ABSTRACT

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Fructose consumption can lead to marked increases in plasma triglycerides in both humans and laboratory animals. We have observed that overnight access to a 16% fructose solution can promote hypertriglyceridemia in rats. Several investigators have suggested that APOC 3 may be implicated in promoting fructose-induced hypertriglyceridemia. Wehave examined the role of APOC 3 in liver and blood taken from rats that had been given access to a fructose solution overnight as a supplement to standard laboratory chow. Hepatic APOC3 mRNA expression from fructose alone resulted in a 14 % reduction compared to control. Interestingly, hepatic APOC3 expression was increased by about 250% in sucrose, high fructose corn syrup and glucose groups. The serum protein levels of APOC3 did not differ across groups. Contrary to our hypothesis, these results indicate that glucose containing sugars increased hepatic APOC3 mRNA expression but no sugar was capable of increasing the serum protein level.

FRUCTOSE INTAKE AND CIRCULATING TRIGLYCERIDES: AN EXAMINATION OF THE ROLE OF APOC 3

By

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Fructose-induced hypertriglyceridemia – a review

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Abstract

Fructose is a sugar that has unique attributes that facilitate its conversion into body fat. We and others have reported that fructose consumption can quickly put into motion several metabolic and regulatory mechanisms that favor increased caloric

intake and abdominal fat stores. The present review focuses on one of the most robust effects of fructose consumption – that of a rapid and significant rise in circulating triglycerides. We have divided this review into three sections. In the first, we summarize some of the ways that fructose affects intake and metabolism. We then contrast the metabolic pathways used by fructose with those used by glucose. The comparison makes it clear how fructose has a unique advantage in promoting hypertriglyceridemia. We finish this review by organizing the existing literature so that the reader can quickly become familiar with specific topics of how fructose can foster increased intake and body fat.

I. Introduction

Nikkila and Ojala[1] were among the first to report that fructose consumption leads to a dramatic increase in circulating triglycerides. Since that time fructose-induced hypertriglyceridemia (FIH) has been reported by dozens of investigators [2-4], many of whom have proposed mechanisms to explain how fructose uniquely and quickly is converted from sugar to circulating fat. This review attempts to summarize the major factors involved in FIH. It is by no means comprehensive, and we apologize if we have inadvertently omitted someone's contribution to the literature. On the other hand, we hope that this introduction to the phenomenon contributes to a deeper understanding of how fructose can be added to the long list of factors promoting obesity, particularly abdominal obesity.

The obesity epidemic that we are experiencing in the US is a national concern that affects all Americans. It does not discriminate among age, race, class, or gender. In 1985 the rate of obesity across our country was under 14%; but by 2010 the rate exceeded 30%[5]. Furthermore, obesity is quickly becoming a leading cause of major health problems and death[6]. The research community, along with policy makers and health care officials are grappling to find the causes of this relatively sudden increase in American's girth. Several researchers have targeted high-fructose corn syrup as a potential accomplice supporting the obesity epidemic[7-10]. They and their supporters have pointed out that fructose has become more prevalent in our diets over the past century. In 1900 the average fructose intake was 15g/day. Fructose was consumed mainly through eating fruits and vegetables, which have the added benefit of fiber[11]. However, as of 2010, fructose consumption had risen to 73g/day and was being

consumed in highly processed forms[11]. One particularly attractive hypothesis linking obesity and fructose consumption is that increased fructose intake can disrupt normal hepatic metabolism leading to increased hepatic lipogenesis[12]. The purpose of this review is to summarize the evidence supporting this hypothesis all the while focusing on one manifestation of this disruption, that of fructose-induced hypertriglyceridemia.

Fructose and Appetite

It is currently believed that normal hunger and satiety signals operate to alert the body when to initiate or end eating. One of these protein signals, leptin, decreases food intake when in excess and is therefore said to have anorexigenic effects[12,13]. Leptin is produced within adipose cells in relation to the amount of adipose in the body and travels to the arcuate nucleus. When excess adipose tissue exists, there is excess leptin produced signaling the body to stop eating. Another signal, ghrelin, is produced in the absence of food in the stomach and stimulates food intake[14,15]. Ghrelin is said to have orexigenic effects and travels through the blood system to the hypothalamus to signal hunger[15]. Recently, we have reported that overnight access to sucrose or fructose solutions can dramatically increase liver 11 β hydroxysteroid dehydrogenase – 1, an enzyme that is linked to omental obesity[16,17]. Further, we have demonstrated that both fructose and sucrose can have significant effects on hypothalamic leptin, ghrelin and several other factors that help to control appetite[18,19]. We believe that dietary obesity arises when normal hunger and satiety signals are disrupted causing food intake disregulation in the form of overeating. While fructose has become a target of many research topics surrounding obesity, it is important to remember that sucrose (table sugar) is comprised of two molecules, one of glucose

and one of fructose. Not surprisingly, sucrose and fructose have both been shown to have similar effects on weight gain.

Fructose can increase the palatability of some foods and as a result can cause normal signals for fullness to be ignored, thereby allowing for increased intake and subsequent weight gain[20,21]. Others have confirmed this effect with other sugars, showing sugary foods motivate intake despite satiety[22]. Rats fed high fructose diets had increased body adiposity after 3-6 weeks[23-25]. Furthermore, rats continually fed fructose develop leptin resistance, which in turn accelerates high-fat induced obesity[26]. Additionally, diet-induced obesity causes leptin resistance in both leptin transport across the blood-brain barrier as well as central leptin signaling in mice[27]. Thus the long-term consequences of fructose consumption and weight gain may override normal satiety signals.

Fructose and Reward

In addition to the effect of fructose on satiety signals, sucrose can induce addiction-like behaviors such as bingeing and dependence[28-30]. Hoebel and his associates have shown that underweight rats release more dopamine in the nucleus accumbens during a sucrose binge, indicating a potential mechanism for how sucrose may induce addiction-like behaviors[31]. Additionally, intermittent sucrose bingeing decreased D2 receptors and μ-opioid receptors in rats, which is an effect similar to that seen with morphine, indicating a role for sucrose in reward via its dopamine pathway[32]. These data make it clear that sugar (either sucrose or fructose) can have a major impact on reward and possibly addiction, as both phenomena are mediated by dopaminergic pathways. Additionally this research may shed light on how sugary

foods override satiety and result in overeating through mechanisms other than directly affecting satiety signals of the hypothalamus.

Fructose and Liver

Sugar also has effects on peripheral metabolic mechanisms that influence intake. For example, fructose may increase lipogenesis in the liver beginning at the molecular level. Miyazaki[33] reported that sterol regulatory element-binding protein-1 (SREBP-1), an integral part of hepatic lipogenesis, was increased in the livers of rats receiving high-fructose diets. This enzyme is a critical factor that transforms excess energy into triglycerides. Thus, when in excess SREBP-1 will increase triglyceride formation. Additionally, elevated levels of glucocorticoids have been linked to visceral obesity and metabolic complications[34]. In a recent study 11-β hydroxysteroid dehydrogenase -1 (11-βHSD-1, a regulator of intracellular glucocorticoids) was increased in the livers of rats after 24-hours of fructose access[17]. Additionally, sucrose increases 11β-HSD1 in adipose tissue, which is a characteristic of human and animal models of obesity[15]. In a study that assessed the effect of fructose and glucose in humans, overweight and obese subjects consuming 25% of their energy (10 weeks) as either glucose or fructose sweetened beverages gained weight regardless of the type of sugar used[34]. Interestingly, in subjects consuming fructose the visceral adipose volume was significantly higher than both control and glucose groups[34]. Rats fed high fructose diets (6 weeks) displayed hypertriglyceridemia, hyperinsulinemia, and became glucose intolerant[35]. Liver X receptors (β and α) are nuclear receptors that

help regulate carbohydrate and lipid metabolism, which decrease and increase respectively in response to chronic fructose consumption[35]. Taken together, these results indicate a potential connection between liver metabolism dysfunction, fructose, and increased visceral obesity.

Based upon all of the above, it seems to be clear that sugars, and particularly fructose, have the potential of being major contributors in supporting at least part of the obesity epidemic. However, not all authorities agree that sugar consumption is solely responsible for it. For example, the American Heart Association concluded that CVD and sugar consumption is not linked and that the effect on triglycerides seen with a high-sugar, high-carb diet can be reduced through exercise. Fried has proposed that long term, randomized clinical studies are needed to assess if there is a threshold at which fructose and sucrose content in diets can cause abnormalities in triacylglycerol metabolism.

The work summarized above reviews how sugars enter our diets and our bodies, and how they bypass some of the controls of intake and metabolism. The following section of this review attempts to outline how fructose is metabolized, and how it uniquely contributes to adiposity via hypertriglyceridemia.

II. Fructose metabolism – An Overview

Before attempting to explain how fructose causes hypertriglyceridemia, a short review of how it is absorbed and metabolized may be of help.

Absorption

Glucose is readily absorbed through the intestinal lumen via a diffusive GLUT-2 transporter protein and an active sodium glucose co-transporter pump

(SGLT) in humans[36-39]. GLUT-2 is found on the basolateral membrane of epithelial cells to transport glucose into circulation[37]. GLUT-2 exhibits a low affinity for fructose and is responsible for a fraction of fructose absorption[37]. In contrast, many investigators reported that intestinal fructose absorption is incomplete in humans[40-42]. Fructose is predominantly taken into the intestinal epithelial cells from the intestinal lumen passively via GLUT-5 protein transporter in humans[42]. GLUT-5 is also expressed on the basolateral side of the intestinal epithelial cells to allow for fructose to exit the epithelial cell and enter into circulation[43]. Some evidence suggests that fructose can exit epithelial cells via GLUT-2 as well as GLUT-5 [44].

