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Research Article

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Refinement in Obesity Model Induced by Fructose with Intragastric Administration

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Abstract Obesity is defined as the accumulation of adipose tissue, overweight and obesity are defined on the basis of body mass index and this is accompanied by an increased prevalence of comorbidities as type 2 diabetes, cardiovascular and metabolic disease in the population. The research related to obesity, overweight and metabolic syndrome use animal models, rats and mice generally, these animal models use hypercaloric diets with fat content ranging from 12% to 49% in fat, in addition to the high-calorie diet with a high content of fats, solutions with a varied concentration of sweeteners up to 30% are used (fructose, glucose, galactose), with duration of treatment with diet and sweetened drinking water that goes from 3 to 5 months, also find genetically manipulated animals, but in most cases they are costly and inaccessible models for researchers. In 1959 William M. S. Russell and Rex L. Burch published the principle of the 3 R's for research (Replace, Reduce, and Refine), in this sense, we proposed to refine the model and to evaluate the intragastric administration of fructose syrup (80%), the results were rats with phenotype metabolically healthy but obesity in eight weeks with adiposity index of 11.4 for fructose syrup group vs 8.0 of water control group (fructose solution as drinking water) and 6.2 for control group, with elevated blood glucose at the fourth and seventh week of treatment.

Keyword: Obesity, 3R’s principle, Glucose Oral Tolerance, Body Mass Index, Adiposity Index

Introduction

The first works with animals date from the 5th century BC, Hippocrates (450 BC) relate aspects of diseases in man with diseases in animals, on the other hand Aristotle (384-322 BC) comparative studies of human and animal organs. The intensive use of animals for scientific experimentation data of the early nineteenth century. With the intensification of the use of animals in scientific research the first animal protection association was founded in England in 1824, and the primary legend for the use of animals in the research was proposed in Great Britain in 1876 - British cruelty To Animal Act, the North American public publication on ethical aspects of the use of animals for experimentation was published 33 years later, in 1909 [1].

In 1959 William M. S. Russell and Rex L. Burch published the principle of the 3 R's for research (Replace, Reduce, and Refine). In the sense of refinement starts from the choice of procedure for the purpose of the investigation [1].

Obesity is the accumulation of excess adipose tissue [2], the excessive consumption of carbohydrates and fats are the main factor that induces it [3]. Overweight and obesity are defined on the basis of body mass index (BMI), this is accompanied by an increased prevalence of comorbidities as type 2 diabetes (MD2), cardiovascular and metabolic disease in the population, included metabolic syndrome [4–7]. Around the world the obesity has increased more of

double since 1980 [8], currently affects 5% of children and 12% of adults [9, 10]. Currently, overweight and obesity is considered one of the main public health problems in the world as result of growing and development of big cities [11]. Special importance have the abdominal adipose tissue, which is strongly predictive of metabolic health [12, 13].

In this sense for the investigation of aspects related to obesity, overweight and metabolic syndrome they use animal models, rats and mice generally[14], with diets of special hypercaloric diets with fat content ranging from 12% to 49% in fat [15, 16], however there are diets with a content of up to 60% of fat, in addition to the high-calorie diet with a high content of fats, solutions with a varied concentration of sweeteners up to 30% are used [15–18], with duration of treatment with diet and sweetened drinking water that goes from 3 to 5 months [15], [16]. They also find genetically manipulated animals, but in most cases they are costly and inaccessible models for researchers.

However, in the animal models of obesity by hypercaloric diet, with standardized food with high fat content, or by diet of "coffee shop" and sweeteners solution, these are the only source of food and drinking water and its consumption in variable by rat and day, being these factors that explain the deviations in this obesity model. The variation in the physiological responses of animals affects the reliability and reproducibility of results, which is why the reliable part of a scientific investigation is reproducibility based on low variability.

In this work, oral administration of 80% fructose syrup is proposed for the induction of obesity in Wistar rats, obtaining good results even with the use of balanced commercial diet commonly used for the maintenance of animals in the animal housing. The time required is from 8 to 10 weeks and with high reproducibility. Refinement consists in carrying out oral administration of a solution with a high concentration of fructose based on the maximum volume that can be administered in this way with respect to weight, thereby making the use of financial resource and time efficient.

Materials and methods

Rats

In this research were used adult male Wistar rats of ten months of age. The rats were bred and acclimated in the vivarium. All experiments complied with the requirements and guidelines established by law respect at proper use, care, and management of laboratory animals.

80% fructose syrup

The fructose syrup at 80% was prepared with fructose powder alimentary grade at hot water bath (final solution with 0.007 ml tween/ml).

