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Effects of dietary high fructose corn syrup on regulation of energy intake and leptin gene expression in rats

Efeitos da ingestão de xarope de milho com alto teor de frutose na regulação da ingestão energética e na expressão gênica de leptina em ratos

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ABSTRACT

Objective

To evaluate in Wistar rats the effect of chronic use of high fructose corn syrup on serum lipids, body weight, energy intake regulation, and expression of associated genes.

Methods

For 11 weeks, male rats were fed a standard diet with either water (control) or 15% high fructose corn syrup solution, or fed a high-fat diet. The rats' food intake and body weight were measured weekly. Expression of leptin and fatty acid synthase genes was quantified in their brain and adipose tissue upon sacrifice at age 119 days using real-time polymerase chain reaction.

Results

The intake of 15% high fructose corn syrup did not affect the rats' weight, only the rats on the high-fat diet gained significant weight. The rats in both diets had lower levels of leptin expression and high levels of fatty acid synthase in the brain, which were associated with high serum triglycerides.

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Conclusion

Fifteen percent high fructose corn syrup intake and the high-fat diet reduced leptin gene expression in the brain of Wistar rats, with differential effects on weight gain.

Keywords: Body weight. Diet, high-fat. Fatty acid synthase gene. Fructose. Leptin.

RESUMO

Objetivo

Avaliar em ratos Wistar *o efeito do consumo crônico de xarope de milho com alta concentração de frutose sobre os lipídeos séricos, peso corporal, regulação da ingestão energética e expressão de genes associados.*

Métodos

Durante 11 semanas, ratos machos foram alimentados com uma dieta padrão com água (controle) ou 15% de xarope de milho com alta concentração de frutose, ou com uma dieta hiperlipídica. A ingestão alimentar e o peso corporal dos ratos foram medidos semanalmente. Os animais foram sacrificados com 119 dias de vida, e as expressões gênicas de leptina e da sintetase de ácidos graxos foram quantificadas no cérebro e no tecido adiposo usando a reação em cadeia da polimerase em tempo real.

Resultados

O consumo de 15% de xarope de milho com alto teor de frutose não afetou o peso dos animais, somente os ratos da dieta hiperlipídica aumentaram de peso significativamente. Nas dietas hiperlipídica e com alto teor de frutose, foram evidentes expressões mais baixas de leptina e mais altas de sintetase de ácidos graxos no cérebro, assim como concentrações mais altas de triacilglicerídeos séricos.

Conclusão

Ingestão de xarope de milho com alta concentração de frutose a 15% ou de dieta hiperlipídica diminuíram a expressão gênica de leptina no cérebro de ratos Wistar*, com diferentes efeitos sobre o aumento de peso.*

Palavras-chave: *Peso corporal. Dieta hiperlipídica. Gene de sintetase de ácidos graxos. Frutose. Leptina.*

INTRODUCTION

In Mexico the use of High Fructose Corn Syrup (HFCS) as a sweetener has increased in the last 20 years from 0.09 kg *per capita* in 1990 to 5.3 kg in 2000 and 12.5 kg in 2013[1]. Fruit juices, nectars, soft drinks, and cookies are foods that compose the diet of Mexican children sweetened with HFCS. High intake of these foods is associated with the nutritional transition toward ever growing prevalences of overweight and obesity in children[2], and obesity and diabetes in adults[3]. Associations between sugar intake from beverages and weight gain[4], type 2 diabetes[5-7], cardiovascular disease, and metabolic syndrome have been reported[8,9].

High fructose corn syrup was initially regarded as a sweetener suitable for diabetic patients because it has low glycemic index compared with glucose[10]. However, HFCS intake may promote the development of chronic diseases, such as diabetes and cardiovascular disease[11], which have been related to hypertriglyceridemia[12] and insulin resistance[13] in patients with prolonged HFCS intake.

