHR with dexamethasone-induced glucose intolerance, which underwent physical training.
5. *SHR dexamethasone flaxseed oil group (SHR-Dexa-OL):* composed of seven SHR with dexamethasone-induced glucose intolerance, supplemented by gavage with flaxseed oil and that were not submitted to physical training.
6. *SHR dexamethasone flaxseed oil trained group (SHR-Dexa-OL-TR):* composed of six SHR with dexamethasone-induced glucose intolerance, supplemented by gavage with flaxseed oil and submitted to physical training.
7. *SHR dexamethasone metformin group (SHR-Dexa-MET):* composed of 10 SHR with dexamethasone-induced glucose intolerance, which were treated with the drug metformin.

The differences in the number of animals by groups are mainly due to losses related to the manipulation of some groups more than in the others. There are also other non-foreseeing factors which occurred more frequently in the more manipulated groups.

Induction of glucose intolerance

SHRs after 14 days of flaxseed oil and/or swimming treatment, received dexamethasone (Dexa) at a single subcutaneous daily dose of 0.1 mg/kg body weight, during four days. Control animals received saline solution (0.9%) vehicle used to dilute Dexa. On the 5th day, after the treatment with Dexa (24 hours after the last administration), the animals were submitted to the glucose tolerance test (GTT). Metformin (MET) was used as the reference drug.

Treatments

Flaxseed oil supplementation

Flaxseed oil was administered for 18 days (14 days without Dexa + 4 days with Dexa) at a dose of 100 mg/kg, orally (gavage). Animals from the group that did not receive treatment with flaxseed oil were given water by gavage. Treatment time and dose were established according to a preliminary study of our group (ELIAS *et al.*, 2012). Farinhas Integrais Cisbra Ltda., located at Panambi-RS, Brazil, supplied the commercial Lino Live brown flaxseed oil.

Physical exercise (swimming protocol)

The physical exercise protocol consisted of daily swimming sessions of 60 minutes for 18 days, with an overload attached to the tail of the animal equivalent to 5% of its body mass, characterized by a predominance of the aerobic component (GOBATTO *et al.*, 2001). This exercise protocol has been classified as a physical activity of low to moderate intensity and long duration, sufficient to stimulate organic adaptations (ANDRADE *et al.*, 2016). The swimming sessions were performed in a rectangular tank, with a capacity of 250 liters capacity. The water temperature was kept at 29 ± 2 °C, in order to insure a neutral condition relative to the animals' body temperature. To avoid contamination by faeces and urine excreted during exercise, the water in the tank was changed at the end of each session.

The animals underwent a five-day adaptation period: on the first day, 15 minutes of swimming without load; on the second day, 30 minutes of unloaded swimming; on the third day, 60 minutes without load; in the fourth day, 30 minutes with load equivalent to 5% of body weight coupled to tail; and finally, on the fifth day, 45 minutes and load of 5%. From the sixth day, the animals started the training in parallel to the administration of flaxseed oil. The animals swam for a period of 23 days (five days of adaptation + 14 days of treatment without Dexa + 4 days with DEX). On the 24th day of experiment, the biochemical tests (GTT and lipid profile) were performed.

Drug treatment

SHR-DEX-MET animals were treated with orally administered metformin (300 mg/kg) in a single daily dose, during four days. Treatment started concomitantly with Dexa administration, and the last day of treatment predated the day of the experiment. Metformin 850 mg (Laboratorios Biosintética Ltda.) was employed as the standard drug.

Intravenous glucose tolerance test (GTT)

Twenty-four hours after the last dose of Dexa, 12-hour fasting animals were submitted to the glucose tolerance test (GTT). The animals were anesthetized with sodium pentobarbital (Hypnol® 3%, 40 mg/kg) intraperitoneally. After laparotomy and exposure of the inferior vena cava, a blood sample of 0.5 mL corresponding to the basal glycemia was collected. Glucose (0.5 g/kg) was then administered via this same route and 0.5 ml blood samples were collected at times 5, 10, 20, 30 and 60 minutes after glucose injection. These blood samples were centrifuged (5 min/3000 rpm) and glycemia determined by the glucose oxidase method (Gold Analisa®) using 20 µl serum aliquots.

Lipid profile

On the 24th day of experiment, blood samples were collected for biochemical analysis. Plasma aliquots were submitted to lipid profile evaluation. Total cholesterol (TC) was determined by the enzymatic cholesterol oxidase method (Gold Analisa®). For HDL-cholesterol (HDL-c) dosage the serum HDL cholesterol precipitation system (Gold Analisa®), which uses phosphotungstic acid and magnesium chloride, was employed for the selective and quantitative precipitation of very low density (VLDL) and low density (LDL) lipoproteins. After centrifugation, HDL-c was determined in the supernatant using the same CT dosing method. Triglyceride (TG) concentrations were determined by the enzymatic method of glycerol-3-phosphate oxidase (Gold Analisa®). All results were expressed as mg/dL.

