THE EFFECTS OF A HIGH FRUCTOSE MEAL ON POSTPRANDIAL SUBSTRATE OXIDATION

By

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CERTIFICATE OF APPROVAL

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LIST OF ABBREVIATIONS AND ACRONYMS

BMI	Body Mass Index
NHANES III	Third National Health and Nutrition Examination Survey
ATP	Adenosine Triphosphate
AMP	Adenosine Monophosphate
ADP	Adenosine Diphosphate
AMP kinase	5'-AMP-activated protein kinase
ACC	Acetyl CoA Caboxylase
AUC	Area Under the Curve
CPT-1	Carnitine Palmitoyltransferase 1
REE	Resting Energy Expenditure
vO_2	Volume of Oxygen Consumed
vCO_2	Volume of Carbon Dioxide Expelled
RQ	Respiratory Quotient
UUN	Urine Urea Nitrogen
OCTRI	Oregon Clinical & Translational Research Center
OHSU	Oregon Health & Science University
NPRQ	Non Protein Respiratory Quotient
Kcal	Kilocalorie
SD	Standard Deviation

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Abstract

Background: The consumption of fructose has increased substantially in recent decades due to the increased use of added sugars, including sucrose and high-fructose corn syrup in food products. This trend of increasing fructose consumption has mirrored the rising trend in obesity rates in the U.S. Current research suggests that excessive consumption of fructose may increase the risk of obesity, cardiovascular disease, and type 2 diabetes.

Objectives: The primary aim of this study was to determine the effects of a high fructose meal on postprandial substrate oxidation in non-obese, healthy males. Fructose does not stimulate insulin release from the pancreas. Lower insulin concentrations lead to lower leptin release from adipose tissue. Because leptin signals the switch from carbohydrate oxidation in the fed state to lipid oxidation postprandially, we hypothesized that a high fructose meal would result in prolonged carbohydrate oxidation and decreased lipid oxidation. The secondary objectives were to determine the effects of a high fructose meal on postprandial triglyceride, glucose, insulin, and leptin levels.

Methods: A randomized, double-blind, crossover study design was used to compare the effects of consuming a high fructose and high glucose meal containing 30% of kilocalories from either fructose or glucose. Triglyceride concentrations, glucose, insulin, leptin, and non protein respiratory quotient were measured at fasting and at one, four, and seven hours postprandially.

Results: There were no differences in baseline values of any of the parameters measured between the two meals. Triglyceride concentrations were significantly higher four hours following the high fructose meal compared to the high glucose meal. Insulin concentrations one hour following the high fructose meal were significantly lower compared to the glucose meal, and area under the curve for insulin was significantly lower following the high fructose meal compared to the high glucose meal. There were no differences in postprandial non protein respiratory quotient, glucose, or leptin concentrations following the high fructose and high glucose meals.

Conclusions: There was no difference in carbohydrate and lipid oxidation during the seven hours following the high fructose meal compared to the high glucose meal. Although the predicted elevated triglyceride concentrations and blunted insulin concentrations were seen following the high fructose meal, the expected responses for glucose and leptin were not. Because there was no difference in the seven hour non protein respiratory quotient following the high fructose and high glucose meals, which was the primary outcome of this investigation, we conclude that a single high fructose meal does not alter postprandial substrate oxidation in non-obese, healthy males. Additional research is needed on the effects of multiple high fructose meals and the effects of fructose in different subject groups to better understand the metabolic effects of a high fructose diet.

Chapter 1 –Overview, Specific Aims and Hypotheses

Overview

Consumption of fructose has risen substantially in recent decades due to the increased use of added sugars, including sucrose and high-fructose corn syrup, in food products. Current research suggests that excessive consumption of fructose may increase the risk of obesity, cardiovascular disease, and type 2 diabetes. It has been demonstrated that consumption of a high-fructose meal increases triglyceride concentrations in both healthy weight and obese subjects, which may have negative effects on cardiovascular health. Also, unlike glucose, fructose does not elicit an insulin response. Insulin release is necessary to induce the release of leptin from adipose tissue. Without episodic insulin peaks, postprandial leptin levels gradually decrease. One of the roles of leptin is to regulate lipid metabolism in muscle cells; the leptin signaling cascade functions to increase fatty acid oxidation. Leptin also acts as a long-term satiety signal in the brain. It has been demonstrated that, in the long term, decreased leptin concentrations lead to increased energy intake and weight gain. Similarly, a high-fructose diet induces greater weight gain and increased visceral adiposity than a high-glucose diet. Thus, a high fructose diet may promote unwanted weight gain by limiting insulin and leptin secretion, which in turn results in reduced fat oxidation and increased storage of fat. Therefore, we hypothesize that a high-fructose meal will lead to increased postprandial carbohydrate oxidation and decreased fat oxidation compared to a high-glucose meal.

Specific Aims and Hypotheses

In a randomized, double-blind, crossover design study of 12 healthy, non-obese men ages 21-31 consuming either a fructose or glucose supplemented mixed-meal, we aimed to:

1. Measure postprandial substrate oxidation following a glucose rich meal versus a fructose rich meal using indirect calorimetry.

   Hypothesis: Non protein respiratory quotient will be higher seven hours following a fructose rich meal compared to a glucose rich meal.
2. Determine the effect of a high fructose meal versus a high glucose meal on fasting and postprandial triglyceride, glucose, insulin, and leptin concentrations.

   Hypothesis: Triglyceride concentrations will be higher and glucose, insulin and leptin levels will be lower after consuming a fructose rich meal compared to a glucose rich meal.
3. Determine the effects of a high glucose meal and a high fructose meal on the relationships difference between change in leptin concentrations and substrate utilization over time.

   Hypothesis: The change over time in non protein RQ will be inversely correlated with change in leptin concentrations.

Chapter 2 - Background

The epidemic of overweight and obesity continues to plague the health of the general population in the United States and other developed and developing countries. As of 2008, 72.3% of men and 64.1% women over the age of 20 are overweight, with a body mass index (BMI) between 25 and 29.9 kg/m^2, or obese, with a BMI greater than 30 kg/m^2 (1). Overweight and obesity are major health concerns because they are associated with increased rates of comorbid conditions including type 2 diabetes, hypertension, coronary heart disease, dislipidemia, liver and gallbladder disease, stroke, sleep apnea and respiratory problems, and certain cancers (2).

The basic cause of overweight and obesity is an imbalance between energy intake and energy expenditure as a result of a combination of genetic, environmental and hormonal factors. The "typical American diet", or a diet high in red meat, high fat dairy, and refined grains, has been linked to increased risk of cardiovascular disease and increased obesity biomarkers (3). In 2004 meat, poultry and fish accounted for 13.4% of caloric intake in the United States, dairy accounted for almost 8.6%, grains for 23.5%, fruits and vegetables 7.8%, added fats and oils 23.9% and sugars and sweeteners 17.3% of total caloric intake (4).

The rate of adult overweight and obesity in the United States began to increase in the mid 1980s. Figure 1 shows the trends in overweight and obesity over the last 45 years. Around the same time, the consumption of fructose began to rise (5). Figure 2 shows the trends in refined sugar and high fructose corn syrup consumption. This

increase in fructose consumption was primarily due to the use of high fructose corn syrup by the food industry as a cheaper and more shelf-stable sweetening alternative to sucrose. While fructose is naturally found in fruit, the main source of fructose in the American diet is from the added sweeteners high-fructose corn syrup and sucrose. High-fructose corn syrup is composed of either 55% fructose and 45% glucose, the form more commonly found in soft drinks, or 42% fructose and 58% glucose, the form more common in processed foods; whereas, sucrose, or common table sugar, is 50% fructose and 50% glucose. High fructose corn syrup and sucrose are found primarily in processed foods like soft drinks and other sweetened beverages, candy, sweetened cereals, yogurt, and pre-made desserts. Results from the Third National Health and Nutrition Examination Survey (NHANES III), performed in 1988 to 1994, showed that the average fructose intake in the U.S. was 54.7 grams per day or 10.2% of total caloric intake compared to 17 grams per day or 8% of total caloric intake from 1977 to 1978 (6). NHANES 1999-2004 data showed an average fructose intake of 49±1.0 grams per day or 9.2% of total caloric intake (7). These percentages (10.2% and 9.2%) of kilocalories from fructose are lower than 30% of kilocalories from fructose or glucose used in our current study. NHANES III also found that adolescents are the highest consumers of high-fructose corn syrup and that people with the lowest incomes consume more high-fructose corn syrup than those with higher incomes (8). Other examinations of NHANES III data have shown that in children and adolescents age 8 to 18 years, low nutrient density foods account for more than 30% of caloric intake with sweeteners and desserts

accounting for 25% of caloric intake (9). Since the mid 1980s, the rise in consumption of high fructose corn syrup has mirrored the rise in obesity in the United States.

Figure 1. Trends in overweight, obesity, and extreme obesity, ages 20-74 years. Data extracted from National Center for Health Statistics report on the prevalence of overweight, obesity and extreme obesity among adults in the U.S. (1)

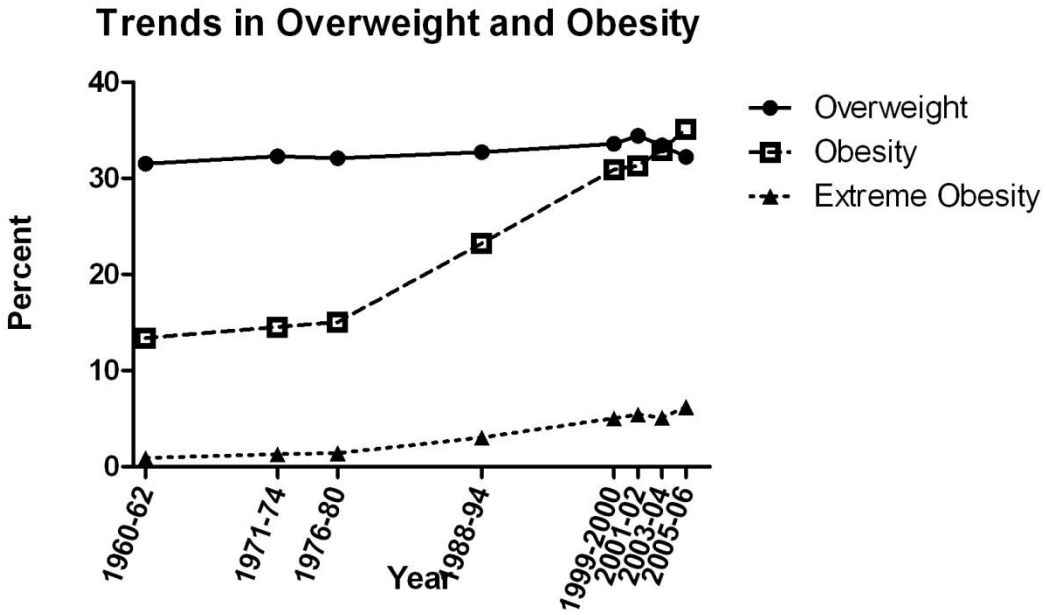
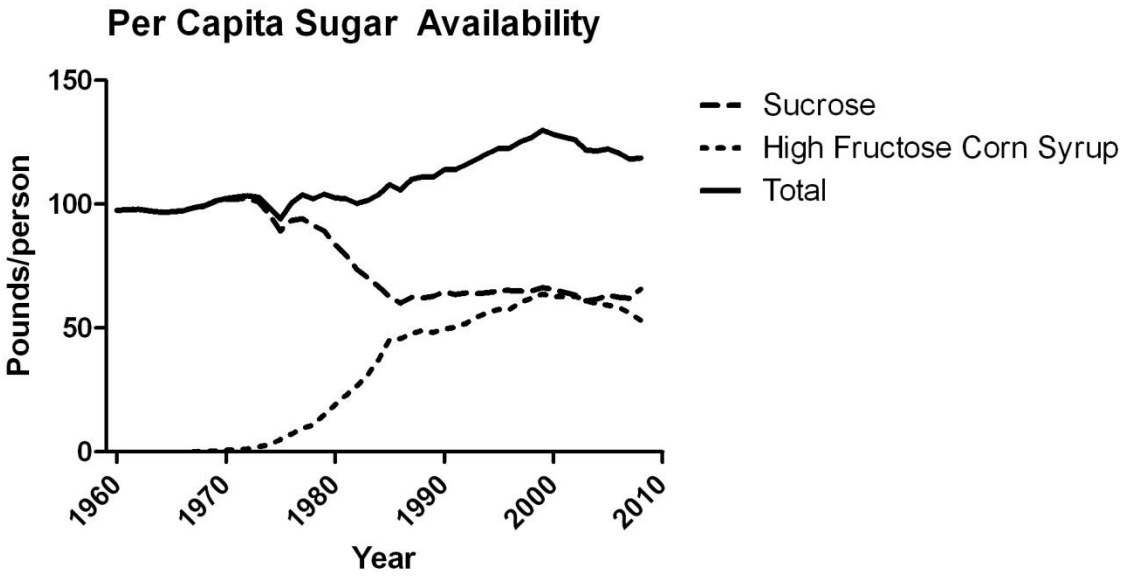


Figure 2. Per capita high fructose corn syrup and refined sugar availability. Data extracted from the U.S. per capita food availability data (5)



Fructose Metabolism

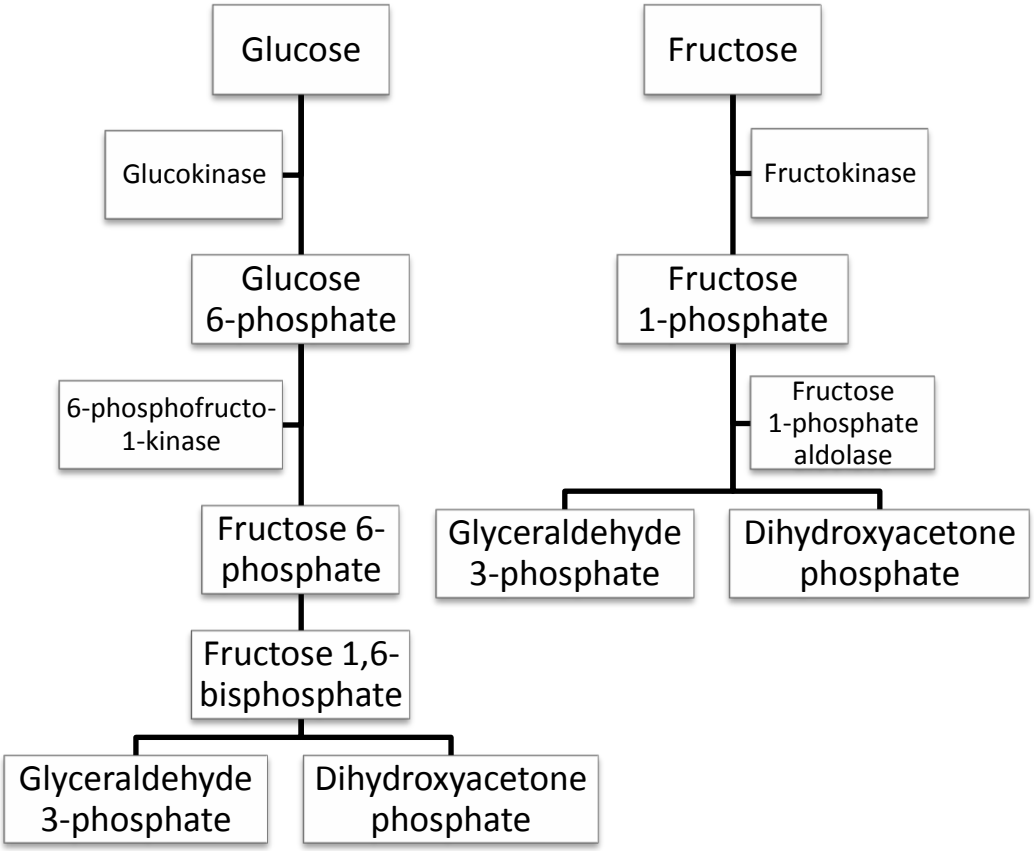
Fructose, like glucose, is a six-carbon sugar. The structures of these two sugars differ only slightly in that fructose contains a keto group at position two of its carbon chain, and glucose contains an aldehyde group at position one. Because of the structural difference between fructose and glucose, absorption and metabolism of the two sugars differ.

High fructose corn syrup is composed of a combination of free glucose and free fructose, but in sucrose the two molecules are bound by a α-1,4 glycosidic bond. This glycosidic bond must be broken by the enzyme sucrase at the brush boarder of the small intestine before the glucose and fructose molecules can be absorbed. It has been proposed that, because of this difference between sucrose and high fructose corn syrup, the two sweeteners may have different effects in the body. Several studies have shown, however, that there is no difference between consumption of fructose and glucose as either high fructose corn syrup or sucrose on blood glucose, insulin, or leptin levels as well as dietary intake and satiety (10), (11), (12).

Once digested into their monosaccharide forms, glucose and fructose are absorbed into the enterocyte via the high affinity transporter SGLT-1 for glucose and passive diffusion facilitated by GLUT-5 for fructose. After absorption, these two monosaccharides are transported into the blood stream by the GLUT-2 transporter protein.