Direct fructose administration into the hypothalamic arcuate nucleus increases food intake in rats and regulates the expression of some genes, such as orexigenic neuropeptide Y and agouti protein[14]. Glucose but not fructose changes blood flow in brain regions associated with appetite and reward pathways, affecting hunger, fullness, and satiety[15]. Hence, fructose may be associated with lower satiety and thus favor weight gain. The objective of this study was to evaluate the effects of chronic fructose intake on weight gain, on leptin gene expression in adipose tissue and the brain, and on metabolic indicators.

METHODS

Male rats aged 21 days were housed in standard polycarbonate shoebox cages under a 12 hour light and 12 hour dark regimen at 24±2°C and 55±10% humidity. The control rats received water *ad libitum* and were fed a standard diet (Formulab Chow 5008, Purina®, Framinghn, Massachusetts, United States). The other groups received defined diets: High-Fructose diet (HFr, standard diet containing 3.5 kcal/g and HFCS 55 solution in deionized water at 15% concentration) and High-Fat diet (HFat, diet containing 6.5 kcal/g with 40% calories from margarine) for 14 weeks.

Body weight, water intake, and food intake were measured weekly. Blood samples were obtained at 45 and 98 days of life to quantify plasma levels of triglycerides, total cholesterol, High-Density Lipoprotein (HDL) cholesterol, and glucose, using commercial enzymatic colorimetric kits (Winer Lab®). All procedures were approved by the *Universidad Autónoma del Estado de Hidalgo* Animal Research Committee.

Gene expression

At 14 weeks of life the rats were killed in the morning after fasting for 8 hours. After dissection, brain tissue and mesenteric adipose tissue were washed in dulbecco's phosphate-buffered saline to eliminate blood contamination and immediately frozen in liquid nitrogen. Total Ribonucleic Acid (RNA) was extracted with TRIzol® reagent (Invitrogen®, Carlsbad, California, United States) supplemented with Ribonuclease (RNAse)-free Deoxyribonuclease (DNAse), and cleaned with the Qiagen RNeasy® mini kit according to the manufacturer's instructions. RNA integrity was assessed by gel electrophoresis. Complementary DNA (cDNA) was synthesized at 42°C for 60 minutes using 2 μg of total RNA and 0.5 μg of Oligo (dT) 18 primers (Invitrogen®) denatured at 72°C for 10 minutes. Then, strand buffer (1X), 0.5 μM dithiothreitol, 500 μM of each deoxyribose-containing Nucleotide Triphosphate (dNTP), and 200 U of moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT) were added (Invitrogen®). Subsequently, MMLV-RT was inactivated at 72°C for 10 minutes.

Polymerase Chain Reaction (PCR) was performed in a Techne® TC-512 Thermal cycler (Stone, Staffordshire, United Kingdom). The relative expression levels of the genes of interest and housekeeping genes (beta-actin and 18S rRNA) were determined by semi-quantitative PCR. Each reaction (20 μL) contained 20 mM Tris/hydrochloric acid, 50 mM potassium chloride, 2 mM $MgCl_2$, 200 μM dNTP, 0.25 pmol/μL specific primers, and 2.5 U of TaqDNA polymerase (Invitrogen®, Dun Looghaire, Dublin, Ireland). Primers were designed with Primer Premiere 5.0 software and the sequences are as follows: Fatty Acid Synthase (FAS) forward (fo) 5'CTC CGT GGA ACA AAG GAG TG 3', FAS reverse (re) 5' GTC AAA GGG CAG AGG CAT AG 3'; Leptin (LEP) fo 5'AAA AGA ACG GGA CAG AAC AAC 3', LEP re: 5'TGA CCA AGG TGA CAT AGC G 3'; Beta-actin fo: 5' ACT GCC GCA TCC TCT TCC TC 3', Beta-actin re: 5' TCTGCTTGCTGATCCACATC 3', 18S fo: CCTGAGAAACGGCTACCACATC and 18 re: CTTTCGCTCTGGGTCGTCTTGC, where A (Adenine), C (Cytosine), G (Guanine), and T (Thymine). The intensity of the amplified bands was analyzed by the AlphaImager® software (San Jose, California, United States, and then normalized with the beta-actin or 18S signal.