Statistical analysis

Results were expressed as mean ± standard error of the mean (sem) and were analysed using Student's *t*-test, when two paired means were compared and analysis of variance (Anova) for multiple comparisons, followed by the Tukey's test. P <0.05 was used as the level of significance. The analysis of the area under the curve was obtained from the curves of the glucose tolerance test, determined by the Graphpad Prism program, version 5.0 (Graphpad Software Inc., Microsoft Corp.).

RESULTS

Effects of the treatments with flaxseed oil on the body weight of SHR-Dexa animals.

Table 1 – Body weight monitoring results during the 24 days of experiment (mean ± SEM)

Groups	Body weight (g)			
	Initial (week 20)	Week 23	Final (week 24)	Δ% BW (g)
NTR	423.2 ± 3.7	468.5 ± 2.5	477.8 ± 3.5*	+12.9
SHR-C	323.2 ± 8.1‡	345.0 ± 9.8‡	350.3 ± 9.1*‡	+8.4

SHR-Dexa-SED	329.3 ± 7.1‡	339.2 ± 6.9‡	305.4 ± 5.9*‡	-7.2
SHR-Dexa-TR	319.9 ± 4.7‡	328.0 ± 4.2‡	325.4 ± 4.1‡	+1.7
SHR-Dexa-OL	322.1 ± 3.9‡	333.7 ± 7.7‡	321.1 ± 6.7‡	-0.3
SHR-Dexa-OL-TR	325.5 ± 2.4ª	335.9 ± 9.4ª	333.4 ± 8.8ª	+2.4
SHR-Dexa-MET	322.6 ± 4.3ª	330.6 ± 5.3ª	333.6 ± 5.5ª	+3.4

Δ% BW (g) – percentage variation of body weight in grams (g) between the initial and final moments.* Significant difference between the initial and final moments ($p < 0.05$; Student's *t*-Test). ‡ Significant difference compared to the NTR group ($p<0,01$; ANOVA).

In the weight evaluation of treated and control animals (**Table 1**), no significant difference was observed in the initial body weight between SHR animal groups. However, the SHR (control and treated) groups had significantly lower body weight throughout the experimental period compared to the NTR group. **Table 1** shows that NTR and SHR-c animals had a significant increase of 12.9% and 8.4% of body weight, respectively, throughout the experimental period. In the SHR-Dexa-SED group, a significant reduction in the mean body weight was observed at the end of the experiment (-7.2%), whereas in the other groups (treated), the weight variation verified was not statistically significant. Treatments provided protection against both weight loss caused by Dexa and natural weight gain due to advancing age (**Table 1**).

Effects of the treatments with flaxseed oil and metformin on the glycemic profile of shr-dex animals submitted or not to exercise

The blood glucose concentration results displayed in **Table 2** demonstrate that the mean values of glucose at time (T) zero (before intravenous glucose injection - 0.5mg/kg) were significantly lower in groups not given Dexa (NTR and SHR-C), thus characterizing an acute hyperglycemia picture in the other groups (injected with dexamethasone).

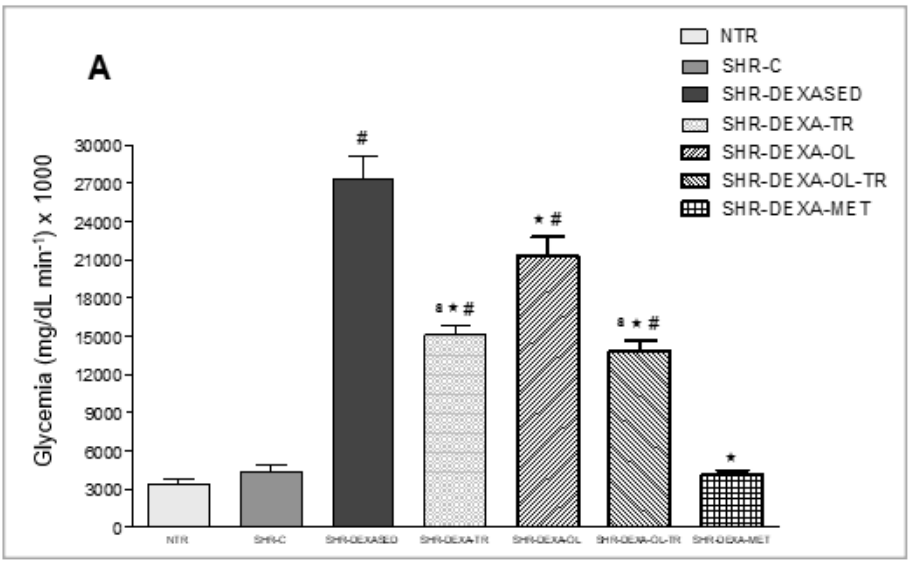
Treatment of SHR-Dexa with flaxseed oil and/or exercise or metformin did not reduce the baseline hyperglycemia promoted by Dexa. However, as shown in **Table 1**, despite the hyperglycemia induced by Dexa, the mean value of glycemia at the zero T of the animals that exercised and were treated with flax oil was significantly lower than the group that remained sedentary. The same tendency was observed at times 5, 10, 20, 30 and 60 minutes after the intravenous injection of glucose, indicating that the treatment through physical exercise associated or not with flaxseed oil promoted a significant decrease of glycemia during the GTT. However, the group treated with the reference drug (MET) presented a more pronounced decrease in glycemia at all times when compared to the other treated groups, with values close to the SHR-C group, what evidences the hypoglycemic effect of MET in animals with Dexa-induced hyperglycemia (**Table 2** and **Figure 2B**).

