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Dry heated whole sorghum flour (BRS 305) with high tannin and resistant starch improves glucose metabolism, modulates adiposity, and reduces liver steatosis and lipogenesis in *Wistar* rats fed with a high-fat high-fructose diet

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ABSTRACT

This study aimed to evaluate the effect of dry heated whole sorghum flour (BRS 305 hybrid) on insulin resistance, glucose tolerance, adiposity, steatosis, and liver lipogenesis in rats fed with a high-fat high-fructose diet (HFHF). Male *Wistar* rats (n = 10/group), 45–50 days old, were fed with AIN93-M diet, HFHF (31% saturated fat and 20% fructose) and HFHF + sorghum flour (replacing 50% of dietary fiber, 100% starch, 19.8% protein and 22.5% lipids in the experimental diet), for 10 weeks. The sorghum flour restored the effect of the HFHF diet, decreasing triglycerides, uric acid, alanine aminotransferase (ALT), liver steatosis, and lipogenesis. Further, sorghum improved insulin sensitivity and glucose tolerance and increasing the concentration of PPAR α protein in the liver. Thus, the BRS 305 whole sorghum flour with high tannin and resistant starch showed potential as a functional food, since it improves glucose metabolism, modulates adiposity, and reduces liver steatosis and lipogenesis in *Wistar* rats fed with a high-fat high-fructose diet.

1. Introduction

Ultra-processed foods and beverages dense in energy, fat, and sugar are present in a Western diet, which combined with a genetic predisposition can trigger metabolic syndrome with weight gain, high plasmatic triglycerides, and lower insulin sensibility. These effects may increase the deposits of fat into adipose tissue and serum levels of free fatty acids, besides negatively affect glucose metabolism, raising plasma glucose, and insulin levels, generating insulin resistance (IR) (Nemati et al., 2017).

The consumption of high-fat high-fructose diet (HFHF) promotes an increase in free fatty acids (FFA) circulating in adipose, muscle, and liver tissues, as well as an increase in fat deposits, providing hypertrophy and hyperplasia of adipocytes (McArdle et al., 2013). Further, the HFHF diet negatively affects glucose metabolism due to high plasma glucose and insulin levels, which results in IR. Thus, in the liver, insulin resistance

leads to an increase in gluconeogenesis and hepatic lipogenesis, due to the excessive accumulation of FFA resulting in liver steatosis (McArdle et al., 2013).

Due to the growing number of individuals with metabolic syndrome, nutritional strategies are essential to combat this condition. Sorghum (*Sorghum bicolor* L.), a cereal native to Africa, has high nutritional and functional value, with potential benefit for human health, able of improving the antioxidant and inflammatory conditions (de Morais Cardoso et al., 2017). There are sorghum hybrids, such as BRS 305, which has brown pericarp and pigmented test with a high content of condensed tannins and resistant starch (RS) (Teixeira et al., 2016). Resistant starch can improve end points related to acute and long-term glucose and insulin responses, including the down-regulation of post-prandial peak glucose, postprandial glucose and insulin at specific points in time, and the incremental area under the curve (iAUC) for postprandial glucose and insulin (Harris, 2019). On the other hand,

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condensed tannins, represented especially by catechin, epicatechin and procyanidin B2, are a class of flavonoids related to the reduction of oxidative stress, which is related to less inflammation and better homeostasis of glucose metabolism (Laddha and Kulkarni, 2019). Further, sorghum tannins can reduce the activity of glycolytic enzymes, reducing the absorption rate and consequently improving the glycemic response (Mkandawire et al., 2013). In this way, the BRS 305 sorghum as a source of both these compounds can be a viable alternative to improve glucose and insulin metabolism.

Recent studies, by our research group, showed that SC 319 sorghum genotype (with a high content of 3-deoxyanthocyanidins, flavones, proanthocyanidins, and dietary fiber), in extruded form, promoted a reduction of fat accumulation in the liver, increasing β -oxidation, besides an anti-inflammatory action, helping to reduce obesity and liver steatosis in *Wistar* rats fed with a high-fat diet (de Sousa et al., 2018). ANUNCIAÇÃO et al. (2019) observed that the daily consumption of a breakfast cereal made with SC 319 sorghum genotype reduced the percentage of body fat and oxidative stress, increasing antioxidant enzymes in overweight men compared to wheat consumption.

However, there are currently no studies relating the intake of BRS 305 sorghum flour, which has high content of condensed tannins (Martinez et al., 2020) and resistant starch (Teixeira et al., 2016) on rats fed with a high-fat high fructose diet (HFHF). Thus, we hypothesize that BRS 305 hybrid sorghum flour can improve sensitivity to insulin and glucose tolerance, and promote the control of liver lipogenesis, increasing β -oxidation in animals fed a HFHF diet. The objective of this study was to evaluate the effect of dry heated whole sorghum flour (BRS 305 hybrid) on insulin resistance, glucose tolerance, adiposity, steatosis, and liver lipogenesis in rats fed with a high-fat high-fructose diet (HFHF).