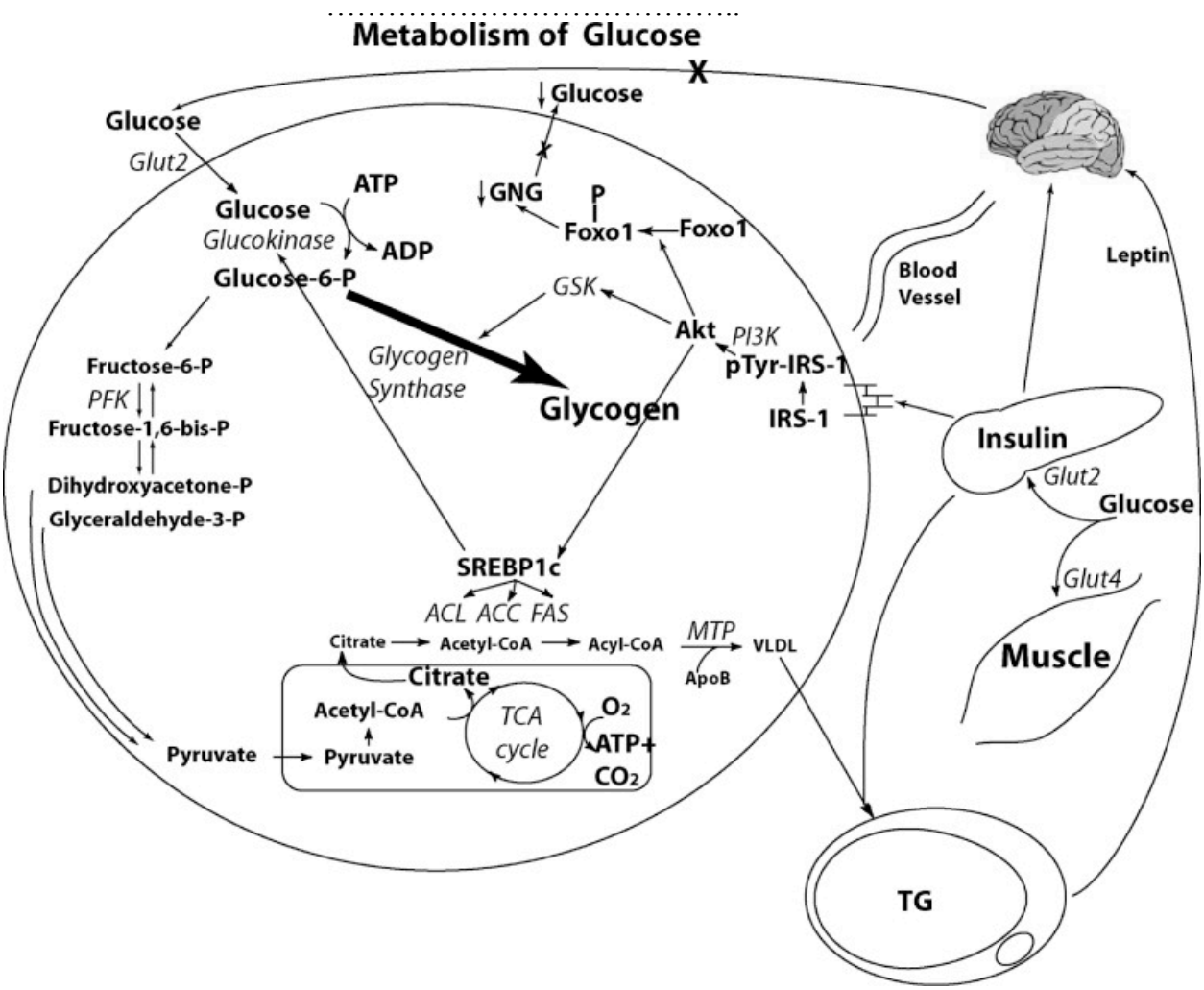
The liver can extract 40-70% of dietary fructose in both rats and humans [45-48]. The high efficiency of fructose extraction by the liver is due to the fact that the liver is the main source of GLUT-5[42]. Anatomically, the liver is the first organ to receive absorbed nutrients via the portal vein and, in that way contributes to its role in fructose extraction. By contrast, the liver accounts for a smaller percentage of glucose extraction for glycogen restoration[49].

Fructose Metabolism

Fructose metabolism is best understood by considering three enzymes: fructokinase, aldolase B, and triokinase. All three of these are only found in the liver and kidneys of rats and humans. In the liver, fructose is rapidly converted to fructose-1-phosphate via fructokinase. Fructose -1-phosphate is then converted into the trioses dihydroxyacetone-phosphate and glyceraldehyde via the enzyme aldolase B. Aldolase B also functions in the liver for normal glycolysis (glucose metabolism). Glyceraldehyde is then converted to glyceraldehyde-3-phosphate via triokinase. Up

until this point in fructose metabolism, there have been no rate limiting steps and as a result there is an increased amount of substrate leading to metabolic pathways from triose phosphate (ie, glycolysis. glycogenesis, glyconeogenesis, and lipogenesis, fatty-acid esterification). Glyceraldehyde-3-phosphate is next metabolized to pyruvate via the rate-limiting enzyme pyruvate kinase. Fructose enhances activation of pyruvate kinase and thus leads to an increased flux of pyruvate into the Kreb's cycle. It should be noted that fructose metabolism does not promote an insulin response.

Illustration of Glucose Metabolism: Lustig Ref #50 For Image



Glucose Metabolism

Glucose typically enters liver cells and is phosphorylated via glucokinase to form glucose-6-phosphate. Glucose-6-phosphate has two fates: conversion to fructose-6-phosphate via phosphoglucose isomerase, or storage in the form of glycogen. Fructose-6-phosphate is further phosphorylated by phosphofructokinase to produce fructose-1, 6-bisphosphate. Phosphofructokinase is the main rate-limiting step in glycolysis. Aldolase, as seen in fructose metabolism, next converts fructose-1, 6-bisphosphate into dihydroxyacetone-phosphate and glyceraldehyde-3-phosphate. Dihydroxyacetone-phosphate is at the crossroads between glucose and fructose metabolism[51,12]. The metabolic fate of glucose relies heavily on the body's energy needs at the time. The integrated pathways of glucose metabolism include glycogenesis, glycogenolysis, glycolysis, pentose phosphate pathway, lipogenesis and the tricarboxylic acid cycle.

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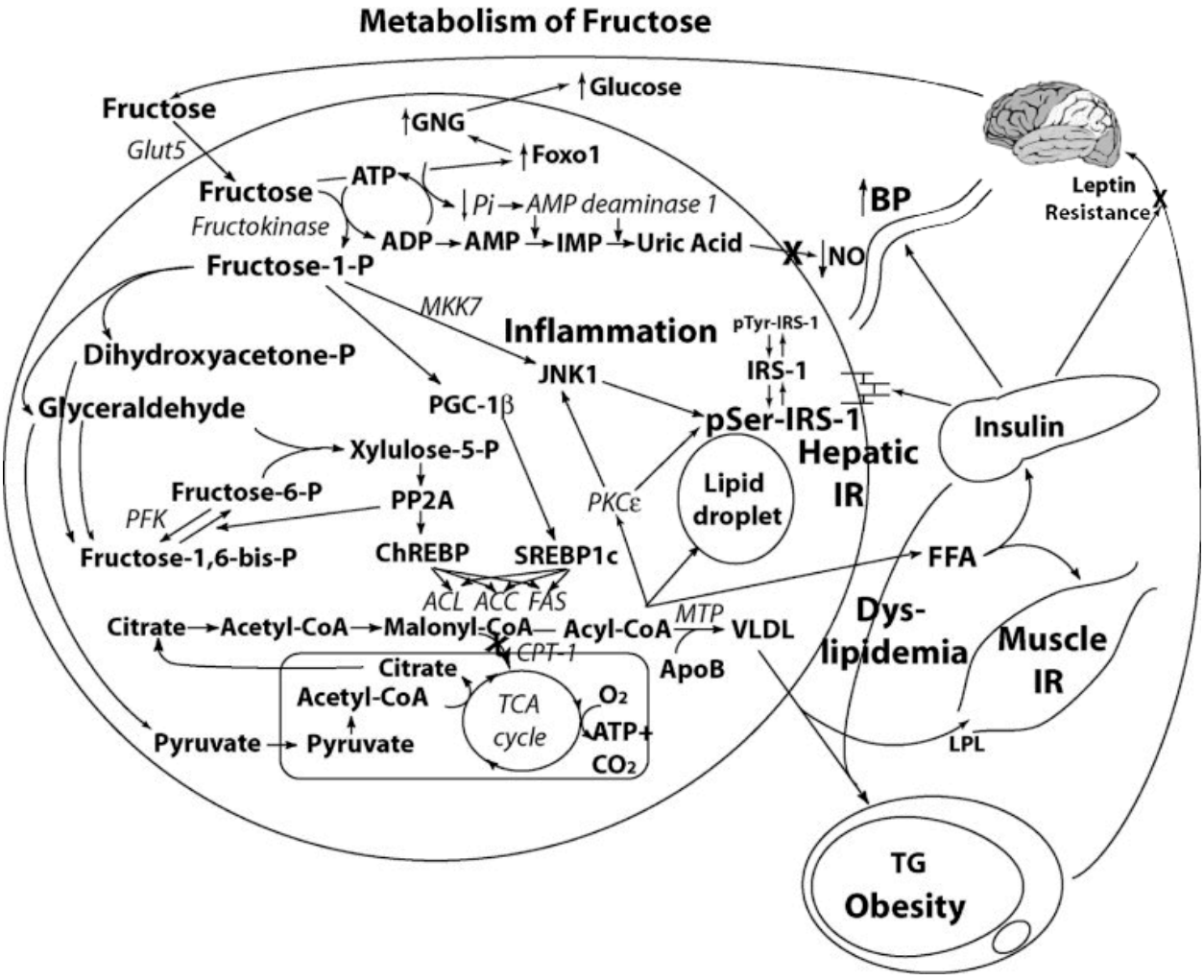

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Theories on Fructose-Induced Hypertriglyceridemia

A diet high in fructose can produce adverse metabolic changes such as increased plasma triglycerides (TG), hepatic insulin resistance, and hepatic steatosis[52]. These changes usually occur in people consuming large amounts of fructose and under hypercaloric conditions[52]. Tappy has suggested that sugar calories should be limited to 140kcal/day for men and 100kcal/day for women[52]. Fructose can alter normal lipid metabolism in the liver by generating unregulated surges of pyruvate. Pyruvate enters the mitochondria via pyruvate dehydrogenase and forms acetyl-CoA. Acetyl-CoA acts as a carbon source for three different pathways: the

citric acid cycle, lipogenesis, and the formation of ketone bodies. In the lipogenic pathways Acetyl-CoA is shuttled across the mitochondrial membrane as citrate and is restored back to Acetyl-CoA in the mitochondrial cytosol via ATP-citrate lyase. Here Acetyl-CoA provides substrate to the production of long-chain fatty acids. Fatty acid synthase is a multi-enzyme complex that helps facilitate the conversion of Acetyl-CoA to long-chain fatty acids. Rats fed 63% fructose for 24-48 hours and then fasted developed liver steatosis because fructose is highly lipogenic[53,54].