Table 2 – Blood glucose concentration during intravenous glucose tolerance test (GTT)

Groups	$T_{0\ min}$	$T_{5\ min}$	$T_{10\ min}$	$T_{20\ min}$	$T_{30\ min}$	$T_{60\ min}$
1. NTR (n=12)	138.5 ± 7.2	318.4 ± 31.9	294.9 ± 12.6	250.7 ± 10.1	212.1 ± 7.5	185.9 ± 5.6
2. SHR-C (n=12)	174.0 ± 10.2	321.4 ± 9.9	350.4 ± 25.7	356.4 ± 31.1	340.8 ± 23.5	201.5 ± 20.4
3. SHR-Dexa-SED (n=14)	910.4 ± 9.5[5]	1313.6 ± 11.3[5]	1333 ± 17[5]	1261.6 ± 9.6[5]	1309 ±16.2[5]	1121.3 ± 59.8[5]
4. SHR-Dexa-TR (n=12)	533.1 ± 30.8[1,3,5]	1114.0 ± 16[1,3,5]	1067.6 ± 22.7[1,3,5]	1023.2 ± 33.5[1,3,5]	849 ± 38.9[1,4,5]	477.3 ± 74.5[2,3,4,5]
5. SHR-Dexa-OL (n=7)	653.9 ± 40.6[1,3,5]	1136 ± 14.8[1,3,5]	1187.6 ± 30.7[1,3,5]	1153.3 ± 45.3[3,5]	1129.1 ± 56[1,3,5]	867.3 ± 78.5[1,3,5]
6. SHR-Dexa-OL-TR (n=6)	513.9 ± 22.8[2,3,5]	1094.4 ± 19[1,3,5]	1051.6 ± 16.6[1,3,5]	1003.3 ± 23.5[1,3,5]	809 ± 18.9[1,3,4,5]	487.3 ± 85.9[1,2,3,4,5]
7. SHR-Dexa-MET (n=10)	278.6 ± 12.5[2,5]	373.9 ± 40.8[2]	377.0 ± 28.3[2]	394.3 ± 25.2[2]	270.1 ± 6.2[2,5]	217.8 ± 16.8[2]

Significant difference in relation to the SHR-C group for p<0.01 (Anova).[1] Significant difference in relation to the SHR-Dexa-SED group for p<0,05 (Anova).[2] Significant difference in relation to the SHR-Dexa-SED group for p<0,01 (Anova).[3] Significant difference in relation to the SHR-Dexa-MET group for p<0,01 (Anova). [4] Significant difference in relation to the SHR-Dexa-OL group for p<0,05(Anova)[5]

Figure 1 – Intravenous glucose tolerance test (GTT) results of the SHR control animals (SHR-C), SHR with sedentary dexamethasone-induced glucose intolerance (SHR-Dexa-TR), dexamethasone-induced SHR with exercise intolerance (SHR-Dexa-TR), SHR with glucose-induced glucose intolerance dexamethasone supplemented with flaxseed oil (SHR-Dexa-OL), SHR with glucose intolerance induced by dexamethasone supplemented with flaxseed oil and submitted to exercise (SHR-Dexa-OL-TR) and SHR with glucose intolerance induced by dexamethasone and treated with metformin (SHR-Dexa-MET), before and after administration of glucose overload



A) Each bar represent the mean ± sem; #Significant difference between NTR and SHR-C groups (p<0,001); *Significant difference from the SHR-DEX-TR group (p<0,01); ªSignificant difference from the SHR-DEX-OL group (p<0,05) - (ANOVA, followed by the Tukey Test).

SHR-Dexa-TR and SHR-Dexa-OL-TR groups showed a significant decrease in glycemia values during GTT at 30 and 60min, in comparison with the SHR-Dexa-OL group. In addition, by analysing the area under the curve (Figure 1) obtained from the glucose tolerance test curves, the area of SHR-Dexa-OL group was larger than areas of SHR-Dexa-TR and SHR-Dexa-OL-TR groups. These results suggest that physical exercise could be more effective in preventing the development of Dexa-induced glucose intolerance in SHR animals compared to treatment with flaxseed oil. Also, there were lower glycemia values at all GTT times in the MET-treated group when compared to the groups that were treated with exercise and flaxseed oil, a result that suggests the greater efficacy of the drug treatment compared to non-pharmacological treatment in preventing the picture of glucose intolerance.

Effects of the treatments with flaxseed oil and metformin on the lipid profile of shr-dexa animals submitted or not to physical exercise

The administration of Dexa in SHR promoted a picture of hypertriglyceridemia (SHR-Dexa-SD = 75.5 ± 1.9 mg/dL) when compared to SHR control group (54.31 ± 0.93 mg/dL). In addition, both SHR-C and SHR-Dexa-SD groups had significantly lower HDL-c values than NTR animals (Table 3). Accordingly, the SHR animals injected with Dexa presented a picture of dyslipidemia.