2. Material and methods

2.1. Material

The sorghum BRS 305 hybrid grains, with brown pericarp and high tannin (Martinez et al., 2020) and resistant starch (Teixeira et al., 2016) developed by Embrapa Milho and Sorgo Sete Lagoas, MG, harvest August 2015, were used. The seeds were manually selected, sieved, to eliminate impurities. Then, the grains were submitted to heat treatments at 105 °C in an air circulation oven, for 30 min, as proposed by Martinez et al. (2020). Then, the sorghum was ground in a knife mill (Brabender®, Dusburg, Germany) with a 1.0 mm stainless steel sieve to obtain the flour.

The chemical analysis of protein, lipids, moisture, ash, fiber (soluble, insoluble, and total), carbohydrates, and condensed tannins in BRS 305 dry heating sorghum were previously carried out and already published in Martinez et al. (2020). The chemical composition of this sorghum was moisture: $9.09 \pm 0.14\%$; protein: $12.97 \pm 0.19\%$; lipids: $5.01 \pm 0.12\%$; ash: $1.13 \pm 0.04\%$; total dietary fiber: $14.28 \pm 0.69\%$; insoluble fiber: $13.95 \pm 0.77\%$; soluble fiber: $0.33 \pm 0.08\%$; carbohydrates: $66.61 \pm 0.69\%$; and condensed tannins 59.53 ± 1.80 mgEC/g (milligrams of catechin per gramme of sample).

2.2. Chemical analysis of resistant starch

The resistant starch content (RS) was determined through simulated digestion with pancreatic α -amylase and amyloglucosidase, using a commercial kit (Resistant star assay kit AACC 32–40, Megazyme), following the instructions of manufacturer. These analyzes were performed after dry heating.

2.3. Identification of phenolic compounds

The 1g of sorghum dry heating flour was added in 5 mL of a methanol/water solution (50:50 v/v), mixed in the vortex for 1 min, and

Table	-	1	

Composition of experii	mental d	iets
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Ingredients (g/kg diet)	AIN93-M	HFHF	HFHF Sorghum ^a
Albumin ^b	136.6	136.6	108.8
Dextrinized starch	155.0	45.0	46.5
Corn starch	463.5	135.0	-
Sucrose	100.0	28.6	31.0
Soybean oil	40.0	40.0	31.1
Sorghum	-	-	195.4
Lard	-	310.0	310.0
Fructose	-	200.0	200.0
Microcrystalline Cellulose	55.8	55.8	27.9
Mineral mix	35.0	35.0	35.0
Vitamin mix	10.0	10.0	10.0
L-cystine	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5
Macronutrients			
Protein (%)	12.6	12.6	12.4
Carbohydrate (%)	82.8	47.1	46.2
Lipids (%)	4.6	40.3	42.4
Energetic density (Kcal/g)	3.8	5.3	5.2

AIN-93M: diet for rodents; HFHF: diet rich in saturated fat and fructose; HFHF + Sorghum: HFHF diet added of sorghum flour.

^a Chemical composition of sorghum in g/100g: carbohydrates: 58.9g; total dietary fiber: 14.3g; lipids: 4.6g; protein: 12.1g; moisture: 9.1g; antioxidant capacity (μ mol Trolox Equivalent/g sample): 296.4; condensed tannins (mgEC. g-1): 59.5 (Martinez et al., 2020).

^b Albumin based on 88% protein content.

placed in a water bath with sonication at 24 $^\circ C$ for 20 min. Then it was mixed again for 1 min and centrifuged (4000g,15 min). The supernatant was filtered with a 0.20 μm Teflon syringe and stored at - 20 $^\circ C$ until use.

The extracts and standards were analyzed by an Agilent 1220 Infinity liquid chromatography (LC) (Agilent Technologies, Santa Clara, CA, USA), coupled to an Advion expression LC compact mass spectrometer (Advion, Ithaca, NY, USA). Then, 10 µL samples were injected and passed through a 3.5 μ m 2.1 \times 100 mm X Bridge Shield RP18 column (Waters, Milford, MA, USA) at 0.6 mL/min with the controlled temperature at 40 $^{\circ}$ C. The mobile phase was ultrapure water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B). The polyphenols were eluted using linear gradients of 84.4% A in 1.50 min, 81.5% A in 2.25 min, 77.0% A in 6.25 min, 55.0% A in 1.25 min, 46.0% A in 2.25 min, 94.0% A in 2.25 min and maintaining 94.0% A for 2.25 min, for a total run time of 18 min. From the column, the flow was directed to an ultraviolet (UV) detector of variable wavelength (265 and 278 nm), and later to an Advion LCMS mass spectrometer, and ESI mass spectrometry was performed in negative ionization mode using selected ion monitoring with a scan time of 200 ms. The temperature and voltage of the capillaries were 250 °C and 180 V, respectively, the voltage of the ESI source and the temperature of the gas were 2.5 kV and 250 °C, with a flow of desolvation gas at 240 L/h. Advion Mass Express™ software (Advidon, Ithaca, USA) was used to control LC, CMS instrumentation, and data acquisition. Individual polyphenols were identified and confirmed by comparing the m/z and LC retention times with authentic standards. Analysis of MS and UV data was performed using the Advion Data Express[™] software.