Fructose metabolism begins in the liver where it is taken up from the blood stream and converted into fructose-1-phosphate by the enzyme fructokinase. It should be noted that, unlike glucose, almost 100% of fructose is taken up from the hepatic portal vein by the liver on the first pass, so that there is little to no increase in serum fructose levels after fructose ingestion (13), (14). As fructose-1-phosphate, fructose enters glycolysis and is converted to glyceraldehyde and dihydroxyacetone phosphate. By entering glycolysis as fructose-1-phosphate, fructose bypasses a critical regulatory step in glucose metabolism controlled by the enzyme 6-phosphofructo-1-kinase. In metabolism of glucose, glucose is phosphorylated to glucose-6 phosphate and then converted to fructose-6-phosphate by 6-phosphofructo-1-kinase. 6-phosphofructo-1-kinase is allosterically inhibited by adenosine triphosphate (ATP) and citrate and stimulated by adenosine monophosphate (AMP) and adenosine diphosphate (ADP). Thus, when large amounts of fructose are ingested, glycolysis remains largely unregulated, because fructose metabolism is unaffected by feedback from ATP, citrate, AMP and ADP. Figure three compares fructose and glucose metabolism in the liver.

Figure 3. Glucose vs. fructose metabolism in the liver



Fructose and Blood Glucose

During fructose metabolism, some carbon from the glycolytic intermediates glyceraldehyde and dihydroxyacetone phosphate is converted to glucose through gluconeogenesis. The newly formed glucose is released slowly from the liver over several hours providing a continuous carbohydrate to peripheral tissues for oxidation. The presence of glucose in skeletal muscle promotes glucose oxidation and inhibits fat oxidation by inhibiting CPT-1B. It is possible that the slow release of glucose from the

liver may in itself lower postprandial fat oxidation and increase glucose oxidation by providing glucose as substrate for a prolonged period of time after the meal.

In contrast to acute fructose consumption causing depressed postprandial glucose levels, chronic high fructose consumption has been shown to raise fasting glucose levels. In a four week study of the effects of a high fructose diet in healthy, normal weight males, investigators found that a diet containing 1.5 grams of fructose per kilogram of body weight resulted in a 5.5% increase in fasting plasma glucose levels (15). Also, subjects consuming a diet with 25% of kilocalories from fructose for 10 weeks significantly increased fasting plasma glucose levels from 82.9±7.2 mg/dL at baseline to 88.3±7.2 mg/dL at two weeks and 10 weeks (16). This effect of fructose on fasting glycemia may indicate hepatic insulin resistance with continued gluconeogenesis which is normally suppressed during hyperglycemia (17).

Fructose and Triglyceride Concentrations

Compared to glucose metabolism, the unregulated metabolism of fructose leads to a greater buildup of pyruvate and glycolytic intermediaries, such as glycerol-3-phosphate, as well as acetyl-coA and citric acid cycle intermediaries, such as citrate and malate. Acetyl-coA also accumulates as the product of citrate conversion in the cytosol. Buildup of these lipogenic substrates increases de novo lipogenesis in the liver, which results in elevated post-prandial triglyceride and circulating lipid concentrations.

In a study conducted by Stanhope et al., overweight and obese subjects were fed 25% of their energy requirements from either fructose or glucose for 10 weeks. The

results of this study showed that, while weight gain was similar between the two groups over the 10 week time period, there was a significant increase in hepatic de novo lipogenesis and 23-hour postprandial triglyceride area under the curve (AUC) in those subjects consuming fructose (18). In another 10 week study of the effects of consuming 25% of kilocalories from fructose in overweight and obese postmenopausal women, postprandial triglyceride levels significantly increased, and the AUC for triglyceride concentrations increased by 141% after 10 weeks (16). Another study found that a four-week diet containing 1.5 g fructose per kg of body weight resulted in a 36% increase in triglyceride concentrations from baseline in healthy, normal-weight males (15). In a two day crossover study, Teff et al. compared the effects of consuming 30% of kilocalories in one day from either fructose or glucose in 12 normal weight women. Researchers found that triglyceride concentrations increased more rapidly, reached higher peak levels, and remained elevated for a longer period of time following fructose intake compared to glucose intake (19). Another crossover study compared the effects of consuming 30% of kilocalories from glucose or fructose over a one day period in 17 obese subjects. Results of this study showed a 200% increase in triglyceride AUC on the fructose day compared to the glucose day (20). In both acute and chronic high fructose intake, in both men and women, a high fructose diet results in increased triglyceride concentrations.

Current research has shown a relationship between fructose consumption and amount of visceral adipose tissue. Stanhope et al. reported that overweight and obese subjects on a diet containing 25% of their energy requirements from fructose for eight

weeks had significant increases in both total abdominal fat and visceral adipose tissue. Subjects who consumed 25% of their energy requirements from glucose did not have significant increases in visceral adipose tissue. The total kilocalories consumed during the eight week ad libitum phase of this study did not differ between the fructose and glucose groups (18). Increased visceral adiposity has been linked to an increased risk for metabolic syndrome, a group of metabolic risk factors including dyslipidemia, hypertension, and insulin resistance (21), (22). Studies have shown that there is a greater decrease in insulin sensitivity associated with increased visceral adiposity than with subcutaneous adiposity (23), (24). In a study conducted by Goodpaster et al., it was found that overall weight loss was associated with improved insulin sensitivity in obese subjects, but loss of visceral adiposity specifically was highly inversely associated with improved insulin sensitivity (25). If high fructose diets increase visceral adipose deposition, they could potentially lead to the development of insulin resistance.

Fructose and Insulin

Insulin is a hormone stimulated by glucose absorption and secreted by the β-cells of the pancreas and is responsible for initiating cellular uptake and metabolism of glucose. A number of short-term studies have reported decreased insulin secretion after fructose consumption compared to glucose. In the 2004 study by Teff et al., the 12 normal-weight women experienced a 65±5% lower average peak insulin response on the day they consumed fructose. The insulin AUC, or overall insulin secretion during the day, was blunted by 49±5% on the high fructose day (19). The 2009 study by Teff et al.

showed similar results in a mixed-gender, obese population. This study found that postprandial insulin levels were significantly lower during the high fructose day compared to the high glucose day. Also, the AUC for insulin was approximately 50% lower on the fructose day (20). Studies have shown conflicting effects of chronic high fructose consumption on insulin. A 10 week study found that a chronic high fructose diet, compared to a chronic high glucose diet, resulted in increased fasting insulin levels, decreased postprandial insulin excursions, and decreased insulin sensitivity (18). On the other hand, another study found that a four-week high fructose diet did not change fasting insulin levels or insulin sensitivity (15). So while chronic effects of a high fructose diet on insulin concentrations are equivocal, acute high fructose ingestion has been shown to blunt postprandial insulin concentrations.

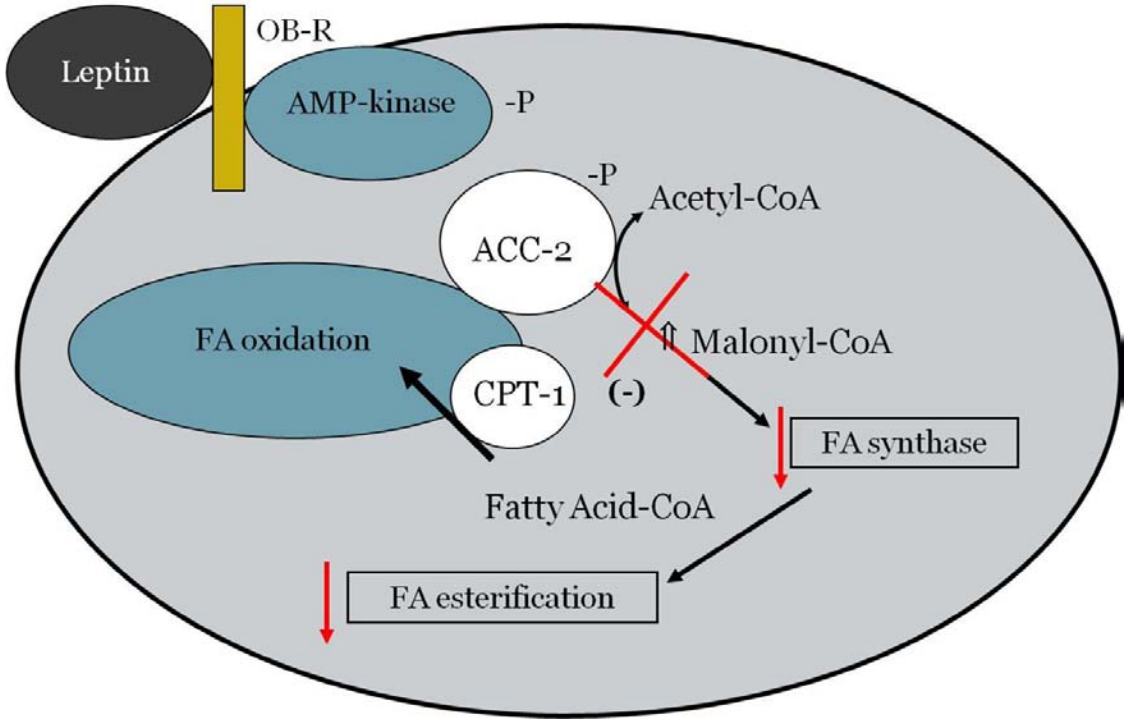
Fructose and Leptin

Leptin is an adipocytocine that is released from adipose tissue in direct proportion to the amount of fat stored in the body (26), (27). This hormone acts in the central nervous system where it functions as a key long-term regulator of hunger and satiety in the body by stimulating proopiomelanocortin (POMC), which suppresses hunger by stimulating the release of α-melanocyte-stimulating hormone, and inhibiting the release of neuropeptide Y, a hypothalamic neurotransmitter responsible for stimulating appetite (28), (29). Normally, if body fat increases, such as during acute overfeeding, leptin release is increased, signaling the hypothalamus to suppress hunger. In the obese however, this rise in leptin secretion from increased adipose stores does not result in

decreased hunger indicating leptin resistance in the body (30). As body fat decreases, such as with dieting, leptin concentrations fall and hunger is increased. Also, insulin plus glucose is associated with the release of leptin from adipocytes; therefore, if a fructose meal affects insulin concentrations, it may also affect leptin concentrations (31), (32). As insulin concentrations increase following a rise in blood glucose postprandially, leptin concentrations increase as well (31). Unlike glucose, fructose does not stimulate insulin secretion from the pancreas, and therefore leptin release is blunted (19), (33), (32).

Recent research has shown that leptin may also play an important role in stimulating fat oxidation in skeletal muscle by activating the enzyme 5'-AMP-activated protein kinase (AMP kinase) (34). When activated, AMP kinase stimulates fatty acid oxidation in muscle cells by inhibiting acetyl coenzyme A carboxylase. Acetyl CoA caboxylase (ACC) is an enzyme bound to the outer mitochondrial membrane that converts acetyl-coA to malonyl-coA. Low levels of malonyl-coA in the cytosol both decrease fatty acid synthesis and storage as well as increase fatty acid oxidation by increasing carnitine palmitoyltransferase I (CPT-1) activity (35). CPT-1 is the rate limiting enzyme for fatty acid oxidation that is essential for fatty acid import into the mitochondria. Thus, when leptin stimulates AMP kinase activity within the muscle cell, fatty acid oxidation is stimulated by increased CPT-1 activity. Figure four illustrates the leptin signaling cascade in skeletal muscle, and figure 5(b) depicts the expected leptin response to the high fructose and high glucose meals in this study.

Figure 4. Leptin signaling pathway in muscle



A fructose rich diet has been shown to decrease postprandial leptin levels. In a crossover study comparing the effects of a one-day diet high in fructose or glucose in normal-weight women, investigators found a significant reduction in 12 hour leptin levels, 24 hour leptin levels, and the diurnal amplitude on the high fructose day compared to the high glucose day (19). A similar one-day cross-over study in obese subjects found that after consuming a diet containing 30% of kilocalories from either fructose or glucose for one day there was a 30% reduction in AUC for leptin on the high fructose day compared to the high glucose day (20). The ability of an acute high fructose diet to lower postprandial leptin concentrations may be related to the diet's similar effects on insulin concentrations. Also, a four week study of a chronic high fructose diet in normal weight, healthy men observed a 48% increase in fasting leptin

levels within one week (10). There was also a 34% increase in carbohydrate oxidation with a trend toward decreased lipid oxidation, although this was not significant. These results are interesting in light of the fact that there was no significant change in energy expenditure or fasting hepatic glucose output. Elevated fasting leptin concentrations seen with a chronic high fructose diet could potentially be related to the increase in visceral adiposity associated with chronic high fructose consumption.

It has also been suggested that consumption of a high fructose diet may result in leptin resistance. A study conducted in rats found that the rats fed a high fructose diet became leptin resistant and, in the presence of a high fat diet, had exacerbated weight gain compared to rats fed a fructose free diet who remained leptin responsive (36). These effects of a high fructose diet may individually, or in combination, result in a lower satiety and increased caloric intake. This increased caloric intake alone may be responsible for the increased adiposity associated with a high fructose diet.

Another possible mechanism by which a high fructose diet may increase adiposity is related to the effects of fructose on satiety. A study on the effects of solutions with varying glucose to fructose ratios found that those beverages with the lowest glucose to fructose ratio (G20:F80, G35:F65) suppressed hunger the least (10). The G20:F80 beverage also resulted in the highest cumulative energy intake of all the beverages tested. Another study found that, in subjects classified as restrained eaters, a one-day high fructose diet led to increased hunger ratings and a compensatory increase in fat intake the following day. The study found no effects of an acute high fructose diet

on unrestrained eaters (19). In contrast, a 10-week study found no difference in energy intake reported by 24-hour diet recalls in subjects consuming a high fructose and high glucose diet (18). While these studies show that the effects of a high fructose diet are equivocal, the increase in adiposity associated with high fructose consumption could be the result of increased hunger, decreased satiety, and increased food intake caused by a high fructose diet.

Because of leptin's role in stimulating fat oxidation in muscle cells and because fructose does not elicit the same insulin response that glucose does, we hypothesized that this metabolic switch from carbohydrate to fat oxidation would be delayed resulting in prolonged carbohydrate oxidation, decreased fat oxidation, and potentially increased fat storage.

Measuring Substrate Utilization

Postprandial substrate oxidation was the primary outcome measurement for this study. The ability to measure substrate utilization and resting energy expenditure (REE) is an important tool in both a research setting and a clinical setting. Accurate measurement of energy expenditure can be crucial in a critical care situation where over or underfeeding could lead to increased risk for morbidity and mortality (37). In a research setting, measuring substrate utilization and REE can provide a wealth of information to researchers on changes in metabolic rate, the effects of an intervention diet, etc.

Indirect calorimetry is a common method for measuring substrate utilization and REE. With this non-invasive technique, oxygen consumption (vO_2) and carbon dioxide production (vCO_2) are measured with a sensor. These values, along with measured urine urea nitrogen (UUN), can then be used to calculate respiratory quotient (RQ) and resting energy expenditure.

Respiratory quotient is a tool used to determine the primary fuel source being used by the body. A high RQ value (close to 1) indicates that carbohydrate is the predominant fuel source. A low RQ value (close to 0.7) indicates fatty acids are the main fuel source. A mid-range RQ value (~0.85) can indicate either protein oxidation or a mix carbohydrate and fat oxidation. If urine urea nitrogen (UUN) is measured, then protein oxidation can be factored out resulting in non protein respiratory quotient (NPRQ). By removing the protein component, NPRQ allows us to see the effects of diet on just carbohydrate and fat oxidation. Figure 5(c) depicts the expected non protein respiratory quotient response to the high fructose and high glucose meals in this study.