Table 3 – Lipid profile of NTR and SHR treated animals and controls

Groups	Total cholesterol	HDL-c	Triglycerides
	Mean ± sem (mg/dL)	Mean ± sem (mg/dL)	Mean ± sem (mg/dL)
NTR-C (n=12)	104.1 ± 2.55#*	36.9 ± 1.19#	157 ± 1.17#*
SHR-C (n=12)	42.6 ± 0.66	28.59 ± 0.99*	54.31 ± 0.93*
SHR-Dexa-SED (n=14)	49.8 ± 1.21	29.7 ± 1.04	75.50 ± 1.9
SHR-Dexa-TR (n=12)	47.9 ± 0.7#	34.6 ± 1.5#ª	43.24 ± 1.4ª
SHR-Dexa-OL (n=7)	49.6 ± 1.83	33.6 ± 0.94#ª	36.04 ± 1.45*
SHR-Dexa-TR-OL (n=6)	50.98 ± 2.02#	39.87 ± 1.29#*	35.79 ± 0.63*
SHR-Dexa-MET (n=10)	44.2 ± 2.2	37.7 ± 1.8#*	45.9 ± 1.8#ª

#A significant difference from the SHR-Dexa-SED and SHR-C groups (p<0,01) – Anova; *Significant difference from the SHR-Dexa-TR group (p<0,05) – Anova; ªSignificant difference from the SHR-Dexa-OL-TR goup (p<0,01) – Anova; sem = standard error of the mean.

Animals treated with flaxseed oil, physical exercise and metformin showed a significant reduction in TG levels and a significant increase in HDL-c levels in relation to the SHR-Dexa-SD and SHR-C groups (Table 3). Regarding the triglyceride levels, the values found for the SHR-Dexa-OL and SHR-Dexa-OL-TR groups were significantly lower than the ones observed for the SHR-Dexa-TR and SHR-Dexa-MET animals; suggesting greater efficiency of the flaxseed oil in comparison with exercise and the standard drug in improving this parameter.

As for HDL-c levels, the values were higher in the SHR-Dexa-OL-TR group compared to the SHR-Dexa-OL and SHR-Dexa-TR groups (Table 3), evidencing an additive effect of the exercise and flaxseed in increasing the concentrations of HDL-c.

DISCUSSION

During the experimental period, we performed a weekly monitoring of animals' body weight (BW). The SHR presented lower weight than the NTR at the beginning of the protocol; this profile did not change during the experimental period (Figure 1, Table 2). Compared to other strains, SHR is resistant to body weight gain, even with hypercaloric interventions (RAUT; BANDAWANE, 2018). SHR administered with Dexa that received no treatment showed a significant reduction in body weight throughout the experiment, while the animals which did not use Dexa (SHR-C) had a significant increase in BW during the same period and the treated groups did not present a significant change in BW. Other authors have verified the decrease in BW of animals treated with Dexa (SAIMITHRA *et al.*, 2018). This anorexic effect has been attributed to a number of factors, including suppression of muscle protein synthesis, increased protein degradation, increased energy expenditure, and decreased food intake (SAIMITHRA *et al.*, 2018).

In the present study, we observed a significant increase in BW in the untrained animals (NTR and SHR-C) after 24 days, which is expected. Nevertheless, the physical training resulted in BW control in the SHR-Dexa-TR and SHR-Dexa-OL-TR groups. There is evidence that physical exercise promotes not only the maintenance but also BW loss in obese rodent models (COQUEIRO *et al.*, 2019). The maintenance of BW in adult SHR-Dexa animals verified in the present study can be explained by an imbalance between lipolysis and lipogenesis (YOSHIMURA *et al.*, 2018), with consequent increase in energy expenditure because of exercise performed. Furthermore, the significant contribution of physical exercise in the treatment of type 2 DM (SHAKIL-UR-REHMAN *et al.*, 2018), arterial and cardiovascular dysfunctions all illnesses that present an intimate relationship with obesity (APPERLEY; NG, 2019), has been extensively reported.

Although the experimental model herein studied did not present obesity, it was verified that the treatments could be related to the animals weight maintenance, what suggests a preventive effect on the genesis of obesity, which is considered a risk factor for the development of other present pathophysiological changes in MetSyn such as dyslipidaemia, SHA, hyperglycaemia and insulin resistance (OWENS; GALLOWAY, 2014).

The relevance of the study of peripheral resistance to insulin is indisputable; as it is associated with several pathologies such as type 2 DM, obesity, hypertension and atherosclerosis (SAMPATH KUMAR *et al.*, 2019). Insulin resistance is characterized by the decreased cellular ability to increase the transport and/or use of glucose in response to insulin action. In this condition, the lower consumption of glucose causes its serum levels to rise, promoting one state of glucose intolerance (NICOLAU *et al.*, 2017).

