ABCDDV/933

ABCD Arq Bras Cir Dig
2013;26(Suplemento 1):17-21

Original Article

OBESITY INDUCTION WITH HIGH FAT SUCROSE IN RATS

Indução de obesidade com sacarose em ratos

Andressa Bressan **MALAFAIA**, Paulo Afonso Nunes **NASSIF**, Carmen Australia Paredes Marcondes **RIBAS**, Bruno Luiz **ARIEDE**, Karen Negume **SUE**, Manuela Aguiar **CRUZ**

From the Post-Graduate Program in Principles of Surgery, Faculdade Evangélica do Paraná / Hospital Universitário Evangélico de Curitiba and Instituto de Pesquisas Médicas, Curitiba, PR, Brazil

ABSTRACT - *Background* - Although is complex to identify the factors responsible for the important growth in obesity all over the world, the main causes are increased consumption of energy, highly saturated fats and sugars, and reduced physical activity. ***Aim*** – To compare rats with normal and supplemented diet with sucrose in relationship to body mass, weight of gonadal and retroperitoneal fat and Lee index. ***Methods*** - Forty rats were divided into two groups: 20 in the control group that received normal chow diet and water for three months, and 20 animals in the experimental group who received the same diet but supplemented with sucrose 300 g/l of water. The animals were weighed once a week during 91 days. At scheduled death, they had measured the naso-anal length, body weight and Lee index. After laparotomy, retroperitoneal and gonadal fat were isolated, dried and the percentage of weight in relation to body weight at the date of death was evaluated. ***Results*** - There was a statistic significant difference between the 14th and 78th day favoring the experiment group indicating that sucrose interferes with weight gain in rats. The average weight was higher in the experimental group in all periods in comparison to initial weight. There was also significant difference in the weight of the gonadal and retroperitoneal fat. There was no significant difference comparing the Lee index. ***Conclusion*** – The body mass index was higher in animals treated with diet supplemented with sucrose and had higher gonadal and retroperitoneal fat, but no difference in the Lee index.

HEADINGS - Obesity. Wistar rats. Sucrose. Abdominal fat.

Correspondence:
Andressa Bressan Malafaia
E-mail: debremalafaia@hotmail.com

Financial source: none
Conflicts of interest: none

Received for publication: 05/02/2013
Accepted for publication: 26/04/2013

RESUMO - ***Racional*** - Embora seja muito complexo identificar o que levou ao grande crescimento da obesidade, as causas principais são o aumento do consumo de alimentos energéticos e ricos em gorduras saturadas e açucares, e a redução das atividades físicas. ***Objetivos*** – Comparar a massa corpórea, o peso da gordura retroperitoneal e gonadal e o índice de Lee em ratos submetidos à dieta normal e à suplementada com sacarose. ***Métodos*** - A amostra foi de 40 animais, divididos em dois grupos: 20 do grupo controle que receberam dieta de ração normal e água por três meses e 20 do grupo experimento que receberam a mesma ração suplementada com sacarose 300 g/l na água. Os animais foram pesados uma vez por semana durante 91 dias. Na data da morte foram aferidos o comprimento nasoanal, peso corporal e foi calculado o índice de Lee. Após, foram submetidos à laparotomia e a gordura retroperitoneal e gonadal foram individualizadas, ressecadas e avaliada a porcentagem do peso dela em relação ao corporal na data da morte. ***Resultados*** - Verificou-se diferença estatisticamente significativa entre o 14º até o 78º dia, quando comparados os grupos indicando que a sacarose interfere no ganho de peso dos ratos. A média de peso foi maior no grupo experimento em todos os períodos, tendo como referência o peso inicial. Verificou-se diferença significativa a favor do grupo experimento no peso da gordura gonadal e retroperitoneal. Não houve diferença significativa da comparação do índice de Lee. ***Conclusão*** - A massa corpórea foi maior nos animais submetidos à dieta suplementada, com maior gordura retroperitoneal e gonadal e sem diferença no índice de Lee.

DESCRITORES - Obesidade. Ratos Wistar. Sacarose. Gordura abdominal

INTRODUCTION

Although it is very complex to identify the reasons for rapid growth of obesity, the main causes are increased consumption of energy and food rich in saturated fats and sugars, and reduced physical activity. Factors such as economic growth, modernization, urbanization and globalization also drive the increase in obesity in society.

