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ABSTRACT

PURPOSE: The overall aim of this study was to examine the effects of a high-fructose diet on postprandial lipemia and hormones associated with glucose control during periods of altered physical activity. **METHODS:** Twenty-two recreationally active males and females participated in this randomized, cross-over design study (age: 21.2 ± 0.6 years; BMI: 22.6 ± 0.6 kg/m²). Subjects ingested 75 g of fructose for 14 days during a period of high physical activity (FR+Active) (>12,500 steps/day) and a period of low physical activity (FR+Inactive) (<4500 steps/day). Prior to and following the 2 wk loading period, a fructose-rich meal challenge was administered (45% carbohydrate [25% fructose], 40% fat, and 15% protein). Blood was sampled at baseline and for 6 h after the meal and analyzed for triglycerides (TG), very-low density lipoproteins (VLDL), c-reactive protein (CRP), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), glucagon-like peptide 1 (GLP-1), glucose-dependent insulintropic peptide (GIP), c-peptide, glucose and insulin concentrations. Area under the curve (AUC) and absolute change from peak to baseline concentrations (Δ peak) were calculated to quantify the postprandial responses. **RESULTS:** TG, VLDL and IL-6 significantly increased in response to the FR+Inactive intervention ($p < 0.05$), while GIP, insulin, c-peptide and GLP-1 (males only) significantly decreased in response to the FR+Active intervention ($p < 0.05$). No changes occurred with glucose, TNF- α , and CRP concentrations for either intervention ($p > 0.05$).

CONCLUSIONS: When an inactive lifestyle is adopted for two weeks, while consuming a high fructose diet, postprandial lipidemia and low grade inflammation occurs. In contrast, two weeks of increased physical activity induces positive changes in hormones associated with glucose control in order to attenuate the deleterious effects of the fructose-rich diet.

**Impact of Physical Activity on Postprandial Lipidemia
and Glycemic Control after Chronic Fructose Ingestion**

By

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M.S. Syracuse University, 2007**

DISSERTATION

**Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy
in Science Education in the Graduate School of Syracuse University**

December 2012

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Introduction:

In the United States and worldwide, the prevalence of obesity is increasing, with approximately 67% of the U.S. population over 20 years of age considered overweight (BMI $\geq 25\text{kg/m}^2$) or obese (BMI $\geq 30\text{kg/m}^2$)¹. Obesity contributes to metabolic abnormalities such as insulin resistance, dyslipidemia, hypertension, and glucose intolerance, all of which are risk factors associated with metabolic syndrome². Individuals with metabolic syndrome have increased risk of type 2 diabetes as well as cardiovascular disease and vascular dysfunction³. The growing prevalence of metabolic syndrome seems to be an end result of our current lifestyle which promotes high caloric, high fat foods and minimal physical activity resulting in a state of positive energy balance⁴. Lifestyle behaviors such as dietary and exercise habits are strongly associated with risk for development of obesity and metabolic syndrome⁵. Increased adiposity and physical inactivity may represent the beginning of the appearance of these risk factors. Understanding the metabolic and cardiovascular disturbances associated with diet and exercise habits is a crucial step towards reducing the risk factors for metabolic syndrome.

With increased availability of palatable food, people tend to overconsume and thus increase caloric intake well in excess of caloric expenditure⁶. Palatability of foods may be altered through an alteration of fat and sugar content. This natural attractiveness toward sweetness is responsible for a substantial consumption of sugars⁷. These sugars may occur naturally or can be manufactured and include sucrose, fructose and glucose. The monosaccharides glucose and fructose are present in small amounts in fruits and honey⁶. Sucrose, a disaccharide formed by one molecule of fructose and one molecule of glucose bonded together with an α 1-4 glycoside bond, is found in sugar cane and beets. Fructose can also be found in high fructose corn syrup (HFCS) which consist of a combination of fructose and glucose, usually in a 55:45 ratio (55% fructose, 45% glucose)⁸. HFCS is a popular additive in

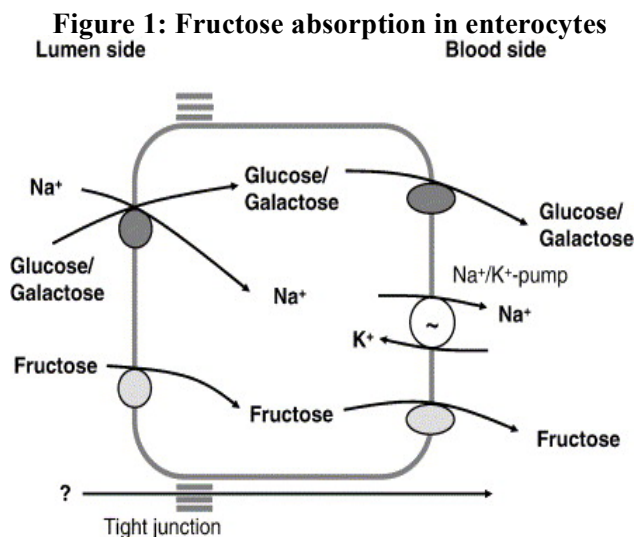
processed food because it adds sweetness, being 100% sweeter than sucrose, enhances color and texture, preserves the food, is a cheaper alternative to sucrose⁶ and can improve sports performance⁹. HFCS is also a lower glycemic food than glucose, making it seem like a beneficial alternative to glucose for diabetics. A consequence of the addition of HFCS to our food supply has resulted in the consumption of fructose to increase by 135% from 1977 to 2001, paralleling the rising rate of obesity over that same time period^{2,10}. Today, the average American consumes 60-70 g/day of HFCS alone^{8,11}; leading many researchers to believe that a causative link between obesity and fructose consumption may exist.