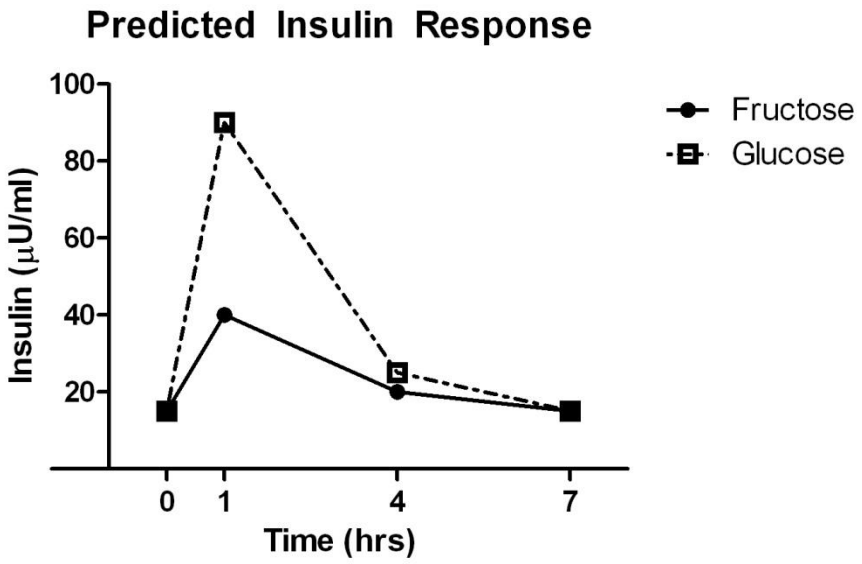
$$NPRQ = [vCO2 - (4 \times UUN_{(g/min)})]/[v02 - (5.9 \times UUN_{(g/min)})]$$

$$RQ = vCO_2/v0_2$$

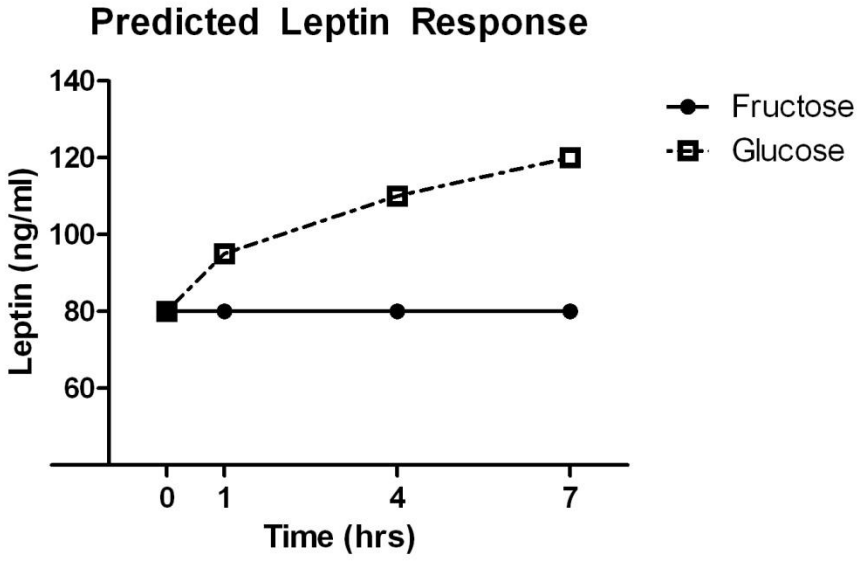
$$REE\ (kcal/d) = [(3.9 \times v0_{2(l/min)} + 1.1 \times vCO_{2(l/min)}) - 2.2 \times UUN_{(g/min)}] \times 1440_{(min/d)}$$

Figure 5. Model of hypothesized outcomes for (a) insulin, (b) leptin, and (c) NPRQ

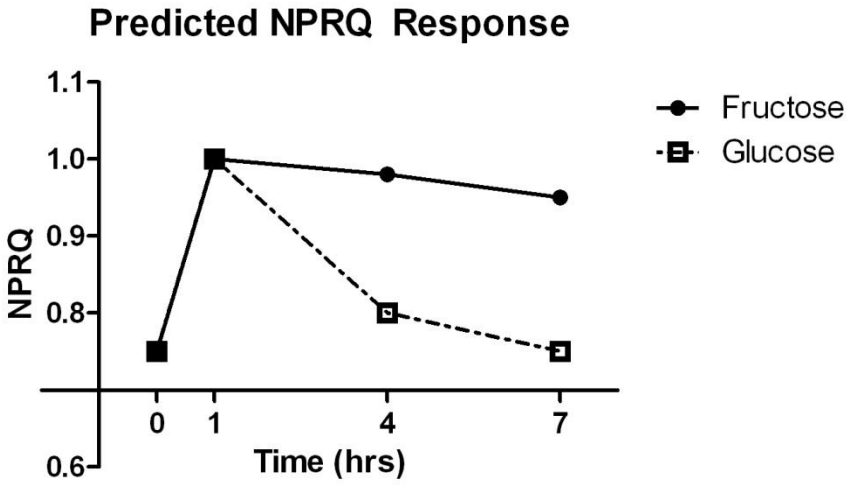
a)



b)



c)


Predicted NPRQ Response
1.1
1.0
0.9
0.8
0.6
NPRQ
0
1
4
7
Time (hrs)
Fructose
Glucose

Chapter 3 - Methods

Study Design

In this randomized, double-blind, crossover design study, we compared the effects of a high-glucose meal and a high-fructose meal on substrate-oxidation following the meal during a seven hour fast. Subjects were admitted to the Oregon Clinical & Translational Research Institute (OCTRI) on two separate days at least one week apart. At each admission, the subjects consumed a meal supplemented with either a fructose- or glucose-beverage, in random order. Respiratory quotient was measured using indirect calorimetry and blood samples and 24-hour urine samples were collected for analysis. All study related procedures were reviewed and approved by the Oregon Health & Science University (OHSU) Institutional Review Board.

Subject Selection

For this study, 12 healthy, non-obese, male subjects aged 21-31 years were recruited. Because of the preliminary nature of this study, only male subjects were studied in order to minimize variation. Inclusion and exclusion criteria are detailed in Table 1. Subjects were recruited from the student body at OHSU. Potential subjects were invited to participate in the study verbally, via email, or through recruitment fliers. Each subject was required to provide written consent before participating in any screening or intervention related activities. A copy of the consent form is provided in appendix A.

Table 1. Inclusion and Exclusion Criteria	
Inclusion	Exclusion
Male	Type 1 and type 2 diabetes mellitus
Age 21-31 years	Liver disease
Good health	Hyperlipidemia
Normal body weight	Glycogen storage diseases
Willing to complete 2 OCTRI admissions	Hereditary fructose intolerance
Willing to abstain from food, beverage (excluding water), and alcohol consumption for 12 hours prior to admission	

Screening

Pre-study screening visits were conducted for each potential participant to determine eligibility for the study. Consent forms were reviewed with each subject by study personnel. Once the potential participant fully comprehended the study requirements and gave written consent, the participant continued with the established screening procedures. Each participant's height and weight were measured at the screening visit. Subjects who were judged to be healthy by self-report and who do not meet any exclusion criteria were considered eligible for participation.

Intervention Protocol

Subjects for this study completed the inpatient protocol twice. Each admission occurred at least one week and up to one month apart, and each inpatient visit lasted up to 10 hours. Subjects arrived at OCTRI early in the morning after fasting and abstaining from alcohol for no less than 12 hours. The study evaluated blood markers and energy expenditure by indirect calorimetry (RQ) at baseline (t=0), immediately

postprandially (t=1 h), four hours postprandially (t=4), and seven hours postprandially (t=7). A 24-hour urine sample was collected for each subject at each admission. Subjects were responsible for continuing their urine collection after discharge and returning the sample within 24 hours of completing the collection. For admission days where two subjects were scheduled, times were staggered to accommodate resource restrictions. A sample schedule for a day where two subjects were scheduled and staggered can be seen in Table 2. If only one patient was admitted on a given day, either of the schedules may have been followed.

Table 2. Sample Schedule for OCTRI Admissions

Patient 1		Patient 2	
0700	Subject arrives at OCTRI	0800	Subject arrives at OCTRI
0730	RN evaluates subject, takes vitals, and performs baseline blood draw Body density is measured	0830	RN evaluates subject, takes vitals, and performs baseline blood draw Body density is measured
0800	Measurement of baseline RQ value	0900	Measurement of baseline RQ value
0845-0900	Meal with 30% of kilocalories from fructose or glucose is consumed	0945-1000	Meal with 30% of kilocalories from fructose or glucose is consumed
1000	Postprandial blood draw Measurement of RQ value	1100	Postprandial blood draw Measurement of RQ value
1300	Postprandial blood draw Measurement of RQ value	1400	Postprandial blood draw Measurement of RQ value
1600	Postprandial blood draw Measurement of RQ value	1700	Postprandial blood draw Measurement of RQ value
1700	RN evaluates subject Optional post study meal Subject discharged	1800	RN evaluates subject Optional post study meal Subject discharged
1700-0845 on Day 2	Subject resumes normal diet Subject collects urine at home	1800-0945 on Day 2	Subject resumes normal diet Subject collects urine at home

Blinding and Randomization

Throughout the data collection phase of the study, both subjects and investigators remained blinded to which of the two interventions was taking place. The OCTRI Bionutrition Unit was responsible for the key to the interventions and also ensured that the interventions were randomized.

Intervention Meals

At each of the two admissions, subjects consumed a meal supplemented with either a fructose- or glucose-containing beverage. All subjects tolerated the meals served and consumed all of the food provided as assessed by visual inspection of food containers. Each study meal consisted of a bagel with cream cheese, an egg bake, and the glucose or fructose sweetened beverage. The planned macronutrient composition of each meal was 55% of energy from carbohydrate, 30% of energy from fat, and 15% of energy from protein. Making up the 55% of energy from carbohydrate was 25% of total energy from complex carbohydrate and 30% of energy from the fructose or glucose containing beverage. Total energy for the meal given with each admission was determined by calculating total daily energy requirements for each participant and dividing by 3, so that the meal provided one third of the participant's energy needs. The total daily energy requirements were determined by using the Harris-Benedict equation and the Mifflin-St. Jeor equation to calculate basal metabolic rate (BMR). BMR was then multiplied by an activity factor of 1.4, and the two numbers were averaged for the total

energy requirements of each subject. These equations were chosen because they are the most common and validated equations in healthy weight subjects.

Harris-Benedict equation (male):

Basal Energy Requirements = 66 + 13.7 (weight in kg) + 5 (height in cm) – 6.8 (age in years)

Mifflin-St. Jeor equation (male):

Basal Energy Requirements = 9.99 (weight in kg) + 6.25 (height in cm) – 4.92 (age in years) - 161

Indirect Calorimetry

Indirect calorimetry was conducted to measure respiratory quotient (RQ) and to determine substrate oxidation before meal consumption, one hour post meal, four hours post meal, and seven hours post meal. For each measurement of indirect calorimetry, subjects rested in a dim, quiet environment for thirty minutes. In conducting indirect calorimetry, we used a metabolic cart (Sensormedics Corp. model 29n, Yorba Linda, CA) to measure each subject's oxygen consumption (vO_2) and carbon dioxide production (vCO_2) for 20-30 minutes at a time. RQ was then calculated and used to determine if the subject was oxidizing carbohydrate, fat, or protein. Once RQ data was collected, we combined the data with the results of the urine analysis to calculate non-protein respiratory quotient (NPRQ) which factors out protein oxidation.

$RQ = vCO_2 / vO_2$

$NPRQ = [vCO_2 – (4 \times UUN_{(g/min)})]/[v0_2 – (5.9 \times UUN_{(g/min)})]$

Blood Samples

Blood samples were taken to evaluate blood markers of metabolic activity (glucose, insulin, leptin, triglyceride concentrations). Samples were drawn before meal consumption, one hour post meal, four hours post meal, and seven hours post meal. An intravenous catheter was placed in the peripheral arm vein of the subjects to minimize harm to the patient from repeated blood draws. A maximum of 30 mL of blood was collected from each patient per admission. From the four blood samples drawn at each admission, glucose, leptin, insulin and triglyceride concentrations were measured. The following tubes were collected at each admission:

Glucose: 1 ml of blood was collected in a grey-top tube for glucose analysis. Whole blood was centrifuged, and plasma was separated and stored at -80^{o}C until analysis. Glucose concentrations were measured in the PI's lab using a glucose oxidase assay (Raichem Inc.).

Insulin: 2 ml of blood were collected in a red-top tube for insulin and leptin analysis. Whole blood was centrifuged, and serum was separated and stored at -80^{o}C until analysis. Insulin concentrations were measured in the OCTRI core lab using an Immulite autoanalyzer. The sensitivity of this assay is 2 mU/L. The intra- and inter-assay coefficients for the assay were 7.01% and 7.91% respectively.

Leptin: Concentrations of leptin were measured in the PI's lab using a leptin radioimmunoassay (Millipore, Inc.). The sensitivity of this assay is 0.5 ng/ml. The calculated intra-assay coefficient of variance was 16%, and the calculated inter-assay coefficients of variance for the two kits used were 1.6% and 6.6%.

Triglyceride Concentrations: 1 ml of blood was collected in a green-top tube for analysis of triglyceride concentrations. Whole blood was sent to the Oregon Health & Science University hospital pathology lab for analysis. Triglyceride concentrations were measured using an enzymatic reaction and autoanalyzer (Beckman Coulter counter). Triglycerides were hydrolzyed to glycerol and fatty acids by lipase. Glycerol was converted to H_2O_2 by glycerol kinase and glycerolphosphate oxidase. Change in absorbance was read at 520 nm.

Urine Samples

Participants' urine was collected over a 24-hour period beginning with each subject's arrival at OCTRI. Subjects continued the urine collection after their discharge from OCTRI and returned the completed sample the following morning. All urine collected was mixed and the volume recorded. An aliquot of each urine sample was measured for urine urea nitrogen (UUN), a marker of protein metabolism, at the OHSU hospital pathology lab. UUN was measured using an enzymatic reaction and autoanalyzer (Beckman Coulter counter). Urea was hydrolyzed to ammonia and CO_2 by urease. Glutamate dehydrogenase condensed ammonia and alpha ketogluterate to

form glutamate and NAD. Change of absorbance of NADH to NAD was measured at 340 nm.

Height and Weight

Body weight was measured in light clothing on the day consent was given using a digital scale (Scale-Tronix, Model 5002, Carol Stream, IL) in the Bionutrition Unit of the OCTRI. On the same day, height was measured without shoes by a Harpendend wall mounted stadiometer (Holtain LTD., UK).

Statistical Analysis

The crossover design of this study allowed for comparing results within subjects receiving different experimental diets and reduced subject-to-subject variability. Changes in plasma glucose, insulin, leptin and triglyceride concentrations were summarized by calculating an area under the curve (AUC) using the trapezoidal method. Calculating AUC allowed us to compare the average response for each parameter over the time course of the study. Total AUCs for each parameter were compared by paired t-test. Post-hoc analysis of differences between individual time points were compared by paired t-tests. Response feature analysis was used to describe the trajectories or patterns of change between the two diets. We analyzed the slope for change in leptin concentration and NPRQ. The slope for leptin was calculated over all four times points, and the slope for NPRQ was calculated over the three postprandial time points. Slopes were compared using paired t-tests. For all statistical analysis, $p \leq 0.05$ was considered significant.

Repeated measures ANOVA is a broad analysis often used to determine if differences exist between the two groups anywhere in the data when the researcher has no a priori expectation of differences and pattern of change. Because we already had predicted responses for our outcomes, changes in outcome parameters between glucose and fructose treatments were not compared by repeated measures ANOVA.

Power

Our primary endpoint was the difference in non protein respiratory quotient (NPRQ) between the glucose and fructose arms of our study. In our preliminary data, the NPRQ difference seven hours after the fructose compared to the glucose meal was 0.07 with a standard deviation of 0.11 giving us an effect size of 0.64. Using a paired analysis, a total sample size of 12 subjects gave 70% power to detect a difference in NPRQ seven hours following the breakfast meal. Six subjects had completed the preliminary study, and six additional subjects were recruited to make a total of 12 study participants.

Compensation

Subjects were compensated $50 for time and travel after completion of the second admission.

OCTRI Bionutrition Lab

All subject meals were prepared in the OCTRI Bionutrition Laboratory. In the bionutrition lab, a bionutritionist prepared meals meeting 1/3 of the daily calorie

requirements calculated for each subject. The bionutritionist also prepared the glucose- and fructose- containing beverages that accounted for 30% of the total kilocalories for each meal. All meals were standardized and controlled for macronutrient content.

Chapter 4 – Results

Sample Characteristics

The effect of a high fructose vs. a high glucose meal on postprandial substrate oxidation was studied in 12 healthy, non-obese males between the ages of 21 and 31. The average age (± SD) of participants was 25.2±2.2 years, and the average (± SD) BMI was 24.4±2.0 kg/m^2. Table 3 presents the characteristics as well as the predicted energy expenditure, kilocalories (kcals) per meal, and kcals per kilogram for each subject. Subjects arrived at the research center fasting.