Study released by the Brazilian Ministry of Health, indicates that overweight and obesity have increased in the country in 2006-2011 period. Pursuant to the Surveillance of Risk and Protective Factors for Chronic Diseases survey, the proportion of overweight people in Brazil increased from 42.7% in 2006 to 48.5% in 2011 - while the percentage of obese rose from 11.4% to 15.8% during the same period.

The high technological standard of the society has been changing the health concept, and the introduction of new chemicals has changed the eating habits of humanity, who is increasingly searching for a healthy lifestyle. For simple esthetic or health problems, the human been is replacing the known and consecrated sugar (sucrose) for products such as composed sweeteners that taste similar to sucrose, but with low or non caloric features.

Carbohydrates, modernly, can also be grouped as having high and low glycemic index.

A few years ago, there was a believe that the high fat ingestion was one of the factors that mostly contribute to obesity. However, today it´s known that reducing the amount of ingested fat does not necessarily result in a decrease in the prevalence of obesity since such a measure is, in most cases, associated with carbohydrates increase consumption. Since years ago, it has been recommended for the general population to decrease the fat ingestion in order to prevent cardiovascular disease, obesity, diabetes mellitus type 2, among other chronic diseases.

The mechanism is regulated by the interaction of several metabolic factors such as glucose, cytokines, hormones leptin, ghrelin, insulin, among others. Leptin levels indicate the amount of fat and metabolic status (triglyceride synthesis) of adipocytes.

As the individuals become obese, the characteristics of fat deposits changes, increasing deposition in intra and retroperitoneal abdominal area, subcutaneous tissue and, in advanced stages, deposition in the muscles, liver and pancreas. The fat storage is accompanied by a progressive increase in the health risk. A quick determination of obesity in rats was described by Lee in 1928. It consists in the division of the cubicle root of the weight in grams by the nasoanal length in millimeters multiplied by 1000. The result sets the nutritional content or Lee index of obesity mensuration. The Lee index and the fat mass have a correlation. It can be used as a fast and accurate way to measure obesity in an experiment subjected to a weight gain method. It becomes necessary the association of an index with others anthropometric data such as waist circumference, nasoanal length and metabolic data.

The bioelectrical impedance observed that anthropometric parameters - body mass index, Lee index and circumference of the abdomen - can be used to estimate body composition in rats.

In laboratory animals the obesity genesis is mostly related with genetic mutations, but it is far from that found in humans. The adoption of hypercaloric or hyperlipidemic diets have been used as a model to induce obesity in animals due to its similarity to the genesis and the metabolic responses arising from obesity in humans.

The goal of this study was to compare the body mass, the fat gonadal and retroperitoneal weight and the Lee index in rats submitted to normal and supplemented diet with sucrose.

METHOD

This study was approved by the Animal Ethics Committee of the Evangelic School of Paraná, Curitiba, PR, Brazil.

Were used 40 male rats, Wistar (*Rattus norvegicus albinus*) with an average weight of 170 g divided into two groups of 20 animals each, being 20 for the Control Group, fed ad libitum with normal chow for the species and water for three months, and 20 for Experimental Group which besides receiving the same diet ad libitum, had water supplemented with sucrose (300 g/l). The animals were individually placed in a container to be weighed once weekly for 91 days. At the scheduled death dates, were measured the nasoanal lengths, the body weight and calculated the Lee indexes between the study groups. This index was calculated by dividing the cube root of body weight (g) by nasoanal length (cm) and multiplying the result by 1000. Xyphopubic median incision was made for resection of retroperitoneal and perigonadal structures (Figure 1) and evaluated the percentage of weight relative to body weight at the time of death.