2.4. Animals and experimental diets

Thirty *Wistar* rats (*Rattus norvegicus*) from 45 to 50 days of age were obtained from the Central Bioterium of Biological and Health Sciences at Universidade Federal de Viçosa. The animals were distributed in individual stainless-steel cages, kept at a temperature of 21 ± 3 °C with a 12-h photoperiod automatically controlled, and received distilled water and their respective experimental diets *ad libitum*. The animals were randomized using the animals' body weight to maintain homogeneity among experimental groups and the experiment was divided into two phases. In phase I, to induced metabolic changes, the animals were

divided into two groups: normal control group (155.98 \pm 17.05g; n = 10), which received the AIN93-M diet (Reeves et al., 1993) and the HFHF group (162.67 \pm 19.07g; n = 20), that received a high-fat and high-fructose diet, with 4% soy oil, 31% lard and 20% fructose, for 8 weeks. At the end of this period, the animals have fasted for 12 h a sample of blood was collected by a caudal puncture to analyze the concentration of glucose and triglyceride. Further, biometric parameters waist circumference and naso-anal length were measure to calculate the Lee index $(\sqrt[3]{P(g)}/CNA(cm))$. In phase II, the animals in the control group were kept with the same diet AIN-93M diet (349.9 \pm 30.71g; n = 10) and the animals from group HFHF were redistributed into two groups: a group that received an HFHF diet (HFHF control) (366.88 \pm 36.90g; n = 10) and HFHF added with sorghum flour (368.44 \pm 34.11g; n = 10) for 10 weeks. The addition of sorghum flour to the diet allowed the replacement of 50% fiber, 100% corn starch, 22.5% soybean oil, and 19.8% protein from sorghum flour (Table 1).

At the end of phase II, the animals were anesthetized with isoflurane (Isoforine, Cristália®) and euthanized by cardiac puncture. Blood was collected in tubes with EDTA heparin anticoagulant and centrifuged (1006g; 10 min) to obtain plasma. The liver of animal and adipose tissue were collected and washed in saline (PBS), weighed and immediately immersed in liquid nitrogen and subsequently stored at -80 °C. A fragment of the liver tissue was fixed in 10% formaldehyde for histological analysis.

The current project was approved by the Animal Use Ethics Committee of the Federal University of Viçosa (CEUA/UFV), protocol No. 38/2019. All the experimental procedures with animals were performed in accordance with Directive 86/609/EEC of November 24, 1986, in compliance with the ethical principles for animal experimentation.

2.5. Food intake and biometric measures

The food intake and body weight were measured weekly. The nasoanal length and waist circumference of the animals were measured using an inelastic measuring tape. The BMI was calculated as body weight/height². Total body adiposity (%) was calculated by the weight of total adipose tissues (epididymal, abdominal, and retroperitoneal), divided by the final body weight, multiplied by 100. The feed efficiency ratio (FER) was calculated by the ratio between body weight gain and total diet consumption (de Sousa et al., 2018).

2.6. Biochemical analyzes

The concentration of glucose and triglycerides was performed at the end of the induction of metabolic changes (phase I) and the end of the intervention (phase II). The animals have fasted for 12 h and the blood was collected by a caudal puncture to analyze the concentration of glucose and triglycerides, using the commercial kits AccuChek Active and Accutrend devices, respectively, both from the Roche Company.

Plasma concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and uric acid were evaluated by colorimetric methods according to the manufacturer's instructions (Bioclin®). Insulin was analyzed by immunoassay according to the protocol (RayBio® Rat Insulin) in serum samples.

2.7. Assessment of glucose and insulin tolerance

The glucose intraperitoneal tolerance test (GTT) was conducted in the ninth week of the experiment (phase II). After fasting for 12 h, a 50% D-glucose solution (2 g/kg body weight) was administered into intraperitoneal cavity of animals. The glucose concentration was determined in blood obtained from the caudal vein at baseline (zero) and after 30, 60, 90, and 120 min. Blood glucose was quantified using a glucometer with an appropriate test strip (Accu-Chek, Roche). The area under the glucose curve (AUC) was calculated using GraphPad Prism 6 (GraphPad Software, San Diego, CA).