Table 3. Subject Characteristics

Subject #	Age (years)	Height (cm)	Weight (kg)	BMI	Predicted Energy Expenditure* (kcal/day)	Kcals/ meal	Kcals/ kg
1	26	178.5	66.8	21.0	2212.5	750	11.2
2	24	166	60.5	22.0	2068.7	700	11.6
3	23	178.4	88.2	27.7	2524.9	850	9.6
4	23	176.25	77.2	24.9	2362.2	800	10.4
5	24	164.8	63.4	23.3	2101.8	700	11.0
6	31	168	66.4	23.5	2114.0	700	10.5
7	25	175	71.4	23.3	2262.4	750	10.5
8	26	191	97.3	26.7	2700.7	900	9.2
9	25	182.5	86.4	25.9	2510.1	850	9.8
10	27	172.4	73.4	24.7	2261.6	750	10.2
11	24	167.4	73.7	26.3	2257.5	750	10.2
12	24	166.5	65.9	23.8	2145.7	700	10.6
Avg ± std. dev.		173.0± 7.9	74.2± 11.2	24.4± 2.0	2293.5± 195.9	766.7 ± 68.5	10.4± 0.7

*Predicted energy expenditure calculated as the average of the Harris-Benedict (66+13.7(weight in kg)+ 5(height in cm)–6.8(age in years)) and the Mifflin St. Jeor (9.99 (weight in kg) + 6.25 (height in cm) – 4.92 (age in years) - 161) prediction equations multiplied by an activity factor of 1.4

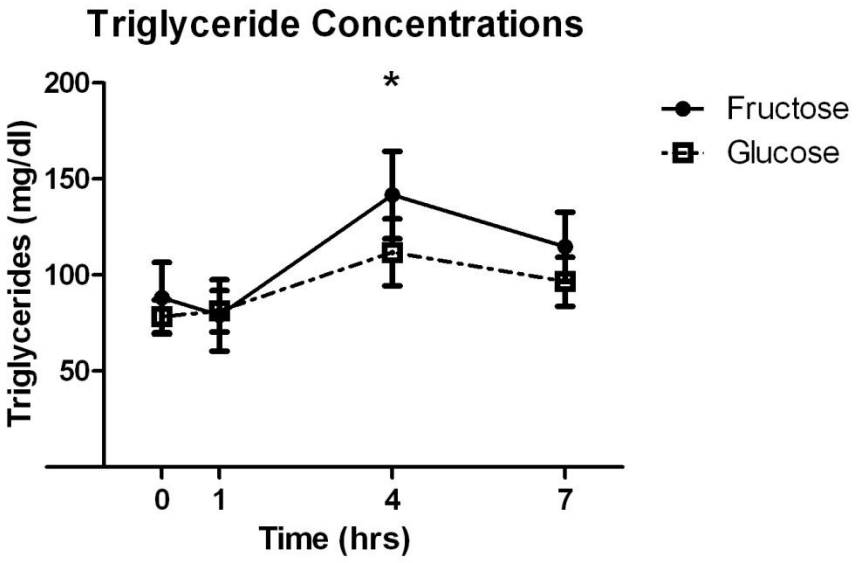
Triglyceride Concentrations

Fasting triglyceride levels were the same between the two admissions. There was not a significant difference between the area under the curve (AUC) for the triglyceride responses to the glucose or fructose meals (p-value = 0.14), and triglyceride levels were similar between the two meals at one hour and seven hours post meal. At the four hour time point however, the triglyceride levels were 26.7% higher following the fructose meal compared to the glucose meal (p-value = 0.05). Figure six shows the change over time for triglyceride concentrations after each meal as well as the AUCs for the triglyceride response to each meal.

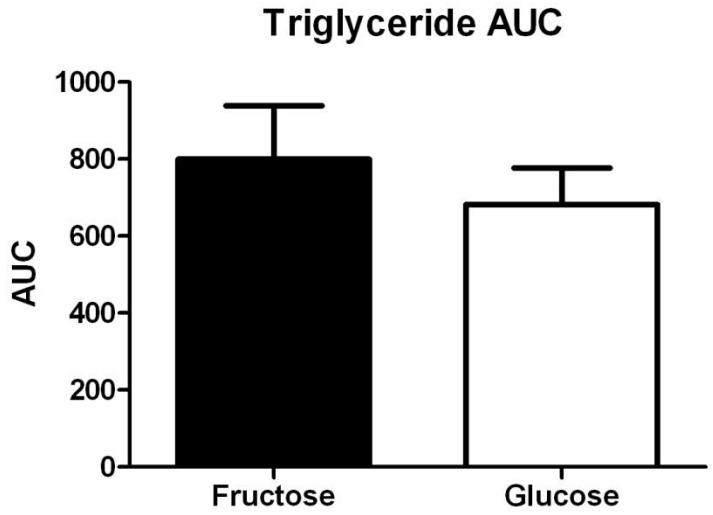
Figure 6. a) Triglyceride response to high fructose and high glucose meals, b) AUC for the triglyceride response after each meal (data shown as mean ± standard error)

*p < 0.05 compared with glucose

a)



b)

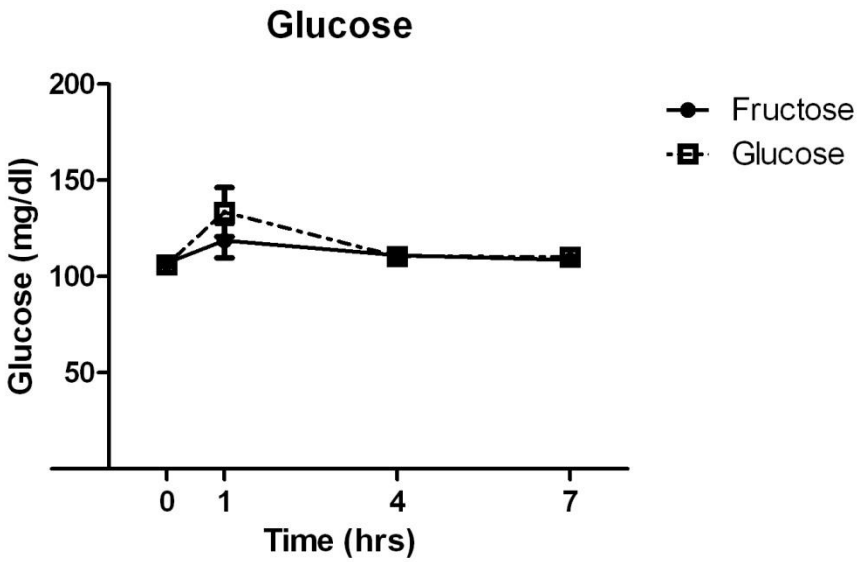


Glucose

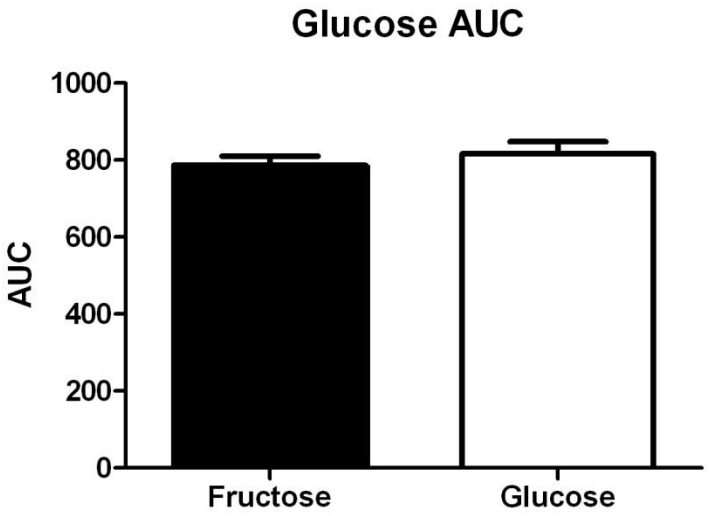
Fasting glucose concentrations were similar between the two admissions as were the AUCs for glucose following the glucose meal compared with the fructose meal significant (p-value = 0.11). At four and seven hours after the meal, glucose concentrations had returned to baseline levels and were not significantly different between treatments. The average glucose concentration at one hour post meal was 12.5% higher following the glucose meal compared to the fructose meal, but the difference was not significant (p-value = 0.08). Figure seven shows the change in blood glucose levels over time for each meal and the glucose AUCs for each meal.

Figure 7. a) Glucose response to high fructose and high glucose meals, b) AUC for the glucose response after each meal (data shown as mean ± standard error)

a)



b)



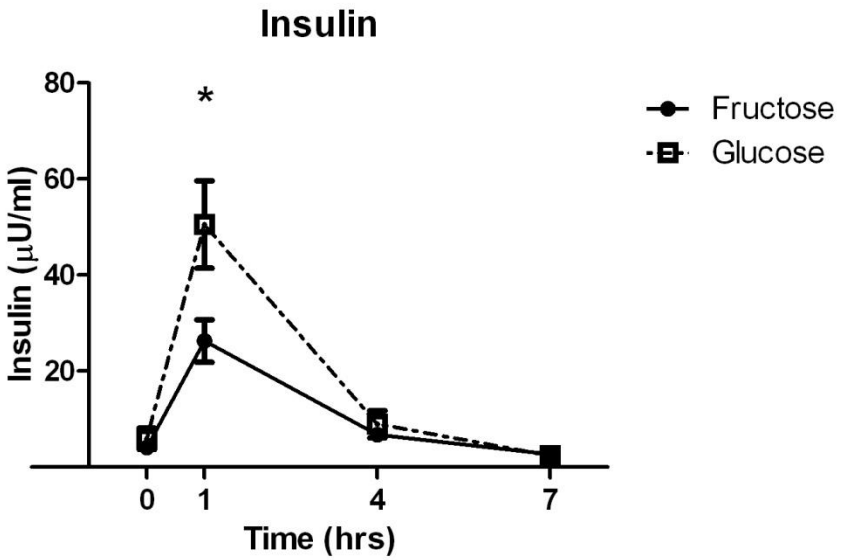
Insulin

Initial fasting insulin values were similar between the fructose and glucose treatment days. The difference in AUC of insulin concentrations between the glucose and fructose treatment was significantly different between meals (p-value = 0.008). The insulin AUC response was approximately 70% higher following the glucose meal compared to the fructose meal. The insulin response at one hour post meal was markedly increased (approximately 92% higher) following the high glucose meal compared to the high fructose meal (p-value = 0.005), but the insulin responses at four and seven hours were not significantly different between treatments (Figure 8).

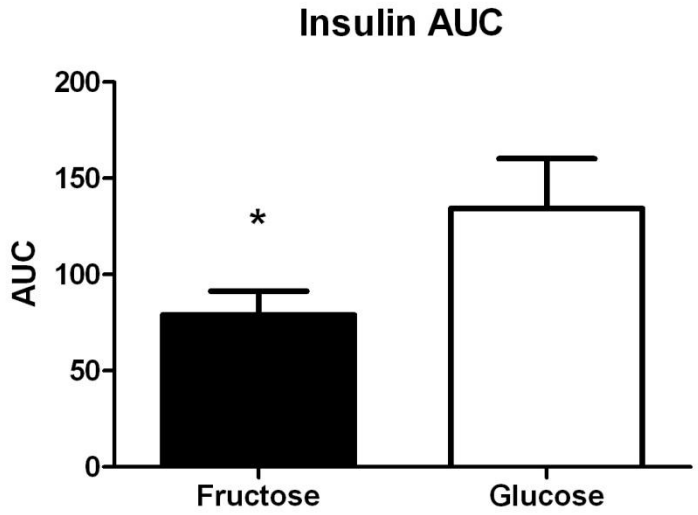
Figure 8. a) Insulin response to high fructose and high glucose meals, b) AUC for the insulin response after each meal (data shown as mean ± standard error)

*p<0.05 compared with glucose

a)



b)

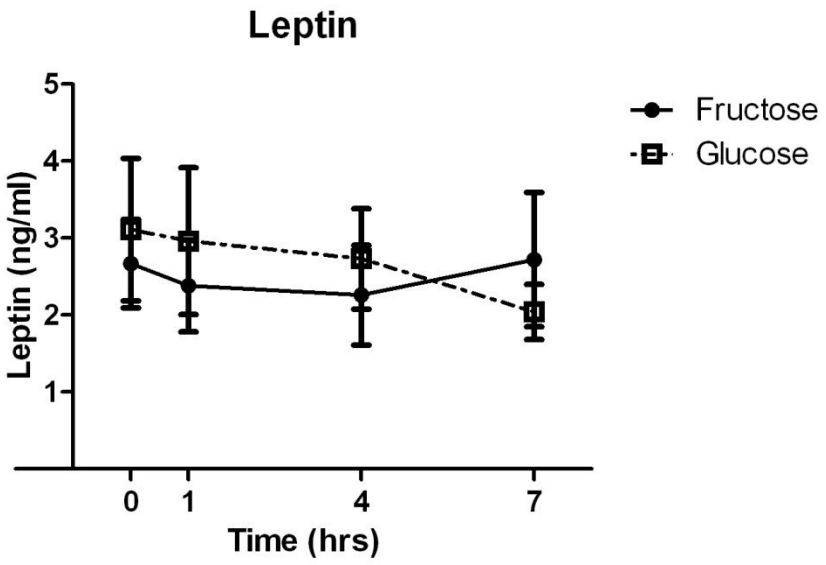


Leptin

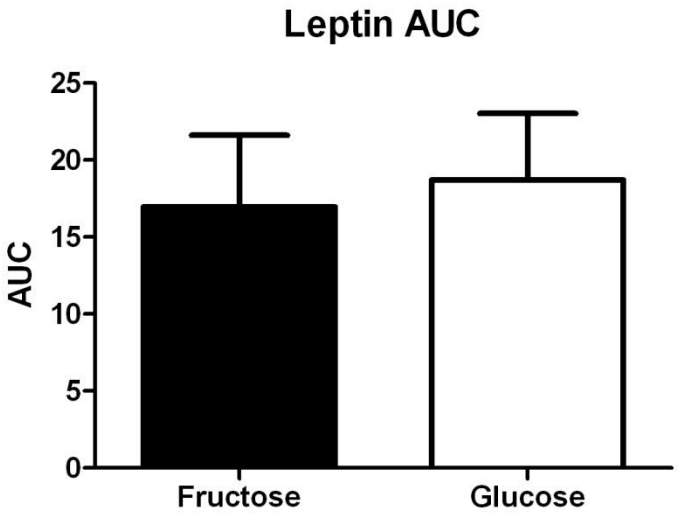
Fasting, AUC, and one, four, and seven hour leptin concentrations were not significantly different between the two treatment days, although there was a trend toward significance at 4 hours post meal (p-value = 0.06). Response feature analysis found no difference in the slope of the leptin responses over the four time points between the fructose and glucose meals (p-value = 0.41). Figure nine shows the leptin response and the leptin AUCs for each of the two meals.

Figure 9. a) Leptin response to high fructose and high glucose meals, b) AUC for the leptin response after each meal (data shown as mean ± standard error)

a)



b)

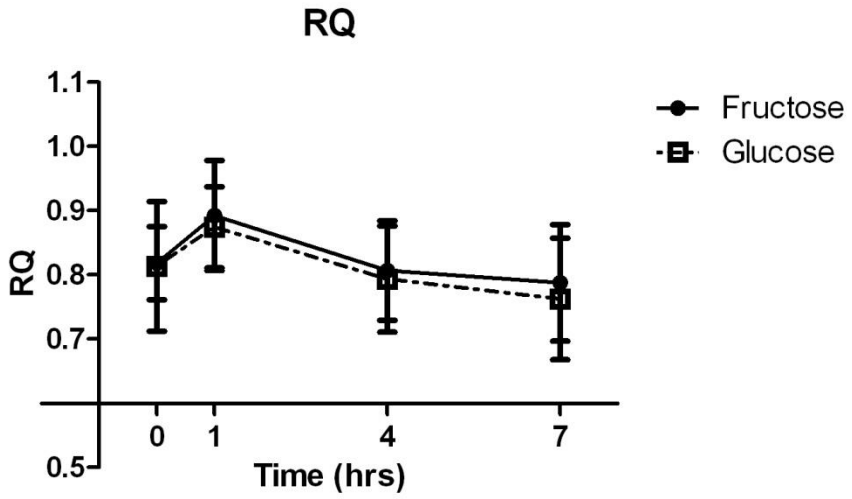


Respiratory Quotient

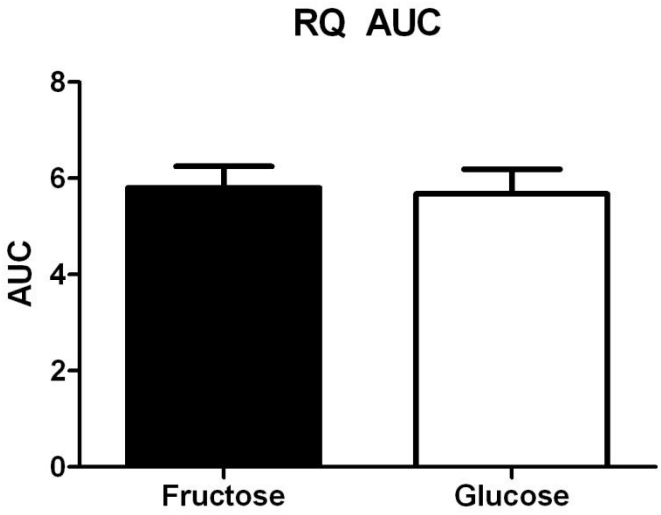
Fasting measurements of respiratory quotient (RQ) were not different between the two treatment days. The difference in AUC for respiratory quotient was not significantly different between the fructose and glucose treatment days. There were no significant differences in respiratory quotient found at any of the post meal time points when the glucose and fructose treatments were compared. Figure ten shows the RQ and the RQ AUC responses following the glucose and fructose meals.

