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Effect of Fructose on lipoprotein lipase in brown adipose tissue

Abstract

The per capita intake of fructose mostly in the form of high fructose corn syrup has increased 4- to 5-fold in recent decades. To determine the impact of dietary fructose on parameters of lipid metabolism in brown adipose tissue in T2DM rats, groups of lean and obese-T2DM rats were fed a nutritionally adequate diet consisting of 54% carbohydrate as either cooked cornstarch (CS) or equal parts CS and fructose (CSF diet) from one until 9 months of age. Measures of initial and final body weight s were recorded. At 9 months of age, measures of interscapular brown adipose tissue mass, and size, number, lipoprotein lipase activity, and lipid content determined. Data were analyzed by ANOVA. The body weights of lean and obese littermates were similar at 4 weeks of age, but the net weight gain of the obese phenotype over the 8 months of observation was twice that of their lean littermates, ($p = < 0.01$). The IBAT mass of obese rats >> than their lean littermates and was not affected by diet in either phenotype. The IBAT number / depot and lipid content / cell and percent lipid / IBAT depot was greater in obese than lean and was not affected by diet. The IBAT LPL activity of obese >> lean and was greater with the CSF than the CS diet in both phenotypes. In conclusion, these results indicate that the obese phenotype results in marked increases in IBAT mass and cellularity independently of diet. LPL activity of lean >> obese and was increased modestly in both phenotypes with the CSF diet. Thus, long term consumption of an isoenergetic diet high in fructose modulates LPL activity and lipid accumulation in brown adipose tissue in a rodent model of insulin resistance and NIDDM. In addition, the expression of obesity in the obese phenotype is more likely a result of the epigenetic metabolic determinants of obesity rather than the specific type of the dietary carbohydrate consumed *per se*.

Keywords: obesity, brown fat, NIDDM, insulin resistance, rat

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Abbreviations: NIDDM, non insulin dependent diabetes mellitus; T2DM, Type 2 diabetes mellitus; LPL, Lipoprotein Lipase; e.c, enzyme nr; HFCS, high fructose corn syrup; CS, cornstartch diet; CSF, cornstarch-fructose diet; IBAT, interscapular brown adipose tissue; ANOVA, analysis of variance; p, level of statistical significance; usually followed by number; SHR/Ntul//*-cp*, Spontaneously hypertensive ray crossed with NIH strain; *tul,* Tulp subcolony of rats as designated by the NIH; *-cp*, corpulent genetic trait for obesity; FFA, free fatty acids; IR, insulin resistance; GLUT4, GLUT5, glucose transporters 4 and 5 respectively; °C, temperature in degrees centigrade; NIH, National Institutes of Health;

Synopsis

Brown adipose tissue of insulin resistant obese-diabetic (NIDDM) rats increased in mass and cellularity compared to their lean littermates when fed zero fructose- vs high fructose diets until 9 months of age. The increases in the lean phenotype fed the high fructose diet were consistent with additional hyperplasia, while the increases in IBAT in the obese phenotype were due to hyperplasia and hypertrophy, yielding a higher tissue lipid content and a greater cell lipid content. In contrast, the high fructose diet resulted in increases in brown adipocyte number in the lean but not in further increases in the obese phenotype. IBAT tissue lipid content was greater and IBAT LPL activity lower in obese than lean littermates, despite similar feeding and environmental environments. These data confirm differential carbohydrate-linked processes of brown adipocyte hyperplasia and hypertrophy development in the lean and obese phenotypes of this strain.