Most of what has been presented above focuses on the potential effects of fructose consumption. We should point out that not only ***can*** these shifts in metabolism take place, but there is growing evidence that they are taking place in the population. For example, the amount of added sugars in the average American diet is currently 477kcal/day[10]. This is in contrast to the recommendation from the WHO that only 10% energy of total daily intake come from added sugars[55]. Stanhope and her associates have shown that leptin production drops by 20-30% when subjects consumed fructose-sweetened beverages (30% caloric intake) compared to glucose-sweetened beverages[56,57]. They hypothesize that the leptin reduction observed during prolonged consumption of diets high in fructose may lead to increased energy intake and/or decreased energy expenditure and weight gain. In fact, in subjects consuming fructose there is a decrease in energy expenditure and fat oxidation when compared to subjects consuming glucose. In rhesus monkeys, the consumption of fructose over a 3 and 6 month period resulted in significant weight gain and decreased energy expenditure[10]. In a steady state fructose feeding study, postprandial levels of VLDL-TG increased in parallel with total TG, and it is likely that delayed TG clearance also

contributes to this rise[10]. Rebollo[55] has reported that sucrose sweetened beverages significantly increases TG deposition in the liver, skeletal muscle, and visceral adipose tissue. Additionally, fructose consumption can increase TG over a 24-hour period, as well as apoB concentration[56]. In a study comparing the effects of consuming glucose, fructose or HFCS (25% energy) TG increased during HFCS and fructose consumption over a 24-hour period[57]. Fasting LDL and apoB concentrations were also increased as a result of fructose and HFCS consumption[57]. In an earlier separate study assessing the effect of fructose and glucose in humans, Stanhope[34] reported that overweight and obese subjects consuming 25% of their energy over 10 weeks as either glucose or fructose sweetened beverages all gained weight regardless of the type of sugar used. Interestingly, in subjects consuming fructose the visceral adipose volume was significantly higher than in both control and glucose groups.

Additional Factors Contributing to Fructose Induced Hypertriglyceridemia

Recently, it has been discovered that hepatic lipogenesis is regulated by several transcription factors, most notably the sterol regulatory element-binding protein (SREBP-1c) and the carbohydrate responsive element binding protein (CHREBP)[58,59]. SREBP-1c can induce expression of the fatty acid synthase complex, which leads to an increase in triglyceride production and secretion[60]. Fructose increases hepatic expression of the SREBP-1 & CHREBP families and thus the lipogenic enzymes responsible for triglyceride synthesis[59,61].

Additional discoveries of peroxisome proliferator-activated receptors alpha and beta provide additional insights into the regulation of fatty acids in the liver[62].

The peroxisome proliferator-activated receptor gamma co-activator-1 (PGC-1β) elicits how SREBP-1c can regulate and influence lipogenesis[63]. PGC-1β is a direct co-activator of the transcription factor for SREBP-1c. Interestingly, Nagai showed that PGC-1β knockout studies protected rats from fructose-induced hyperlipidemia[61].

Peroxisome proliferator-activated receptor alpha (PPAR-α) is a liver transcription factor that enhances fatty acid β-oxidation. Fructose directly suppresses PPAR-α, which leads to less fatty acid oxidation and in that way promotes hypertriglyceridemia. In rats fed 10% fructose solutions PPAR-α was reduced while these changes were not observed in glucose fed rats[64]. Additionally, fructose reduced levels of hepatic fatty oxidation and increased levels of the pro-inflammatory transcription factor, nuclear factor K-β (NFK-β)[64]. Many compounds, such as fibrates and some fatty acids successfully enhance PPAR-α activity and remedy fructose-induced hypertriglyceridemia[61].

No list of factors promoting fructose-induced hypertriglyceridemia would be complete without including the forkhead transcription factor (FOXO1), which is involved in carbohydrate and lipid metabolism. FOXO1 is required for the activation of gluconeogenic gene expression in hepatic cells[65]. Fructose can stimulate FOXO1 expression, which favors a gluconeogenic state[66]. Additionally, it has been suggested that FOXO1 increases hepatic expression of a potent lipoprotein lipase (LPL) inhibitor, Apolipoprotein C-III (APOC3)[67]. APOC-3 is produced in the liver and released into circulation attached to very low-density lipoproteins[68]. In the presence of insulin LPL plays a pivotal role in the clearance of plasma triglycerides[69]. Thus, it

seems that fructose is a dual threat to plasma triglyceride clearance; by its ability to activate APOC3 and its inability to produce significant insulin release.

Lastly, protein tyrosine phosphatase-1B (PTP-1B) is a receptor localized on the cytoplasmic side of endoplasmic reticulum. It negatively regulates insulin signaling in vivo [70,71]. Normally, PTP-1B will regulate insulin signaling to match that of the body's demand. The greatest expression of PTP-1B occurs in the absence of insulin and in insulin resistant states. Overexpression of PTP-1B leads to a desphosphorylation of insulin receptors and a down regulation of insulin signaling molecules[72]. Chronic fructose feeding causes PTP-1B overexpression in hamsters[73] and mice[74]. PTP-1B overexpression shifts hepatic lipid metabolism to favor an increase in lipoprotein secretion by increasing apoB100, a required substrate for lipoprotein production [74]. In that way, fructose promotes increased VLDL synthesis and release, and contributes to FIH.

III. Therapeutics

Recently, some researchers have focused on finding therapeutic drugs to ameliorate fructose-induced hypertriglyceridemia. Several exogenous compounds have been tested for their ability to interfere at different steps in the fructose metabolism pathway. Some of these findings are discussed below.

Insulin requires nitric oxide to stimulate glucose uptake. Fructose raises uric acid levels and uric acid inhibits nitric oxide bioavailability. Allopurinol is a zanthene oxidase inhibitor, and in that way can interfere with uric acid synthesis. Nakagawa reported that Allopurinol effectively reduced uric acid even in combination with a HFCS diet[75]. Additionally, it inhibited hyperinsulinemia,

hypertriglyceridemia, and hyperuricemia seen in rats fed only HFCS diets without the drug. These data provide evidence that uric acid may be important in the connection between fructose consumption and metabolic syndrome development.

Vila has reported that atorvastatin can inhibit triglycerides in plasma, and was effective in preventing increased plasma and liver TG accumulation in rats fed a high fructose diet[76]. Atorvastatin also reduced fatty acid beta oxidation. It also increased several genes that control fatty acid catabolism, prevented activation of CREBP transcriptional activity, blunted liver incorporation of fructokinase and prevented histological damage by fructose-induced liver inflammation. Rasineni[77] has shown that C. roseus (a subshrub also known as Lochnera rosea) can prevent fructose-induced hypertriglyceridemia and lower hepatic fructokinase activity. Another therapeutic, Enicostemma littorale Blume extract (EL), successfully lowered TG levels by ameliorating glucose tolerance[78]. EL has HMG-CoA reductase activity that allows it to have a hypolipidemic effect as well as beneficial effects on platelet aggregation and blood coagulation[78].

Finally, the antisense oligonucleotide ChREBP ASO (a therapeutic aimed at reducing TGs) successfully decreased plasma TG concentrations compared with control ASO treatments in rats fed high fat and high fructose diets[79]. Additionally fructose fed rats treated with ChREBP ASO had increased plasma uric acid, alanine transaminase and aspartate aminotransferase concentrations due to decreased fructose aldose and fructokinase expression[79].

Fibrates are a class of plasma triglyceride lowering drugs that are used routinely in clinical practice[80]. The fibrates act as ligands for PPAR-α and induce

expression of their associated fatty acid beta-oxidation genes. Additionally, fibrates also act to increase the sensitivity of LPL by reducing the expression of the LPL inhibitor, APOC3[81]. These three actions by fibrates can lower plasma triglycerides by increasing fatty acid beta-oxidation, by interfering with lipolysis, and by increasing LPL sensitivity, respectively. Several investigators have reported that fibrates can ameliorate fructose-induced hypertriglyceridemia in animal models[61,66,82,83]. Thiazolidinediones (TZD) are a class of anti-diabetic drugs that target peroxisome proliferator activated receptor-gamma (PPAR-γ) in adipocytes to improve insulin sensitivity . TZD's can reverse and/or prevent fructose-induced hypertriglyceridemia is animal models[84-87].

IV. Summary

Long-term exposure to dietary fructose (as opposed to glucose) results in marked increases in several enzymes that lead to metabolic dysfunction in rats and humans. The increases in gene expression such as those noted above, as well as a lack of key regulatory steps in initial fructose metabolism favors de novo lipogenesis (synthesis of triglycerides) in the liver. As a consequence, there is an overproduction of very low-density lipoprotein and triglycerides that are released into circulation, leading to hypertriglyceridemia [10,55,56]. In contrast, glucose does not increase gene expression that facilitates de novo lipogenesis, and thus spares a massive unregulated surge of metabolites favoring lipid production. One of the questions for future research deals with the safety of the country's continued use of fructose as a principal sweetening agent in our food supply. As attributed to Paracelsus "The dose makes the poison". Is there a safe level of exposure? If so, how does it vary across the life-

span? Also, there are financial implications to altering a major component of the country's diet that need to be factored in to making such a decision. We look forward to several years of scientific debate.

The review of fructose intake and metabolism presented above has been written to facilitate a thorough examination of FIH. Presented below is a summary of the work that has been reviewed. The list is by no means exhaustive, but is offered to the reader as an initial guide into an important literature as we continue to grapple with developing a thorough assessment of the effects of our use of sugars in our diets. These summaries are divided into three types of FIH papers: reviews (like this one), papers that focus on some component of the pathway (from fructose intake to circulating triglycerides) and papers that focus on assessing the efficacy of exogenous compounds that suppress circulating triglycerides.

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References

References

1. Nikkilä EA, Ojala K. Acute effects of fructose and glucose on the concentration and removal rate of plasma triglyceride. *Life Sci.* 1966; **5**:89–94

2. Storlein LH, Oakes ND, Pan DA, Kusunoki M, Jenkins AB. Syndromes of insulin resistance in the rat. Inducement by diet and amelioration with benfluorex. *Diabetes* 1993; **42**: 457-62.

3. Kanarek RB, and Orthen-Gambill N. Differential effects of sucrose, fructose, and glucose on carbohydrate-induced obesity in rats. *Journal of Nutrition* 1982; **112**: 1546-54.

4.Bar-On H, and Stein Y. Effect of glucose and fructose administrations on lipid metabolism in the rat. *Journal of Nutrition* 1967; **94**: 95-105.

5. State Obesity Prevalence, http://www.cdc.gov/obesity/data/adult.html. CDC, Published May 2011, Accessed 22 May 2013.

6. Cawley, John, and Chad Meyerhoefer. The medical care costs of obesity: an instrumental variables approach. *Journal of health economics* 2012; **31**: 219-230.

7. Institute of Medicine (US). Committee on Accelerating Progress in Obesity Prevention, & Glickman, D. (2012). *Accelerating Progress in Obesity Prevention: Solving the Weight of the Nation*. National Academies Press.

8. Bocarsly ME, Powell ES, Avena NM, Hoebel BG. High-fructose corn syrup causes characteristics of obesity in rats: Increased body weight, body fat and triglyceride levels. *Pharmacol Biochem Behav* 2010; **97**: 101–6.

9. Malik VS, Hu FB. Sweeteners and risk of obesity and type 2 diabetes: the role of sugar-sweetened beverages. *Curr Diab Rep* 2012; **12**: 195–203.