Figure 10. a) RQ response to high fructose and high glucose meals, b) AUC for the RQ response after each meal (data shown as mean ± standard error)

a)



b)



Non-Protein Respiratory Quotient

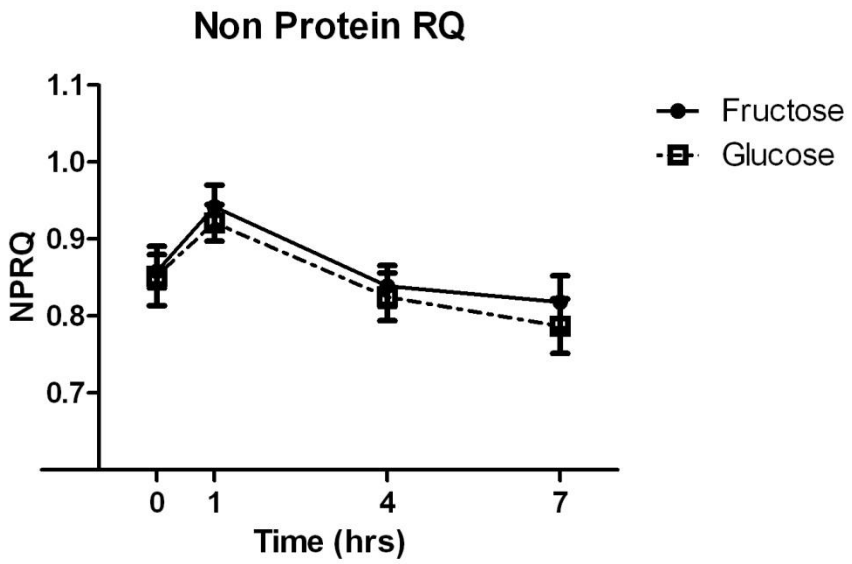
Protein oxidation was estimated based on urea excretion over 24 hours. Total protein oxidation was not significantly different following the fructose (80.5±35.6 g/d) and the glucose meals (80.2±14.8 g/d) (p-value = 0.97). Protein oxidation was used to calculate non protein respiratory quotient.

There was not a difference in fasting concentrations or AUC for non-protein respiratory quotient (NPRQ) between meals. There were no significant differences in NPRQ between the glucose and fructose meals at one, four, or seven hours post meal. Response feature analysis found no difference in slope over the three postprandial time points between the fructose meal and glucose meal (p-value = 0.77). Figure 11 shows the change in NPRQ with the meal and the NPRQ AUCs on the fructose and glucose meals. There was a trend toward a significant positive association between the

difference in NPRQ response between the two meals and the subject BMI such that subjects with a grater BMI had a greater difference in NPRQ between meals (p-value = 0.06). Figure 12 shows this association.

Figure 11. a) NPRQ response to high fructose and high glucose meals, b) AUC for the NPRQ response after each meal (data shown as mean ± standard error)

a)



b)

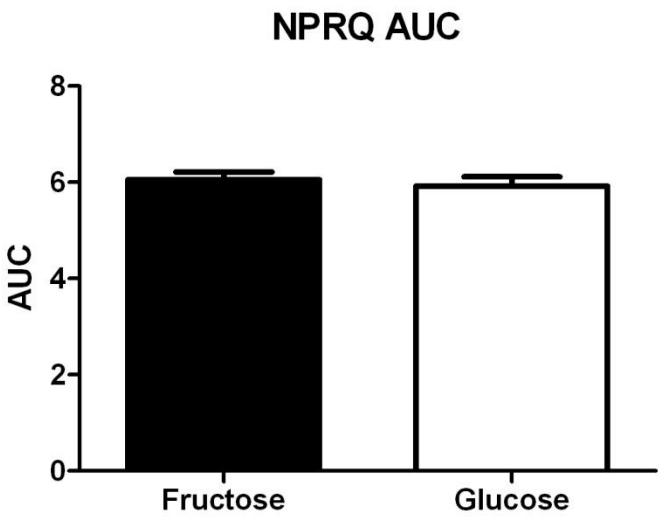
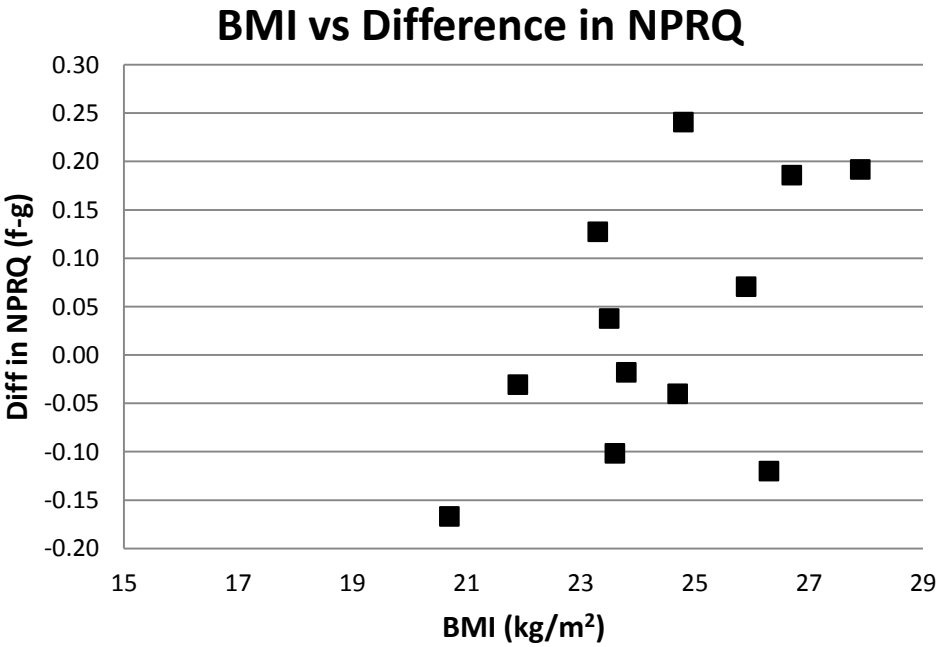


Figure 12. The association between subject BMI and the difference in NPRQ following the fructose and glucose meals.



Chapter 5 – Discussion

This randomized, double-blind, crossover study was conducted in 12 healthy, non-obese males to measure the difference in substrate oxidation and blood markers following a high fructose meal and a high glucose meal. The primary hypothesis was that non protein respiratory quotient would be significantly higher following the high fructose meal compared to the high glucose meal suggesting more carbohydrate oxidation and less fat oxidation following a high fructose meal. While we did observe differences in postprandial triglyceride and insulin concentrations between the high fructose meal and the high glucose meal, we did not observe a difference in postprandial NPRQ for this study which suggests that one meal high in fructose does not alter postprandial substrate oxidation in non-obese, healthy men.

No significant differences were found in post meal blood glucose concentrations between the fructose and glucose meals. This differs from previous studies which have shown a difference in blood glucose concentrations between a high fructose and a high glucose diet. Teff et. al. found that blood glucose AUC was approximately 50% lower in obese subjects consuming a one-day high fructose diet than the AUC of those subjects consuming a one-day high glucose diet (20). Other short- and long-term studies conducted by Teff (19) and Stanhope (38), Bantle (39), and Reiser (40) showed similar decreases in postprandial glucose excursions with a high fructose diet. It's possible that the blood draws may not have been frequent enough which may have caused us to miss the true glucose peaks between meal consumption and one hour post meal.

Another potential explanation for this difference in glucose response is that because the subjects in our study were healthy, non-obese, and young, they may have been more insulin sensitive than obese subjects in previous studies and thus able to maintain euglycemia with either a fructose or glucose meal. We assessed insulin resistance in our subjects using the homeostatic model (HOMA-IR). A HOMA-IR score less than 2.5 is indicative of insulin sensitivity (20). The average ± SD HOMA-IR for our subjects was 1.33 ± 1.49 indicating that our subjects were very insulin sensitive. A study that compared blood lipids in insulin sensitive and insulin resistant subjects on a 0, 7.5, or 15% fructose diet for 5 weeks found that the insulin resistant subjects had a significant increase in plasma triglyceride concentrations on both the 7.5 and 15% fructose diets; whereas, the insulin sensitive group had no change in triglyceride concentrations on any of the diets (41). A very similar study found that these same fructose diets resulted in much more pronounced increase in fasting and postprandial glucose concentrations in insulin resistant subjects compared to insulin sensitive (42). A study comparing the effects of fructose versus starch in diabetic and non-diabetic subjects found that a meal containing 1 gram of fat plus 0.75 grams fructose per kilogram of body weight lowered postprandial glucose and insulin and increased postprandial triglyceride concentrations in both diabetic and non-diabetic subjects compared to the same diet with 0.75 grams of starch/kg body weight instead of fructose (43). It appears that fructose elicits different responses from insulin sensitive and insulin resistant subjects.

The insulin response following the high glucose day was markedly increased compared to the insulin response following the high fructose day which is consistent with known fructose metabolism. Previous research has shown a similar insulin response to a high fructose diet. Acute fructose ingestion has been shown to decrease insulin in both normal-weight and obese subjects (19), (20). Effects of chronic fructose consumption on insulin concentrations are equivocal. A study of chronic fructose consumption showed that a six-week high fructose diet lead to an 18% reduction in postprandial insulin AUC compared to a glucose diet (39). In contrast, another long term study found that both fasting insulin and the insulin excursion after an oral glucose tolerance test were increased after 10 weeks of a high fructose diet compared to a high glucose diet in overweight and obese subjects (18). This is most likely the result of increased insulin resistance which would lead to increased fasting and postprandial insulin concentrations. However, another study found that despite an increase in fasting glucose concentrations after four weeks of a high fructose diet, there was no change in fasting insulin levels (15). Our results are consistent with previous reports of the acute effects of fructose on insulin concentrations.

Insulin concentration is thought to play a role in modulating substrate oxidation. Elevated insulin concentrations in the bloodstream promote carbohydrate oxidation and suppress fatty acid oxidation in the skeletal muscle by stimulating the uptake of glucose into the cell (47). Insulin concentrations were significantly lower one hour after the fructose meal but not different at four or seven hours between meals. Despite

significantly lower insulin concentrations following the fructose meal, we did not observe a difference in NPRQ between meals.

In this study, triglyceride concentrations were measured as a control outcome with an expected outcome based on previous research on the effects of fructose on triglyceride concentrations. Triglyceride response to the fructose meal was higher at four hours postprandially than the triglyceride response to the glucose meal. This post meal increase in triglyceride response after fructose ingestion is also consistent with results from two studies by Teff et. al. (19), (20), the first in normal weight women and the second in obese men and women, both of which found that short term consumption of a high fructose diet resulted in a significantly elevated postprandial triglyceride response compared to a short term high glucose diet. The study in normal weight women found an approximate 26% increase in triglyceride concentrations 24 hours after consuming a one day high fructose diet compared to a one day high glucose diet. This is quite comparable to the approximate 27% increase in triglyceride concentrations at four hours after consuming one high fructose meal compared to a high glucose meal in the current study. Another study conducted in normal weight men found that a beverage containing 50 grams of glucose plus 15 grams of fructose increased triglyceride concentrations by 38.7% compared to a beverage containing only 50 grams of glucose during 30 minutes of aerobic exercise (44).

Similar results for triglyceride concentrations were also found in longer term studies. Stanhope et. al. found that consuming a high fructose diet for 10 weeks

increased postprandial triglyceride concentrations as early as two weeks after starting the diet (18). Swarbrick et. al. found that a 10 week high fructose diet resulted in a progressive increase in postprandial triglyceride concentrations (16). Another study by Bantle et. al. found that a six week high fructose diet led to increased fasting and postprandial triglyceride concentrations in normal weight men but not women (39). In contrast, a study in 11 normal weight men and women age 29-65 years found no difference in triglyceride concentrations after 14 days of a high fructose diet (45). Similarly, a 28 day study of a diet with 20% of kcals from fructose found no difference in fasting, peak, or integrated serum triglyceride concentrations at any point during the diet, and peak postprandial triglyceride concentrations were significantly higher than the comparison high starch diet only on day 1 of the study (46). These differences in reported effects of long term high fructose diets could be due to differences in study duration; age, weight, and sex of subject; percentage of kilocalories from fructose; or a combination of these.

The previous studies finding effects of fructose on triglyceride concentrations also found significant increases in triglyceride AUC after consuming a high fructose diet, whereas our study did not find a difference in triglyceride AUC. The moderate change in triglyceride concentrations observed in our study was not related to a lower fructose content of the meals, because the percentage of kilocalories from fructose was comparable between our study and previous studies (16), (18), (50). It is possible that if our study protocol had included multiple high fructose meals, as these other studies did, we would have observed a significant difference in triglyceride AUC. It is also possible

that the magnitude of the triglyceride response to a high fructose diet is greater among the overweight and obese, and we did not observe a significant difference in triglyceride AUC because our study included only non-obese subjects.

We found no significant difference in plasma leptin concentrations between the two meals. This finding differs from results of other studies which have found that high fructose diets result in significantly lower postprandial leptin concentrations compared to high glucose study diets. A 2004 study in normal weight women found that a one day high fructose diet consisting of three meals resulted in an approximate 24% decrease in peak amplitude of leptin compared to a one day high glucose diet (19). This study also showed a 24% lower AUC for leptin on the high fructose diet compared to the high glucose diet (19). A similar study conducted in obese men and women found that a one day high fructose diet consisting of three meals resulted in a leptin AUC that was 30% lower than a high glucose diet (20). As in our study, both of these studies fed subjects 30% of kcals from fructose or glucose. There are a few potential explanations for these differing results. It is possible that because the current study compared only the effects of one high fructose meal rather than three meals as in the other studies, the amount of fructose may not have been enough to induce a significant change in postprandial leptin concentrations. Another explanation is that the expected diet induced changes in leptin didn't appear until later in the day and fell outside the time frame of the study. We may have found a significant difference in leptin responses between the two meals had we continued measurements into the night when leptin concentrations peak (26), (27). It is also possible that the difference in leptin findings results from a difference in subject

populations. Research has shown that leptin concentrations and pulsitility differ between obese and non obese subjects as well as between men and women regardless of adiposity (48), (26). Therefore, high fructose consumption may affect leptin in non-obese males differently than in normal weight women or obese subjects.

Our finding of a lack of difference in non protein respiratory quotient at any post meal time points following the fructose meal compared to the glucose meal does not support our hypothesis that the high fructose meal would result in a higher NPRQ at seven hours compared to the glucose meal. An early study from 1986 found that consumption of 75 grams of fructose significantly increased carbohydrate oxidation and significantly decreased lipid oxidation postprandially in normal weight, healthy men and women. The study also found that fructose increased postprandial carbohydrate induced thermogenesis (49). Another study found that consumption of a beverage containing 75g of fructose or sucrose resulted in higher peak and integrated total carbohydrate oxidation compared to glucose or corn starch. The study also found that fat oxidation was significantly decreased following the fructose beverage compared to the glucose beverage (50). Two other short term studies looked at the effects of a liquid mixed meal containing 75g of either fructose or glucose on substrate oxidation in both healthy men and women and obese women. The studies found that in healthy men and women, the fructose meal significantly increased carbohydrate oxidation and significantly decreased fat oxidation at 30, 60, and 90 minutes post-meal. However, because fat oxidation increased between 120 and 240 minutes following the fructose meal, overall fat oxidation was not different between the two meals (51). In obese

women, the fructose diet significantly increased carbohydrate oxidation and significantly decreased fat oxidation postprandially (52). Another study of the effect of acute fructose consumption on substrate oxidation found that consumption of a fructose-sweetened beverage significantly increased carbohydrate oxidation and significantly decrease lipid oxidation in normal weight, healthy males but had no effect on substrate oxidation in normal weight, healthy, premenopausal females (53). A study of the effects of overfeeding with fructose or glucose found that both the one-week hypercaloric fructose and glucose diets increased carbohydrate oxidation and decreased lipid oxidation in normal weight, healthy males (54). When the metabolic effects of high sucrose and high starch meals were compared, Daly et. al., found that the high sucrose meals significantly increased carbohydrate oxidation and decreased lipid oxidation compared to the high starch meal for 150 minutes after the meals (55).

The lack of difference in NPRQ between the fructose and glucose meals at seven hours could be due to the fact that leptin levels were not different between the two diets. Leptin has been shown to increase fatty acid oxidation in skeletal muscle by activating AMP kinase and inhibiting ACC activity (34). Therefore, because leptin concentrations were not different, we might not expect NPRQ at seven hours to be significantly different. We correlated the difference between one and seven hour NPRQ and the difference between the one and seven hour leptin concentration. This analysis found no difference in y-intercept or slope between the glucose and fructose meals. However, the range of the data was so narrow it was difficult to determine any relationship in the data.

Alternately, the unexpected lack of change in NPRQ could be due to inadequate duration of the high fructose and high glucose diets. A recent study that looked at the effects of a four week high fructose diet found that after one week of the diet, carbohydrate oxidation had increased by 34% with a trend toward decreased lipid oxidation (15). Another study of the effects of a seven day high fructose diet (3.0 g fructose/kg body weight) on lipid metabolism found that the high fructose diet decreased fat oxidation postprandially and after a lipid load compared to a control diet (56). Based on these previous findings, had we extended our feeding and sampling period, it is possible that this study may have found a significant change in NPRQ.