Data were expressed as mean ± standard deviation. Means between groups were assessed by one-way Analysis of Variance (Anova), followed by a *post-hoc* comparison to determine differences between or within the groups. The two groups were compared by the Student's *t* test. All statistical analyses were by the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, United States) for Windows. *P* values below 0.05 were defined as significant.

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RESULTS

Intake regulation and weight gain

After 11 weeks of treatment, the mean weights of the groups differed significantly (Figure 1); however, calorie intake *per* kilogram (kcal/g weight) at 77 days of treatment was similar in all study groups because only the distribution of energy nutrients varied between the groups (Table 1). Rats fed the HFat diet were heavier by the end of the treatment (429.3±25.6 g). Rats fed the HFr diet presented the same pattern of weight gain as the control rats. This similar pattern of weight gain occurred despite different protein intakes (27% in the standard diet and 18% in the HFr diet). Rats fed the HFat diet consumed 10% of their energy as protein in week 14 of life.

Intake of a 15.0% HFCS solution *ad libitum* for 11 weeks did not cause the rats to lose their energy intake regulation (kcal/g weight), the same was observed when they were fed with HFat diet (Table 1). The animals in the HFr group increased their carbohydrate consumption by consuming a larger volume of the HFCS solution (1.2 mL/g weight/week *versus* 0.6 mL/g weight/week in the control group, $p<0.001$ Student's *t* test). The 15.0% HFCS solution in week 14 of life accounted for 33.5±4.1% of the total calorie intake of animals in the HFr diet. Animals fed 15.0% HFCS solution preferred the sweet solution, while water intake was similar in animals fed the standard and HFat diets (Table 1).

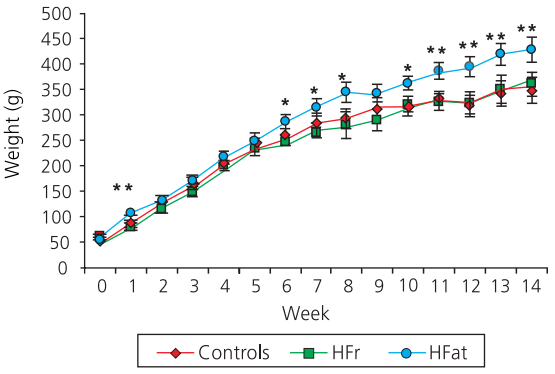

Figure 1. Body weight gain of weaned (21 days of life) male Wistar rats fed High-Fat Diet (HFat), High-Fructose Diet (HFr), or standard diets. The results are presented as mean, standard deviation with six animals *per* group.

Note: Differences are in relation to the standard diet, *$p<0.05$, **$p<0.01$; one-way analysis of variance, Fisher's least significant difference *post hoc* test.

Biochemical indicators

Table 2 shows the circulating levels of glucose, total cholesterol, HDL-cholesterol and triglycerides of the three groups. In the HFr group the effect of consuming the 15% HFCS solution on the metabolic indicators was present at 98 days (14 weeks) of treatment as serum triglycerides increased and HDL-cholesterol decreased. Animals in the HFat group had higher levels of glucose than the control group ($p<0.01$, Student's *t* test).

Both treatment groups had higher plasma triglyceride levels than the control group ($p<0.01$, one-way Anova) but as expected, the HFr group had the highest levels. HDL-cholesterol levels decreased in both treatment groups (Table 2).