Treatment

Three groups of 7 rats were formed, control group (CG) with water and feed ad livitum, the intragastric introduction of feeding needles 10 seconds but without administration was did, the drinking water control group (WCG)was administeredv.o. with vehicle (water-tween 80®, 0.007 ml tween/ml water, 10 ml/kg) and fructose solution (30%) as drinking water, and feed with standard rodent diet. The fructose syrup group (FSG) was administrated v.o. with fructose syrup (80%, 10 ml/kg), and fructose solution (30%) as drinking water and feed with standard rodent diet. The food used was the Rodent Laboratory Chow 5001® ad livitum. The treatment was administrated by eight weeks.

Consumption of food and water

Every day was weighed the feed intake (Rat Chow 5001®) with bascule of 0.1 g precision. The consumption of fructose solution (30% as water drinking) was measured every day with probate of 2 ml precision.

Variation of weight

The rats was weighted daily in the morning with bascule of 1.0 g precision.

Weekly glucose

The blood glucose was recorded weekly in mg/dl by glucometer determination [19].

Body mass index and adiposity index

Body mass index was calculated with the weight (kg)/ square height (m^2). The adiposity index was used as an indicator of obesity because it enables the precise evaluation of body fat percentage [3]. This was evaluated in the 8th week with intraperitoneal fat calculated in 4 rats per group (this included epididimal fat + retroperitoneal fat + visceral fat) by X-ray with densitometer HOLOGYC® model DISCOVERY, the image was analyzed with HOLOGIC´s software. The adiposity index was obtained as (intraperitoneal fat calculated/body weight) X 100 [20].

Statistical analysis

Data was tested in normality and equal variances for parametric analysis, if any of this conditions not existed then was realized non parametric analysis.

Results

Weekly consumption of food and water

The table 1 and 2 shows that weekly food consumption and water (vehicle) diminished by oral administration of vehicle or fructose syrup (80%) and fructose solution (30%) as drinking water. The oral administration of fructose syrup (80%) generate the highest reduction of consumption of balanced food and water.

Table 1: Median food weekly consumption (gr)

Group	Week							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
CG*	21±1	22±2	25±1	24±1	26±1	25±1	25±1	25±1
WCG*	14±1	12±1	11±1	11±1	11±1	10±1	15±5	12±1
FSG*	9±1	9±1	8±1	7±1	7±1	8±0	8±0	7±0

Groups with n=7. CG: Control group; WCG: Water control group; FSG: Fructose syrup group. Median ± Standard error. ANOVA by ranks. Holm-Sidak Post-Hock test. *P<0.05.

Table 2: Median weekly water consumption (ml)

Group	Week							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
CG*	40±3	45±3	47±2	50±2	51±2	54±2	53±1	52±1
WCG*	27±2	41±3	40±2	44±3	44±3	44±2	46±3	45±3
FSG*	25±3	35±2	27±2	29±2	28±4	28±2	27±2	24±2

Groups with n=7. CG: Control group; WCG: Water control group; FSG: Fructose syrup group. Median ± Standard error. ANOVA by ranks. Holm-Sidak Post-Hock test. *P<0.05.

Variation of weight

The figure 1 shows the effect of oral administration of emulsion with fructose solution (30%) and fructose solution (with oral administration of emulsion vehicle) on percentage of variation of weight. Both groups have a percentage weight reduction compared with intact control group, but without differences between them.

Table 3: Median weekly weight(gr).

Group	Week								
	Day 1	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
CG*	472±12	472±12	472±10	472±10	472±11	479±13	486±13	490±13	489±12
WCG*	612±15	612±15	594±17	589±17	584±17	583±17	584±18	583±18	580±16
FSG*	545±24	545±24	526±15	530±14	521±12	517±11	526±11	532±12	530±11

Groups with n=7. CG: Control group; WCG: Water control group; FSG: Fructose syrup group.Median ± Standard error. ANOVA. Holm-Sidak Post-Hock test. *P<0.05.

Blood glucose weekly

The figure 1 shows the blood glucose weekly, the fructose solution (30%) as drinking water increased the blood glucose weekly, this effect was greater with oral administration of fructose syrup (80%). The results shows an increase of blood glucose in the fourth week, but is greater in the fructose syrup group, the blood glucose low in fifth and sixth week but upped again in seventh week, the fructose syrup group was again of the greater register.