10. Stanhope KL. Role of fructose-containing sugars in the epidemics of obesity and metabolic syndrome. *Annu Rev Med* 2012; **63**: 329-43.

11. Vos, Miriam B., et al. Dietary fructose consumption among US children and adults: the Third National Health and Nutrition Examination Survey. *The Medscape Journal of Medicine* 2008;**10**: 160.

12. Havel PJ. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. *Nutr Rev* 2005; **63**: 133–57.

13. Farooqi IS, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher PC. Leptin Regulates Striatal Regions and Human Eating Behavior. *Science* 2007; **317**: 1355.

14. Wren AM, Small CJ, Ward HL, et al. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000; **141**: 4325–8.

15. Wren AM, Small CJ, Abbott CR, et al. Ghrelin causes hyperphagia and obesity in rats. *Diabetes* 2001; **50**: 2540–7.

16. London E, Lala G, Berger R, et al. Sucrose access differentially modifies 11β-hydroxysteroid dehydrogenase-1 and hexose-6-phosphate dehydrogenase message in liver and adipose tissue in rats. *J Nutr* 2007; **137**: 2616–21.

17. London E, Castonguay T. High fructose diets increase 11β-hydroxysteroid dehydrogenase type 1 in liver and visceral adipose in rats within 24-h exposure. *Obesity* 2010; **19**: 925–32.

18. Colley DE, London B, Jiang J, Khural, and Castonguay TW. Fructose and the control of food intake. In: *Fructose and the Synthesis, Functions and Health Implications.* Johnston BM and Collins AB (eds). Nova Science Publishers Hauppage, NY. Pp 129-143.

19. Colley D and Castonguay TW. The effects of sugar solutions on hypothalamuc appetite regulation. *J. Nutrition (submitted).* 2013.

20. Pelchant, ML. Of human bondage: food craving, obsession, compulsion, and addiction. *Physiol Behav* 2002; **76**: 347–52.

21. Erlanson-Albertson C. How palatable food disrupts appetite regulation. *Basic Clin Phamacol Toxicol* 2004; **97**: 61–73.

22. Zheng, H et al. Meal patterns, satiety, and food choice in a rat model of Roux-en-Y gastric bypass surgery. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 2009; **297**: R1273-R1282.

23. Rizkalla SR, Hellal I, and Boillot J. Comparative metabolic effects of 10 week starch, glucose, fructose in normal rats. *Insulin and the Cell Membrane* 1990; 443-450.

24. Rizkalla SW, et al. Comparative effects of 6 week fructose, dextrose and starch feeding on fat-cell lipolysis in normal rats: effects of isoproterenol, theophylline and insulin. *Molecular and cellular biochemistry* 1992; **109**: 127-132.

25. Rizkalla SW, et al. Effects of chronic dietary fructose with and without copper supplementation on glycaemic control, adiposity, insulin binding to adipocytes and glomerular basement membrane thickness in normal rats. *British Journal of Nutrition* 1993; **70**: 199-209.

26. Shapiro A, Mu W, Roncol C, Cheng K, Johnson RJ, Scarpace PJ. Fructose-induced leptin resistance exacerbates weight gain in response to subsequent high-fat feeding. *Am J Physiol Regul Integr Comp Physiol* 2008; **295**: R1370–5.

27. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest* 2000; **105**: 1827–.

28. Avena, Nicole M. Examining the addictive-like properties of binge eating using an animal model of sugar dependence. *Experimental and clinical psychopharmacology* 2007; **15**: 481.26. Colanutoni (2002)

29. Grimm, Jeffrey W., Amber M. Fyall, and Dan P. Osincup. Incubation of sucrose craving: effects of reduced training and sucrose pre-loading. *Physiology & behavior* 2005; **84**: 73-79.

30. Wideman, C. H., G. R. Nadzam, and H. M. Murphy. Implications of an animal model of sugar addiction, withdrawal and relapse for human health. *Nutritional neuroscience* 2005; **8**: 5-6.

31. Avena NM, Rada P, Hoebel BG. Underweight rats have enhanced dopamine release and blunted acetylcholine response in the nucleus accumbens while bingeing on sucrose. *Nueroscience* 2008; **156**: 865–71.

32. Spangler R, Wittkowski KM, Goddard NL, Avena NM, Hoebel BG, Leibowitz SF. Opiate-like effects of sugar on gene expression in reward areas of the rat brain. *Brain Res Mol Brain Res* 2004; **124**: 134–42

33. Miyazaki M, Dobrzyn A, Man WC, et al. Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated introduction of lipogenic gene

expression by sterol regulatory element-binding protein-1c-dependent and – independent mechanisms. *J Biol Chem* 2004; **279**: 25164–71.

34. Björntorp, P. "The regulation of adipose tissue distribution in humans."*International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity* 1996; **20**: 291.

35. Stanhope KL, Schwarz JM, Keim NL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest* 2009; **119**: 1322–34.

36. Kruse MS, Rey M, Vega MC, Coirini H. Alterations of LXRα and LXRβ expression in the hypothalamus of glucose-intolerant rats. *J Endocrinol* 2012; **215**: 51–8.

37. Groen J. (1937). The absorption of hexoses from the upper part of the small intestine in man. *J Clin Invest* 1937; **16**: 245.

38. Kellett GL, Helliwell PA. The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane. *Biochemical Journal* 2000; **350**: 155

39. Semenza G, Kessler M, Hosang M, Weber J, Schmidt U. Biochemistry of the Na+, D-glucose cotransporter of the small-intestinal brush-border membrane. The state of the art in 1984. *Biochim Biophys Acta* 1984; **779**: 343.

40. Ravich WJ, Bayless TM, Thomas M. Fructose: incomplete intestinal absorption in humans. *Gastroenterology* 1983; **84**: 26.

41. Kneepkens CM, Vonk RJ, Fernandes J. Incomplete intestinal absorption of fructose. *Archives of disease in childhood* 1984; **59**: 735–38.

42. Truswell AS, Seach JM, Thorburn AW. Incomplete absorption of pure fructose in healthy subjects and the facilitating effect of glucose. *Am J Clin Nutr* 1988; **48**: 1424–30.

43. Burant CF, Takeda J, Brot-Laroche E, Bell GI, Davidson NO. Fructose transporter in human spermatozoa and small intestine is GLUT5. *J Biol Chem* 1992; **267**: 14523–6.

44. Blakemore SJ, Aledo JC, James J, Campbell FC, Lucocq JM, Hunda HS. The GLUT5 hexose transporter is also localized to the basolateral membrane of the human jejunum. *Biochem J* 1995; **309**: 7.

45. Kellett GL, Brot-Laroche E. Apical GLUT2 a major pathway of intestinal sugar absorption. *Diabetes* 2005; **54**: 3056–62.

46. Topping DL, Mayes PA. Comparative effects of fructose and glucose on the lipid and carbohydrate metabolism of perfused rat liver. *Br J Nutr* 1976; **36**: 113–26.

47. Topping DL, Mayes PA. The concentrations of fructose, glucose and lactate in the splanchnic blood vessels of rats absorbing fructose. *Nutr Metab* 2008; **13**: 331–8.

48. Mendeloff AI, Weichselbaum TE. Role of the human liver in the assimilation of intravenously administered fructose. *Metabolism* 1953; **2**: 450.

49. Radziuk J, McDonald TJ, Rubenstein D, Dupre J. Initial splanchnic extraction of ingested glucose in normal man. *Metabolism* 1978; **27**: 657–69.

50. Lustig, Robert H. Fructose: metabolic, hedonic, and societal parallels with ethanol. *Journal of the American Dietetic Association* 2010; **110**: 1307-1321.

51. Mayes PA. Intermediary metabolism of fructose. *The American J. of Clin Nutr* 1993; **58**: 754S-765S.

52. Tappy L, Le KA, Tran C, Paquot N. Fructose and metabolic diseases: new findings, new questions. *Nutrition* 2010; **26**: 1044–9.

53. Castro GSF, Cardoso JFR, Vannucchi H, Zucoloto S, Jordao AA. Fructose and NAFLD: metabolic implications and models of induction in rats. *Acta Cir Bras* 2011; **26**: 45–50.

54. Castro MC, Massa ML, Schinella G, Gagliardino JJ, Francini F. Lipoic acid prevents liver metabolic changes induced by administration of a fructose-rich diet. *Biochim Biophys Acta* 2013; **1830**: 2226–32.

55. Rebollo A, Roglans N, Alegret M, Laguna JC. Way back for fructose and liver metabolism: bench side to molecular insights. *World J Gastroenterol* 2012; **7**; 6552–9.

56. Teff, Karen L., et al. "Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. *Journal of Clinical Endocrinology & Metabolism* 2004; **89**: 2963-2972.

57. Teff, Karen L., et al. Endocrine and metabolic effects of consuming fructose-and glucose-sweetened beverages with meals in obese men and women: influence of insulin resistance on plasma triglyceride responses. *Journal of Clinical Endocrinology & Metabolism* 2009; **94**: 1562-1569.

58. Stanhope KL, Bremer AA, Medici V, et al. Consumption of fructose and high fructose corn syrups increases postprandial triglycerides, LDL-cholesterol, and apolipoprotein-B in young men and women. *J Clin Endocrinol Metab* 2011; **96**: E1596–605.

59. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002; **109**: 1125–31

60. Lizuka K, Bruick RK, Liang G et al. Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. *Proceedings of the National Academy of Sciences of the United States of America* 2004; **101**: 7281-7286.

61. Moon YA, Shah NA, Mohapatra S, Warrington JA, Horton JD. Identification of a mammalian long chain fatty acyl elongase regulated by sterol regulatory element-binding proteins. *J Biol Chem* 2001; **276**: 45358–66.

62. Nagai Y, Nishio Y, Nakamura T, Maegawa H, Kikkawa R, Kashiwagi A. (2002). Amelioration of high fructose-induced metabolic derangements by activation of PPARα. *Am J Physiol Endocrinol Metab* 2002; **282**: E1180–90.

63. Braissant O, Foufelle F, Scotto C, Dauca M, Wahli W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* 1996; **137**: 354–66.

62. Lin J, Yang R, Tarr PT, et al. Hyperlipidemic effects of dietary saturated fats mediated through PGC-1β coactivation of SREBP. *Cell* 2005; **120**: 261–73.

64. Roglans N, Vila L, Farre M, et al. Impairment of hepatic Stat-3 activation and reduction of PPARalpha activity in fructose fed rats. *Hepatology* 2007; **45**: 778–88.

65. Matsumoto M, Han S, Kitamura T, Accili D. Dual role of transcription factor FoxO1 in controlling hepatic insulin sensitivity and lipid metabolism. *J Clin Invest* 2006; **116**: 2464–72.