Expression of the leptin and FAS genes

Levels of gene expression are shown in Figure 2. The HFat diet had higher leptin gene expression in adipose tissue than the control diet ($p<0.05$, Student's *t* test) or the HFr diet, by 1.5 times in the latter. Leptin expression in the adipose tissue of rats fed the HFat diet contrasted expression in the brain, which was the lowest of the study groups ($p<0.01$, one-way Anova). Animals fed the HFr diet had lower leptin expression in adipose tissue and brain than the control group ($p<0.01$, Student's *t* test).

Leptin expression was lowest in the brains of the HFat and HFr groups with the increased expression of FAS in the brain. Compared with the control group, they presented 2.7- and 3.1-fold changes, respectively ($p<0.01$, one-way Anova).

Table 1. Comparison of effects of High Fructose Corn Syrup Solution (HFCS) and vegetable fat on food energy intake, liquid intake and energetic nutrient distribution in male rats.

Week	Control		HFr		HFat	
	M	SD	M	SD	M	SD
	Energy intake (kcal/g weight/week)					
2	3.6	0.25[a]	4.7	0.32[b]	4.0	0.52
4	3.1	0.18	3.5	0.24	3.3	0.28
8	2.2	0.12	2.2	0.13	2.4	0.17
10	1.8	0.10	2.3	0.14	2.2	0.13
11	1.8	0.12	2.3	0.23	1.9	0.39
	Protein intake (g/g weight/week)					
2	0.24	0.02[a]	0.26	0.02[a]	0.16	0.01[b]
4	0.21	0.02[a]	0.17	0.01[b]	0.09	0.01[bc]
8	0.14	0.01[a]	0.09	0.01[b]	0.06	0.004[bc]
10	0.13	0.10[a]	0.14	0.14[a]	0.05	0.003[b]
11	0.12	0.12[a]	0.10	0.13[a]	0.05	0.39[b]
	Energetic nutrient distribution (Ch-L-P %)					
2	56-17-27		69-12-19		21-69-10	
4	56-17-27		69-12-19		21-69-10	
8	56-17-27		72-11-17		21-69-10	
10	56-17-27		68-12-20		21-69-10	
11	56-17-27		71-11-18		21-69-10	
	Liquid intake (mL/g weight/week)					
	Water		Fr (15%)		Water	
2	1.23	0.22[b]	2.38	0.35[a]	1.4	0.26[b]
4	0.97	0.13[b]	1.50	0.14[a]	0.7	0.17[b]
8	0.68	0.12[b]	1.32	0.13[a]	0.7	0.17[b]
10	0.52	0.11[b]	1.17	0.12[a]	0.7	0.13[b]
11	0.6	0.12[b]	1.2	0.13[a]	0.6	0.19[b]

Note: Data not sharing a common superscript (a, b, c) are significantly different (p<0.05, one-way analysis of variance, least significant difference *post hoc* test).

HFr: High-Fructose diet; HFat: High-Fat diet; M: Mean; SD: Standard Deviation; Ch: Carbohydrates; L: Lipids; P: Proteins.

Table 2. Biochemical markers of metabolic syndrome in Wistar rats. The data represent serum levels ± standard deviation at 24 and 77 days of treatment (n=6).

Groups	Glucose mg/dL		Cholesterol mg/dL		Triglycerides mg/dL		HDL-c mg/dL	
	M	SD	M	SD	M	SD	M	SD
	24 days of treatment							
Control	104.8	17.1	94.1	7.2[a]	140.9	15.8	41.6	4.5
HFr	95.5	13.4	123.2	11.1[b]	143.9	16.7	34.3	2.1
HFat	103.2	21.7	111.4	13.2	133.5	13.7	46.2	7.1
p	0.222		0.018		0.263		0.168	
	77 days of treatment							
Control	94.5	6.5[a]	102.1	8.0	105.5	12.1[a]	56.6	4.6[a]
HFr	96.2	3.8	94.8	7.2[a]	210.3	17.1[b]	27.3	3.3[b]
HFat	142.1	12.9[b]	121.3	7.6[b]	183.2	18.2[b]	27.5	3.9[b]
p	≥0.001		0.005		≥0.001		≥0.001	

Note: Data not sharing a common superscript (a, b, c) are significantly different (p<0.05, one-way analysis of variance, least significant difference *post hoc* test).