In the tenth week (phase II), a week before the euthanasia of animals, the intraperitoneal insulin tolerance test (ITT) was performed with the animals fasted for 12 h. Glycemia was measured at baseline (zero) and 5, 10, 15, 20, 25 and 30 min, after insulin injection intraperitoneally (Novolin®, Novo Nordisk), at the rate of 0.75 U/kg of body weight. The mean of the glycemia decay constant (KTTII) was calculated using the formula: [0.693/(t1/2)], with t1/2 being the time needed to have baseline blood glucose. The glucose t1/2 was calculated from the slope of the glucose decay curve during the linear phase.

2.8. Quantification of PPARa

The extraction of the nuclear fraction from liver samples was performed using a specific kit (NE-PER TM Nuclear and Cytoplasmic Extraction Reagents - Thermo Scientific), following the manufacturer's instructions. The quantification of the protein concentration of the peroxisome proliferator-activated receptor alpha (PPAR α) was performed using the Rat PPAR- α ELISA kit (Elabscience, USA) by reading the absorbance of the samples in an ELISA reader at 450 nm (Multiskan Microplate Photometer, ThermoFisher Scientific, MA, USA).

2.9. Gene expression

Expression of mRNA levels was performed using the Quantitative Polymerase Chain Reaction technique in real-time (RT-qPCR). Briefly, 1 µL of cDNA (1:10), 3 mL H₂O, 5 µL of Applied FastStart Master SYBR Green Biosystems (Foster City, CA), and oligonucleotides at a final concentration of 100 nM were mixed to a final volume of 10 µL. Quantification was performed on the StepOnePlus TM Real-Time PCR System, using the SYBR-Green fluorescence quantification system and Primer Express software (Applied Biosystems, Foster City, CA). Sense and antisense oligonucleotide sequences (Sigma-Aldrich Brasil Ltda) were used: AMPK: 5'-TGA AGC CAG AGA AGG TGT TG-3' (forward) and 5'-ATA ATT TGG GGA TCC ACA-3' (reverse); AKT: 5'-GGG CCA CGG ATA CCA TGA AC-3' (forward) and 5'-AGC TGA CAT TGT GCC ACT GA-3' (reverse); PFK: 5'-CCA CCT GGA GGC CAT TGT AGA-3' (forward) and 5'-GGG ATG ACG CAC ATG ACGA-3' (reverse); PEPCK: 5'-GTT GCA GGC CCA GTT GTT GA-3' (forward) and 5'-CTC ACC TCT GGC CCA GAT TGG TA-3' (reverse). The β -actin was used as an endogenous control to normalize the relative expression of mRNA.

2.10. Histological analyzes of the liver tissue

The liver tissue samples were fixed in 10% formaldehyde and then embedded in resin. The sections were cut to 3 µm thick with 12 cuts of the distance between them and set up on glass slides and stained with gomori and picrosirius trichrome using the hematoxylin/eosin technique. The analyzes were performed and photographed under a bright field microscope (Olympus AX 70 TRF, Tokyo, Japan) in a 40× objective.

In the images of the histological sections, the cytoplasm, lipid vesicles, and collagen type I, III, and total were quantified using Image J® 1.48v software (National Institute of Health, USA). Ten liver fields were selected for each animal, with each histological field with a 266-point reticulum on the images until reaching the sum of 1064 points per animal in a 20x increase. The degree of liver steatosis was assessed semiquantitatively according to the grade scale: grade 0, if the fat percentage was absent or <5%; Grade 1, if \geq 5% and <25%; Grade 2, if \geq 25% and <50%; Grade 3, if \geq 50% and <75%.

2.11. Molecular docking

The main phenolic compounds of sorghum (Catechin, Procyanidin B1, Procyanidin B2, Quercetin, Quercetin 3-glucoside, Quercetin, and 3-rutinoside) were used as potential ligands for PPARα. The Protein Data



Fig. 1. Glycaemia and insulin resistance of animals fed with a HFHF diet and treated with BRS 305 sorghum flour after 10 weeks. Glucose and insulin tolerance were accessed through intraperitoneal glucose tolerance test (iGTT) and insulin tolerance test (ITT). Area under the curve of the glucose tolerance test (A), glucose tolerance test (B), area under the curve of the insulin tolerance test (C), insulin tolerance test (D). Values are represented by means and standard deviation (n = 10), means followed by the same letter do not differ by the Newman-Keuls test at 5% probability. AIN-93M: standard diet for rodents; HFHF: high-fat high-fructose diet; HFHF Sorghum: HFHF diet plus sorghum flour.