Introduction

The incidence of overweight and obese conditions has increased significantly in recent decades and is now approaching epidemic proportions in much of Westernized societies worldwide.[1,2] The increase in dietary fructose consumption has also increased more than 4-to 5-fold over the same duration, in large part due to the introduction of high fructose corn syrup sweetener in foods and beverages, where fructose may contribute over 70 % of the available carbohydrate in the HFCS sweetened products.[3] Fructose is calorically equivalent to ordinary table sugar, has a similar chemical structure, but has a greater sweetness index than ordinary sugar.[4] It is often preferred more than many other natural sweeteners, and often preferred as an ingredient in commercial food and beverage manufacturing.[3] The widespread of inclusion HFCS in manufactured foods is now commonplace, with *per capita* fructose consumption 4- to 5-fold greater than in past generations.[3] Because the per capita consumption of fructose in Western society has increased so dramatically since the introduction of high fructose corn syrup as a sweetener, it now contributes to its availability toward extreme levels in some ordinary diets, where dietary intakes may exceed 80-100 grams per day.[3,5] Thus, HFCS as a sweetening agent occurs in many foods and beverages, and has been proposed to be linked to overweight, obesity and a number of metabolic disorders including gout.[3,5–10] In the fructose hypothesis, the impairments in ATP and energy metabolism are presumed contributors to excess body fat accretion and in the development of obesity and some of its metabolic sequala.[5]

The various metabolic roles of fructose in energy metabolism span multiple organ systems, including the adipose tissue depots.[5,11]

In a recent article, the merits of brown adipose tissue as an efficient potential energy buffer enhances the capacity for energy expenditure but that when thermogenic activity in brown adipose tissue is deficient, it may be a contributor to excess fat accretion and in the development of obesity.[12,13] As such, any activity that can enhance metabolic activity of brown adipose tissue offers the potential to become a target tissue for therapies or diets that could be developed as adjuncts in the management and treatment of excess body fat accretion. Because of the metabolic link between excess fat accretion and the development of adult-onset Type 2 diabetes (also referred to as NIDDM or T2DM), effective dietary strategies to manage the disorder and limit the pathophysiologic magnitude become important considerations. Fructose consumption is of interest because it can bypass the insulin dependent GLUT-4 receptor pathway during its absorption in peripheral tissues, and at least theoretically would be predicted to decrease the dependence on insulin for its peripheral absorption and oxidation.[6] Thus, an investigation was conducted to determine the effects of long term substitution of dietary carbohydrate with isoenergetic content of a fructose-starch diet in an animal model highly predisposed to early onset obesity and T2DM.

Methods

To determine the effects of dietary fructose consumption on development and cellularity of brown adipose tissue in NIDDM, groups of congenic lean and obese SHR/Ntul//*-cp* rats (*Rattus rattus*) demonstrating insulin resistance (IR) were fed diets containing 54% (w/w) carbohydrate as cornstarch (CS diet) or equal parts CS plus fructose (CSF diet) plus essential proteins fats, vitamins, minerals, and dietary fiber from one to nine months of age. The SHR/Ntul//*-cp* rat is a congenic animal model in which the only genetic difference between the phenotypes is the epigenetic expression of the obese (*-cp*) trait, where it is accompanied by the development of chronic IR and non-insulin dependent Type 2 diabetes (NIDDM or T2DM) soon after weaning in the obese phenotype of the strain.[14–17] The strain was established at the small animal genetic laboratory of the Veterinary Resources Branch of the NIH by Hansen by mating the spontaneously hypertensive SHR rat with an NIH (N) background strain that contained the -cp trait originally derived from the Koletsky rat.[14–16]