66. Qu S, Su D, Altomonte J, et al. PPARα mediates the hypolipidemic action of fibrates by antagonizing FoxO1. *Am J Physiol Endocrinol Metab* 2007; **292**: E421–34.

67. Altomonte J, Cong L, Harbaran S, Richter A, Xu J, Meseck M, Dong HH. Foxo1 mediates insulin action on apoC-III and triglyceride metabolism. *J Clin Invest* 2004; **114**:1493–1503.

68. Jong MC, Hofker MH, Havekes LM. Role of ApoCs in lipoprotein metabolism functional differences between ApoC1, apoC2, and ApoC3. *Arterioscler Thromb Vasc Biol* 1999; **19**: 472–84.

69. Eckel RH. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N Engl J of Med* 1989; **320**: 1060.

70. Elchebly M, Payette P, Michaliszyn E, Cromlish. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene.*Science* 1999; **283:** 1544-1548.

71. Haj, F. G., Verveer, P. J., Squire, A., Neel, B. G., & Bastiaens, P. I. Imaging sites of receptor dephosphorylation by PTP1B on the surface of the endoplasmic reticulum. *Science Signaling*, 2002; **295**: 1708.

72. Cheng A, Dube N, Gu F. Coordinated action of protein tyrosine phosphatases in insulin signal transduction. *European Journal of Biochemistry* 2002; **262** 1050-1059.

73. Taghibiglou, C, Rashid-Kolvear, F, Van Iderstine, SC. et al. Hepatic very low density lipoprotein-ApoB overproduction is associated with attenuated hepatic insulin signaling and overexpression of protein-tyrosine phosphatase 1B in a fructose-fed

hamster model of insulin resistance. *Journal of Biological Chemistry* 2002; **277**: 793-803.

74. Qiu W, Avramoglu RK, Dubé N et al. Hepatic PTP-1B Expression Regulates the Assembly and Secretion of Apolipoprotein B–Containing Lipoproteins Evidence From Protein Tyrosine Phosphatase-1B Overexpression, Knockout, and RNAi Studies.*Diabetes* 2004, **53**: 3057-3066.

75. Nakagawa T, Hu H, Zharikov S, et al. A casual role for uric acid in fructose-induced metabolic syndrome. *Am J Phyisol Renal Physiol* 2006; **290**: F625–31.

76. Vila L, Rebollo A, Adalsteisson GS, et al. Reduction of liver fructokinase expression and improved hepatic inflammation and metabolism in liquid fructose-fed rats after atorvastatin treatment. *Toxicol Appl Pharmacol* 2011; **15**: 32–40.

77. Rasineni K, Bellamkonda R, Singareddy SR, Desireddy S. Abnormalities in carbohydrates and lipid metabolism in high-fructose dietfed insulin resistant rats: amelioration by Catharanthus roseus treatments. *J Physiol Biochem* 2013.

78. Bhatt NM, Chavda M, Desai D, et al. Cardioprotective and antihypertensive effects of Enicostemma littorale Blume extract in fructose-fed rats. *Can J Physiol Pharmacol* 2012; **90**: 1065–73.

79. Erion DM, Popov V, Hsiao, et al. The role of the carbohydrate response element-binding protein in male fructose-fed rats. *Endocrinology* 2013; **154**: 36–44.80.

80. Staels B, Schoonjans K, Fruchart JC, Auwerx J. The effects of fibrates and thiazolidinesdiones on plasma triglyceride metabolism are mediated by distinct peroxisome proliferator activated receptors (PPARs). *Biochimie* 1997; **79**: 95-99.

81. Malmendier CL, Lontie JF, Delcroix C, Dubois DY, Magot T, De Roy L. Apolipoproteins C-II and C-III metabolism in hypertriglyceridemic patients Effect of a drastic triglyceride reduction by combined diet restriction and fenofibrate administration." *Atherosclerosis* 1989; **77**: 139-149.

82. Ackerman Z, Oron-Herman M, Rosenthal T, Pappo O, Link G, Sela BA, Grozovski M. Effects of amlodipine, captopril, and bezafibrate on oxidative milieu in rats with fatty liver. *Digestive diseases and sciences* 2008; **53**: 777-784.

83. Bastow MD, Durrington PN, and Ishola M. Hypertriglyceridemia and hyperuricemia: effects of two fibric acid derivatives (bezafibrate and fenofibrate) in a double-blind, placebo-controlled trial. *Metabolism* 1988; **37** : 217-220.

84. Sharabi Y, et al. P-543: Effect of PPAR-Γ agonist-rosiglitazone-on adiponectin level in the metabolic syndrome. *American Journal of Hypertension* 2005; **18**: 204A-204A.

85. Collino M, et al. Pioglitazone improves lipid and insulin levels in overweight rats on a high cholesterol and fructose diet by decreasing hepatic inflammation. *British journal of pharmacology* 2010; **160**: 1892-1902.

86. Saltiel AR and Olefsky JM. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes* 1996; **45**: 1661-1669.

87. Lee M, et al. Metabolic effects of troglitazone on fructose-induced insulin resistance in the rat. *Diabetes* 1994; **43**: 1435-1439.

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Chapter 2: Fructose intake and circulating triglycerides: an examination of the role of APOC 3

Introduction

Many recent review articles have focused on the increase in fructose consumption over the last century and suggest large amounts of fructose in the diet can lead to hypertriglyceridemia (HTG) [1-2]. This phenomenon has been replicated in both human and animal studies [3-6]. Literature searchers on "fructose AND hypertriglyceridemia" reveal that there are several plausible mechanisms that link fructose and HTG. In particular, this study will focus exclusively on the examination on the role of apolipoprotein CIII (APOC3) in promoting HTG in less than 24 hours.

The obesity epidemic that we are experiencing in the US is a national concern that affects all Americans. It does not discriminate among age, race, class, or gender. In 1985 the rate of obesity across our country was under 14%; but by 2010 the rate exceeded 30% [7]. Furthermore, obesity is quickly becoming a leading cause of major health problems and death in the US [8]. The research community, along with policy makers and health care officials are grappling to find the causes of this relatively sudden increase in American's girth. Several researchers have targeted high-fructose corn syrup as a potential accomplice supporting the obesity epidemic [9-12]. They and their supporters have pointed out that fructose has become more prevalent in our diets over the past century. In 1900 the average fructose intake was

15g/day. Fructose was consumed mainly through eating fruits and vegetables, which have the added benefit of fiber. However, as of 2010, fructose consumption had risen to 73g/day and was being consumed in highly processed forms (13).

The undesirable HTG seen via fructose metabolism is best understood by comparing it to the non-lipogenic sugar, glucose. First, fructose metabolism is under minimal kinetic control when compared to glucose and can increase metabolites that favor triglyceride synthesis (de novo lipogenesis) [14]. This is in part due to the fact that all of fructose metabolism occurs in the liver and the liver is the site for triglyceride synthesis. Secondly, fructose has been shown to alter genetic expression of lipogenic genes that favor increased triglyceride synthesis and reduced plasma clearance when compared to glucose [9, 15-17]. Furthermore, fructose does not simulate a significant postprandial insulin release, which reduces the livers ability to restore homeostasis after a large bolus of fructose when compared to glucose [18]. Taken together, it becomes increasingly clearer how fructose can pose a threat to the metabolic homeostasis of the liver.

Apolipoprotein C-III (APOC3) is a lipoprotein that is expressed in the liver of humans and rodents. The active APOC3 protein consists of 79 amino acids and has a molecular mass of 8.8 kDa. APOC3 is also the most abundant apolipoprotein in plasma with an average concentration of about 12 mg/dL. Several studies have shown a positive correlation between plasma APOC3 concentration and elevated levels of plasma triglycerides & VLDL triglycerides. In addition, several studies have shown that a transgenic mouse model that overexpressed or lacked the APOC3 gene resulted in hyperlipidemia or was protected from hyperlipidemia, respectively [30].

An attractive hypothesis to the lipogenic nature of fructose is in its ability to alter normal hepatic gene expression favoring an increase in lipogenic gene expression [19]. APOC3 has become an increasingly popular mechanistic approach that links fructose with HTG [17]. One study has replicated this phenomenon in chronic fructose feeding (>4 week) [20] but never has it been shown before in an acute fructose bolus (<24 hours). Keep in mind that it has been demonstrated already that fructose can elicit HTG in 24 hours or less [21]. The question addressed by the following experiment is "Is it APOC3 that is responsible for the HTG model in 24 hours or less?"

Fructose can indirectly increase hepatic expression of APOC3. APOC-3 is produced in the liver and released into circulation attached to very low-density lipoproteins [22]. APOC3 is a potent inhibitor of lipoprotein lipase (LPL) [17]. In the presence of insulin LPL plays a pivotal role in the clearance of plasma triglycerides [23]. However, fructose alone does not stimulate a significant insulin release [18]. Thus, it seems that fructose is a dual threat to plasma triglyceride clearance; by its ability to activate APOC3 and its inability to produce significant insulin release.

The purpose of this study is to test various types of fructose-containing sugars (fructose, sucrose and high fructose corn syrup) with the intent of replicating our earlier observations that overnight access to fructose can promote HTG as well as to examine the role that apolipoprotein C3 (APOC3) in promoting HTG . Since pure fructose is the most lipogenic of all the sugars, we hypothesize that the treatment of pure fructose will elicit the greatest perturbation in APOC3 message and protein levels.

Materials and Methods

Animals

Adult male Sprague-Dawley (CD strain) rats (Charles River Laboratories, Wilmington, MA) with a mean weight of approximately 300 grams were used. Upon arrival, all animals were individually housed and maintained on a 12h light/dark cycle with a room temperature of 22° C ± 1° C. During the 1 wk acclimation period the rats were given free access to the control diet and water. Animals were weighed and 24h food intake and sugar solution intake (when appropriate) were measured daily at 0900h throughout the experiment.

All rats were killed by slow replacement of air in a specialized chamber with pure CO_2 followed by rapid decapitation and exsanguination. This method has been approved for use by the Panel on Euthanasia of the American Veterinary Medical Association as well as the UM IACUC. All procedures described herein are in compliance with the University of Maryland's IACUC guidelines.

Animal Diets

The control diet is a nutritionally complete low fat diet [Rodent diet 7012] prepared by Harlan Teklad (Bethlehem, PA) and provides 3.41 metabolizable kcal/g of diet. Per gram of chow, 2.14 kcal were derived from carbohydrate, 0.79 kcal was

derived from protein, and 0.51 kcal was derived from fat. All animals were given free access to water throughout the experiment.