Bank (PBD) website (http://www.rcsb.org/pdb/home/home.do) was used to obtain the 3D crystal structures of PPARα. The binding sites of inhibitors or co-crystallized substrates were selected as the center of the anchorage area. The structures of ligants Catechin, Procyanidin B1, Procyanidin B2, Quercetin, Quercetin 3-glucoside, Quercetin, and 3rutinoside recovered from the PubChem Compound database (htt ps://pubchem.ncbi.nlm.nih.gov/). The binder files were opened in AutoDock Tools to add partial loads of Gasteiger and detect the root of each rotating link in the set of structures. The dimensions of the survey space, the center point, and the flexible torsions were assigned with AutoDock Tools and the coupling calculations were performed using AutoDock Vina (Oleg and Arthur, 2010). Several different runs per ligand were performed, and the pose with the highest binding affinity, with the least binding energy (BE)



Fig. 2. Gene expression of biomarkers related to insulin resistance of animals fed with a HFHF diet and treated with BRS 305 sorghum flour after 10 weeks. Adenosine monophosphate-activated protein (AMPK) (A); protein kinase B (AKT) (B); phosphofructo kinase (PFK) (C); peroxisome proliferator-activated by receptor alpha (PPARa) (PEPCK) (D). Values are represented by means and standard deviation (n = 10), means followed by the same letter do not differ by the Newman-Keuls test at 5% probability. AIN-93M: standard diet for rodents; HFHF: high-fat high-fructose diet; HFHF Sorghum: HFHF diet plus sorghum flour.



Fig. 3. Histological analysis of liver tissue of animals fed with a HFHF diet and treated with BRS 305 sorghum flour after 10 weeks. Histological images of liver from experimental groups (A); cellular components (B); degree of steatosis (C); cellular markers of hepatic steatosis (D). Values are represented by means and standard deviation (n = 10), means followed by the same letter do not differ by the Newman-Keuls test at 5% probability. Colorant: Gomori trichrome; N: Hepatocyte nucleus; F: Fat vesicles; C: cytoplasm; V: Blood vessels. Bar = 50 μ m.

being saved. Protein-ligand interactions and binding modes were visualized in Discovery Studio Client (Dassault Systèmes Biovia Corp).

2.12. Statistical analysis

The normality of the data was assessed by the Kolmogorov-Smirnov test. The data that did not present a normal distribution were transformed into Log_{10} before the parametric statistical analysis. The results were submitted to analysis of variance (ANOVA) and *post-hoc* of Newman-Keuls test, at 5% probability. The area under the curve (AUC) for insulin and blood glucose was calculated using the trapezoidal rule. A significance level of 5% was considered. All statistical analyzes were performed using the Graphpad Prism version 7.0 program.

3. Results

3.1. The BRS 305 hybrid sorghum flour resistant starch and phenolic composition

The dry heated whole BRS 305 sorghum flour showed 37.2 \pm 1.5 g/100g of resistant starch. The mains phenolics found were the condensed tannins catechin, procyanidin B1, procyanidin B2, quercetin, quercetin 3-glucoside, and quercetin 3-rutinoside, and they concentrations were 45.5 \pm 2.2; 29.9 \pm 7.8; 47.5 \pm 5.5; 2.0 \pm 0.5; 0.1 \pm 0.0; and 0.1 \pm 0.0 nmol/g, respectively.

3.2. Food intake and body composition

The food intake of the HFHF and HFHF sorghum group was lower (p < 0.05) than that of the AIN-93M group (Supplementary Figure S1A).



Fig. 4. The in silico interaction of the quercetin-3-rutinoside and PPARα. Dates analyzed by AutoDock Vina® and visualized by Discovery Studio (2016) Client®. PPAR-α: Peroxisome proliferator-activated α receptor.

The food efficiency ratio (FER), energy efficiency ratio (EER), and body weight were similar (p > 0.05) between the experimental groups (Supplementary Figure S1B, S1C, and S1D). The epididymal, retroperitoneal, and visceral adipose tissues increased (p < 0.05), as well as, in the percentage of adiposity in the HFHF group compared with the AIN93-M group (Supplementary Figures S1E, S1F, S1G, and S1H). Sorghum flour reduced (p < 0.05) the retroperitoneal and visceral adipose tissues and the percentage of adiposity relative to the AIN93-M group (p > 0.05) (Supplementary Figures S1E, S1F, S1G, and S1H). However, no difference was observed for epididymal adipose tissue between the HFHF groups and the sorghum flour (p > 0.05) (Supplementary Figure S1E). Further, BMI and Lee's index did not differ among the experimental groups (p < 0.05) (data not shown).

3.3. Biochemical analyses

The levels of AST did not differ among experimental groups (p > 0.05) (Supplementary Table S1). The HFHF group increased the levels of ALT, uric acid, TG, glycemia, and insulin, while the sorghum flour reduced these parameters become similar to the AIN93-M group (Supplementary Table S1). In the glucose tolerance test, the AUC of the HFHF group was higher than the group fed with sorghum flour (p < 0.05), demonstrating that the sorghum was able to recover glucose tolerance (Fig. 1A and B). Further, the HFHF group showed a lower insulin tolerance test than the AIN93-M group (p < 0.05) (Fig. 1C and D). The glycemia of the animals fed with sorghum flour was lower (p < 0.05) relative to the HFHF group, at 30 min of glucose tolerance test (Fig. 1B).