Glucocorticoids exert a number of metabolic effects that involve several physiological systems and trigger important clinical changes. The influence of these hormones on carbohydrate metabolism has been demonstrated in other experiments of our group, when we studied the effects of the administration of Dexa in normoglycemic NTR (MOLENA-FERNANDES *et al.*, 2010). The metabolic

effects observed after the administration of Dexa were a state of glucose intolerance during GTT, in addition to an increase in triglyceride concentration and hyperglycaemia. These diabetogenic effects promoted by glucocorticoids point to pathophysiological mechanisms that involve alterations in hepatic and peripheral metabolism of glucose, resulting in the development of peripheral resistance to insulin and in the increase of hepatic gluconeogenesis (GEER; ISLAM; BUETTNER, 2014). The progression of glucose intolerance associated with insulin resistance may be similar to diabetes. Metformin is an antihyperglycemic agent, which has no effect on the secretory cells of the pancreas, but increases tissue sensitivity to insulin (YANG *et al.*, 2017). We observed a significant reduction in glycemia values by treatment with metformin during GTT, indicating that this drug may reduce the severity of hyperglycaemia and insulin resistance caused by dexamethasone, a result that corroborates previous reports (ROGACKA *et al.*, 2018). As the metformin treatment corrected these alterations, this medicament is potentially effective in treating the glucose intolerance and probably the acute insulin resistance observed in the hyperglycaemic SHR group.

The effects of aerobic physical training on endocrine-metabolic changes in experimental SHR models with insulin resistance induced by the administration of synthetic glucocorticoids have been poorly investigated. The GTT performed in our study suggests antihyperglycemic outcomes of the physical training in SHR with Dexa-induced glucose intolerance. It is well established that physical exercise promote immediate physiological and metabolic adjustments (acute adaptation) and long-term adjustments (chronic adaptations) so that the body can meet the highest energy demand and maintain homeostasis (FELIG; WAHREN, 1975; TEO *et al.*, 2018). Oxygen consumption during exercise increases about twenty times. To meet this demand glucose uptake increases by 7 to 20 times compared to basal levels in exercised muscles (BARTHOLOMAE *et al.*, 2018), a fact that can justify the decrease in blood glucose in swimming animals. Moreover, the effects of physical exercise on glycaemic behaviour herein observed may be related to the reduction of tissue glucose, what have been verified in diabetic experimental models (PAN *et al.*, 2018). We also verified that the trained animals had a tendency to lower glucose concentrations at all GTT times when compared to sedentary animals, indicating a potent action of regular physical exercise in increasing the uptake of glucose by peripheral tissues and hepatic function. Thus, our results confirm the positive effect of physical training on insulin sensitivity, which have been reported by several authors (MOLENA-FERNANDES *et al.*, 2015).

Among glucocorticoids, Dexa has been prescribed and successfully applied in the treatment of numerous diseases. However, its use triggers side effects such as hepatic and muscular resistance to insulin; thus being a potential agent in the installation of type 2 DM (CHRUVATTIL *et al.*, 2017). Wherefore, it is possible to assume that the swimming training protocol applied in our study was beneficial and may be important as a preventive measure in the development of glucose intolerance and consequently type 2 DM in hypertensive patients who use glucocorticoid drugs. The treatment with flaxseed oil was the least effective in preventing glucose intolerance in SHR-Dexa; however, data from Table 2 show that the glycaemia of these animals during GTT was lower than SHR-Dexa-SED.

Hence, flaxseed oil also had a beneficial effect on glucose intolerance induced by Dexa. In addition, the Dexa administration promoted an increase in the triglyceride rate of SHR, a fact that can be attributed to the glucose intolerance and the effect of glucocorticoids on adipose tissue. According to the literature, Dexa promotes an increase in lipolysis of this tissue, mobilization of fatty acids, and an increase in hepatic triglyceride synthesis (HARASIM-SYMBOR; KONSTANTYNOWICZ-NOWICKA; CHABOWSKI, 2016). Another study from our group showed that flaxseed oil used for the treatment of dyslipidaemia and hyperglycaemic animals at doses of 50 mg/kg and 100 mg/kg over a period of seven and 14 days reduced the hyperglycaemia and hypertriglyceridemia of the animals (ELIAS *et al.*, 2012).

The reduction in the risk of cardiovascular disease attributed to flaxseed consumption is related to its remarkable content of omega-3 polyunsaturated fatty acids (OM3). In recent decades, the importance of OM3 in the prevention and treatment of several diseases has been proven due to its anti-inflammatory action (FERGUSON *et al.*, 2019). These fatty acids also lower triglyceride and cholesterol levels and lower blood pressure (ELAGIZI *et al.*, 2018). However, work on the study of the action of OM3 and more specifically on flaxseed intake on glycemic metabolism in glucose intolerant SHR is scarce.

In the present study, SHR-Dexa treated with 100 mg/k flaxseed oil during 18 days, associated or not to exercise had a significant reduction in serum triglyceride concentrations when compared to SHR-DEX-SD animals. Such effect could be attributed to the facilitation of transport of triglycerides to the liver for catabolization and excretion (ELIAS *et al.*, 2012) studied the effect of flaxseed oil intake on normotensive rats with Triton WR-1339-induced dyslipidemia and found that the dose of 100 mg/kg over the seven-day period had antihyperglycemic and antihyperglycemic effects.

