Metabolic profile of wistar rats after consumption of flour prepared with grains and fruits

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The occurrence of chronic and degenerative diseases, such as obesity, diabetes, cardiovascular disease (CVD), and cancer is increasing sharply. In the last years, there has been an increase in interest in the consumption of foods with functional properties that are related to weight loss and disease prevention. For these reasons, this study aimed to evaluate the effects of flour prepared with grains and fruits on the metabolic profile of Wistar rats. Female rats were randomly divided into four groups: G1: control group; G2: Animals that received condensed milk and sugar; G3: Animals that received food supplemented with a mixture of powdered grains and fruits; G4: Animals that received condensed milk, sugar and rat food supplemented with a mixture of powdered grains and fruits. The authors results showed that the use of the flour of grains and fruits promoted a significant reduction in abdominal circumference, visceral fat, and triglycerides levels in the animals from G3 compared with G1. However, the use of the flour of grains and fruits can be beneficial in association with a balanced diet. When the association is carried out at the same time that there is the consumption of foods rich in sugars, the beneficial effects was not observed.

Key words: Obesity, grains, fruits, cardiovascular disease.

INTRODUCTION

The incidence of chronic and degenerative diseases is increasing sharply and is accountable for about 70% of all deaths around the world. In many cases, the lifestyle is responsible for these conditions (Zujko et al., 2020; Samuel-Hodge et al., 2020 Barbalho et al., 2020). The regular intake of some foods has been associated with a decrease in the risk of chronic degenerative diseases. Several studies suggest that the practice of

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physical exercises and a diet rich in fibers and bioactive compounds reduce glucose intolerance, dyslipidemia, and obesity (da Silva et al., 2020; He et al., 2020).

The presence of polyphenols, vitamins, and other compounds with antioxidant and anti-inflammatory effects found in green and red tea, orange, lemon, apples, soy, eggplant, green banana, and several other vegetables, have been reported to improve hyperglycemia, body weight, diabetes, and metabolic syndrome (Kumar et al., 2020; Marrelli et al., 2020).

Grains and fruits are also rich in fibers that are dietetic compounds resistant to digestion and absorption in the small intestine and may be classified as soluble (mucilage, pectin, and some hemicelluloses) and insoluble (lignin, cellulose, and some hemicelluloses). They can produce a luminal gel capable of reducing the absorption of nutrients leading to better glycemic and lipid profiles. Furthermore, fibers can decrease the appetite both in obese and non-obese subjects. For these reasons, these dietetic compounds are associated with the reduction of obesity, diabetes, and cardiovascular complications (Fornari et al., 2020; Rosado et al., 2020).

Recently they observe an increase in the consumption of foods with functional properties that are related to weight loss and disease prevention, and it is known that the consumption of some plant-based food, especially those rich in fibers, polyphenols, carotenoids, and polyunsaturated fatty acids that are rich in grains and fruits, can help. In many countries, it is possible to find a plethora of natural products that promise the reduction of body weight, glycaemia, and plasma lipids. For these reasons, this study intended to evaluate the effects of flour prepared with grains and fruits on the metabolic profile of Wistar rats fed a hyper-caloric diet.

METHODS

Forty adult female Wistar rats (50 days; weight: 180 g) were obtained from the Animal Models Experimentation Center of the University of Marília (UNIMAR) – Marília, São Paulo, Brazil. The animals went through an adaptation period of seven days (pre-experimental period) in the room where the experiment was performed, during which water and feed were offered ad libitum (the authors limited the use of female since in this group there is a greater concern with the maintenance of body weight due to numerous hormonal changes and the articles found in the literature are usually performed with male animals).

During the experimental protocol (sixty consecutive days), all animals were maintained in plastic boxes (40x30x17 cm with five animals per box) with pine shavings suitable for the breed and kept in an environment with a 12 h light / dark cycle, temperature of 22 ± 2°C and relative humidity of 60±5% until the end of the experimental protocol. The animals were divided into four groups (n=10 for group) at random.

Group 1 (G1): Control group. The animals received water and commercial feed (Nuvilab® ad libitum for 60 days (Table 1). Group 2 (G2): The animals received condensed milk and sugar mixed in the water (for each liter of water, 495g of condensed milk plus 100g of white sugar were used) and commercial feed (Nuvilab® ad libitum) for 60 days (Table 1). Group 3 (G3): The animals received water and commercial rat food (Nuvilab® ad libitum) for 30 days and commercial rat food supplemented with a mixture of powdered grains and fruits (20%) for more 30 days (Table 1).

Group 4 (G4): The animals received condensed milk and sugar mixed in the water (for each liter of water, 495g of condensed milk plus 100g of white sugar were used) and commercial rat food (Nuvilab® ad libitum for 30 days and commercial rat food supplemented with a mixture of powdered grains and fruits (20%) for more 30 days (Table 1).