3.4. Gene expression and histomorphometry measurements in liver tissue

Sorghum flour up-regulated the mRNA levels (p < 0.05) of AMPK and AKT, which was higher than both control groups (Fig. 2A and B) and PFK expression was similar to the AIN-93M and HFHF groups (Fig. 2C). The sorghum flour added to the HFHF diet increased the protein levels of PPAR α relative to the HFHF group (p < 0.05) (Fig. 2D).

3.5. Histomorphometry measurements in the liver

The sorghum flour increased the percentage of cytoplasm compared to the control groups (Fig. 3A and B). The percentage of fat in the liver of animals fed an AIN93-M diet was 12.28% (Fig. 3A and B), classified as grade 1 liver steatosis (Fig. 3C). The HFHF group increased the percentage of fat in the liver to 28.66% (Fig. 3A and B), classifying it in grade 2 liver steatosis (Fig. 3C). Besides, the consumption of sorghum reversed the liver steatosis to grade 1, with a percentage of 11.28% (Fig. 3A and B), similar to the AIN-93M control (p > 0.05). The sorghum flour added to the HFHF diet reduced inflammatory infiltrate, collagen type I, II, and total compare to the HFHF group (Fig. 3D).

3.6. Molecular docking

The *in silico* analysis of the interaction between phenolic compounds Catechin, Procyanidin B1, Procyanidin B2, Quercetin, Quercetin 3glucoside, Quercetin, and 3-rutinoside and PPAR α showed that every phenolic compound founded in sorghum flour had interaction with PPAR α (Supplementary Table S2), mainly quercetin-3-rutinoside, which had the highest interaction (Fig. 4).

4. Discussion

The present study provides new knowledge about the properties of the dry heated BRS 305 hybrid sorghum flour, rich in condensed tannins and resistant starch (RS), on insulin sensitivity, glucose tolerance, and modulation of liver adipogenesis and lipogenesis in animals fed with a high-fat high-fructose diet (HFHF). We use the heat treatment on sorghum, since this method has been shown efficient in maintaining antioxidant levels (Cardoso et al., 2014) and resistant starch (Teixeira et al., 2016) compared to the wet heat treatment in soghum.

The HFHF diet was effective in promoting metabolic changes, which increased tissue adiposity, triglycerides, and glycemic levels decreased insulin sensitivity and glucose intolerance. Furthermore, HFHF diet increased alanine aminotransferase (ALT) and uric acid levels, markers related to liver and kidney integrity, respectively (Ozer et al., 2008).

However, the presence of BRS 305 sorghum flour in a HFHF diet decreased the plasma levels of ALT, uric acid, triglycerides (TG), glycemia, and insulin, improving insulin sensitivity and glucose intolerance. Further, sorghum flour decreased the degree of liver steatosis and adiposity and increased PPAR α , an important marker for mitochondrial β -oxidation (Wan et al., 2020).

The HFHF diet contributes to a pro-inflammatory state associated with possible damage to hepatocytes. In our study, we observed an increase in plasma ALT levels in the HFHF group, which are more common in hyperlipidemia and hyperglycemia conditions (Kim et al., 2008). The decrease in TG and glycemia by sorghum flour may have contributed to the reduction in ALT levels in HFHF sorghum group, as well as recovery the insulin sensitivity in this group. The significant relationship between TG and glycemia and ALT activity is demonstrated in literature (Kim et al., 2008), as observed in our study. The maintenance of AST values between the experimental groups may be due to its short half-life of 12h compared to 40–60 h for ALT and the rapid normalization in the blood. In addition, ALT is cytosolic and AST is cytosolic and mitochondrial, making it difficult to detect (Ramaiah, 2007).

The HFHF group in our study showed a hyperglycemic effect, decreasing glucose tolerance and promoting insulin resistance (Arbex et al., 2018). However, the BRS 305 sorghum flour in a HFHF diet improved glucose tolerance, showing a hypoglycemic effect. The reduction of glucose and insulin plasma concentration, probably due to the compounds present in this sorghum hybrid, such as phenolics, condensed tannins, dietary fiber, and resistant starch (RS). Dietary fibers and RS can reduce the rate of digestion and activity of the enzyme amylase preventing the increase of glucose level, insulin, and decreasing post-meal plasma glucose (Carvalho et al., 2019). Further, sorghum tannins can partially inhibit the activity of α -amylase (pancreatic and salivary) and α -glucosidase during enzymatic carbohydrate hydrolysis, reducing the absorption rate and consequently improving the glycemic response (Mkandawire et al., 2013), as observed in other study with animals (Arbex et al., 2018). Thus, sorghum flour rich in condensed tannin and RS improves glucose metabolism under high-fat high-fructose diet conditions.