Study Design

After an initial 1 wk acclimation period (during which they had ad lib access to food and water), the rats (N=40) were randomly assigned to one of five weight-matched groups (n=8). Rats assigned to the first group were given ad libitum access to control diet (Harlan rodent diet 7012) only, and served as the control group. Rats assigned to the second group had ad libitum access to the control diet and free access to a 16% weight/volume fructose (Tate & Lyle, Decatur, IL) solution. Rats assigned to the third group had ad libitum access to the control diet plus free access to a 16% glucose weight/volume (Sigma Aldrich, St Louis MO) solution. Rats assigned to the fourth group had ad libitum access to the control diet and free access to 16% high fructose corn syrup (HFCS) weight/volume (Tate & Lyle, Decatur, IL), Finally, rats assigned to the fifth group had ad libitum access to the control diet and free access to a 16% weight/volume sucrose (Domino Foods, Baltimore, MD) solution. All sugar solutions were prepared 24h in advance and stored at 4°C. The rats were maintained on their respective diets for 24h and then sacrificed. At the time of sacrifice, the lobus lateralis sinister of each liver was dissected and flash frozen for storage at -80°C.

Plasma Measures

A Rat/Mouse Insulin ELISA Kit was purchased from Milipore, (Cat.# EZRMI-13K) and used to quantify insulin in rat plasma. Plasma glucose concentrations were measured enzymatically (Smith-Kline Beecham Laboratories). A

Dimension clinical chemistry system Flex reagent cartridge (Siemens Healthcare Diagnostics, Newark, DE) was used with a Dade Behring Dimension Xpand automated system to quantify plasma triglyceride levels. A rat APOC3 ELISA kit was purchased from NeobioLab (Cat.#RA0523) and used to quantify APOC3 protein levels in serum. All plasma measurements were run in duplicate.

RNA Extraction

Total RNA was extracted from each liver sample according to RNeasy Mini and RNeasy Lipid (QIAGEN) kit directions. The samples were purified with DNA-*free* (Ambion) and analyzed spectrophotometrically (Nanodrop) to determine concentration and check for quality. cDNA template was created for each sample from 500 ng of purified RNA using SuperScript III Reverse Transcriptase (Invitrogen).

cDNA Synthesis

cDNA was made using the SABiosciences RT² First Strand Kit. A genomic DNA elimination mixture was first created using 15 μg RNA samples from each group (equal quantity from each animal) that was then mixed with 2.0 μl of 5X qDNA elimination buffer and RNase-free H_20 to a final volume of 10 μl. Contents were mixed gently, incubated at 42˚C for 5 minutes and chilled on ice. An RT cocktail was then prepared, containing 5X RT Buffer 3, Primer & External Control Mix, RT Enzyme Mix 3, and RNase free H_20. 10 μl of the RT cocktail was added to each genomic DNA elimination mixture, mixed well, and incubated at 42˚C for

exactly 15 min and then immediately stopped by heating at 95˚C for 5 min. 91 μl of ddH_20 was added to each 20 μL of cDNA synthesis reaction and the solution was mixed well and stored at -20˚C.

Measurement of Gene Expression

Changes in gene expression were measured in each liver sample, n = 40, using RT-PCR array. IQ SYBER Green Supermix (BIORAD Cat # 170-8882) was used on a C1000 BIO-RAD thermal cycler (CFX96 Real-Time System) to quantify gene expression.

A "housekeeper" or reference gene is used in RT-PCR to enable quantification of a target gene. Numerous attempts were made to elucidate a "housekeeper" gene that was not influenced by our treatments, including beta-ACTIN, RPLP1, and GAPDH. Unfortunately, the Ct values of each identified "housekeeper" were influenced by the treatments. To correct/normalize the "housekeeper" gene in each group a weighting factor was assigned. The weighting factor that transformed each sample to match the beta-actin control groups "housekeeper" value was selected. In addition, the same weighting factor was applied to the target genes Ct values. A sample of how the weighting was conducted is provided in table 1.

Cycle threshold (Ct) values obtained through RT-PCR for all samples are used to calculate fold change of gene expression. Fold change was calculated as 2^(-ddCt). Fold change of 0.5 or less or of 1.5 or greater were considered 'robust'.

Custom Primer Design and Optimization

Primers were designed using Beacon Designer 7 software default parameters (PREMIER Biosoft, Palo Alto, CA). These primers include beta-ACTIN, RPLP1, GAPDH, and APOC3.

Primers were evaluated using the CFX96 (BioRad) protocol, using a gradient that was centered around predicted optimum annealing temperature (TaOpt). The protocol for primer verification PCR is as follows: 95°C, 3 min, and 40 cycles at 95°C for 10 sec, at a gradient of temperatures for 30 sec with subsequent melt curve of 65°- 95°C at increments of 0.5°C for 5 sec. Housekeepers were selected based on the least variation in cycles between groups. See 2 for primer sequences.

Results

Body Weight

Twenty-four hour access to any of the four sugar solutions used failed to promote significant differences in body weight ($p<0.05$) ($F=0.0571$) ($df=(4, 38)$). Refer to body weight data presented in Table .

There was no significant correlation in the amount of sugar consumed and the observed body weight after treatment ($R^2= 0.12$) ($F= 3.9297$).

Intake

Chow

Food intake did not differ between groups during the six-day acclimation period leading up to the treatment day. The average amount of chow consumed by all animals during the acclimation period was 25 grams. However, during the treatment period when the rats had access to sugar, the average chow consumption plummeted to 18.5 grams. All treatment groups but high fructose corn syrup and fructose ate significantly less chow on the treatment day when compared to the control (water) group ($P<0.05$) ($F = 4.1631$)($df = 4, 37$). There were no significant differences between any of the treatment groups. See Figure 1 for values.

Sugar

No differences were observed in the amount of sugar consumed amongst each treatment group ($P<0.05$)($F=1.9691$)($df=3, 30$). See Table 4 for intake data.

Total Intake

No differences were observed in the amount of total caloric intake amongst each group, including control. See table 4 for total intake data ($P<0.05$) ($F=0.2190$) ($df=4, 37$).

Plasma Analyses

Fructose, HFCS, and sucrose consumption caused significant upregulation in circulating triglycerides ($P<0.05$)($F=13.69$)($df=(4,38)$) . No differences exist among the five groups in plasma insulin ($P<0.05$) ($F=0.12$)($df=(4,35)$), plasma glucose ($P<0.05$)($F=1.3$)($df=(4,35)$), and plasma APOC3 ($P<0.05$)($F=1.35$)($df=(4,38)$). Refer to plasma data presented in table 5.

Housekeeper Selection

It has long been recognized that the selection of a housekeeper gene should be determined by that gene's resistance to treatment effects. We examined several standard housekeepers and found that all were significantly affected by sugar solution consumption (Duncan's new multiple range test).The housekeepers that were tested include beta-actin ($P<0.05$)($F=18.5$)($df=(4,37)$), Ribosomal protein large protein1 (RPLP1) ($P<0.05$)($F=20.9$)($df=(4,37)$), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) ($P<0.05$)($F=18.9$)($df=(4,37)$). Refer to housekeeper selection data presented in figure 2.

It was discovered that our treatment groups could influence all of the housekeepers Ct values by a particular replicable pattern. The Ct values of both the Fructose and the control groups were always significantly higher than the other groups but did not differ from one another. This observation was the result of all available housekeepers tested. Refer to housekeeper selection data presented in Table 6.

APOC3 Gene Expression

After normalizing the housekeepers Ct values and adjusting the weighting factor for each group, there were some differences in gene expression among the treatment groups. The water group was our control and reference group for which APOC3 gene expression fold change was measured. The sucrose, & glucose groups had similar significantly positive fold changes at 2.68, and 2.59, respectively when compared to control ($P<0.05$)($F=7.1$)($df=(4,37)$). High-fructose corn syrup had a

positive fold change of 2.40 but was not considered significant by the means ANOVA. Fructose elicited a negative fold change at 0.86 but was not considered significant but the means ANOVA. Refer to APOC3 gene expression presented in figure 3.

Discussion

There is an alarming obesity epidemic in this country. The obesity problem is particularly worrying not only in terms of human health and quality of life but also in terms of dollars currently spent and will be spent on healthcare in the treatment of obesity related comorbidities. Obesity has become the nation's most preventable health problem [24-25].

Despite it's prevalence, there is no known cure for this disease or consensus about how it develops. One hypothesis for the cause of obesity is that dietary intake patterns of Americans have significantly shifted over the past fifty years by including unprecedented amounts of refined sugar [26].

The purpose of this experiment was to examine this hypothesis by evaluating how sugars affect triglyceride metabolism in the liver. Animals were given 24-hour access to either a dilute glucose, sucrose, HFCS, or fructose sugar solution. Intake of both chow and sugar was measured. Hepatocyte tissue was collected and changes in APOC3 gene expression were measured using a RT-PCR assay for the different treatment groups.

Intake

Previous studies have shown that initial presentation of a sugar solution will result in rats consuming excess calories from sugar. Over time however this result washes out and total caloric intake returns to a level that resembles control [27-28].

These findings did not support previous studies that showed an increase in kcal upon initial presentation of sugar access. Our findings suggest that all animals were able to selectively regulate their overall total intake regardless off the type of sugar presented. All sugar-fed animals consumed the same amount of total calories and there were no differences in percent kcal from sugar for groups consuming fructose-containing sugars (sucrose, HFCS, fructose).

The rats in this experiment did not increase intake of glucose-fructose disaccharides (sucrose and HFCS) in such a way as to match the amount of fructose consumed in the fructose group. In other words rats did not have a threshold for fructose consumption and compensate by consuming 50% more sucrose or 45% more HFCS to match the total consumption of fructose in the fructose fed group. We applied the same model as Collier and Bolles did in their 1968 sugar dilution studies. Looking at intake of various sugar dilutions in rats, Collier and Bolles found that regardless of the type of sugar presented, rats will increase their intake of a dilute sugar so as to take in a certain threshold amount of total grams of sugar [29]. In the current study, this model was applied to dilutions of fructose intake. For example, sucrose fed animals had a solution that was 50% less fructose than the fructose animals. Replicating Collier and Bolles' findings, there was not set level for fructose intake as animals consuming sucrose and HFCS, which have less fructose, consumed

the same amount of total kcal from sugar. These findings also show that fructose having a sweeter taste than glucose did not impact intake.

Plasma

Fructose, high-fructose corn syrup, and sucrose caused a significant and robust increase in triglycerides with only 24 hours of access. Others have demonstrated that fructose alone could elicit HTG in 24 hours but never has sucrose and high-fructose corn syrup resulted in HTG in only 24 hrs. The importance of this finding is that hypertriglyceridemia occurred with only 24-hour access to fructose and in addition with other fructose containing sugars.

No differences in plasma insulin, glucose, and APOC3 were observed, as all blood samples were collected under ad libitum feeding conditions.

Gene Expression

The reoccurring pattern of group differences for each housekeeper inhibited our ability to quantify gene expression using traditional methods. Traditionally, a housekeeper is defined by not being affected by the treatment itself and is used as a reference in 2^(-ddCt) equation. Some collaboration with experts in using RT-PCR was required before concluding that a weighting factor was a viable option. The control group in our experiment became the reference Ct value that was needed to correct for all other grouped beta-actin & APOC3 values and eventually applied to the 2^(-ddCt) equation.