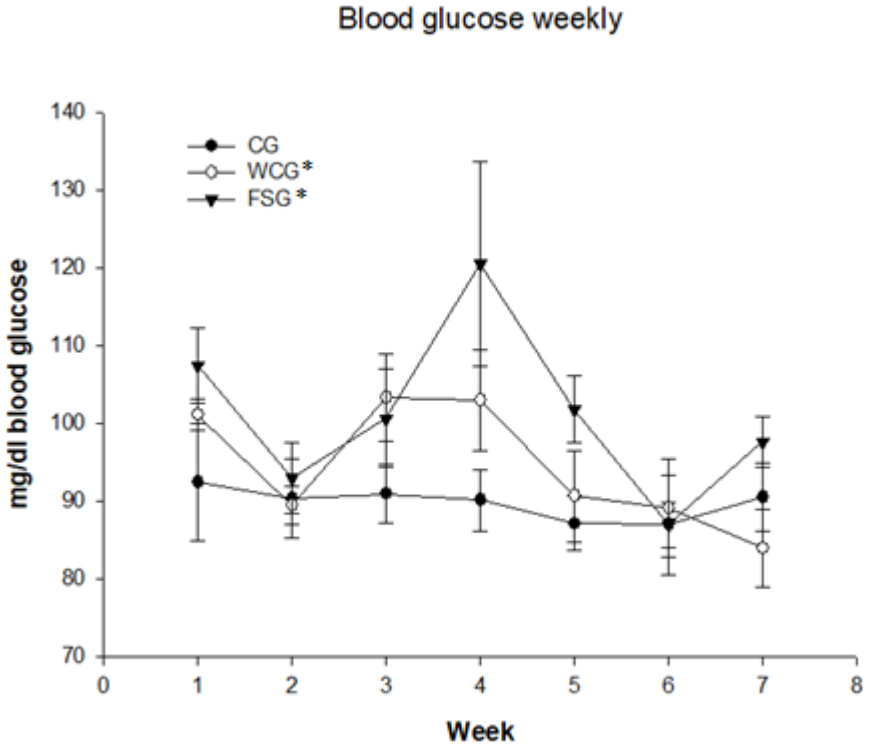

*Figure 1: Blood glucose weekly. CG: Control group; WCG: Water control group; FSG: Fructose control group. *P<0.05*

Body mass index and adiposity index

The table 4 shows the percentage of visceral fat calculated by X-ray (figure 3) and adiposity index. The percentage of visceral fat presented statistical difference the sham group and emulsion group with respect at control group but no have differences between them. On adiposity index the sham group and emulsion group present statistical difference with respect to control group and between them.

Table 4: Body mass index and adiposity index

Group	BMI	AI
CG	7.6±0.6*	6.2±0.7*
WCG	9.2±0.1	8.0±0.1*
FSG	8.7±0.2	11.4±0.3*

Groups with n=4. BMI: Body mass index. Adiposity index (AI). CG: Control group; WCG: Water control group; FSG: Fructose syrup groupMedian ± Standard error. ANOVA, Holm-Sidak Post-Hock test. *P<0.05.

Discussion

The consumption of feed (table 1) and water (table 2) of control group is compeered with the consumption reported by Francisqueti et al 2017 with rats of 10 weeks of age with 6, 12 and 24 weeks of feeding of an intact control group, and the consumption of our WCG is compared with the groups administered with fructose solution [3]. The

group with oral administration of fructose syrup (80%) take around a third rations of feed and a half of fructose solution (30%) as drinking water with respect to CG.

The current animal models with special foods and any sweetener (glucose, fructose, galactose) to increased body weight, body mass index and visceral fat with increase in triglycerides, cholesterol and glucose serum, that is, they generate a models of obesity with metabolic disorders and elevated weight [5, 14, 21–24]. The fructose syrup (80%) evaluated in this work, generate a loss weight light (table 3) compared with others treatments reported, but increased fatty tissue compared with control group and fructose solutions as drinking water, it is shown in table 4 with body mass index (BMI) and adiposity index (AI). Nevertheless, adiposity index in FSG increase almost double with respect to control group ($p>0.05$), inclusive more than WCG (Table 4). Also is important de glucose blood in the time, the FSG and WCG increased the blood glucose into fourth week, but is greater in FSG, the blood glucose decreased into fifth and sixth weeks and increased in seventh week, is probably that the adipocity tissue diferencition and some methabolic mechanisms were activated or desactivated in the fourt and seventh weeks, the perspective of this research is to research the molecular process that will let to know the metabolic mechanims in those weeks.

The study and understanding of obesity causes are important, the subjects with obesity without overweight represent more than 20% of the obese population, and understanding their causes will allow developing appropriate therapies for the treatment of obesity and for the timely diagnosis of the development of this [25–29].

Conclusion

The intragastric fructose syrup (80%) let to generate obesity in rats without overweight with glucose blood altered in fourth and seventh weeks.

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