There are several other factors known to affect substrate oxidation. Fatty acid oxidation is correlated with plasma free fatty acid concentrations. When free fatty acid concentrations are high, fatty acid oxidation is high, and when free fatty acid concentrations are low, fatty acid oxidation is low. We did not measure free fatty acid concentrations in our subjects, but previous short term studies have shown no difference in postprandial free fatty acid levels between high glucose and high fructose treatment (19), (20). A longer term study, however, found that a 10 week high glucose diet significantly increased 24 hour free fatty acid levels whereas the high fructose diet had no effect on free fatty acids (18). This would suggest that there was increased fat oxidation following the high glucose diet. Given the short term nature of our current study, it is possible that there was no difference in free fatty acid levels between the two meals and which could contribute to the lack of difference in fatty acid oxidation as measured by NPRQ.

Glucose availability also affects substrate oxidation. Sufficient glucose availability will result in greater carbohydrate oxidation, whereas low glucose stores will result in a switch to lipid oxidation (47). During fructose metabolism, some fructose is converted to glucose in the liver through gluconeogensis and released slowly over several hours. Thus there is a prolonged release of glucose from the liver after a high fructose meal. This prolonged glucose release may not be detectable by measuring total blood glucose but has been measured using isotopes in previous reports (57), (54), (53). Even a subtle increase in glucose output may promote increased carbohydrate oxidation postprandially. We did not measure hepatic glucose output in this study. However, because of the association between blood glucose concentrations and substrate oxidation, the fact that this study saw no difference in glycemic response between the two meals could help explain the lack of difference in NPRQ.

Limitations

As a preliminary study, only male subjects were studied in order to minimize variation. This resulted in an inability to generalize the results to women, obese or other populations. Also, because this study tested the effects of pure fructose and pure glucose, the results cannot be applied to high fructose corn syrup or sucrose, which are much more likely to be consumed in a typical diet than pure fructose or glucose. The study may have been limited by the brevity of the study diets. Had the diets been extended to include more meals, more significant results may have been found. Also, it's possible that our results were limited by the infrequency of the blood draws. More

frequent blood draws may have resulted in more significant results, particularly for glucose concentrations between baseline and one hour post meal. Finally, this study may not have been adequately powered to detect the difference we were expecting. Initial calculations gave the study 70% power to detect an average difference in 7 hour NPRQ of 0.07±0.11 (effect size = 0.64) with 12 pairs and a one-sided α of 0.05. Based on our actual results, the study only had 20% power to detect a difference of 0.03±0.17 (effect size = 0.18) in NPRQ at seven hours. A total of 150 pairs would be needed to reach 80% power.

Future Directions

The next step in the investigation of the effects of fructose on substrate oxidation would be to increase the feeding time frame for the high fructose and high glucose diets. The time frame could be expanded to a one day, three meal high fructose and high glucose diet, or it could be expanded into a longer term study that covered several weeks. Expanding the feeding time frame in these ways could result in a significant effect of fructose on postprandial substrate oxidation. The study could also be expanded to include analysis of free fatty acid, lactate, and pyruvate concentrations.

Other future directions in the study of the effects of fructose on substrate oxidation could include conducting similar investigations in different study populations. Some studies have shown lesser effects of a high fructose diet in women compared to men, and others have shown no effect of a high fructose diet in women at all. Couchepin et. al. found that normal weight men on a six day high fructose diet had

increased fasting glucose, insulin, triglyceride concentrations, endogenous glucose production, and carbohydrate oxidation and decreased free fatty acids and lipid oxidation. Normal-weight women on the same diet had increased fasting glucose and triglyceride concentrations only. The increase in triglyceride concentrations was significantly higher in men than in women (58). Bantle et. al. found that in men a high fructose diet increased triglyceride AUC by 32% compared to a high glucose diet but had no effect on triglyceride concentrations in women (39). Another study found that diets high in high-fructose corn syrup or sucrose resulted in significantly higher triglyceride concentrations in men than in women. That study also found that these diets resulted in higher leptin concentrations in women than in men; however, sex differences in leptin concentrations have been previous established so this was not unexpected (38), (48), (26). Still other research has shown a significant effect of fructose in women. Swarbrick et. al. found that a 10-week high fructose diet increased fasting glucose and postprandial triglyceride AUCs and decreased postprandial glucose and insulin AUCs in overweight and obese women (16). Teff et. al. found that a one-day high fructose diet decreased postprandial glucose, insulin, and leptin concentrations and increased triglyceride concentrations compared to a high glucose diet in normal-weight women (16), (19). Conducting this or a similar study in women or obese subject groups would result in a better understanding of the effects of fructose on a variety of populations.

Conclusions

This study found that consuming a high fructose meal did not result in a higher non protein respiratory quotient compared to a high glucose meal seven hours following the meal in young, healthy men. Some predicted effects of the high fructose meal were seen: postprandial insulin levels were lower and postprandial triglyceride levels were higher following the fructose meal compared to the glucose meal. These results were consistent with our original hypotheses. In contrast to our predictions however, there was no difference in postprandial glucose or leptin concentrations following the high fructose and high glucose meals. There was also no effect seen on non protein respiratory quotient following the high fructose and high glucose meals. Because there was no difference in the seven hour NPRQ levels following the high fructose and high glucose meals, which was the primary outcome of this investigation, we conclude that a single high fructose meal does not alter substrate oxidation in healthy, non-obese men.

References

1. Anonymous Prevalence of overweight, obesity and extreme obesity among adults: United States, trends 1976-80 through 2005-2006. NCHS Health and Stats, 2008.

2. Anonymous Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity In Adults: The Evidence Report. National Institutes of Health; National Heart, Lung, and Blood Institute, 1998.

3. Fung TT, Rimm EB, Spiegelman D, et al. Association between dietary patterns and plasma biomarkers of obesity and cardiovascular disease risk. Am J Clin Nutr 2001;73:61-7.

4. Hiza HAB, Bente L. Nutrient Content of the U.S. Food Supply, 1909-2004. U.S. Department of Agriculture, 2007. no. 157.]

5. Anonymous U.S. per capita food availability data: Sweeteners by individual caloric sweetener. USDA: Economic Research Service,

6. Vos MB, Kimmons JE, Gillespie C, Welsh J, Blanck HM. Dietary Fructose Consumption Among US Children and Adults: The Third National Health and Nutrition Examination Survey. Medscape Journal of Medicine 2008;10:160.

7. Marriott BP, Cole N, Lee E. National Estimates of Dietary Fructose Intake Increased from 1977 to 2004 in the United States. The Journal of Nutrition 2009;139:1228S-35S.

8. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. American Journal of Clinical Nutrition 2004;79:547-3.

9. Kant AK. Reported Consumption of Low-Nutrient-Density Foods by American Children and Adolescents. Arch Pediatr Adolesc Med 2003;157:789-96.

10. Akhavan T, Anderson GH. Effects of glucose-to-fructose ratios in solutions on subjective satiety, food intake, and satiety hormones in young men. American Journal of Clinical Nutrition 2007;86:1354-63.

11. Melanson K, J., Zukley L, Lowndes J, Nguyen V, Angelopoulos TJ, Rippe JM. Effects of high-fructose corn syrup and sucrose consumption on circulating glucose, insulin, leptin, and ghrelin and on appetite in normal-weight women. Nutrition 2007;23:103-12.

12. Soenen S, Westerterp-Plantenga MS. No differences in satiety or energy intake after high-fructose corn syrup, sucrose, or milk preloads. American Journal of Clinical Nutrition 2007;86:1586-94.

13. Macdonald I, Turner IJ. Serum fructose levels after sucrose or its constituent monosaccharides. The Lancet 1968;1:841-3.

14. Smith LH, Ettinger RH, Seligson D. A comparison of the metabolism of fructose and glucose in hepatic disease and diabetes mellitus. Journal of Clinical Investigation 1953;32:273-82.

15. Le K, Faeh D, Stettler R, et al. A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans. American Journal of Clinical Nutrition 2006;84:1374-9.

16. Swarbrick MM, Stanhope KL, Elliott SS, et al. Consumption of fructose-sweetened beverages for 10 weeks increases postprandial triacylglycerol and apolipoprotein-b concentrations in overweight and obese women. British Journal of Nutrition 2008;100:947-52.

17. Sacca L, Hendler R, Sherwin RS. Hyperglycemia Inhibits Glucose Production in Man Independent of Changes in Glucoregulatory Hormones. Journal of Clinical Endocrinology and Metabolism 1978;47:1160-4.

18. Stanhope KL, et. al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. Journal of Clinical Investigation 2009;1-13.

19. Teff KL, et. al. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. Journal of Clinical Endocrinology and Metabolism 2004;86:2963-72.

20. Teff KL, Grudziak J, Townsend RR, 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 and Metabolism 2009;94:1562-9.

21. Hayashi T, et. al. Visceral adiposity, not abdominal subcutaneous fat area, is associated with an increase in future insulin resistence in Japanese Americans. Diabetes 2008;57:1269-75.

22. Abate N, Garg A, Peshock RM, Stray-Gundersen J, Grundy SM. Relationships of Generalized and Regional Adiposity to Insulin Sensitivity in Men. Journal of Clinical Investigation 1995;96:88-98.

23. Bolinder J, Kager L, Ostman J, Arner P. Differences at the receptor and postreceptor levels between human omental and subcutaneous adipose tissue in the action of insulin on lipolysis. Diabetes 1983;32:117-23.

24. Mittelman SD, Van Citters GW, Kirkman EL, Bergman RN. Extreme Insulin Resistance of the Central Adipose Depot in Vivo. Diabetes 2002;51:755-61.

25. Goodpaster BH, Kelley DE, Wing RR, Meier A, Thaete FL. Effects of Weight Loss on Regional Fat Distribution and Insulin Sensitivity in Obesity. Diabetes 1999;48:839-47.

26. Saad MF, Riad-Gabriel MG, Khan A, et al. Diurnal and ultradian rhythmicity of plasma leptin: effects of gender and adiposity. Journal of Clinical Endocrinology and Metabolism 1998;83:453-9.

27. Schoeller DA, Cella LK, Sinha MK, Caro JF. Entrainment of the Diurnal Rhythm of Plasma Leptin to Meal Timing. Journal of Clinical Investigation 1997;100:1882-7.

28. Cowley MA, Smart JL, Rubinstein M, et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 2001;411:480-4.

29. Smith FJ, Campfield LA, Moschera JA, Bailon PS, Burn P. Feeding inhibition of neuropeptide Y. Nature 1996;382:307.

30. Enriori PJ, Evans AE, Sinnayah P, et al. Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. Cell Metabolism 2007;5:181-94.

31. Kolaczynski JW, Nyce MR, Considine RV, et al. Acute and chronic effects of insulin on leptin production in humans: studies in vivo and in vitro. Diabetes 1996;45:699-701.

32. Levy JR, Stevens W. The Effects of Insulin, Glucose, and Pyruvate on the Kinetics of Leptin Secretion. Endocrinology 2001;142:3558-62.

33. Curry DL. Effects of Mannose and Fructose on the Synthesis and Secretion of Insulin. Pancreas 1989;4:2-9.

34. Minokoshi Y, et. al. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 2002;415:339-43.

35. Rasmussen BB, Holmback UC, Volpi E, Morio-Liondore B, Paddon-Jones D, Wolfe RR. Malonyl coenzyme A and the regulation of functional carnitine palmitoyltransferase-1 activity and fat oxidation in skeletal muscle. Journal of Clinical Investigation 2002;110:1687-93.

36. Shapiro A, Mu W, Roncal C, Cheng K, Johnson RJ, Scarpace PJ. Fructose-induced leptin resistance exacerbates weight gain in response to subsequent high-fat feeding. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 2008;295:R1370-5.

37. Uehara M, Plank LD, Hill GL. Components of energy expenditure in patients with severe sepsis and major trauma: A basis for clinical care. Critical Care Medicine 1999;27:1295-302.

38. Stanhope KL, Griffen SC, Bair BR, Swarbrick MM, Keim NL, Havel PJ. Twenty-four-hour endocrine and metabolic profiles following consumption of high-fructose corn syrup-, sucrose-, fructose-, and glucose-sweetened beverages with meals. American Journal of Clinical Nutrition 2008;87:1194-203.

39. Bantle JP, Raatz SK, Thomas W, Georgopoulos A. Effects of dietary fructose on plasma lipids in healthy subjects. American Journal of Clinical Nutrition 2000;72:1128-34.

40. Reiser S, Powell AS, Scholfield DJ, Panda P, Fields M, Canary JJ. Day-long glucose, insulin, and fructose responses of hyperinsulinemic and nonhyperinsulinemic men adapted to diets containing either fructose or high-amylose cornstarch. American Journal of Clinical Nutrition 1989;50:1008-14.

41. Hallfrisch J, Reiser S, Prather E, S. Blood lipid distribution of hyperinsulinemic men consuming three levels of fructose. The American Journal of Clinical Nutrition 1983;37:740-8.

42. Hallfrisch J, Ellwood KC, Michaelis OE, Reiser S, O'Dorisio TM, Prather EM. Effects of dietary fructose on plasma glucose and hormone responses in normal and hyperinsulinemic men. Journal of Nutrition 1983;113:1819-26.

43. Abraha A, Humphreys SM, Clark ML, Matthews DR, Frayn KN. Acute effect of fructose on postprandial lipaemia in diabetic and non-diabetic subjects. British Journal of Nutrition 1998;80:169-75.

44. Fernandez JM, da Silva-Grigoletto ME, Ruano-Ruiz JA, et al. Fructose modifies the hormonal responses and modulates lipid metabolism during aerobic exercise after glucose supplementation. Clinical Science 2009;116:137-45.

45. Crapo P, A., Kolterman OG. The metabolic effects of 2-week fructose feeding in normal subjects. The American Journal of Clinical Nutrition 1984;39:525-34.

46. Swanson JE, Laine DC, Thomas W, Bantle JP. Metabolic effects of dietary fructose in healthy subjects. American Journal of Clinical Nutrition 1992;55:851-6.

47. Yki-Jarvinen H, Bogardus C, Howard BV. Hyperglycemia stimulates carbohydrate oxidation in humans. American Journal of Physiology 1987;253:376-82.

48. Havel PJ, Kasim-Karakas S, Dubuc GR, Meuller W, Phinney SD. Gender differences in plasma leptin concentrations. Nature Medicine 1996;2:949-50.

49. Tappy L, Randin J, Felber J, et al. Comparison of thermogenic effect of fructose and glucose in normal humans. American Journal of Physiology 1986;250:718-24.

50. Blaak EE, Saris WHM. Postprandial Thermogenesis and Substrate Utilization After Ingestion of Different Dietary Carbohydrates. Metabolism 1996;45:1235-42.

51. Schwarz J, Schutz Y, Froidevaux F, et al. Thermogenesis in men and women induced by fructose vs glucose added to a meal. American Journal of Clinical Nutrition 1989;49:667-74.

52. Schwarz J, Schutz Y, Piolino V, Schneider H, Felber J, Jequier E. Thermogenesis in obese women: effect of fructose vs. glucose added to a meal. American Physiological Society 1992;262:393-401.

53. Tran C, Jacot-Descombes D, Lecoultre V, et al. Sex differences in lipid and glucose kinetics after ingestion of an acute oral fructose load. British Journal of Nutrition 2010;104:1139-47.

54. Tudor Ngo Sock E, Le K, Ith M, Kreis R, Boesch C, Tappy L. Effects of a short-term overfeeding with fructose or glucose in healthy young males. British Journal of Nutrition 2010;103:939-43.

55. Daly ME, Vale C, Walker M, et al. Acute fuel selection in response to high-sucrose and high-starch meals in healthy men. American Journal of Clinical Nutrition 2000;71:1516-24.

56. Abdel-Sayed A, Binnert C, Le K, Bortolotti M, Schneiter P. A high-fructose diet impairs basal and stress-mediated lipid metabolism in healthy male subjects. British Journal of Nutrition 2008;100:393-9.

57. Faeh D, Minehira K, Schwarz J, Periasami R, Seongsu P, Tappy L. Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. Diabetes 2005;54:1907-13.

58. Couchepin C, Le K, Bortolotti M, et al. Markedly blunted metabolic effects of fructose in healthy young female subjects compared with male subjects. Diabetes Care 2008;31:1254-6.

Oregon Health & Science University
Consent Form

IRB#: 5013

Protocol Approval Date: 12/02/2010

MED. REC. NO. _____
NAME _____
BIRTHDATE _____

Complete this section only if clinical services are provided.

OREGON HEALTH & SCIENCE UNIVERSITY

Consent Form

TITLE: Effect of A High Fructose versus a High Glucose Meal on Postprandial Metabolic Flexibility

PRINCIPAL INVESTIGATOR: Kent Thornburg, Ph.D (503) 494-2382
Melanie Gillingham, Ph.D., R.D. (503) 494-1682

CO-INVESTIGATORS: Anne Smeraglio, Emily Kennedy

SPONSOR: Oregon Health & Science University, School of Medicine**,** Oregon Clinical and Translational Research Institute (OCTRI)

PURPOSE:

The purpose of this study is to compare the effects of different sugars on the ability to burn fat after a meal. Our hypothesis is that eating a high-fructose meal will delay the switch from metabolizing carbohydrates to metabolizing fat compared with a high glucose meal. You are being invited to participate in this research study because you are a normal weight, healthy, male student.