An obvious limitation to the APOC3 gene expression results includes the manipulation of data as seen in the weighting factor that was applied. However, many attempts were made to elucidate a housekeeper gene that would allow the use of the more traditional method in gene expression studies. Each housekeeper was tested twice with duplicate sample data. In addition, the selection of housekeepers to be tested came from literature searchers that suggested they are widely utilized in the scientific community.

However limited the results may be, they are much different than expected. The available literature suggests that APOC3 is a possible link to eliciting hypertriglyceridemia via increased mRNA gene expression in liver that leads to increased protein expression in circulation. The literature also suggests chronic fructose consumption leads to an increase in APOC3 mRNA and Protein levels. Therefore, the current studies hypothesis was that treatment groups with the highest fructose concentration would have highest levels of APOC3, both message and protein. Interestingly, the results from this study tell quite a different story. The groups containing glucose (glucose, sucrose, and high-fructose corn syrup) elicited the highest APOC3 mRNA concentration in the liver compared to the control. The pure fructose treatment group, which was thought to increase APOC3 message the most, deviated from the hypothesis by slightly decreasing the message compared to control.

Conclusion

This study contributed a few novel items to the literature on fructose-induced HTG. First, this study confirmed that fructose-induced HTG could be elicited in 24 hrs. or less in many of the readily available fructose containing sugars (fructose, sucrose, and high-fructose corn syrup). Not to mention that in the experimental design the animals had free access to sugars for the first time and could control their own intake, which makes it physiologically relevant. Secondly, this study tested the validity of APOC3 and its role in the fructose-induced HTG mechanism in 24 hrs or less. Contrary to other studies that suggest APOC3 is responsible for HTG, here we conclude that there is an alternative mechanism to elicit HTG in 24 hrs or less that has not yet been identified. To be certain a more comprehensive study needs to be designed that could measure the ratios of other lipoproteins involved in newly synthesized VLDL and test their relative proportionalities to APOC3. This could confirm or deny APOC3's involvement in the 24 h mechanism to elucidate HTG.

Table 1. Example of weighting factor used for the “housekeeper” beta-actin

Rat #	Ct value	Group	Water avg. Ct	Water avg. Ct / Ct value		
1A	22.57	W	21.87	*Represented as a % and added to APOC3 Ct	APOC3 Ct	Corrected APOC 3
7A	22.23	W	21.87		17.04	
10A	22.13	W	21.87		16.16	
15A	20.54	W	21.87		15.61	
9A	19.14	S	21.87	*1.14	16.71	19.09
18A	18.92	S	21.87	*1.16	13.69	15.83
19A	19.30	S	21.87	*1.13	14.48	16.40
3A	21.75	HF	21.87	*1.01	16.19	16.27
11A	21.46	HF	21.87	*1.02	15.04	15.32
16A	19.97	HF	21.87	*1.09	14.95	16.36
20A	19.00	HF	21.87	*1.15	13.99	16.10

Rat # is used for identification purposes. Ct value is obtained via RT-PCR and represents relative threshold amount of amplified beta-actin mRNA. The Group identifiers are W (water, control), S (sucrose, treatment), and HF (high fructose corn syrup, treatment). The water average Ct value represents the “housekeeper” value for the control group. Water avg. Ct / Ct value is represented as a percentage, which is used as the weighting factor for subsequent use. APOC3 Ct is multiplied by the weighting factor to produce the corrected APOC3 Ct value as seen in the last column. Please find a more simplified equation here; Y (Corrected APOC3)= [X (avg ct value of water group) / Z (ct value of sample)]*A (APOC3 ct value) ; Y= [X/Z] * A.

Table 2. Primers used in RT-PCR analyses.

Gene	Accession Number	Sense Primer	Anti-sense Primer
APOC 3	NM_012501	ATGGACAATCGCT TCAAA	TCAAGAGTTGGT GTTGTT
B ACTIN	NM_031144	TGTCACCAACTGG GACGATA	GGGGTGTTGAAG GTCTCAAA
GAPD H	NM_017008	TCCCATTCTTCCA CCTTT	TAGCCATATTCAT TGTCATACC
RPLP1	NM_001007 604	GAAGAATCCGAG GATGACA	CAGGTTCAGCTCT TTATTGG

Primers were designed using Beacon Designer 7 unless otherwise noted in Methods section.

Table 3. Average body weight of rats following 24-hour sugar access.

	Control	Glucose	Sucrose	HFCS	Fructose
Average BW (g)	**293.8[a]**	**292.2[a]**	**295.1[a]**	**292.7[a]**	**289.8[a]**
SEM	**+8.8**	**+8.4**	**+8.0**	**+7.4**	**+8.7**

Average body weight in grams. Rats were weighed prior to sacrifice. Averages that share a common superscript are not different from one another ($F=0.0571$; $df=(4,38)$. Standard error of the mean (SEM) is presented below the averages for each group. No significant correlation existed between sugar intake (g) and body weight (g) ($R^2 = 0.12$).

Table 4. Average sugar, chow and total intake in kcal following 24-hour sugar access.

Group	Sugar-Kcal	SEM	%Kcal from sugar	Chow-kcal	SEM	%Kcal from chow	Total kcal	SEM
Control	na	na	0	93.1[a]	+10.8	100	93.1[a]	+10.8
Sucrose	52.7[a]	+3.9	51.5	49.6[b]	+7.4	48.5	102.3[a]	+7.8
HFCS	44.6[a]	+3.4	43.8	57.2[b]	+8.7	56.2	101.8[a]	+7.0
Fructose	38.8[a]	+5.7	40.2	57.7[b]	+12.1	59.8	96.5[a]	+15.2
Glucose	46.2[a]	+2.2	50	46.2[b]	+5.2	50	92.4[a]	+4.8

Mean intake of kilocalories derived from sugar and chow on treatment day. There are no significant differences in the amount of kcals derived from the sugar treated groups ($F=1.9691$)($df=3, 30$) or total kcals from sugar + chow ($F=0.2190$) ($df=4, 37$). Standard error of the mean (SEM) is presented beside the averages for each group. Superscripts with different letters indicate a significant difference at the $P<0.05$ level.

Table 5. Average Triglycerides, Insulin, Glucose, and APOC3 in Plasma following 24-hr sugar access.

Group	Insulin ng/mL	SEM	Triglycerides	SEM	Glucose mg/dL	SEM	APOC3 ng/mL	SEM
Water	**4.2^{a}**	**+0.6**	**73.4^{c}**	**+20.7**	**150.0^{a}**	**+2.0**	**321.9^{a}**	**+53.7**
Sucrose	**4.5^{a}**	**+0.7**	**257.4^{ab}**	**+30.3**	**149.9^{a}**	**+2.1**	**333.8^{a}**	**+71.1**
HFCS	**4.2^{a}**	**+0.9**	**286.2^{a}**	**+31.6**	**148.8^{a}**	**+3.6**	**243.9^{a}**	**+64.4**
Fructose	**4.5^{a}**	**+0.9**	**311.6^{a}**	**+41.6**	**155.7^{a}**	**+2.9**	**412.7^{a}**	**+87.4**
Glucose	**4.4^{a}**	**+1.1**	**132.1^{bc}**	**+20.6**	**147.2^{a}**	**+3.4**	**208.4^{a}**	**+57.0**

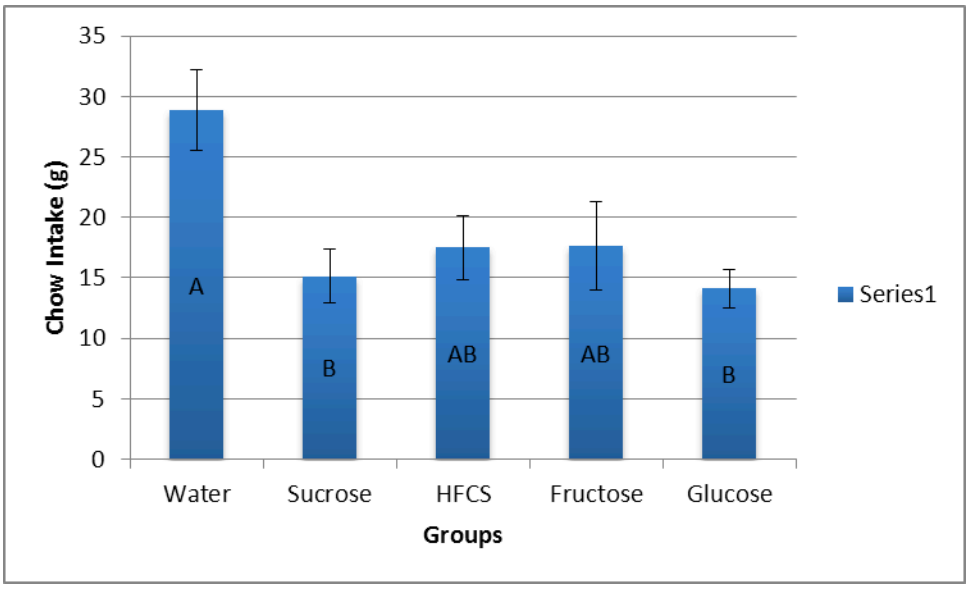
Group means are presented here for plasma insulin, triglycerides, glucose, and APOC3. Superscripts with different letters indicate a significant difference at the $P<0.05$ level. The treatments were only effective in eliciting a significant difference in the triglyceride assay as indicated by the subscripts ($P<0.05$)($F=13.69$)($df=(4,38)$). The statistics for insulin, glucose and APOC3 are ($F=0.12$)($df=(4,35)$), ($F=1.3$)($df=(4,35)$), and ($F=1.35$)($df=(4,38)$), respectively. Standard error of the mean (SEM) is presented beside the averages for each group.