HFr: High-Fructose diet; HFat: High-Fat diet; HDL-c High-Density Lipoprotein-cholesterol; M: Mean; SD: Standard Deviation.

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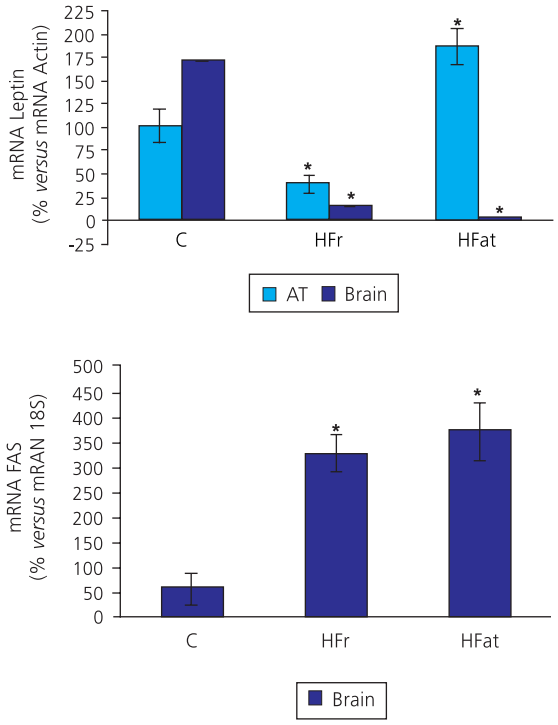

Figure 2. Relative expression of the leptin and Fat Acid Synthase (FAS) genes in adipose tissue and brain of 3.5-month-old male Wistar rats fed after weaning for 98 days (n=6) standard diet (C), standard diet plus 15% high-fructose corn syrup solution (High-Frutose diet, HFr), or diet with 40% margarine (High-Fat diet, HFat).

Note: *Different from control group ($p<0.05$, Student's *t* test).

AT: Adipose Tissue; mRNA: Messenger Ribonucleic Acid.

DISCUSSION

Growing obesity rates in Mexico have been linked to changes in eating habits and physical activity, along with higher intake of sweetened beverages, beginning in the first years of life[16]. HFCS is widely used by the food industry to sweeten a large variety of foods consumed mainly by children and adolescents[17]. Chronic HFCS and excess fat intakes by humans[4,18] and rodents[19,20] promotes weight gain, further altering blood metabolic indicators, such as triglycerides and glucose[21].

To determine whether the changes in weight are explained by loss of energy intake regulation or by nutrient distribution, we used an animal model that allowed intake of a 15% HFCS *ad libitum*. The energy intake of rats fed a high-fat diet, high-fructose diet, or standard chow was quantified and changes in body weight were measured.

The results indicate that consumption of 15% HFCS did not affect energy intake regulation, only the animals fed the HFat diet gained weight after 11 weeks of treatment. This weight difference was not solely related to dietary protein intake because protein intake was lower in the HFat diet group. The data suggest that male rats fed with a 15% HFCS solution and a standard diet after weaning can maintain a body weight similar to that of control rats.

In several studies there is controversy regarding the amount of fructose that promotes weight gain. Intake of 70% fructose, but not glucose, negatively affects weight gain in Wistar rats fed the same amount of protein[22]; however, weight gain in female rats fed a solution of 13% HFCS *ad libitum* for 8 weeks has also been reported[20]. HFCS may have little effect on females at the 30th week of life but still favor significant weight gain in males (8% HFCS)[19]. The data suggest that weight gain or loss is related to the proportion of HFCS consumed. Weight changes are not explained solely by protein intake; total glucose intake can also affect weight gain. The amount of glucose in the diet of animals consuming fructose should not be less than 23% of total energy intake[22]. In our study, the glucose proportions in the control and HFr diets were 36 and 37%, respectively, thus same glucose intake could explain the equal weight gain of the animals.