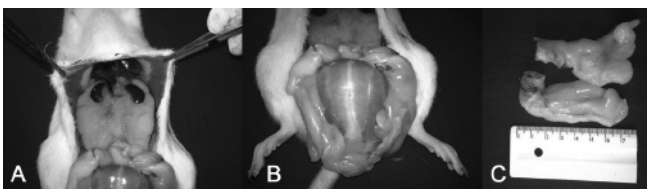

FIGURE 1 - A) Retroperitoneal fat; B) gonadal fat; C) adipose tissue

Data were analyzed with the Statistica v.8.0 software. All data collected were analyzed and the results posted in the database form of Excel spreadsheet. To compare the groups with respect to the weights evaluated at every moment and in relation to weight differences in the initial weight, was considered the Student t test for independent samples. P values <0.05 were considered statistically significant.

RESULTS

Of the 40 animals selected at the beginning of the study, 38 were evaluated. Two of the Control Group and one from the Experimental Group died in the first half of the study for non determined reasons.

When comparing the control and experimental groups regarding weight, there was significant difference between the 14th and 78th days, indicating that sucrose affected the Experimental Group weight gain (Table 1).

TABLE 1 – Difference in weight between the Control and Experimental Groups

Weight (g)	Group	n	Mean	Median	Minimum	Maximum	Standard deviation	p*
Initial	GC	18	166,7	167,0	145,0	188,0	14,2	
	GE	19	165,1	170,0	137,0	192,0	16,9	0,748
7 days	GC	18	210,2	214,0	162,0	241,0	20,5	
	GE	19	220,9	228,0	182,0	258,0	22,6	0,138
14 days	GC	18	251,3	253,0	205,0	280,0	20,1	
	GE	19	267,1	266,0	231,0	304,0	22,0	0,030
21 days	GC	18	274,3	274,0	238,0	303,0	19,1	
	GE	19	303,4	299,0	270,0	335,0	20,0	<0,001
28 days	GC	18	304,8	306,5	270,0	337,0	21,8	
	GE	19	337,2	340,0	292,0	380,0	22,8	<0,001
35 days	GC	18	312,4	314,5	265,0	350,0	25,3	
	GE	19	347,3	342,0	298,0	392,0	26,0	<0,001
42 days	GC	18	314,4	315,0	276,0	366,0	25,6	
	GE	19	357,9	351,0	314,0	402,0	25,3	<0,001
49 days	GC	18	337,1	334,5	302,0	380,0	23,3	
	GE	19	374,7	382,0	321,0	431,0	26,5	<0,001
56 days	GC	18	343,7	332,0	300,0	393,0	25,7	
	GE	19	387,6	384,0	322,0	458,0	34,8	<0,001
63 days	GC	18	365,1	356,5	338,0	400,0	23,1	
	GE	19	405,7	406,0	362,0	488,0	34,3	<0,001
71 days	GC	18	360,3	354,0	308,0	410,0	29,3	
	GE	19	415,3	412,0	347,0	510,0	38,2	<0,001
78 days	GC	18	376,6	370,5	325,0	423,0	26,6	
	GE	19	424,7	432,0	313,0	486,0	38,8	<0,001
85 days	GC	18	383,3	381,0	329,0	437,0	26,2	
	GE	19	418,6	423,0	333,0	500,0	45,0	0,007
92 days	GC	18	400,2	388,5	338,0	462,0	33,6	
	GE	19	444,3	443,0	371,0	509,0	37,0	0,001

*Student t test for independent samples, p<0,05. GC = Control Group. GE = Experiment Group

Weight changes in relation to the initial assessment were compared between the control and experimental groups. Regarding these variations there was a significant difference between the groups in all periods with reference to the initial weight (Dif 7 days -Table 2).

The gonadal and retroperitoneal fat weight showed a significant difference favoring the experimental group (Table 3).

TABLE 2 – Weight differences between Experiment and Control Groups with respect to Dif 7 days