Groups of male lean and congenic obese SHR/Ntul//*-cp* rats originally obtained from the congenic breeding colony at the NIH small animal genetics research unit (n=11-12 rats/group) were fed an isoenergetic diet from ~4 weeks until 9 months of age. Initial body weights of the lean and obese animals were 90.35±5.9 vs 102.65± 8.0 respectively (p=n.s.). The diet consisted of (w/w) 54% CHO as cooked cornstarch (CS diet) or equal parts CS and fructose (CSF diet) plus 20% protein (equal pats casein and lactalbumin) 16 %fat (as equal parts lard, corn oil, beef tallow and coconut oil) plus essential vitamins, minerals, (AIN vitamin /mineral mix), and non-nutritive fiber fed *ad libitum*, from ~1.5 until 9 months of age.[16,17] All animals experienced the same environmental conditions, including being housed in plexiglass shoebox cages in littermate pairs (I lean plus 1 obese), 20-22°C room temperature and 50% relative humidity with a reverse light cycle (light 2000-0800 hrs.). Live body weights were obtained initially and periodically throughout the study. At the end of the study, rats were sacrificed by cervical dislocation with a small animal guillotine, truncal bloods collected for later study and the interscapular brown fat depots carefully dissected free of white adipose tissue, weighed to the nearest 0.1 mg to determine fat pad mass, and measures of brown adipocyte size and number determined in representative sections of the tissue as described previously.[18] Briefly, the tissue aliquots were fixed in 10% buffered formalin for 24-48 hours, post-fixed with 4% osmium tetroxide for an additional 48-72 hours, washed and sieved with Nitex filters to remove extraneous debris, and counted in a Coulter Model B particle counter.[18] Cell and tissue lipid content were determined gravimetrically in weighed aliquots of tissue with the method of Dole and Meinertz as originally described by Hirsh and Gallian performed in our laboratory and expressed a micrograms of lipid per cell and as a percent lipid in the tissue fragment.[18–20] Measures of lipoprotein lipase activity per gram of tissue were determined via the method of Shirai and Jackson[21] and expressed as uMols of FFA released per gram of tissue per hour at physiologic temperatures (37°C). Data were analyzed via standard descriptive and statistical methods including ANOVA and tend analysis.[22,23] This study was approved by the Institutional Ethics, Animal Care and Use Committee (IEACUC) of the University of Science Arts and Technology, Montserrat.

Results

The effects of the diet on initial and final body weights are presented in Table 1 and indicate that the weight gain in the obese phenotype was markedly greater than occurred in their lean littermates. (p=<0.001). In addition the fructose diet resulted in only a small but not significant trend toward increases in weight gain in the lean phenotype. While the CSF vs the CS diet was associated with a modestly greater mean body weight in the obese phenotype, the trend also failed to reach a statistically significant level of increase. The sum of the epididymal, retroperitoneal and dorsal fat pad mass in the 4 groups is also depicted in Table 1 and indicates that the combined fat pad mass is also markedly treater in the obese than the lean phenotype. In addition, the significant CSF diet effects occurred in the lean but not the obese phenotype. When the combined fat pad mass is expressed as a proportion of final body weight, the obese phenotype was significantly greater than their lean littermates, irrespective of diet consumed.

The effects of diet and phenotype on Interscapular brown adipose tissue parameters are presented in Table 2, and indicate that the IBAT mass of the obese phenotype was markedly greater than occurred in the lean phenotype. In addition the diet did not result in differences in IBAT mass in either phenotype. The percent lipid in the IBAT depots was also greater n the obese than the lean phenotype but was unaffected by diet. The cell number per IBAT depot was also markedly greater in the obese than the lean phenotype, and also was not impacted by the diet in either phenotype. Brown adipocyte lipid content is shown in the far right column of Table 2, and indicates that cellular lipid content was significantly greater in the obese than the lean phenotype and while it was not affected by diet in either phenotype, the diet was associated with a significant trend toward lower cellular lipid content in the obese phenotype when fed the CSF than the CS diet.

Measures of lipoprotein lipase activity are depicted in Figure 1 below, and indicate that the release of FFA was greater in the lean than the obese phenotype, and was significantly decreased in animals fed the CSF than the CS diet in both phenotypes. This represented a fructose diet effect increase of ~35 % in the lean phenotype and an increase of ~70% in the obese phenotype.