This study requires two, ten-hour admissions to the Clinical and Translational Research Center (OCTRI). The two admissions will be at least 1 week and up to 4 weeks apart.

PROCEDURES:

If you agree to be in this study, you will arrive at the OCTRI early in the morning. You must not eat or drink anything but water 12 hours prior to the start your appointment. You must not drink alcohol 12 hours prior to the start of your appointment.

If you can continue in the study, a series of tests will be completed. Each test is explained below. All of the tests listed will be completed while you are admitted to the OCTRI.

24-hour Urine collection: During your stay, you will collect all your urine in a jug. Toilet caps and collection jugs will be provided to make this process easy. You will be responsible for collecting your own urine until 8:00 AM the following day. The urine must be returned to OCTRI within 24 hours of completing the collection.

IV Catheter: An intravenous (IV) catheter or tube will be placed in your arm to draw blood. If the IV nurse is unable to get the IV started after 4 tries, you will be able to go home. We will not complete the remainder of the study.

Blood Samples: Once the catheter is in place, the nurse will take an initial blood sample prior to breakfast. We will take about 1 teaspoon of blood from your arm at this time. Blood will be collected again 1, 4, and 7 hours after breakfast. Breakfast will be provided after the first blood draw. We will take a total of 4 teaspoons or 30 ml of blood during each admission.

We will store your blood and urine samples in the core laboratory of the OCTRI. Samples will be stored with your name and medical record number for purposes of this study. When the study is over the principal investigator would like to store these samples for future research. Should you agree to this storage, your name and medical record number will be removed and the samples will be given a unique code. The samples will then be stored in the principal investigator's locked laboratory indefinitely to be used by the principal investigator or other researchers. You do not have to agree to the storage of your samples for future research to be in this study.

Body Composition Analyzer (BIA), Model 310e:
Body composition will be measured by passing a very small electrical current through your body. You should not feel any pain involved with this test. You will have two electrodes placed on your left hand and two electrodes placed on your left foot. It will take less than one minute to perform the test.

Energy Expenditure Test: Your energy expenditure will be measured using Indirect Calorimtry. Energy expenditure is how many calories you use during the day. A clear, colorless, Plexiglass canopy (bubble) will be placed over your head and chest while you rest on a bed. Samples of the air that you breathe out will be collected for about 30 minutes. A trained research assistant from the graduate programs at OHSU will perform

this test in a quiet room to make you feel comfortable and relaxed. This test will be performed before breakfast and again 1, 4 and 7 hours after breakfast.

Breakfast: After your 1st blood sample and energy test, you will be given a standardized breakfast to consume. The meal will be accompanied with either a glucose or fructose drink. When you return for the second admission to the OCTRI, you will receive the opposite drink (if you got fructose the first day, you will get glucose the second breakfast day). The first drink will be chosen for you at random by the investigators. The content of the drinks (glucose or fructose) will be unknown to you and to the investigators – i.e., this is a double-blind study. Neither you nor the investigators will know which drink you received on the first stay and which you received on the second stay.

Once your visit day is completed you will be evaluated by the RN and offered a standard hospital meal. You will then return home and be allowed to resume your normal diet.

Below is a summary of the timeline of the study.

Example Schedule of Trial Day 1:

7:00 AM	A nurse will evaluate you, place a IV in your arm and draw your blood Your Body Composition will be measured. Research Staff will perform an Energy Expenditure Test.
8:00 AM	Breakfast
9:00 AM	Blood draw and Energy Expenditure Test.
12:00 PM	Blood draw and Energy Expenditure Test
3:00 PM	Blood draw and Energy Expenditure Test.
3:45 PM	The nurse will evaluate you, remove your IV and give you a post study meal

Your length of stay at OCTRI may be to be up to 10 hours.
You will need to continue collecting your 24hr urine until 8am the following morning. Urine samples must be returned to OHSU within 24hours of finishing the collection.

The fasting period on the trial day lasts a total of 7 hours, during which you will remain at OCTRI. You will complete the above study schedule twice, once with glucose and once with fructose, at least one week apart. The order of the two trials will be randomly assigned. Blood is collected four times in each trial. Approximately one teaspoon (5mL) of blood will be taken with each blood draw. The total volume of blood collected in one trial will not exceed two tablespoons (30mL).

If you have any questions regarding this study now or in the future, please contact Melanie Gillingham, Ph.D., R.D. at (503) 494-1682.

RISKS AND DISCOMFORTS:

Participation in this study involves some risks, discomforts, and inconveniences. These include:

Participation requires up to a 10-hour stay at the OHSU OCTRI that can be inconvenient if you have a busy schedule.

Estimation of Resting Energy Expenditure (calorie use): There are no risks associated with having energy expenditure measured. Some individuals with claustrophobia (fear of closed spaces) may find the canopy of the indirect calorimeter too confining. The procedure takes about 30 minutes to complete and will be performed in a private room.

Blood Samples/IV Catheter: You may feel some pain when your blood is drawn. There is a small chance the needle will cause bleeding, a bruise, or an infection at the puncture site. You may get an infection where the tube is placed. This will cause swelling, redness, and pain. You may bleed or get a bruise.

BIA: This is a painless, risk-free process that takes less than one minute to complete. Although there are electrodes, the amount of electricity used is that of a heart monitor on a standard treadmill at most gyms.

BENEFITS:

You may not benefit from being in this study. However, by serving as a subject, you may help us learn how to benefit patients in the future.

ALTERNATIVES:

You may choose not to be in this study.

CONFIDENTIALITY:

We will not use your name or your identity for publication or publicity purposes. Research records may be reviewed and copied by the OCTRI, the OHSU Institutional Review Board, the Office for Human Research Protections, and the National Center for Research Resources.

COSTS:

It will not cost anything to participate in the study. You will be compensated $50 for completing the study.

LIABILITY:

If you believe you have been injured or harmed while participating in this research and require immediate treatment, contact Melanie Gillingham, Ph.D., R.D. at (503) 494-1682.

You have not waived your legal rights by signing this form. If you are harmed by the study procedures, you will be treated. Oregon Health & Science University does not offer to pay for the cost of the treatment. Any claim you make against Oregon Health & Science University may be limited by the Oregon Tort Claims Act (ORS 30.260 through 30.300). If you have questions on this subject, please call the OHSU Research Integrity Office at (503) 494-7887.

It is not the policy of the U.S. Department of Health and Human Services to compensate or provide medical treatment for human subjects in the event the research results in physical injury.

PARTICIPATION:

If you have any questions regarding your rights as a research subject, you may contact the OHSU Research Integrity Office at (503) 494-7887.

You do not have to join this or any research study. If you do join, and later change your mind, you may quit at any time. If you refuse to join or withdraw early from the study, there will be no penalty or loss of any benefits to which you are otherwise entitled.

You may be removed from the study if:
- the investigator stops the study
- the sponsor stops the study
- you do not follow study instructions.

The participation of OHSU students or employees in OHSU research is completely voluntary and you are free to choose not to serve as a research subject in this protocol for any reason. If you do elect to participate in this study, you may withdraw from the study at any time without affecting your relationship with OHSU, the investigator, the investigator's department, or your grade in any course.

We will give you a copy of this form.

SIGNATURES:

Your signature below indicates that you have read this entire form and that you agree to be in this study.

- I give my consent for my blood samples to be stored and used for this study only.
- I give my consent for my blood samples to be used for this study and stored for possible use in future studies, but I wish to be contacted for permission prior to any future use.
- I give my consent for my blood samples to be used for this and future studies and do not need to be contacted for permission in the future.

OREGON HEALTH & SCIENCE UNIVERSITY

INSTITUTIONAL REVIEW BOARD

PHONE NUMBER (503) 494-7887
CONSENT/AUTHORIZATION FORM APPROVAL DATE

Dec. 2, 2010

Do not sign this form after the

Expiration date of: 12/01/2011

Subject's Signature: _____ Date: _____

Person Obtaining Consent Signature: _____ Date: _____

APPENDIX B – Evidence Table

Study Identification	Participants	Study Duration	Interventions	Outcomes
Teff, Karen L.; Grudziak, Joanne; Townsend, Raymond R.; et. al. (2009) 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 Response *Journal of Clinical Endocrinology and Metabolism*, May, 1562-1569	17 obese men and women age 19-49 years with BMI > 30 kg/m2	24 hours	Crossover design where subjects were fed three mixed nutrient meals over one day with 30% of kcals from either a fructose- or glucose-sweetened beverage AUC for glucose, lactate, insulin, leptin, ghrelin, uric acid, triglyceride concentrations, and free fatty acids were measured HOMA-IR was used to classify subjects by insulin sensitivity and to determine if metabolic differences existed between insulin resistance and insulin sensitive subjects on the two diets	AUC for glucose ↓ by 50% on the fructose day compared to the glucose day. AUC for lactate was > five times higher on the fructose day than the glucose day. Trend toward ↓ free fatty acids following the fructose day, but no significant difference in FFA. Peak insulin concentrations were significantly ↓ on the fructose day. Insulin AUC was 50% ↓ on the fructose day. Leptin AUC was ↓ by 30% on the fructose day compared to the glucose day. No difference in ghrelin or uric acid levels between the two diets. AUC for triglyceride concentrations was almost 200% on the fructose day compared to the glucose day. Insulin resistant subjects had greater reduction in insulin and leptin on the high fructose diet. No difference between glucose/fructose diet on the increase in tgs in those classified as insulin resistant.

Teff, Karen L.; Elliott, Sharon S.; Tschop, Matthias; et. al. (2004) Dietary Fructose Reduces Circulating Insulin and Leptin, Attenuates Postprandial Suppression of Ghrelin, and Increases Triglycerides in Women *Journal of Clinical Endocrinology & Metabolism*, 2963-2972	12 normal weight women age 19-33 years with a BMI between 19.8 and 26.7	24 hour controlled diet with study beverage, followed by a 12 hour ad libitum diet	Crossover design where subjects were fed three mixed nutrient meals over one day with 30% of kcals from either a fructose- or glucose-sweetened beverage. Blood samples were taken every 30-60 minutes and analyzed for glucose, insulin, leptin, ghrelin, GIP, GLP-1, triglyceride concentrations and free fatty acids. Hunger ratings and food intake were also measured.	Glucose excursions lower after each fructose meal compared to glucose meals, particularly after dinner. 12- and 24-hour glucose AUCs ↓ by 55±7% and 39±9% on the high fructose day compared to the high glucose day. Average peak insulin response ↓ by 65±5% and the 24 hour AUC ↓ by 49±5% on the high fructose day compared to the high glucose day. 12- and 24-hour leptin AUCs were 33±7% and 24±7% smaller on the fructose day. Suppression of ghrelin after high glucose meals was > following the high fructose meals. GIP levels higher on the glucose day than the fructose day. Integrated GLP-1 concentrations > following the fructose meals compared to the glucose meals. Triglyceride levels more rapidly, achieved higher peak levels, and remained elevated longer following the fructose meals compared to the glucose meals. No difference in free fatty acid response between the two diets. Subjects classified as restrained eaters reported an increased hunger rating on the day of the treatment and consumed more fat the following day than unrestrained eaters.

Stanhope, Kimber L.; Schwarz, Jean Marc; Keim, Nancy L.; et. al.; 2009 Consuming Fructose-Sweetened, Not Glucose-Sweetened, Beverages Increases Visceral Adiposity and Lipids and Decreases Insulin Sensitivity in Overweight/Obese Humans Journal of Clinical Investigation, 1-13	32 overweight and obese men and women age 40-72 years with BMI between 25-35 kg/m2	12 weeks	Parallel-arm study with matched subjects. 2 week inpatient - subjects fed energy balanced diet followed by an 8 week outpatient - subjects consumed either fructose or glucose beverage providing 25% of energy needs + ad libitum diet, followed by a 2 week inpatient - subjects consumed the same beverages with an energy-balanced diet. DEXA and CT scans were done to measure total body fat, visceral and subcutaneous adipose tissue. Glucose, insulin, lipid and lipoproteins, and FFA were analyzed at baseline, 2, 8, and 10 weeks. Insulin sensitivity measured by OGTT	Increase in body weight, fat mass, and waist circumference the same between groups. SAT with glucose diet & VAT with fructose diet. Lipid & lipoproteins with fructose, not glucose, consumption. 23-hour Tg AUC, Tg exposure, & postprandial Tg peak increased with fructose and not glucose consumption. Insulin sensitivity & glucose tolerance ↓ with fructose treatment. Fasting insulin & insulin excursions from baseline at nine weeks. Total & % increases in fat mass and intraabdominal fat volume and Tg response was greater in men on the fructose diet than women. Women on the fructose diet had greater reduction in insulin sensitivity than men.

Le, Kim-Anne; Faeh, David; Stettler, Rodrigue; et. al.; 2006 A 4-wk High-Fructose Diet Alters Lipid Metabolism Without Affecting Insulin Sensitivity or Ectopic Lipids in Healthy Humans *American Journal of Clinical Nutrition*, 1375-1379	7 normal weight males, average age 24.7 years, BMI between 19 and 25 kg/m2	4 weeks	Isoenergetic diet + fructose beverage with 1.5 g fructose/kg body weight (beverage consumed 3x/day with meals). Energy expenditure, insulin sensitivity, intrahepatocellular lipids, & intramyocellular lipids were measured at baseline, and after 1 and 4 weeks on the diet. Fasting blood samples collected at baseline, 1, 2, 3, & 4 weeks.	No change in body weight or composition, IMCL, IHCL, insulin sensitivity, fasting insulin, glucagon or total cholesterol. Within 1 week, increase in fasting VLDL tgs, total tgs, & leptin & ↓ FFAs. Within 2 weeks, increase in fasting lactate. Within 4 weeks, increased fasting glucose. CHO oxidation increased after 1 week with a trend toward ↓ fat oxidation.

Stanhope, Kimber L.; Griffen, Steven C.; Bair, Brandi R., et. al.; 2008 Twenty-Four-Hour Endocrine and Metabolic Profiles Following Consumption of High-Fructose Corn Syrup-, Sucrose-, Fructose-, and Glucose-Sweetened Beverages with Meals *American Journal of Clinical Nutrition*, 1194-1203	34 male & female subjects age 20-50 years	24 hours	All subjects completed two one-day trials where they consumed three meals with 25% of kcals from sucrose or HFCS beverage. 8 male subjects completed two more trials where they consumed the same meals with either a fructose or glucose beverage. Glucose, insulin, leptin, ghrelin, tgs, and FFA were measured.	No difference between sucrose & HFCS on glucose, leptin, ghrelin, tgs, FFA, cholesterol, LDL, or HDL. Insulin AUC increased slightly after sucrose than HFCS. ↓ glucose & insulin with fructose than glucose with sucrose & HFCS in between. Increased tgs with fructose, HFCS, & sucrose compared to glucose.

Blaak, E.E.; Saris, W.H.M.; 1996 Postprandial Thermogenesis and Substrate Utilization After Ingestion of Different Dietary Carbohydrates *Metabolism*, Oct, 1235-1242	10 normal-weight male subjects average age 27.8 years, average weight 74.8 kg	7 hours	Subjects admitted on 4 occasions and fed a carbohydrate beverage containing 75g of glucose, fructose, sucrose, or corn starch. Baseline energy expenditure was measure and measured again for 6 hours after consuming the carbohydrate. Blood samples taken to determine glucose, insulin, and FFAs.	Peak glucose was lower after fructose than glucose, sucrose, or corn starch. Integrated glucose AUC highest for glucose and corn starch, intermediate with sucrose, and lowest with fructose. Peak insulin and integrated insulin AUC highest with glucose, intermediate with sucrose and corn starch, and lowest with fructose. No difference in FFA between carbohydrates. Significantly higher peak CHO oxidation and integrated total CHO oxidation with fructose and sucrose compared to glucose and corn starch. Increased decrement in fat oxidation with fructose compared to glucose.

Swarbrick, Michael M.; Stanhope, Kimber L.; Elliot, Sharon S.; et. al; 2008 Consumption of Fructose-Sweetened Beverages for 10 Weeks Increases Postprandial Triacylglycerol and Apolipoprotein-B Concentrations in Overweight and Obese Women *British Journal of Nutrition*, 947-952	7 overweight or obese women age 50-72 years with BMI between 26.8 and 33.3 kg/m2	10 weeks (4 week baseline, 10 week intervention)	4 week baseline with subjects consuming a weight-maintaining diet. 10 week intervention where subjects were fed 100% of energy requirements with 25% of kcals from fructose. Subjects studied at baseline and the 2nd and 10th week of the intervention. Blood draws collected over 14 hours on these days.	Increased fasting glucose & apolipoprotein B. ↓ postprandial glucose excursions & insulin AUC. postprandial tgs excursions and AUC.