Variable	Group	n	Mean	Median	Minimum	Maximum	Standard deviation	p*
Dif 7 days	GC	18	43,4	46,5	16,0	73,0	12,6	
	GE	19	55,9	57,0	36,0	66,0	7,7	0,001
Dif 14 days	GC	18	84,6	84,5	59,0	101,0	11,9	
	GE	19	102,0	104,0	82,0	123,0	10,0	<0,001
Dif 21 days	GC	18	107,6	111,0	78,0	146,0	16,1	
	GE	19	138,3	142,0	117,0	158,0	12,4	<0,001
Dif 28 days	GC	18	138,1	139,0	116,0	157,0	12,7	
	GE	19	172,2	171,0	143,0	200,0	14,7	<0,001
Dif 35 days	GC	18	145,7	148,0	107,0	168,0	17,6	
	GE	19	182,2	181,0	148,0	226,0	19,4	<0,001
Dif 42 days	GC	18	147,7	145,0	115,0	178,0	17,9	
	GE	19	192,9	198,0	162,0	236,0	21,1	<0,001
Dif 49 days	GC	18	170,4	165,5	150,0	198,0	14,4	
	GE	19	209,6	206,0	172,0	265,0	22,5	<0,001
Dif 56 days	GC	18	177,0	174,0	150,0	211,0	16,6	
	GE	19	222,5	229,0	159,0	292,0	31,9	<0,001
Dif 63 days	GC	18	198,4	194,5	182,0	223,0	13,6	
	GE	19	240,6	244,0	186,0	322,0	31,8	<0,001
Dif 71 days	GC	18	193,6	190,0	158,0	233,0	21,3	
	GE	19	250,2	249,0	196,0	344,0	37,7	<0,001
Dif 78 days	GC	18	209,9	209,0	163,0	247,0	19,7	
	GE	19	259,7	259,0	142,0	315,0	42,8	<0,001
Dif 85 days	GC	18	216,6	219,0	167,0	254,0	20,4	
	GE	19	253,5	252,0	156,0	330,0	45,7	0,004
Dif 92 days	GC	18	233,4	229,0	176,0	308,0	28,4	
	GE	19	279,2	279,0	194,0	347,0	37,9	<0,001

* Student t test for independent samples, p<0,05. GC = Control Group. GE = Experiment Group

TABLE 3 – Weight differences in gonadal and retroperitoneal fat on Control and Experimental Groups

Peso	Group	n	Mean	Median	Minimum	Maximum	Standard deviation	p*
Gonadal fat weight (g)	GC	18	8,9	8,7	3,2	14,1	2,7	0,005
	GE	19	12,4	12,4	6,1	22,5	4,3	
Retroperitoneal fat weight (g)	GC	18	7,5	7,8	1,9	13,2	2,9	0,003
	GE	19	11,9	11,1	5,3	27,7	5,1	

* Student t test for independent samples, p<0,05. GC = Control Group. GE = Experiment Group

In Table 4 is shown a comparison of Experiment and Control Groups regarding the Lee index, with no significant difference between the groups.

TABLE 4 – Lee index difference in Control and Experimental Groups

	Group	n	Mean	Median	Minimum	Maximum	Standard deviation	p*
Lee index	GC	18	322,3	325,5	295,5	337,8	11,4	
	GE	19	323,9	321,7	309,7	353,3	12,3	0,688

* Student t test for independent samples, p<0,05. GC = Control Group. GE = Experiment Group

DISCUSSION

The great similarity and homology between the genomes of rodents and humans make these animal models important tool for the study of conditions

that affect humans and can be simulated in rats. Obesity can be induced in animals by neuroendocrine, dietary or genetics. The models used to induce obesity in rats are injury hypothalamic nucleus - by administration of monosodium glutamate or direct electrical injury -, ovariectomy, hypercaloric diet feeding and genetic manipulation[20].

Hariri and Thibault[6] demonstrated in epidemiological studies positive relationship between fat intake and obesity. Since mice and rats showed similarity, they are considered appropriate model for the study of obesity diets.

Kanazawa et al.[9] examined the gain in body weight with sucrose feeding on plasma triglycerides and stress tolerance in rats. Feeding with sucrose diet (60%) for two weeks did not induce higher body weight gain compared to the standard diet. In the present study using sucrose (30%) from the second week there was a greater weight gain in the sucrose group compared to control (standard diet).

Kanazawa et al.[9] related that sucrose diet did not induce obesity in mice. The diet increased plasma triglyceride amount in lean and obese mice. In this study, differently, was found that the addition of sucrose in the diet induced obesity in rat.

Kawasaki et al.[11], as in this study using sucrose diet (30%), investigated in long-term if it causes hyperglycemia in male rats; at the end, it showed body weight increase and glucose intolerance in normal male rats. In relation to weight gain this study also differs from the results of these authors.