Measures of total glycated hemoglobin fractions are depicted in Figure 2 and indicate that in both phenotypes, the total glycated hemoglobins were elevated in both phenotypes. Diet-specific effects were only observed in the obese phenotype however, where the percent total hemoglobin fractions were decreased by ~ 22% compared to their normally fed obese littermates (p = < 0.05, students't' test for unpaired comparisons).[24,25]

Table 1 Effect of fructose intake on body weight and relative adiposity

Group	n	Initial	Final	Gain	Fat pad mass, g/3 depots	Rel adiposiy
Lean CS	6	90.8±6.0	454.8±7.7	364±8.0	17.5±0.3	3.85±0.08
Lean CSF	6	89.9±5.8	471.1±7.0	381.1±10.2	20.6±0.3	4.37±0.11
Obese CS	6	98.0±9.0	820.0±32.0	720.0±35.0	137.9±2.5	16.81±0.08
Obese CSF	6	107.3±8.0	865.0±36.0	758.0±38.0	143.9±3.0	16.64±-0.13
ANOVA F		1.24	92.64***	70.56***	136.70***	114.67***
PHENOTYPE		-	277.04***	211.19***	207.9***	117.5***
DIET		-	1.81*	1.16	3.19*	n.s.
INTERACTION		-	0.42	0.16	0.36 -	n.s.

CS = starch diet; CSF = starch-fructose diet; *** = p = < 0.001; + p = trend. - = n.s. Fat pad mass = sum of epididymal, retroperitoneal and dorsal subcutaneous adipose tissue depots.

Table 2 Effects of fructose intake on brown adipose tissue cellularity

Group	n	IBAT Mass, g	IBAT % lipid	IBAT cell nr/depot	IBAT cell size, µg.cell
Lean CS	6	0.65±0.1	51.7±2.3	2.4±0.1	0.23±0.06
Lean CSF	6	0.76±0.0	54.9±1.5	3.4±1.0	0.24±0.08
Obese CS	6	5.93±0.5	77.4±2.6	11.7±8.0	0.85±0.26
Obese CSF	6	5.87±0.8	81.5±5.8	11.6±3.0	0.62±0.15t
ANOVA F		57.36***	26.12***	5.30**	2.68 *
PHENOTYPE		171.12***	77.67***	15.59***	2.60*-
DIET		0.00 -	1.50 -	0.03 -	-
INTERACTION		0.04 -	0.02 -	0.04 -	-

Data are mean ± 1 SEM, n= 8 rats/treatment group. CS = starch diet; CSF = starch-fructose diet; *** = p = < 0.001; + p = trend. - = n.s. ᵗ = trend.

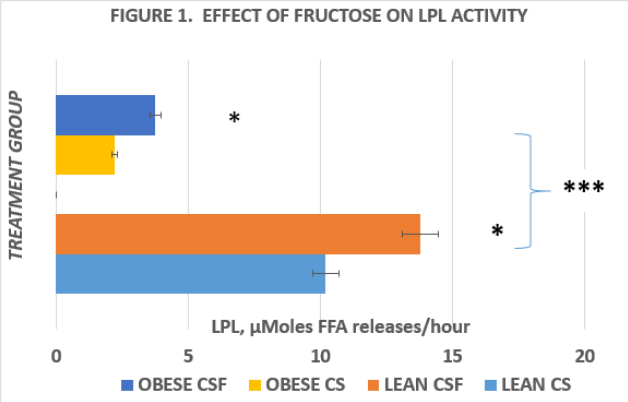

Figure 1 Data are mean ± 1 SEM, n= 6-8 rats/treatment group. CS = starch diet; CSF = starch-fructose diet; * = p = < 0.05; *** = p = < 0.001. LPL = lipoprotein lipase, expressed a µMols of free fatty acids (FFA) released per hour at 37 °C.