There is a growing body of evidence in animal and human models that high dietary intake of fructose is an important nutritional factor associated with development of insulin resistance¹², fatty liver¹³, elevated uric acid¹⁴, hyperlipidemia^{15,16} and hypertension¹⁷ and an increase in small-dense low-density lipoproteins¹⁸; all risk factors associated with metabolic syndrome. Fructose overfeeding has also been linked to hepatic insulin resistance, reduced lipid oxidation and increased triglyceride formation in healthy males¹⁹. Researchers therefore, postulate

that increased consumption of fructose may be one environmental factor contributing to the development of obesity and abnormalities associated with metabolic syndrome².

The metabolic consequences of chronic fructose consumption can be attributed to the unique metabolism of fructose.

When fructose is present in the gut, either as free fructose or from HFCS, it is transported freely



Breves et al Volume 109, Issues 1-3; pgs 4-

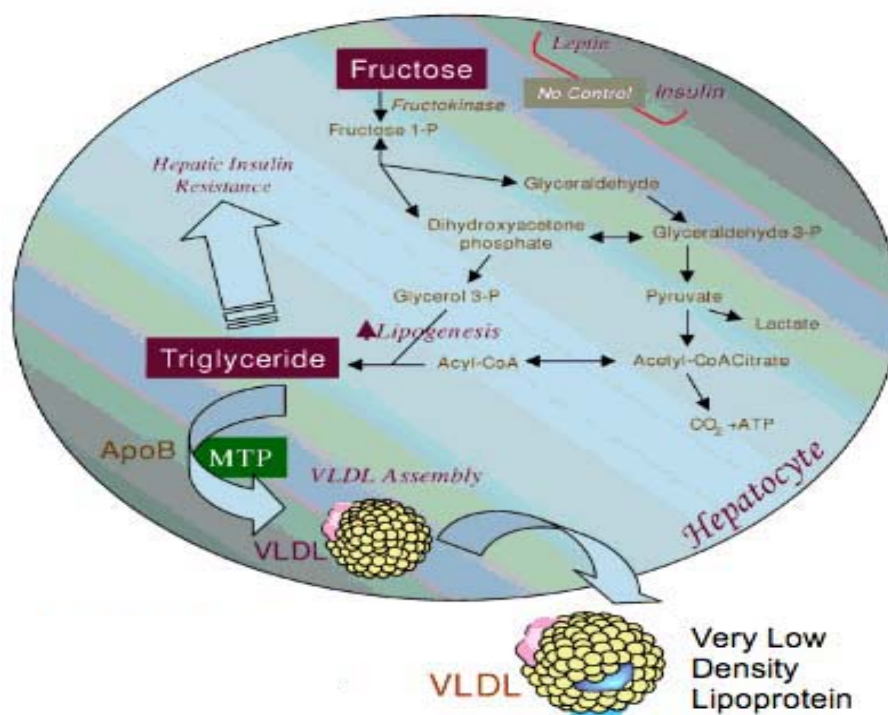
into the enterocytes via GLUT 5 transporters whereas the transport of glucose requires ATP hydrolysis and is sodium-dependent at the brush boarder of the intenstines ⁶ (Figure 1). Free fructose is quantitatively limited to absorption rates ranging from 5-50 g. When fructose is consumed with glucose however, as is the case with sucrose and/or HFCS, fructose abosprtion rates are considerably higher ^{20, 21}.

Following the absorption from the intestine, the fructose that is present in the hepatic portal blood is rapidly taken up by the liver via GLUT 2 transporters. Glucose metabolism is limited by glucokinase and, once phosphorylated, is regulated by the energy status of the cell via phosphofructokinase, yet fructose does not have a rate-limiting enzyme in hepatic metabolism ¹⁹. When large amounts of fructose are consumed, significant quantities of fructose enter the glycolytic pathway distal to phosphofructokinase, facilitating a very-low density lipoprotein (VLDL) and triglyceride production in liver, resulting in *de novo* lipogenesis (DNL) ²¹. Hence, the ability of

fructose to stimulate VLDL synthesis is unregulated which makes fructose a highly lipogenic macronutrient.

Given the unique metabolism of fructose, consumption of

Figure 2: Fructose Metabolism in the Liver



Basciano et al Nutr.Metab.(Lond), 2005, 2, 1, 5

fructose results in increased postprandial triglyceride formation (Figure 2)²². The elevated release of TG from the liver after fructose feeding increases systemic VLDL-TG concentrations which can enhance fat accumulation in adipocytes^{6,22}. Excess adipose tissue accumulation increases fatty acid flux to other tissues such as the muscle as well as various other peripheral tissues²².