20 g of condensed milk with high calorie (280 KJ) and widely consumed in Brazil contains 11 g of carbohydrates, 1.5 g of protein, 1.6 g of fats, 20 mg of sodium without dietary fiber.

The supplemented feed was prepared with commercial feed (Nuvilab®) ground in an electric mill (4500 rpm, Marconi) and then added with the flour (80:20). The mixture was modeled so that the final shape is similar to the commercial feed. After pelletization, a forced air circulation oven at a temperature of 65°C was used for approximately 6 h for drying. The feed already processed and correctly identified was kept refrigerated (5°C) until its use. The composition of the mixture of grains and powdered fruits includes white beans, passion fruit, blackberry, tangerine, green banana, eggplant, goji berry, papaya, lemon, plum, grape, ginger, golden linseed, chia, carrot, black soy, apple, hibiscus and psyllium. The energy value is 25kcal/100g; 1% of carbohydrates, 6% of proteins, 0% of lipids, and 8% of fiber.

The flour of fruit and grains that was used to treat the animals was obtained from local markets. According to the manufacturer, the product contains a mixture of dehydrated fruit and grain (white beans, black soy, grape, carrot, plum, apple, papaya, orange, lemon, passion fruit, eggplant, and green banana) plus hydrolysate collagen, agar-agar, green tea, and red tea.

Condensed milk mixed in water (plus white sugar) was obtained from local markets, and according to the manufacturer. Each 20 g of the product consists of 65 kcal, 11 g of carbohydrates, 1.4 g of proteins, and 1.6 g of total fats.

The weight of each animal the consumption of water and feed were evaluated every three days to calculate the percentage and specific rate of weight gain and Lee index.

At the end of the experimental protocol, animals were anesthetized with an overdose of thiopental (200mg/Kg). Immediately after the death, blood samples were collected from the inferior vena cava and atherogenic indices. Furthermore, abdominal adipose tissue was removed, and the cranial-caudal length was measured.

Atherogenic Index (AI), Atherogenic Coefficient (ATC), and Cardiac Risk Ratio 1 (CRR1), were calculated according to Erejuwa et al. (2016); Ahmadvand et al. (2016); AI = log (TG/HDL-с); ATC = (TC-HDL-с)/HDL-с; CRR1 = TC/HDL-с, and CRR2 = LDL-с/HDL-с. The results were expressed as mean ± standard deviation from the mean and subjected to analysis of variance using the GraphPad Prism 5 program.

RESULTS

In the Table 1 it is possible to see that the initial weight was the same between the groups. Moreover, it is possible to see that the group treated with the flour of grains and fruits (G3) did not show a significant reduction in body weight when compared with the control and did not prevent the body weight gain in animals a hypercaloric diet. The flour produced a significant reduction in the abdominal circumference and the visceral fat G3, but not in animals fed with a hypercaloric diet (G4). The Lee index was higher in the G4 when
Table 1. Distribution of the groups of animals and the treatments.

| Group | Treatment 1                  | Duration of treatment 1 | Treatment 2                                     | Duration of treatment 2 |
|-------|------------------------------|-------------------------|-------------------------------------------------|-------------------------|
| G1    | Water and food              | 60 days                 | -                                               | -                       |
| G2    | Condensed milk and sugar mixed in the water | 60 days     | -                                               | -                       |
| G3    | Water and food              | 30 days                 | Water and food supplemented with a mixture of powdered grains and fruits | 30 days                 |
| G4    | Condensed milk and sugar mixed in the water | 30 days               | Condensed milk and sugar mixed in the water, and and food supplemented with a mixture of powdered grains and fruits | 30 days                 |

compared to the other groups, The BMI in G2 and G4 was higher than the other groups. Regarding the thoracic circumference, there were no significant changes between groups (Table 2).

Table 3 shows a significant reduction in triglycerides levels in the group treated with the flour when compared to the other groups (G1, G2, and G4). Regarding glycemia, it is possible to observe that there were no changes in different diets. The same happened with total cholesterol, HDL-c, and LDL-c.

In Table 4 it is possible to see that the intake of the flour improves the atherogenic indices except in the group treated with the flour and a hypercaloric diet (G4). The hypercaloric diet increased the AIC risk but the use of flour was not able to exert protective effects, the same happened with the risks AI, CCR1 and CCR2.

DISCUSSION

The results showed that the use of the mix of grains and fruits promoted a significant reduction in the abdominal circumference, visceral fat, triglycerides levels, and atherogenic indices. Albeit these results are similar to other studies that investigated the use of the isolated compounds of the flour, it is crucial to note that the benefits promoted by the use of the mix are not observed in the animals that consumed a hypercaloric diet (Tables 2 to 4).