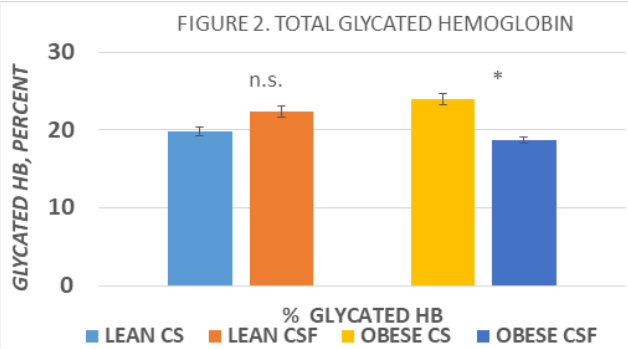

Figure 2 Total glycated hemoglobin fractions in rats. Data are mean ± 1 SEM, N-5-8 rats/group. * = p = < 0.05.

Discussion

The results of this study indicate that net increases in body weight were markedly greater in the obese than the lean phenotype of this strain and were associated with a proportionately greater mass of the principal fat pads when compared to their genetically lean littermates. When comparing the effects of the high fructose vs the cornstarch-based diet, the differences in weight gain and on relative adiposity were modest, despite the prolonged duration of the high fructose diet. While fructose has a greater sweetness index than cornstarch in human trials, direct comparisons to rodents remain unclear. In previous studies, rats were observed to strongly prefer a partially hydrolyzed cornstarch preparation over sucrose when either were added to their drinking water.[26] In the present study, both diets were offered *ad libitum*, and based on casual disappearance observations review appeared to be consumed in similar proportions. In a related study, factors of phenotype but not fructose diet were significant in white adipose tissue depot cellularity, and Song et al noted that adipocyte size was positively correlated with the magnitude of insulin resistance, particularly in visceral adipose tissue. In the present study, measures of lipoprotein lipase activity were decreased in the obese phenotype, consistent with the greater degree of insulin resistance common to this and other rodent and murine strains of obesity.[15]

The development of brown diose tissue typically occurs primarily prior to adulthood in this and other strains of obesity likely as a consequence of nutritional and hormonal stimuli that contribute to its development. Early overnutrition prior to sexual maturity in the rat has long been shown to result in a 2- to 3-fold increase in the mass and cellularity of brown adipose tissue, in association with increases in the capacity for non-shivering thermogenesis in response to typical stimuli of cold exposure and dietary manipulation when imposed on genetically lean rats.[12–15] Typical expression of nonshivering thermogenesis requires the combined, complementary actions of both the sympathetic and thyroidal organ systems.[27–30] In contrast,

in genetically obese rodents, both the thyroidal and sympathetic responses to diet and environment are impaired, enabling animals a more efficient feed efficiency, and a presidposition toward greater fat accretion with an onset during the early postweaning growth stage.[27] Early overnutrition typically results in a greater capacity for brown fat development and energy wastage via brown adipose tissue, while in the obese phenotypes, the impaired thyroidal and sympathetic responses contribute to less active non shivering thermogenesis and early onset excess fat accretion.[27–30] In the present study, the obese phenotype was found to have expressed marked increases in brown adipose tissue cellularity, including phenotypic increases in both cell number per depot and cell lipid content, while brown adipocyte lipid content was unaffected by diet in the lean phenotype, and modest decreases in IBAT cellular lipid content in the fructose-fed obese phenotype. The decreases in IBAT cellular lipid content are consistent with the decreased lipoprotein lipase activity in the same animals, indicative of partial improvements on insulin sensitivity with the added fructose availability. Fructose is taken up by insulin independent membrane associated GLUT 1 and GLUT 5 transporters in peripheral tissues, while glucose uptake is dependent on insulin dependent GLUT4 transporters in most tissues.[6] Nonetheless, LPL activity of lean animals was greater in the lean than the obese phenotype, consistent with a mor efficient FFA metabolism in those animals, and consistent with the established contributions of insulin dominance in the regulation and suppression of LPL activity in adipose tissues.