Tittelback, Thomas J.; Mattes, Richard D.; Gretebeck, Randall J.; 2000 Post-Exercise Substrate Utilization after a High Glucose vs. High Fructose Meal During Negative Energy Balance in the Obese *Obesity Research*, 496-505	14 obese men and women, average age 26 years, average BMI 30.3 kg/m2	7 days	Each subject admitted four times for seven days each . First six days consisted of either energy balanced diet or negative engery balanced diet. Seventh day consisted of subject exercising for 40 minutes then consuming a high glucose (50g) or high fructose (50g) beverage. Substrate utilization was measured and blood samples taken.	2 glucose diets resulted in postprandial glucose excursions at 30 & 60 minutes compared to 2 fructose diets. At 30 minutes, insulin was higher for the energy balanced glucose diet than the two fructose diets, and at 60 minutes, insulin was higher for both glucose diets compared to the two fructose diets. Energy expenditure not different between the four trials. Greater carbohydrate oxidation after the energy balanced fructose diet compared to the two glucose diets. Decreased fat oxidation with energy balanced fructose diet compared to two glucose diets. *Negative energy balance same as two glucose diets in terms of substrate oxidation*

Abdel-Sayed, Andrew; Binnert, Christophe; Le, Kim-Anne; et. al.; 2008 A High-Fructose Diet Impairs Basal and Stress-Mediated Lipid Metabolism in Healthy Male Subjects *British Journal of Nutrition*, 393-399	6 normal mails average age 24.7 years, average BMI 23.1 kg/m2	7 days	Crossover design where subjects consumed a control diet or same diet supplemented with 3.0g fructose/kg body weight for 7 days. On the 6th day of the study a lactate infusion was administered and indirect calorimetry performed. On the 8th day a lipid load was administered and a mental stress test performed to stimulate lipolysis. Blood samples were collected both days.	Fructose diet ↓ fasting FFAs by 19.5%, β-hydroxybutyrate by 78.2% and lipid oxidation by 21.3% compared to the control diet. Lipid oxidation remained lower after lipid loading on the fructose diet compared to control. Lower FFA concentration and exogenous lipid oxidation following mental stress with the fructose diet compared to the control diet. Fructose diet increased lactate production.
Bantle, John P.; Raatz, Susan K.; Thomas, William; Georgopoulos, Angeliki; 2000 Effects of Dietary Fructose on Plasma Lipids in Healthy Subjects *American Journal of Clinical Nutrition*, 1128-1134	24 healthy men and women average age 41 years, average BMI 25 (12 men & 12 women; 6 men $\geq$40 years & 6 women $\geq$40 years)	6 weeks	Crossover design with controlled diet providing 17% of kcals from fructose or glucose. Fasting blood samples were taken every 7 days and on day 42, 24-hour metabolic profiles were obtained.	Fasting tgs increased in men on fructose diet. No change in fasting tgs in women on either diet. On day 42 of the fructose diet, men had 32% higher tgs response on fructose than glucose. No difference in women. On day 42, glucose and insulin both lower 90 minutes after breakfast on fructose diet, but AUCs not different for glucose but insulin AUC 18% lower on fructose day (all subjects).

Akhavan, Tina; Anderson, G. Harvey; 2007 Effects of Glucose-to-Fructose Ratios in Solutions on Subjective Satiety, Food Intake, and Satiety Hormones in Young Men *American Journal of Clinical Nutrition*, 1354-1363	12 (experiment 1) and 19 (experiment 2) males aged 18-35 with BMI between 20 and 26 kg/m2	< 1 day	Repeated measures design where subjects were given sugar solutions containing sucrose, HFCS (G45:F55), G20:F80, and G80:F20 (exp1) and G20:F80, G35:F65, G50:F50, and G80:F20 (exp2). Average appetite, glucose, & food intake were measured. Satiety hormones were measured in 7 subjects in exp2.	G20:F80 and G35:F65 were the only solutions that did not suppress food intake more than the water control. G20:F80 had the highest cumulative energy intake. G80:F20 and G20:F80 had the highest and lowest blood glucose levels. G80:F20 had the highest and G20:F80 and G35:F65 had the lowest insulin response.
Tappy, Luc; Randin, Jean-Pierre; Felber, Jean-Pierre; et. al.; 1986 Comparison of Thermogenic Effect of Fructose and Glucose in Normal Humans American Journal of Physiology, 718-724	17 subjects (6 male, 11 female), age 19-50 years, weight range 48-78 kg	< 1 day	Subjects consumed beverage containing either 75 g fructose or glucose. Indirect calorimetry was performed for four hours following sugar load.	Rate of increase & absolute rate of CHO oxidation was greater for fructose in the first 90 minutes. Total CHO oxidation was greater for fructose than glucose. Rate of decrease & absolute decrease of lipid oxidation was greater for fructose in the first 90 minutes. Total lipid oxidation was greater for fructose than glucose. Plasma glucose & insulin significantly great after glucose. No different in FFA levels. CHO induced thermogenesis greater after fructose.

Schwarz, Jean-Mar; Schutz, Yves; Froidevaux, Francoise; et.al; 1989 Thermogenesis in men and women induced by fructose vs glucose added to a meal *American Journal of Clinical Nutrition*, 667-674	20 normal weight men and women with average age of 23 and average BMI of 20.8kg/m2	7 hours	Crossover design where subjects consumed a mixed nutrient beverage with either 75 g of fructose or glucose. Indirect calorimetry was performed for 30 minutes prior and 6 hours after consumption of beverage. Blood samples taken every 30 minutes.	Postprandial change in energy expenditure greater after fructose meal than glucose. Diet induced thermogenesis greater after fructose. Both were greater in men than women. RQ > after fructose meal. Total carbohydrate oxidation was higher after fructose meal. CHO oxidation was higher and lipid oxidation lower at 30, 60, & 90 min postprandial after fructose, but overall lipid oxidation was not different between the two meals. Postprandial glucose and insulin greater with fructose. No difference in FFA response.
Schwarze, J.M.; Schutz, Y.; Pionlino, V.; et. al.; 1992 Thermogenesis in Obese Women: Effect of Fructose vs. Glucose Added to a Meal *American Physiological Society*, 394-401	13 obese (>27 kg/m2), 10 lean (<25 kg/m2) women age 18-40	7 hours	Crossover design where subjects consumed a mixed nutrient beverage with either 75 g of fructose or glucose. Indirect calorimetry was performed for 30 minutes prior and 6 hours after consumption of beverage. Blood samples taken every 30 minutes.	Thermogenic response higher after fructose meal for lean and obese. Mean RQ and cumulative CHO oxidation higher after fructose. Cumulative lipid oxidation was lower after fructose compared to glucose.

Tran, Christel; Jaco-Descombes, Delphine; Lecoultre, Virgile; et. al.; 2010 Sex Differences in Lipid and Glucose Kinetics After Ingestion of An Acute Oral Fructose Load *British Journal of Nutrition*, 1139-1147	9 male and 9 female (pre-menopausal) average age 24.45, BMI between 19 and 25 kg/m2	6 hours	Subjects consumed a beverage containing 0.3 g/kg per fat free mass of fructose. Substrate oxidation was measured for 6 hours following fructose ingestion.	Carbohydrate oxidation increased and lipid oxidation decreased after fructose ingestion in males, but neither changed in females. Similar gluconeogenesis and total fructose oxidation between males and females
Tudor Ngo Sock, Emilienne; Le, Kim-Anne; Ith, Michael; et. al. 2010 Effects of a Short-Term Overfeeding with Fructose or Glucose in Healthy Young Males *British Journal of Nutrition*, 939-943	11 males average age 24.6 years, with BMI between 19 and 25 kg/m2	1 week	crossover design where subjects followed three separate diets: 1) weight maintenance diet, 2) weight maintenance diet supplemented with 3.5 g/kg FFM fructose, 3) weight maintenance diet supplemented with 3.5 g/kg FFM glucose	Subjects gained weight on both hypercaloric diets. VLDL and IHCL increased after fructose. IMCL increased after glucose. Carbohydrate oxidation increased and lipid oxidation decreased after both hypercaloric diets.

Crapo, Phyllis A.; Kolterman, Orville G.; 1984 The Metabolic Effects of 2-Week Fructose Feeding in Normal Subjects *The American Journal of Clinical Nutrition*, 525-534	11 subjects - 7 female, 4 male; average weight 66.4 kg, average age 39.5 years	14 days	3-4 day baseline period where subjects fed 24% of total carbohydrate from sucrose. Subjects then fed two-week weight-maintenance diet with 24% of total carbohydrate from fructose. Glucose, insulin, pyruvate, and lactate measured during an OGTT at 3 and 14 days. Tgs, FFAs, cholesterol, HDL and uric acid also measured.	No difference from baseline in glucose or insulin responses to OGTT at 3 and 14 days. No difference from baseline in glucose or insulin response to 50g fructose load at 3 and 14 days. Following consumption of breakfast and lunch meals, there was a significant decrease from baseline in glucose and insulin after lunch only on days 3 and 14. FFAs were not different from baseline at 3 days, but were significantly lower at 14 days with a delayed rebound to preprandial levels. No change in tgs. Significant ↓ in cholesterol and HDL during fructose feeding.
Hallfrisch, Judith; Reiser, Sheldon; Prather, Elizabeth S.; 1983 Blood Lipid Distribution of Hyperinsulinemic Men Consuming Three Levels of Fructose *The American Journal of Clinical Nutrition*, 740-748	12 hyperinsulinemic men and 12 control with average weight of 178 pounds and average age 39.7 years	5 weeks	Three leg crossover design where subjects consumed diet with 0, 7.5 and 15% of kcals from fructose.	Tgs increased significantly as fructose increased in hyperinsulinemic men but not in controls. Total and LDL cholesterol were significantly greater with 7.5 or 15% fructose than with no fructose but did not differ between groups. FFA did not change with diet or group.

Hallfrisch, Judith; Ellwood, Kathleen C.; Michaelis IV, Otho E.; et. al.; 1983 Effects of Dietary Fructose on Plasma Glucose and Hormone Response in Normal and Hyperinsulinemic Men *Journal of Nutrition*, 1819-1826	12 hyperinsulinemic men and 12 control with average weight of 178 pounds and average age 39.7 years	5 weeks	Three leg crossover design where subjects consumed diet with 0, 7.5 and 15% of kcals from fructose. Metabolic effects of 2g/kg sucrose load measured at the end of each 5 week period.	Insulin responses were higher in hyperinsulinemic subjects at all time points. Glucose responses higher in hyperinsulinemic group at 1 hour post sucrose load. Postprandial glucose was higher with 15% fructose than 0% and 7.5% was intermediate. Fasting insulin was higher in hyperinsulinemic group and were higher with the 15% fructose diet than the other two. Fasting glucose levels were not different between groups but were higher with the 7.5 and 15% fructose diets than the 0% fructose.

Couchepin, Caroline; Oboni, Jean-Baptiste; Kim-Anne Le; 2008 Markedly Blunted Metabolic Effects of Fructose in Healthy Young Female Subjects Compared with Male Subjects *Diabetes Care*, 1254-1256	8 male and 8 female subjects average age 22.7 years, average BMI 21.8 kg/m2	6 days	Two leg study design with 6 day isoenergetic control diet and 6 days of the same diet supplemented with 3.5g fructose per kg fat free mass. Metabolic assessment on the 6th day of each diet.	Male subjects had significant increase in fasting glucose, insulin, tgs, alanine aminotransferase, and lactate and decrease in FFAs, β-hydroxybutyrate, and glucagon. Males also had increased endogenous glucose production and CHO oxidation and decreased lipid oxidation. Female subjects had signficant increase in fasting glucose and triglyceride concentrations and decrease in β-hydroxybutyrate and glucagon. The increase in endogenous glucose production and tgs and the decrease in β-hydroxybutyrate were significantly lower in females than males.
Fernandez, Juan M.; da Silva-Grigoletto, Marzo E.; Ruano-Ruiz, Juan A.; 2009 Fructose Modifies the Hormonal Response and Modulates Lipid Metabolism During Aerobic Exercise After Glucose Supplementation *Clinical Science*, 137-145	20 men average age 26 years, average BMI 23.45 kg/m2	<1 day	Crossover design where subjects consumed beverage containing 50g glucose or 50g glucose + 15g fructose prior to 30 minutes of aerobic exercise. Hormonal and lipid responses measured.	Insulin, tgs, lipoperoxides, & oxidized LDL concentrations higher with fructose. Urinary catecholamines, FFAs, perceived exertion and heart rate were lower after fructose. No difference in lactate.

Daly, Mark E.; Vale, Catherine; Walker, Mark; et. al.; 2000 Acute Fuel Selection in Response to High-Sucrose and High-Starch Meals in Healthy Men *American Journal of Clinical Nutrition*, 1516-1524	7 males average age 38.4 years, average BMI 24.9 kg/m2	<1 day	3 leg crossover design where subjects consumed meal meeting 40% of daily energy requirements. One meal supplied 50% of energy from starch and the two others from sucrose. Substrate oxidation and serum concentrations measured.	CHO oxidation was significantly increased and lipid oxidation decreased until 150 minutes post meal with the sucrose compared to starch. Glucose had a more rapid rise to a slightly higher peak and a more rapid drop to below baseline after sucrose. Similar for insulin. FFAs decreased postprandially after sucrose and starch but began rising and returned to baseline much sooner with sucrose.
Faeh, David; Minehira, Kaori; Schwarz, Jean-Marc; et. al.; 2005 Effect of Fructose Overfeeding and Fish Oil Administration on Hepatic De Novo Lipogenesis and Insulin Sensitivity in Healthy Men *Diabetes*, 1907-1913	7 males aged 22-31, BMIs 20.2-25.4 kg/m2	variable	4 leg crossover study: 1) subjects consumed 7.2 g fish oil/day for 28 days, 2) 3 g fructose/kg body weight/day for 6 days, 3) fish oil + fructose, 4) control. After each diet, fasting DNL, fasting endogenous glucose production, and insulin sensitivity were evaluated.	Fasting EGP and fasting CHO oxidation were higher and fasting FFAs and fasting lipid oxidation lower after fructose and fructose+fish oil compared to fish oil alone and control. Fasting tgs were lower on the fructose+fish oil compared to fructose alone, but both were higher than control and fish oil alone. Fructose did not change whole body insulin sensitivity.

Abraha, Arefaine; Humphreys, Sandy M.; Clark, Mo L.; et. al.; 1998 Acute Effect of Fructose on Postprandial Lipaemia in Diabetic and Non-Diabetic Subjects *British Journal of Nutrition*, 169-175	6 non-diabetic subjects (3 male, 3 female) 30-61 years old and BMI of 17.6-26.3 kg/m2 6 T2DM subjects (4 male, 2 female) age 43-54 years, BMI 23.1-33 kg/m2	<1 day	Crossover design where subjects consumed a diet containing 1 g fat/kg body weight with either .75 g fructose/kg body weight or .75 g starch/kg body weight	Postprandial glucose and insulin levels were lower after the fructose meal compared to starch in both subject groups (glucose peaked later in diabetics). In both groups FFAs decreased more with fructose in the first 240 minutes and then increased to a higher level than starch. Postprandial tgs higher with fructose in both groups.
Reiser, Sheldon; Powell, Andrea S.; Scholfield, Daniel J.; et. al.; 1989 Day-Long Glucose, Insulin, and Fructose Responses of Hyperinsulinemic and Nonhyperinsulinemic Men Adapted to Diets Containing Either Fructose or High-Amylose Cornstarch *American Journal of Clinical Nutrition*, 1008-1014	10 hyperinsulinemic men average age 47.4 years, average BMI 25.7 kg/m2 11 non-hyperinsulinemic men average age 38.1, average BMI 24.4 kg/m2	5 weeks	Crossover design with a 5 week diet with 20% of kcals from fructose or cornstarch. Fasting and postprandial samples taken at the end of each 5 week period.	Fructose diet increased fasting glucose and decreased postprandial glucose as 60 and 120 minutes after meal. No difference between subject groups. Fructose decreased postprandial insulin levels at 60 minutes. Insulin concentrations were always higher in hyperinsulinemic subjects. Postprandial fructose concentrations always higher on fructose diet. Fructose levels higher at 60 and 90 minutes in hyperinsulinemic group compared to nonhyperinsulinemic.

Swanson, Joyce E.; Laine, Dawn C.; William, Thomas; Bantle, John P.; 1992 Metabolic Effects of Dietary Fructose in Healthy Subjects *American Journal of Clinical Nutrition*, 851-856	14 subjects (7 men, 7 women) age 19-60	28 days	Crossover design where subjects consumed diet with 20% of energy from fructose or an isoenergetic diet with <3% fructose	No difference in hemoglobin A1c, serum glycosylated albumin, fasting lactate, fasting glucose, please plasma glucose, or integrated glucose. Fasting cholesterol was higher at day 28 and fasting LDL cholesterol was higher at days 14 and 28 on the fructose diet compared to starch. Peak serum tgs were higher on day 1 of the fructose diet, but there was no other difference between peak tgs, fasting tgs, or integrated tgs between diets.