Table 6. Housekeeper Ct Values

Group	Beta-Actin	SEM	RPLP-1	SEM	GAPDH	SEM
Water	**21.9[a]**	**+0.3**	**19.7[a]**	**+0.2**	**18.6[a]**	**+0.1**
Fructose	**22.2[a]**	**+0.2**	**19.5[a]**	**+0.3**	**18.8[a]**	**+0.3**
Glucose	**20.2[b]**	**+0.2**	**18.3[b]**	**+0.2**	**17.1[b]**	**+0.2**
HFCS	**20.7[b]**	**+0.4**	**18.2[b]**	**+0.2**	**17.2[b]**	**+0.3**
Sucrose	**19.4[c]**	**+0.1**	**17.6[c]**	**+0.2**	**16.2[c]**	**+0.2**

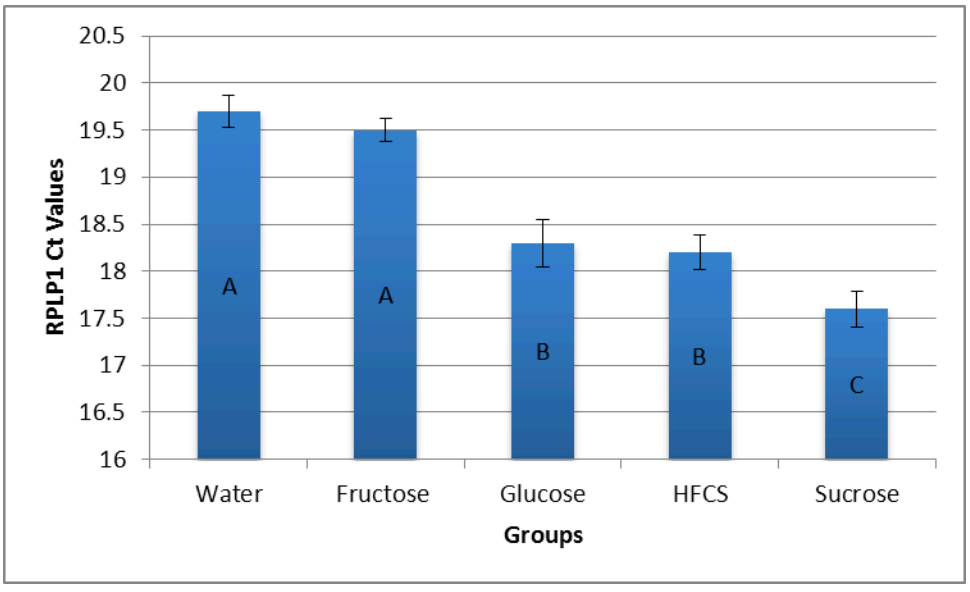
Group means are presented here for three different housekeepers. The housekeepers that were tested include beta-actin ($P<0.05$)($F=18.5$)($df=(4,37)$), Ribosomal protein large protein 1 (RPLP1) ($P<0.05$)($F=20.9$)($df=(4,37)$), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) ($P<0.05$)($F=18.9$)($df=(4,37)$). Superscripts with different letters indicate a significant difference at the $P<0.05$ level. Each housekeeper produced the same pattern for group differences. Standard error of the mean (SEM) is presented beside the averages for each group.

Figure 1. Twenty-four hr. Chow Intake on Treatment Day



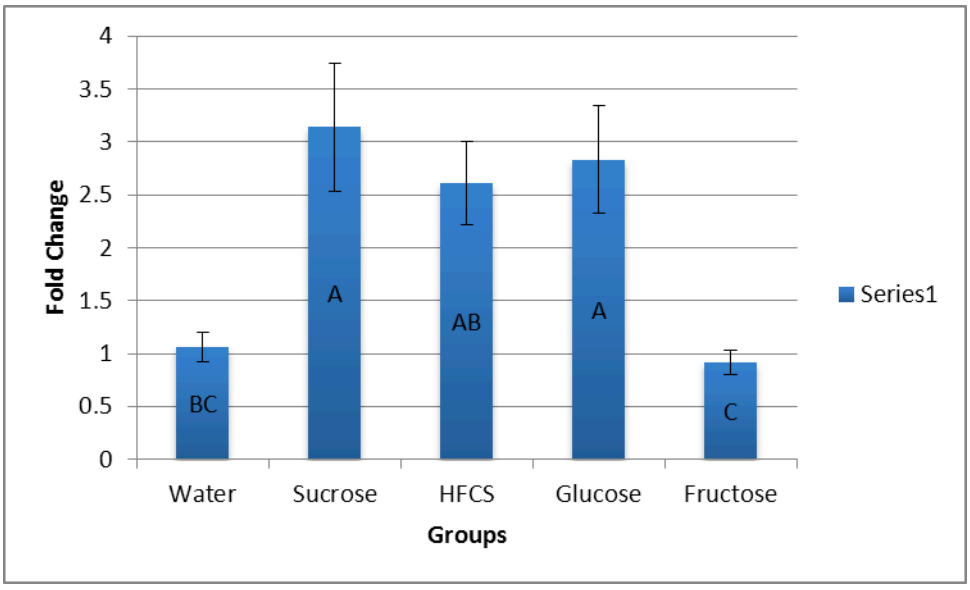
The sucrose and glucose groups ate significantly less chow on the treatment day when compared to the control (water), ($F = 4.1631$)($df = 4, 37$. There are no significant differences between any of the sugar treated groups. Means sharing the same letter are not significantly different from one another.

Figure 2. Example of Reoccurring Housekeeper Data



Group mean Ct values for RPLP1 are presented here. Groups sharing the same letter are not significantly different from one another, ($P<0.05$)($F=20.9$)($df=(4,37)$).

Figure 3. APOC3 mRNA Gene Expression



APOC3 mRNA gene expression is presented here with regards to fold changes using the 2^(-ddCt) equation. Only the glucose containing sugars sucrose, HFCS, and glucose were able to positively increase the mRNA expression of APOC3 in a significant manner ($P<0.05$)($F=7.1$)($df=(4,36)$).

References

[1]. Havel PJ. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. *Nutrition reviews*. 2005; *63*(5): 133-157.

[2]. Tappy L, and Lê KA. Metabolic effects of fructose and the worldwide increase in obesity. *Physiological reviews*. 2010; *90*: 23-46.

[3]. Storlein LH, Oakes ND, Pan DA, Kusunoki M, Jenkins AB. Syndromes of insulin resistance in the rat. Inducement by diet and amelioration with benfluorex. *Diabetes* 1993; **42**: 457-62.

[4]. Kanarek RB, and Orthen-Gambill N. Differential effects of sucrose, fructose, and glucose on carbohydrate-induced obesity in rats. *Journal of Nutrition* 1982; **112**: 1546-54.

[5]. Bar-On H, and Stein Y. Effect of glucose and fructose administrations on lipid metabolism in the rat. *Journal of Nutrition* 1967; **94**: 95-105.

[6]. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, Havel PJ. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *The Journal of clinical investigation*. 2009; *119*(5): 1322.

[7]. State Obesity Prevalence, http://www.cdc.gov/obesity/data/adult.html. CDC, Published May 2011, Accessed 22 May 2013.

[8]. Cawley J, and Meyerhoefer C. The medical care costs of obesity: an instrumental variables approach. *Journal of health economics* 2012; **31**: 219-230.

[9]. Institute of Medicine (US). Committee on Accelerating Progress in Obesity Prevention, & Glickman, D. (2012). *Accelerating Progress in Obesity Prevention: Solving the Weight of the Nation*. National Academies Press.

[10]. Bocarsly ME, Powell ES, Avena NM, Hoebel BG. High-fructose corn syrup causes characteristics of obesity in rats: Increased body weight, body fat and triglyceride levels. *Pharmacol Biochem Behav* 2010; **97**: 101–6.

[11]. Malik VS, Hu FB. Sweeteners and risk of obesity and type 2 diabetes: the role of sugar-sweetened beverages. *Curr Diab Rep* 2012; **12**: 195–203.

[12]. Stanhope KL. Role of fructose-containing sugars in the epidemics of obesity and metabolic syndrome. *Annu Rev Med* 2012; **63**: 329-43.

[13]. Vos MB. Dietary fructose consumption among US children and adults: the Third National Health and Nutrition Examination Survey. *The Medscape Journal of Medicine* 2008;**10**: 160.

[14]. Schwarz JM, Neese RA, Schakleton C, Hellerstein MK. De novo lipogenesis during fasting and oral fructose ingestion in lean and obese hyperinsulinemic subjects. Diabetes. 1993;42(suppl 1):A39.

[15]. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002; **109**: 1125–31

[16]. Moon YA, Shah NA, Mohapatra S, Warrington JA, Horton JD. Identification of a mammalian long chain fatty acyl elongase regulated by sterol regulatory element-binding proteins. *J Biol Chem* 2001; **276**: 45358–66.

[17]. Altomonte J, Cong L, Harbaran S, Richter A, Xu J, Meseck M, Dong HH. Foxo1 mediates insulin action on apoC-III and triglyceride metabolism. *J Clin Invest* 2004; **114**:1493–1503.

[18]. Chong MF, Fielding BA, Frayn KN. Mechanisms for the acute effect of fructose on postprandial lipemia. *The American journal of clinical nutrition*. 2007; *85*(6): 1511-1520.

[19]. Basciano H, Federico L, and Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutrition & Metabolism*. 2005; *2*(1): 5.

[20]. Qu S, Su D, Altomonte J, Kamagate A, He J, Perdomo G, Dong HH. PPARα mediates the hypolipidemic action of fibrates by antagonizing FoxO1. *American journal of physiology. Endocrinology and metabolism*. 2007; *292*(2): E421.

[21]. Colley DE, London B, Jiang J, Khural, and Castonguay TW. Fructose and the control of food intake. In: *Fructose and the Synthesis, Functions and Health Implications.* Johnston BM and Collins AB (eds). Nova Science Publishers Hauppage, NY. Pp 129-143

[22]. Jong MC, Hofker MH, Havekes LM. Role of ApoCs in lipoprotein metabolism functional differences between ApoC1, apoC2, and ApoC3. *Arterioscler Thromb Vasc Biol* 1999; **19**: 472–84.

[23]. Eckel RH. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N Engl J of Med* 1989; **320**: 1060.

[24]. Jia H, Lubetkin EI. Trends in quality-adjusted life-years lost contributed by smoking and obesity. Am J Prev Med. 2010;38:138-44.

[25].Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. JAMA. 2010;303:235-41.

[26]. Paeratakul S, York-Crowe EE, Williamson DA, Ryan DH, Bray GA. Americans on diet: results from the 1994-1996 Continuing Survey of Food Intakes by Individuals. J Am Diet Assoc. 2002;102:1247-51.

[27]. London E, Lala G, Berger R, Panzenbeck A, Kohli AA, Renner M, Jackson A, Raynor T, Loya K, Castonguay TW. Sucrose access differentially modifies 11beta-hydroxysteroid dehydrogenase-1 and hexose-6-phosphate dehydrogenase message in liver and adipose tissue in rats. J Nutr. 2007;137:2616-21.

[28]. Kanarek RB, Orthen-Gambill N. Differential effects of sucrose, fructose and glucose on carbohydrate-induced obesity in rats. J Nutr. 1982 Aug;112:1546-54.

[29]. Collier G, Bolles R. Some determinants of intake of sucrose solutions. J Comp Physiol Psychol. 1968 Jun;65:379-83.

[30]. Jong MC, Hofker MH, and Havekes LM. Role of ApoCs in lipoprotein metabolism functional differences between ApoC1, apoC2, and ApoC3. *Arteriosclerosis, thrombosis, and vascular biology*. 1999; *19*(3): 472-484.