Previous studies have reported that Wistar rats treated with condensed milk (de Souza et al., 2015; de Almeida et al., 2016; Ubeda et al., 2017) or a cafeteria diet (Do Bonfim et al., 2021) can increase the deposition of visceral fat one of the best predictors of CVD. The increase in visceral fat has deleterious effects on the biochemical and anthropometric parameters of the animals that to insulin resistance, diabetes, dyslipidemias, and hypertension (Panchal et al., 2011).

One of the components of the flour is green and red tea (Camellia sinensis) that contains a plethora of flavonoids (catechins), including phenolic acid, theophylline, theobromine, and caffeine. Epigallocatechin-3-gallate represents the most relevant catechin found in green tea leaves, but others such as epicatechin, epigallocatechin, and epicatechin-3-gallate (ECG) are also present. These compounds induce significant anti-inflammatory and antioxidant properties helping the prevention and treatment of obesity, diabetes, heart diseases, cancer, and other chronic inflammatory conditions (Barbalho et al., 2019).

The intake of different citrus juices can promote hypoglycemic and hypolipidemic effects both in animal models and humans. These findings included the consumption of Citrus paradise, Citrus medica, and Citrus bergamia (Menichini et al., 2011; Menichini et al., 2016).

The use of soy (Glycine max) is related to the improvement of glycemia, insulin levels, HOMA-IR (Homeostatic Model Assessment - assessing β-cell function and insulin resistance), and lipid levels both in humans and in animal models. The presence of proteins and compounds such as flavonoids and genistein are related to these effects (Malihe et al., 2019; Sidorova et al., 2019). Similar results are found with beans (Phaseolus vulgaris) once these seeds can reduce the risk factors of the occurrence of Metabolic Syndrome, hepatic steatosis, and hepatic and heart lipid peroxidation. It is related to the increase of the expression of the antioxidant enzymes catalase and glutathione reductase (Micheli et al., 2019; Mattei et al., 2011).

The effects of grapes are known for a long time. Both in natura, juice or wine consumption improve the metabolic profile of men and women at different stages of life and are recognized as beneficial in the control and prevention of cardiovascular events. The effects that may be observed are the reduction in glycemia, total cholesterol, triglycerides, LDL-c, and LDL-c oxidation.

Furthermore, a significant augment in the levels of
Table 2. Anthropometric parameters of G1-G4.

| Parameter     | G1               | G2               | G3               | G4               |
|---------------|------------------|------------------|------------------|------------------|
| Weight (1) (g)| 240.9±25.83      | 261.5±15.14      | 242.5±18.78      | 267.8±25.32      |
| Weight (2) (g)| 240.9±15.83      | 261.5±15.14      | 242.5±18.78      | 267.8±25.32*     |
| Lee index     | 29.73±0.936      | 30.58±0.55       | 29.57±0.56       | 33.85±10.70*     |
| BMI (kg/cm²)  | 0.55±0.02        | 0.60±0.03*       | 0.54±0.03        | 0.80±0.63*       |
| TC (cm)       | 10.82±0.60       | 11.13±0.56       | 10.41±0.55       | 11.00±0.86       |
| AC (cm)       | 13.05±0.68       | 13.78±0.52       | 12.05±0.86*      | 13.18±0.72       |
| Visceral fat (g)| 4.58±2.26        | 7.09±1.54        | 2.80±1.42*       | 7.06±1.79        |

G1: Control group; G2: animals received condensed milk and sugar; G3: animals received food supplemented with a mixture of powdered grains and fruits; G4: animals received condensed milk, sugar and rat food supplemented with a mixture of powdered grains and fruits; Weight (1): weight of the animals at the beginning of the experimental protocol; Weight (2): weight of the animals at the end of the experimental protocol; BMI: Body mass index; TC: Thoracic circumference; AC: Abdominal circumference. *p<0.5; **p<0.01 at a level of 5% of significance.

Table 3. Biochemical parameters of G1-G4.

| Parameter      | G1               | G2               | G3               | G4               |
|----------------|------------------|------------------|------------------|------------------|
| Glycaemia (mg/dL)| 138.7±55.05      | 149.4±37.14      | 135.7±18.46      | 144.6±40.19      |
| Triglycerides (mg/dL)| 112.3±14.54* | 135.0±32.01*     | 84.8±8.22        | 121.7±18.09*     |
| Cholesterol (mg/dL)| 155.4±5.12      | 155.2±3.70       | 154.3±1.53       | 159.5±3.62       |
| HDL-c (mg/dL)   | 66.2±7.40        | 59.5±6.89        | 59.1±9.80        | 50.5±5.87        |
| LDL-c (mg/dL)   | 70.7±6.85        | 72.7±2.23        | 75.2±5.00        | 83.5±4.90        |

G1: Control group; G2: animals received condensed milk and sugar; G3: animals received food supplemented with a mixture of powdered grains and fruits; G4: animals received condensed milk, sugar and rat food supplemented with a mixture of powdered grains and fruits. *p<.05 at a level of 5% of significance.