Nascimento et al[14]. induced obesity in rats with normal and hypercaloric diet. They promote obesity and found several characteristics commonly associated with human obesity.

Hariri and Thibault[6], described the use of high-fat diets to induce obesity in animals, aiming to clarify the consequences of changing the amount and type of fat in weight gain, body composition and adipose tissue cellularity; also explored the contribution of genetics, gender, individual biochemistry and roles of hormones (leptin, insulin and ghrelin) in animal models. They concluded that more studies are needed to prove that the body weight can be regulated by fatty acid profile in high-fat diets.

Feijo used standard diet, saccharin (0.3%) and sucrose (20%). Weight gain was significant between groups showing better performance on the 8th week. In this study, weight gain between normal diet group and sucrose occurred on 2nd week.

Nemosek, et al[16]. compared a diet based on honey with sucrose evaluating weight biomarkers (insulin, leptin and adiponectin) and better blood lipid profile. The body weight gain was 14.7% lower ($p \leq 0.05$) in the group with a diet based on honey. Weight of epididymal fat was 20.1% lower ($p \leq 0.05$) for rats fed with diets based on honey. Here, comparing the weight of the gonadal fat, it was higher in the group with standard diet with addition of sucrose (30%) compared to the standard diet group.

The Lee index can be used as a fast and accurate way to determine obesity in rats subjected to a treatment weight gain. Bernardis and Patterson, described the determination of obesity in rats proposed by Lee in 1928. It consists in dividing the cube root of the weight in grams and nasoanal length in millimeters and multiplied by 1000. The result sets the nutritional content or Lee index, as measure of obesity. The results below 0.300 are considered normal.

Kanarek and Marks-Kaufman[8], evaluated daily caloric intake and body weights measured from weaning at 70 days of age in male rats with standard diet and water, or a standard diet and the 32% sucrose and water. Lee index and levels of fasting blood glucose levels were determined at 46, 57 and 70 days of age. The sucrose and control groups did not differ in body weight. Although there were no differences in body weights between the two groups, the Lee index was significantly higher in sucrose animals than controls, as early at 46 days of age. In this study, the Lee index was determined 91 days and showed no significant difference between groups.

Nascimento et al.[15] evaluated rats randomly divided into two groups: normal diet (3.5 kcal/g) and high caloric diet (4.6 kcal/g). The variables analyzed were body weight, body composition, body weight in relation to length, Lee index, body mass index and the probability of misclassification. The results of this experiment showed that the probability of classification error occurs when the manipulation of the diet is used to promote obesity in animals. This misjudgment varies from 19.49% to 40.52% on high calorie diet and 18.94% to 41.30% in the normocaloric.

Angéloco, et al.[2] used bioelectrical impedance analysis in rats fed diets - high in fat and sucrose -, and correlated with the analysis of biochemical and anthropometric parameters. The bioelectrical impedance was not sensitive enough to detect changes in body composition; however, anthropometric parameters - body mass index, index of Lee and circumference of the abdomen - can estimate body composition in rats.

The main factors that contribute to dietary obesity (hyperphagia, energy density and postprandial effects of high fat diet) are still in scientific discussion. Interesting area for future research is to investigate whether different diets in animals before obesity can be predictors of prone or resistant phenotypes and assess feeding on circadian rhythm differences.

CONCLUSIONS

The body mass index was higher in animals treated with diet supplemented with sucrose and had higher gonadal and retroperitoneal fat, but no difference in the Lee index.