Furthermore, BRS 305 Sorghum flour increased the expression of the AMPK gene in liver tissue, relative to control groups. AMPK is an energy status sensor that plays a significant role in cellular energy homeostasis, associated with insulin resistance and metabolic disorders; its activation stimulates glucose uptake through the translocation of GLUT2 and GLUT4 to the cell surface in the liver and muscle, which can reduce blood glucose levels, improve insulin sensitivity, and modulate glucose homeostasis (Enes et al., 2020). It is observed that AMPK activity is downregulated in obesity and metabolic syndrome and under conditions of an unbalanced diet, which generates an energy imbalance and leads to insulin resistance (Enes et al., 2020). In addition, sorghum flour increased levels of AKT mRNA, a protein with the ability to phosphorylate and activate many metabolic targets, which can stimulate glucose uptake and glycogen synthesis (Ren et al., 2018). The AKT mediates most of the metabolic effects of insulin, regulating lipolysis and decreasing the levels of circulating fatty acids in adipose tissues (Jeong and Kim, 2019). These results show that BRS 305 sorghum flour has effects on the modulation of glycolysis and gluconeogenesis, associated with the activation of AKT and AMPK, and may represent an essential to control glucose metabolism.

The presence of BRS 305 sorghum flour in the HFHF diet decreased the level of uric acid compared to the HFHF diet. The uric acid, a biomarker of metabolic disorder, can directly stimulate the accumulation of liver fat, promoting pro-inflammatory and pro-oxidative effects, causing endothelial dysfunction and intracellular oxidative stress (King et al., 2018). The increase in uric acid in the HFHF diet probably occurred due to fructose, which is phosphorylated into fructose 1-phosphate by fructokinase C, reducing intracellular levels of ATP (adenosine triphosphate), GTP (guanosine triphosphate), and phosphate, leading to the accumulation of adenosine monophosphate (AMP) and fructose 1-phosphate (King et al., 2018). The drop in intracellular phosphate activates the enzyme AMP deaminase, which converts AMP into inosine monophosphate (IMP), resulting in the replacement of purine nucleotides that culminates in the formation of uric acid, using amino acid precursors (King et al., 2018). The phenolic acids (Arbex et al., 2018) present in sorghum can interfere in this metabolic pathway, reducing the levels of uric acid.

The food intake of the HFHF and HFHF sorghum groups was lower than the AIN-93M group. However, the final weight of the animals was similar. This result can be explained by the higher energy density of the HFHF diet compared to the AIN-93M, which increases satiety, reducing consumption, but without changing body weight. Furthermore, the consumption of sorghum flour was effective to reduce adiposity in animals, probably due to its RS content. The RS can improved lipid metabolism disorder and inhibit the accumulation of TG into adipose tissue since after intake of resistant starch the fat synthesis speed is significantly reduced (Shen et al., 2015). Moreover, the RS can regulate the hormones leptin and adiponectin, which are involved in regulating food intake and body weight control (Stolarczyk, 2017). Thus, the consumption of sorghum could improve the lipid metabolism disorder caused by the HFHF diet and consequently decrease serum TG, reducing adiposity. In addition, phenolic compounds present in sorghum can also help to reduce adiposity by the ability to inhibit the expression of adipogenic genes, as observed by Arbex et al. (2018).

Sorghum flour in the HFHF diet increases the concentration of PPARa, decreasing liver lipogenesis. The effects of sorghum on PPARa occurs due to the interaction between the phenolic compounds of sorghum and the active site of PPARα, mainly quercetin-3-rutinoside, as the in silico analyzes demonstrated. Despite not being the phenolic compound present in greater quantity in dry heated whole sorghum, quercetin-3-rutinoside demonstrated the greatest interaction with PPAR-alpha in in silico analyzes. However, it was observed that all sorghum condensed tannins interact with PPAR-alpha, which probably explains the beneficial effects of sorghum on the genetic expression of this marker and the others related to the pathway. PPAR α is a key regulator of hepatocyte metabolism, especially the β -oxidation of fatty acids (Navidshad and Royan, 2016). This effect of sorghum flour is promising since PPAR α increases the expression of genes involved in the peroxisomal and mitochondrial β-oxidation pathways, as well as transport proteins (CPT-1) (Mello et al., 2016), playing an essential role in the oxidation of liver fatty acids decreasing TG. de Sousa et al. (2018) showed that the extruded sorghum flour can increase PPAR α even in a diet rich in saturated fat. This effect was attributed to 3-deoxyanocyanidin (3-DXA) (luteolinidine, apigeninidine, 7-methoxy-apigeninidine, and 5-methoxy-luteolinidine).