Due to the higher excretion of cholesterol, coupled with deficiencies in enteric uptake and molecular transport, SHRs are hypocholesterolemic compared to other strains such as Wistar (YUAN; KITTS, 2002). However, treatment with Dexa promoted a significant increase in triglycerides when compared to the SHR-C group, and treatment with flaxseed oil reduced triglyceride levels, even at levels significantly lower than SHR-C (potential hyperlipemic and hypolipemic effects). The PUFAs profile of the oil used in the present investigation may be related to such effect, since they stimulate the hepatic uptake of cholesterol (BAE *et al.*, 2017).

It is also important to emphasize the benefit of physical exercise in lipid metabolism. In our experiment, the trained SHR-Dexa group showed a significant reduction in TG levels and an increase in HDL-c levels when compared to the SRH-Dexa-SED group, evidencing the action of physical exercise in the prevention of dyslipidaemia. However, when associated with the flaxseed oil treatment, the training protocol performed in this study on SHR-Dexa promoted an even more significant effect on the lipid profile when compared to the isolated treatments. Although the results on the lipid profile of the SHR-Dexa evidence the effectiveness of all the treatments tested, the animals treated with the flaxseed oil associated with the exercise showed superior levels for HDL-c in relation to the other treatments, therefore indicating a synergistic effect. In relation to triglycerides, there was a reduction in all treatments; however, the association of exercise and

flaxseed oil promoted the best effects. Actually, this combined treatment was even more effective than the metformin treatment in improving the lipid profile of the SHR-Dexa group.

Among functional foods, flaxseed is the most abundant source of OM3, also being more accessible and economical than other natural products (ZANQUI *et al.*, 2015). In this regard, the features of flaxseed as a functional food position it better than pharmacological supplements, under all prisms, considering cost, availability and tolerance. In a recent clinical trial, the daily supplementation of ground flaxseeds (28 g/d) during 8 weeks resulted in significant reductions in waist circumference as well as lipid peroxidation, in addition to increased plasma NOx concentrations (vasoprotective effect), with perfect tolerance and no adverse effects (RICKLEFS; JOHNSTON; SWEAZEA, 2015). Therefore, flaxseed can be extremely useful both in the prevention and treatment of the metabolic alterations characteristic of MetSyn (ABDELKAREM; FADDA, 2017).

Taking into account the broad set of pathologies associated to the human MetSyn, namely obesity, insulin resistance, type 2 diabetes mellitus, dyslipidaemia and hypertension (NISHITSUJI *et al.*, 2018), this study successfully established an experimental model that combines three of them (hypertension, dyslipidaemia and insulin resistance). All the treatments tested in this study in SHR demonstrated a protective effect on the development of insulin resistance and dyslipidaemia induced by Dexa. In this regard, regular physical exercise and ingestion of omega-3 PUFAs are a good alternative for the prevention of cardiovascular complications present in diabetic and/or MetSyn patients, since the results herein reported suggest their effectiveness against insulin resistance, hyperglycemia, and associated lipid changes. Despite of it we must recognize that the present experimental model has its limitations mainly related to the possibilities of an experimental model to mirror a complex phenomenon like the metabolic syndrome.

CONCLUSION

The ingestion of flaxseed oil associated or not to the regular practice of physical exercise, promoted a significant improvement on hyperglycemia and glucose intolerance in SHR-Dexa. As for the lipid profile, treatments with flaxeed oil and exercise promoted reduction of triglyceride levels and increase of HDL-c; moreover, the association of these non-pharmacological interventions presented significantly superior results than the isolated treatments. The effects on animals' weight gain troughout the experiment period indicated an important preventive action of flaxseed oil and exercise on the SHR-Dexa weight evolution. The herein verified efficacy of the treatments using metformin, exercise protocol and flaxseed oil supplementation in correcting such metabolic alterations allows us to suggest a beneficial effect of all three treatments as prophylactic measures against MetSyn.

ACKNOWLEDGMENTS

The authors thank the Araucaria Foundation of Scientific Support and Technological Development of Paraná (Call 02/2009, Protocol 16215) for the financial support.

REFERENCES

ABDELKAREM, H. M.; FADDA, L. H. Flaxseed and quercetin improve anti-inflammatory cytokine level and insulin sensitivity in animal model of metabolic syndrome, the fructose-fed rats. *Arabian Journal of Chemistry*, v. 10, p. S3015-S3020, 2017.

ANDRADE, E. F. *et al.* Exercise and beta-glucan consumption (Saccharomyces cerevisiae) improve the metabolic profile and reduce the atherogenic index in type 2 diabetic rats (HFD/STZ). *Nutrients*, v. 8, n. 12, 2016.

APPERLEY, L. J.; NG, S. M. Increased insulin requirement may contribute to risk of obesity in children and young people with Type 1 Diabetes Mellitus. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, v. 13, n. 1, p. 492-495, 2019.