REFERENCES

1. Angeloco LRN, Deminice R, Leme IA, Lataro RC, Jordão AA. Bioelectrical impedance analysis and anthropometry for the determination of body composition in rats: effects of high-fat and high-sucrose diets. Rev. Nutr. 2012; 25(3):331-339.
2. Bernardis LL, Patterson BD. Correlation between 'Lee index' and carcass fat content in weanling and adult female rats with hypothalamic lesions. J Endocrinol. 1968 Apr;40(4):527-8.
3. Bray GA, Popkin BM. Dietary fat intake does affect obesity! Am J Clin Nutr 1998;68(6):1157-73
4. Feijo FM. Efeito da suplementação com sacarina e sacarose no ganho de peso e consumo energético em ratos Wistar com dieta não restrita. [dissertação] Universidade Federal do Rio Grande do Sul - UFRS, Porto Alegre, 2010
5. Guttierres APM, Alfenas RCG. Efeitos do índice glicêmico no balanço energético. Arq Bras Endocrinol Metab. 2007; 51(3): 382-388.
6. Hariri N, Thibault L. High-fat diet-induced obesity in animal models. Nutr Res Rev. 2010 Dec;23(2):270-99.
7. Heikal AH, Badawy OM, Hafez AM. Genetic relationships among some Stevia (Stevia Rebaudiana Bertoni) accessions based on ISSR analysis. Research Journal of Cell and Molecular Biology, 2008; 2(1):1-5.
8. Kanarek RB, Orthen-Gambill N. Differential effects of sucrose, fructose and glucose on carbohydrate-induced obesity in rats. J Nutr. 1982 Aug;112(8):1546-54.
9. Kanazawa M, Xue CY, Kageyama H, Suzuki E, Ito R, Namba Y, Osaka T, Kimura S, Inoue S. Effects of a high-sucrose diet on body weight, plasma triglycerides, and stress tolerance. Nutr Rev. 2003 May;61(5 Pt 2):S27-33.
10. Kaushik R, Narayanan P, Vasudevan V, Muthukumaran G, Usha A. Nutrient composition of cultivated stevia leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts. J Food Sci Technol. 2010 Jan;47(1):27-33.
11. Kawasaki T, Kashiwabara A, Sakai T, Igarashi K, Ogata N, Watanabe H, Ichiyanagi K, Yamanouchi T. Long-term sucrose-drinking causes increased body weight and glucose intolerance in normal male rats. Br J Nutr. 2005 May;93(5):613-8.
12. Lancha Jr A. Obesidade: uma abordagem multidisciplinar. Rio de Janeiro: Guanabara kogan, 2006.
13. Lucas RWC. Metabolismo energético e nutrição aplicados a dermato funcional. In: Nutrição aplicado à fisioterapia. Edição Digital. 2ed. 2010.
14. Nascimento AF, Sugizaki MM, Leopoldo AS, Lima-Leopoldo AP, Nogueira CR, Novelli EL, Padovani CR, Cicogna AC. Misclassification probability as obese or lean in hypercaloric and normocaloric diet. Biol Res. 2008;41(3):253-9.
15. Nascimento AF, Sugizaki MM, Leopoldo AS, Lima-Leopoldo AP, Luvizotto RA, Nogueira CR, Cicogna AC. A hypercaloric pellet-diet cycle induces obesity and co-morbidities in Wistar rats. Arq Bras Endocrinol Metabol. 2008 Aug;52(6):968-74.
16. Nemoseck TM, Carmody EG, Furchner-Evanson A, Gleason M, Li A, Potter H, Rezende LM, Lane KJ, Kern M. Nemoseck TM, Carmody EG, Furchner-Evanson A, Gleason M, Li A, Potter H, Rezende LM, Lane KJ, Kern M. Nutr Res. 2011 Jan;31(1):55-60.
17. Polacow VO, Lancha Junior, AH. Dietas hiperglicídicas: efeitos da substituição isoenergética de gordura por carboidratos sobre o metabolismo de lipídios, adiposidade corporal e sua associação com atividade física e com o risco de doença cardiovascular. Arq Bras Endocrinol Metab. 2007; 51(3):389-400.
18. Portal Brasil. Pesquisa indica que quase metade dos brasileiros está acima do peso. Acessado em: 10/11/2012. <http://www.brasil.gov.br/noticias/arquivos/2012/04/10/pesquisa-indica-que-quase-metade-dos-brasileiros-esta-acima-do-peso>
19. Romero CEM, Zanesco A. O papel dos hormônios leptina e grelina na gênese da obesidade. O papel dos hormônios leptina e grelina na gênese da obesidade. Rev Nutr. 2006;19(1):85-91
20. Von Diemen V, Trindade EN, Trindade MR. Experimental model to induce obesity in rats. Acta Cir Bras. 2006 Nov-Dec;21(6):425-9.