Thus, in this study, the intake of sorghum flour in an HFHF diet prevent lipogenesis *de novo* and promote β -oxidation. These effects were confirmed since sorghum flour decrease liver steatosis caused by HFHF diet, grade 2 (28.7% liver lipid) to grade 1 (11.3% liver lipid), decreasing lipotoxicity in the animal, as well as increase the percentages of cytoplasm and reduced inflammatory infiltrate, collagen type I/II and total. Inflammatory infiltrates, collagen type I/II, and total represents the typical fibrotic markers characterized by failure of degradation and excessive synthesis of extracellular matrix components (Zaki et al., 2019). Sorghum phenolic compounds are able to decrease the deposition of the excessive extracellular matrix, thus reversing the progression of liver fibrosis. The AIN93-M group had steatoses grade 1, which could be explained by the composition of the diet, which had 77.2% carbohydrate, generating an increase in the synthesis of hepatic fatty acids and channeling excess acetyl resulting from glucose catabolism for fat biosynthesis in the liver by a process known as lipogenesis de novo (Ferramosca et al., 2013). Furthermore, the reduction of glycemia and postprandial hyperinsulinemia may decrease the storage of fat, reducing the synthesis of fatty acids from carbohydrates (Ye, 2013).

Then, in this study, we observed that the consumption of HFHF increased glucose and insulin levels, which promoted insulin resistance.



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Fig. 5. Schematic diagram proposing the mechanisms of dry heat sorghum flour, BRS 305 hybrid to improving the metabolic changes caused by high-fat highfructose diet (HFHF) in adult Wistar rats, after 10 weeks of intervention. Consumption HFHF diet induce hypertrophy and hyperplasia of adipose tissue that increase monocyte infiltration into adipose tissue and lipolysis, leading to the secretion of glycerol and free fatty acids (FFA) from the adipose tissue into the circulation (Nieto-Vazquez et al., 2008). In addition, the HFHF diet increased glucose and insulin levels, which promoted insulin resistance. In the liver, insulin resistance increased liver lipogenesis and excessive accumulation of FFA resulted in liver steatosis. Sorghum flour reduced plasma levels of ALT, uric acid, TG, glycemia, and insulin, recovering insulin sensitivity and glucose tolerance. Further, sorghum flour decreased the degree of liver steatosis and adiposity and increased PPARa, an essential marker for inducing β -oxidation. FA: Fatty acids; PPAR-α: Peroxisome proliferator-activated α receptor; ALT: alanine aminotransferase; TG: triglycerides;

FFAs: free fatty acid.

This condition increased liver lipogenesis and excessive accumulation of free fatty acid that results in liver steatosis. Sorghum flour increased PPAR α , which recovering insulin sensitivity and glucose tolerance and collaborate to reducing the degree of liver steatosis and adiposity. Further, the consumption of sorghum reduced plasma levels of ALT, uric acid, TG, glucose, and insulin, confirming the beneficial effects of sorghum on insulin resistance and hepatic metabolism (Fig. 5).

5. Conclusion

The intake of sorghum flour improves glucose metabolism by act directly on insulin sensitivity and glucose tolerance. In addition, sorghum flour promotes the control of adipogenesis and liver lipogenesis, reducing the accumulation of fat in the liver and the adipose tissues of animals fed an HFHF diet, which in turn may have contributed to the improvement of insulin resistance and glucose metabolism. These observed results may have been due to the composition of sorghum, as a rich source of resistant starch and condensed tannins that reestablished the normal metabolism of the animals that had been altered by the HFHF diet. Thus, sorghum demonstrates a good potential to control the fatty liver and insulin resistance and to prevent adiposity.

Authors contributions

ODMM: Conceptualization; Data curation; Investigation; Formal analysis; Roles/Writing-original draft; Software. **JMVT:** Data curation; Formal analysis. **MG:** Formal analysis; Visualization; Writing - review & editing. **RCLT:** Data curation; Formal analysis; Investigation; Methodology; Supervision; Validation; Visualization; Writing - review & editing. **VAVQ:** Conceptualization; Funding acquisition; Investigation; Project administration; Resources; Writing - review & editing. **FARB:** Conceptualization; Data curation; Funding acquisition; Investigation; **HSDM:** Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing.

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Oscar David Medina Martinez: Conceptualization, Data curation, Formal analysis, Writing – original draft, Roles/Writing-original draft, Software. **Jaqueline Maciel Vieira Theodoro:** Data curation, Formal analysis. **Mariana Grancieri:** Formal analysis, Visualization, Writing – review & editing. **Renata Celi Lopes Toledo:** Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. **Valéria Aparecida Vieira Queiroz:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Writing – review & editing. **Frederico Augusto Ribeiro de Barros:** Conceptualization, Resources, Supervision, Writing – review & editing. **Hércia Stampini Duarte Martino:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Roles/Writing - original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2021.103201.

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