Table 4. Atherogenic indices of G1-G4.

| Parameter      | G1               | G2               | G3               | G4               |
|----------------|------------------|------------------|------------------|------------------|
| AtC            | 1.54±0.39*       | 1.92±0.32*       | 1.49±0.31        | 2.22±0.47*       |
| AI             | 0.24±0.13*       | 0.39±0.11*       | 0.17±0.06        | 0.37±0.08*       |
| CCR1           | 2.54±0.39*       | 2.92±0.32*       | 2.48±0.31        | 3.22±0.47**      |
| CCR2           | 0.55±0.56*       | 0.68±0.74*       | 0.45±0.54        | 1.02±0.95**      |

G1: Control group; G2: animals received condensed milk and sugar; G3: animals received food supplemented with a mixture of powdered grains and fruits; G4: animals received condensed milk, sugar and rat food supplemented with a mixture of powdered grains and fruits; AI: Atherogenic Index; AtC: Atherogenic Coefficient; CRR1: Cardiac Risk Ratio 1; CRR2: Cardiac Risk Ratio2. *p<0.5; **p<0.01 at a level of 5% of significance.

HDL-c is also observed. These effects are due to the presence of numerous antioxidant substances such as phenolic compounds (anthocyanidins, quercetin, epicatechin and resveratrol, and others). Other fruits like plum also have these substances that occur mainly in the skin (Barbalho et al., 2020; Lupoli et al., 2020; Noratto et al., 2015).

Apples (Malus domestica) are among the most consumed fruits around the world. A recent review showed that the consumption of 100 and 150 g/day of whole fruit could be associated with decreased risk of cardiovascular death, abdominal aortic calcification, stroke mortality, and all-cause mortality, due to its ability to reduce the levels of cholesterol, LDL-c, systolic blood pressure, and inflammatory cytokines. It is also related to increase HDL-c levels and improved endothelial function (Sandoval-Ramirez et al., 2020).

The consumption of carrots (Daucus carota) is associated with significantly augmented levels of plasma total antioxidant capacity and reduced plasma malondialdehyde production, possibly due to the presence of high amounts of fibers, vitamins C and E,
carotenoids, and phenolic compounds such as caffeic, p-coumaric, and chlorogenic acids. For these reasons, it can help in the protection of the cardiovascular system due to the increase of total antioxidant status and reduced lipid peroxidation (Potter et al., 2011).

In an exciting review, Santana et al. (2019) showed that the pulp of Carica papaya is rich in fibers, vitamins such as A, B, C, and E, and minerals, such as magnesium and potassium. Phenolic compounds are also found in the seeds. Due to this composition, this fruit can be beneficial to the cardiovascular system and in the prevention of the harm produced by free radicals. Furthermore, it is helpful in the reduction of body weight, glycemia, cholesterol, hypertension, and could be useful in the prevention or treatment of obesity and related metabolic disorders. Similar results are found by studies that investigate the use of passion fruit (Passiflora sp) (De Faveri et al., 2020; Salles et al., 2020).

Similar and controversial effects are shown for the use of eggplant (Solanum melongena) once some studies shows effects in reducing plasma lipids and other show no interference (Sudheesh et al., 1999; Nwanna et al., 2019; Da Silva et al., 2020).

The use of green banana has been shown to improve body mass index and lipid and glycemic parameters. These effects are linked to the presence of resistant starch that shows excellent potential for reducing metabolic disorders, especially diabetes complications.

**Conclusion**

Authors have found that a diet supplemented with green banana can prevent oxidative damage in both liver and kidney of animals with induced type 1 diabetes mellitus due to the reduction of the oxidative stress and protection of tissues, avoiding symptoms of inflammatory diseases. Moreover, literature shows that animals fed a high-fat diet and green banana exhibited lower body weight gain when compared with animals without supplementation. They also observed an almost 30% reduction in liver steatosis and 93% in triacylglycerol in the liver. Besides, they found also observed an almost 30% reduction in liver steatosis compared with animals without supplementation. They reached alarming incidence in poor populations (Marrelli et al., 2020; Silva et al., 2016).

Chronic degenerative diseases are reaching epidemic proportions mainly in developed countries, but it is also reaching alarming incidence in poor populations (Marrelli et al., 2020). For this reason, the search for healthier products has been considered an option for the prevention of these conditions. As demonstrated by the study, the use of natural supplements can be beneficial in association with a balanced diet. However, this not the case when this balanced diet is combined with food rich in sugar.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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