Glycated hemoglobin fractions are a valid reflection of the non-enzymatic, mass action kinetics of glycation of hemoglobin proteins, and are deemed a recognized indicator of average glycemic status during the lifespan of the erythrocyte. Both glucose and fructose are active glycation sugars with respect to glycation reactions in erythrocytes and other tissue proteins.[18] Once glycation occurs the reaction typically becomes irreversible, resulting in permanent changes in protein structure and function, where is impairs oxygenation capacity in peripheral tissues where heme is involved, as oxygen delivery is an essential activity for tissue regeneration and wound healing.[26] In the present study, the net glycosylated fractions were elevated in both phenotypes, suggestive of grazing feeding behaviour common to rodents. The glycation was unaffected by the type and proportions of carbohydrates in the diet in the lean phenotype but was modestly improved in obese animals that consumed the fructose enriched diet regimen. This improvement may be a reflection of the shift from starch derived glucose to fructose residues, and the lower insulin requirements for the fructose enriched regimen.

The observation of differences in IBAT lipoprotein lipase activity are a new finding in the obese-NIDDM/T2DM of this strain. LPL is hormonally controlled in large part by actions of insulin and catecholamines including epinephrine and norepinephrine: Insulin acts to stimulate lipogenesis, and LPL acts to mobilize the stored triglycerides as free fatty acids during conditions of energy needs.[21] In contrast, catecholamines facilitate the mobilization of metabolic fuels including glucose and free fatty acids, both of which are essential for the molecular expression of thermogenic activity in brown fat. The differences in LPL activity of brown adipose tissue of the present study are consistent with the marked differences in IBAT tissue lipid content, and the previously reported incidence and magnitude of insulin resistance in the obese phenotype of this strain.[14-17] Insulin resistance, in concert with glucocorticoid dysregulation are a hallmark of obesity and NIDDM, and collectively exert negative influences on the intracellular formation, mobilization, intracellular transport, and transmembrane actions regarding the insulin-dependent GLUT4 glucose transporters. In contrast, fructose enters calls via GLUT 1 or GLUT 5 transporters, thereby bypassing the insulinogenic effects.[4–6] Once internalized, however, both glucose and fructose can impinge on glycolytic and lipogenic pathways, with fructose essentially resulting in a glucose-sparing effect. In high dietary doses of fructose however, fructose may overburden lipogenic pathways in addition to contributing to alterations in renal dynamics, and adversely impacting on pyrimidine metabolism resulting in the formation of excess uric acid, with its attendant adverse effects on the development and progression of the pathophysiologic symptoms of gout and cardiovascular disease.[5] Thus, the consumption of excess fructose whether in the form of free sugar, as a hydrolytic digestive product of sucrose, or via excess consumption of high fructose corn syrups poses a potential dose-related risk not only to renal and cardiovascular parameters, but may also impact on brown adipose tissue dynamics, especially when consumed in excess during early post weaning life in the rat. Observations during dissections revealed a high incidence of renal calculi of unknown composition in the obese+NIDDM animals fed the CSF diet, consistent with the published effects of excess fructose consumption on renal parameters in clinical observations.[14–15]

In conclusion, the results of this study indicate that the obese phenotype results in marked increases in IBAT mass and cellularity independently of diet. In addition, the LPL activity of lean rats was greater than occurred in the obese phenotype and was increased modestly in both phenotypes with the CSF diet. Thus, long term consumption of an isoenergetic diet high in fructose contributes to the modulation of LPL activity and lipid accumulation in brown adipose tissue in a rodent model of insulin resistance and NIDDM. and may be linked to an improvement in insulin sensitivity with the high fructose diet. In addition, the expression of obesity in the obese phenotype is more likely a result of the epigenetic metabolic determinants of obesity rather than the specific type of the dietary carbohydrate consumed *per se*.

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Conflicts of interest

Authors declare that there is no conflict of interest exists.

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