BAE, J. S. *et al.* Amelioration of non-alcoholic fatty liver disease with NPC1L1-targeted IgY or n-3 polyunsaturated fatty acids in mice. *Metabolism: Clinical and Experimental,* v. 66, p. 32-44, 2017.

BARTHOLOMAE, E. *et al.* Reducing glycemic indicators with moderate intensity stepping of varied, short durations in people with pre-diabetes. *Journal of Sports Science and Medicine*, v. 17, n. 4, p. 680-685, 2018.

CHRUVATTIL, R. *et al.* Dexamethasone Alters the Appetite Regulation via Induction of Hypothalamic Insulin Resistance in Rat Brain. *Molecular Neurobiology*, v. 54, n. 9, p. 7.483-7.496, 2017.

COQUEIRO, R. DA S. *et al.* Therapeutic and preventive effects of exercise on cardiometabolic parameters in aging and obese rats. *Clinical Nutrition Espen*, v. 29, p. 203-212, 2019.

DRAGANESCU, D. *et al.* Flaxseed lignan wound healing formulation: Characterization and in vivo therapeutic evaluation. *International Journal of Biological Macromolecules*, v. 72, p. 614-623, 2015.

EIBEL, B. *et al.* B-Blockers Interfere With Cell Homing Receptors and Regulatory Proteins in a Model of Spontaneously Hypertensive Rats. *Cardiovascular Therapeutics,* v. 36, n. 4, 2018.

ELAGIZI, A. *et al.* Omega-3 Polyunsaturated Fatty Acids and Cardiovascular Health: A Comprehensive Review. *Progress in Cardiovascular Diseases*, v. 61, n. 1, p. 76-85, 2018.

ELIAS, R. *et al.* Efeito de diferentes doses de óleo de linhaça no tratamento da dislipidemia em ratos. *Revista Brasileira de Nutrição Esportiva,* v. 6, n. 35, p. 1, 2012.

FELIG, P.; WAHREN, J. Fuel Homeostasis in Exercise. *New England Journal of Medicine*, v. 293, n. 21, p. 1.078-1.084, 1975.

FELIG, P.; WAHREN, J. Fuel homeostasis in exercise. *N Engl J Med.* v. 293, n. 21, p. 1078-1084, 1975. DOI: 10.1056/NEJM197511202932107.

FERGUSON, J. F. *et al.* Omega-3 polyunsaturated fatty acids attenuate inflammatory activation and alter differentiation in human adipocytes. *Journal of Nutritional Biochemistry,* v. 64, p. 45-49, 2019.

GADAU, C. *et al.* Prevalence, characteristics and predictive factors of microalbuminuria in resistant systemic arterial hypertension. *Revista de Chimie*, v. 69, n. 9, p. 2.425-2.429, 2018.

GEER, E. B.; ISLAM, J.; BUETTNER, C. Mechanisms of glucocorticoid-induced insulin resistance: Focus on adipose tissue function and lipid metabolism. *Endocrinology and Metabolism Clinics of North America,* v. 43, n. 1, p. 75-102, 2014.

GHEZZI, A. C. *et al.* Metabolic syndrome markers in wistar rats of different ages. *Diabetology and Metabolic Syndrome,* v. 4, n. 1, 2012.

GOBATTO, C. A. *et al.* Maximal lactate steady state in rats submitted to swimming exercise. *Comparative Biochemistry and Physiology – A Molecular and Integrative Physiology,* v. 130, n. 1, p. 21-27, 2001.

HARASIM-SYMBOR, E.; KONSTANTYNOWICZ-NOWICKA, K.; CHABOWSKI, A. Additive effects of dexamethasone and palmitate on hepatic lipid accumulation and secretion. *Journal of Molecular Endocrinology,* v. 57, n. 4, p. 261-273, 2016.

KAUR, N.; KISHORE, L.; SINGH, R. Therapeutic effect of Linum usitatissimum L. in STZ-nicotinamide induced diabetic nephropathy via inhibition of AGE's and oxidative stress. *Journal of Food Science and Technology*, v. 54, n. 2, p. 408-421, 2017.

MILLAR, C. L. *et al.* Effects of freeze-dried grape powder on high-density lipoprotein function in adults with metabolic syndrome: A randomized controlled pilot study. *Metabolic Syndrome and Related Disorders*, v. 16, n. 9, p. 464-469, 2018.

MOLENA-FERNANDES, C. *et al.* Effects of exercise and metformin on the prevention of glucose intolerance: A comparative study. *Brazilian Journal of Medical and Biological Research*, v. 48, n. 12, p. 1.101-1.108, 2015.

MOLENA-FERNANDES, C. *et al.* Avaliação dos efeitos da suplementação com farinha de linhaça (Linum usitatissimum L.) marrom e dourada sobre o perfil lipídico e a evolução ponderal em ratos Wistar. *Revista Brasileira de Plantas Medicinais*, v. 12, n. 2, p. 201-207, 2010.

NICOLAU, J. *et al.* Rheumatoid arthritis, insulin resistance, and diabetes. *Joint Bone Spine*, v. 84, n. 4, p. 411-416, 2017.