As expected, the animals that consumed HFCS had hypertriglyceridemia. De novo lipogenesis promoted by high fructose intake is a primary mechanism in the development of hepatic steatosis[23]. Several animal studies indicate that Sterol Regulatory Element-Binding Protein 1 (SREBP1c) is a major mediator of lipogenesis in the liver when high-carbohydrate diets are consumed[24,25]. In rats' liver, hypertriglyceridemia from fructose is related to activation of the transcription factor SREBP-1c, which activates

transcription of genes required for fatty acid synthesis, such as acetyl coenzyme A, carboxylase, fatty acid synthase, and stearoyl coenzyme-A desaturase-1[26]. Sirtuin 1 (SIRT1) is a deacetylase protein involved in the cellular metabolism of lipids and glucose[27]. Downregulation of SIRT1 expression resulted in a significant increase of triglyceride accumulation in 3T3-L1 cells[28]. In humans' and rats' liver cells, fatty acid oxidation is inhibited by fructose by reducing peroxisome proliferator-activated receptor alpha expression and activity, through a mechanism involving SIRT1 downregulation[29]. This evidence suggests that high triglycerides in the serum of animals fed the HFr diet is mediated by SREBP-1 and SIRT1.

Elevated triglyceride levels may inhibit the transport of leptin across the blood-brain barrier of rodents[30], and high-fructose diets (60%) induced hepatic triglycerides synthesis[31] and hypothalamic leptin resistance[32], so there is a relation between dietary fructose and leptin. The study animals fed the HFr or HFat diet had hypertriglyceridemia and low levels of leptin expression in the brain. These results suggest that, in the brain, the leptin gene is regulated by fat and fructose availability. Diet-induced obesity is associated with lower levels of leptin receptor expression in the hypothalamus yet high serum leptin[33]. Leptin mRNA levels in the hypothalamus, cortex, and pituitary were undetectable in fasted rats[34], which may have had fasting-induced hypertriglyceridemia. Haring & Harris[35] described peripheral leptin resistance in rats fed fructose (40%). High fat diets also induced central leptin resistance[36]. Lower leptin gene expression by HFCS intake also may be associated with leptin resistance.

Lower leptin expression compared with controls was associated with higher FAS expression in rats' brains. Fructose intake (10%) increases the expression of the FAS gene up to six times from the seventh day of intake[29], and fructose directly inhibits the oxidation of fatty acids in rats' livers[37]. This process is mediated by an increase in xylulose-5-phosphate, which activates protein phosphatase 2A, which affects leptin signaling, ultimately producing a fatty acid oxidation deficit in the liver[38]. These data suggest that the effect of fructose on fatty acid oxidation is mediated by leptin and that resistance to it and/or low leptin signaling may be associated with a higher level of fatty acid synthesis.

CONCLUSION

In conclusion, 15% HFCS did not affect weight gain in Wistar rats, possibly because the animals in the HFCS and control groups had the same glucose intake. The two experimental diets reduced the expression of leptin in the brain. This lower expression may be associated with hepatic synthesis of fatty acids, thereby producing the hypertriglyceridemia observed in the animals. Furthermore, the 15% HFCS in the HFr diet related to lower leptin expression in adipose tissue. The metabolic basis of low leptin expression in rats fed high-fructose diets has not been determined. It would be interesting if future studies determined the effect of HFCS intake on the expression of other genes associated with the regulation of hunger and satiety.

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CONTRIBUTORS

G LÓPEZ-RODRÍGUEZ designed the study, performed the data analysis, and wrote the study report. SK OSUNA and T SUÁREZ DIEGUEZ conducted the experimental analysis. M GALVÁN GARCÍA contributed to the writing and editing of the manuscript.

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