NISHITSUJI, K. *et al.* Effect of coffee or coffee components on gut microbiome and short-chain fatty acids in a mouse model of metabolic syndrome. *Scientific Reports,* v. 8, n. 1, 2018.

OWENS, S.; GALLOWAY, R. Childhood obesity and the metabolic syndrome. *Current Atherosclerosis Reports,* v. 16, n. 9, 2014.

OZEMEK, C. *et al.* An Update on the Role of Cardiorespiratory Fitness, Structured Exercise and Lifestyle Physical Activity in Preventing Cardiovascular Disease and Health Risk. *Progress in Cardiovascular Diseases*, v. 61, n. 5-6, p. 484-490, 2018.

PAN, B. *et al.* Exercise training modalities in patients with type 2 diabetes mellitus: A systematic review and network meta-analysis. *International Journal of Behavioral Nutrition and Physical Activity,* v. 15, n. 1, p. 1-14, 2018.

PARIKH, M.; NETTICADAN, T.; PIERCE, G. N. Flaxseed: Its bioactive components and their cardiovascular benefits. *American Journal of Physiology – Heart and Circulatory Physiology*, v. 314, n. 2, p. H146-H159, 2018.

RAUT, S. K.; BANDAWANE, D. A Systematic Review on Animal Models of Metabolic Syndrome. *Int J Pharma Res Health Sci*, v. 6, n. 1, p. 2.089-2.098, 2018.

RICKLEFS, K.; JOHNSTON, C.; SWEAZEA, K. Therapeutic use of ground flaxseeds for type 2 diabetes. *Faseb Journal,* v. 29, n. 1 Meeting Abstracts, 2015.

ROGACKA, D. *et al.* Metformin overcomes high glucose-induced insulin resistance of podocytes by pleiotropic effects on SIRT1 and AMPK. *Biochimica et Biophysica Acta - Molecular Basis of Disease,* v. 1.864, n. 1, p. 115-125, 2018.

ROLIM, L. P. *et al.* Effects of diabetes mellitus and systemic arterial hypertension on elderly patients' hearing. *Brazilian Journal of Otorhinolaryngology*, v. 84, n. 6, p. 754-763, 2018.

SAIMITHRA, T. *et al.* Catechin, an active constituent of green tea, preserves skeletal muscle activity in dexamethasone induced cachexia by increasing acetylcholine sensitivity in muscles of wistar rats. *Indian Journal of Experimental Biology*, v. 56, n. 5, p. 314-321, 2018.

SAMPATH KUMAR, A. *et al.* Correlation between basal metabolic rate, visceral fat and insulin resistance among type 2 diabetes mellitus with peripheral neuropathy. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, v. 13, n. 1, p. 344-348, 2019.

SENAPHAN, K. *et al.* Ferulic Acid Alleviates Changes in a Rat Model of Metabolic Syndrome Induced by High Carbohydrate, High-Fat Diet. *Nutrients,* v. 7, n. 8, p. 6.446-6.464, 2015.

SHAKIL-UR-REHMAN, S. *et al.* Response to a Supervised Structured Aerobic Exercise Training Program in Patients with Type 2 Diabetes Mellitus – Does Gender Make a Difference? A Randomized Controlled Clinical Trial. *Journal of the National Medical Association*, v. 110, n. 5, p. 431-439, 2018.

STRELKOVA, S. N.; OVSYANNICOV, K. V.; UTKINA, N. I. Role of non-drug therapies of metabolic syndrome: Challenges and prospects. *Rational Pharmacotherapy in Cardiology*, v. 12, n. 6, p. 725-732, 2016.

TEO, S. Y. M. *et al*. Exercise Timing in Type 2 Diabetes Mellitus: A Systematic Review. *Med Sci Sports Exerc*., v. 50, n. 12, p. 2.387-2.397. DOI:10.1249/MSS.0000000000001732. PMID: 30067587.

VELLOSO, L. A. Novo modelo, velho remédio. *Arquivos Brasileiros de Endocrinologia & Metabologia,* v. 53, n. 4, p. 389-390, 2009.

YANG, X. *et al.* Metformin, beyond an insulin sensitizer, targeting heart and pancreatic β cells. *Biochimica et Biophysica Acta – Molecular Basis of Disease*, v. 1.863, n. 8, p. 1.984-1.990, 2017.

YOSHIMURA, S. *et al.* Short- and long-term effects of high-fat diet feeding and voluntary exercise on hepatic lipid metabolism in mice. *Biochemical and Biophysical Research Communications*, v. 507, n. 1-4, p. 291-296, 2018.

YUAN, Y. V.; KITTS, D. D. Dietary fat source and cholesterol interactions alter plasma lipids and tissue susceptibility to oxidation in spontaneously hypertensive (SHR) and normotensive Wistar Kyoto (WKY) rats. *Molecular and Cellular Biochemistry*, v. 232, n. 1-2, p. 33-47, 2002.

ZANQUI, A. B. *et al.* Subcritical extraction of flaxseed oil with n-propane: Composition and purity. *Food Chemistry*, v. 188, p. 452-458, 2015.