Consequently, the hypertriglyceridaemia which can be induced by fructose feeding, is associated with increased atherogenic small-dense low-density lipoproteins (sdLDL)²³. These molecules have been shown to increase the atherogenic potential of the plasma and promote low-grade inflammation²³. Furthermore, sdLDL particles become highly atherogenic when oxidized due to the ability to up-regulate scavenger receptors on active monocytes, transforming them into macrophages and eventually leading to foam cell formation²³. Foam cells are highly atherogenic after modification. The ability of fructose to lead to low-grade inflammation may result in endothelial dysfunction given that fructose has the ability to increase hepatic production of VLDL's and systemic sdLDL's⁸. Increased endogenous triglyceride-rich lipoproteins (TRL) such as VLDL that are present with excessive fructose ingestion² as well as exogenous TG and chylomicrons can cause an increase in neutrophil production with concomitant production of pro-inflammatory cytokines and oxidative stress, all leading to endothelial dysfunction²³.

High fructose consumption has also been linked to hyperuricemia, an added risk factor for endothelial dysfunction and metabolic syndrome. Fructose-induced hyperuricemia is a consequence of fructose depleting ATP when it is metabolized from fructose to fructose 1-phosphate resulting in phosphate depletion and stimulation of AMP deaminase. AMP-deaminase eventually leads to uric acid formation through the xanthine oxidase pathway⁸. Elevated uric acid concentration inhibits endothelial nitric oxide synthase (eNOS), a stimulator of endothelial

vasodilation ⁶, and the ensuing vasoconstriction can lead to hypertension and impaired glucose uptake to skeletal muscles. Moreover, increased uric acid levels can increase oxidative stress, increase pro-inflammatory markers and decreased adiponectin levels, all factors which can lead to insulin resistance ²⁴.

Although the increased consumption of fructose is a public health concern, finding alternative interventions to help combat the side effects of this nutrient should become a public health priority to combat the risks associated with fructose consumption. Physical activity has been demonstrated as an effective alternative treatment for metabolic syndrome and dyslipidemia ⁴. Both high density lipoproteins (HDL) and serum TG favorably respond to changes in habitual physical activity such that HDL increase and serum TG levels decrease relative to the volume of exercise and/or intensity ²⁵. Moreover, physical activity alters the metabolic makeup of an individual by increasing hepatic lipid oxidation, increasing skeletal muscle lipoprotein lipase (LPL) activity and promoting clearance of TG ⁴. Physical activity also increases mitochondrial density, improving oxidation rates of long and medium chained fatty acids, reduces ceramide production and hence increasing insulin sensitivity and modulating hepatic lipase activity thereby reducing triglyceride-rich remnant proteins ²⁶. All of these responses impact postprandial lipemia and atherogenic dyslipidemia. Hence, increased physical activity may be a viable option to counteract the deleterious effects of a diet high in fructose, particularly in individuals considered to be sedentary.

Regular physical activity has been shown to improve postprandial TG clearance due to enhanced fat oxidation, elevated LPL response and increased energy expenditure ²⁷. The exercise-induced attenuation of postprandial TG is also partly mediated by a reduction in hepatic TG synthesis and VLD-TG secretion ²⁸. Hence, the combined effect of regular physical activity

and fructose ingestion are of interest since high physical activity may protect against fructose ingestion and low physical activity may exacerbate the effects of fructose. Consequently, increased physical activity may be a viable lifestyle change to minimize the susceptibility to fructose-induced hyperlipidemia and systemic low-grade inflammation.

There is limited research regarding physical activity and chronic fructose consumption, although the effects of physical activity on postprandial lipemia are well known^{27, 28, 30}. Previous research regarding exercise and fructose consumption has been aimed at determining the efficiency of fructose in improving athletic performance²⁹, however the average American who is consuming fructose may not be participating in regular, high intensity exercise. Additionally, these studies examined the importance of fructose ingestion during exercise and have not focused on the ramifications of a high fructose diet combined with low physical activity on a person's overall health.

Although considerable research has been conducted linking HFCS to the increased prevalence of obesity and metabolic syndrome risk factors, these studies have mainly been performed on animals^{31, 32}, and/or in post-absorptive state³³. Additionally, numerous studies are not without controversy; such as the specific dose of fructose used, which can significantly alter the outcome of such studies³⁴. Further, the magnitude of the effect of fructose may depend on other aspects of the diet, including the total amount of carbohydrates and fats in the diet and the overall consumption of the meal. *Therefore, the overall aim of this study was to examine the effects of a diet high in fructose on postprandial lipemia, inflammatory markers and glucose tolerance in a young, healthy population and to investigate whether increased physical activity alters such effects.*

Specific Aims:

1. To investigate if an increase in fructose consumption over a two week period increased postprandial lipemia and whether increased physical activity (>12,500 steps) altered this response. *Hypothesis:* We hypothesized that a diet rich in fructose increased postprandial lipidemia and increased physical activity attenuated these increases as compared to a sedentary lifestyle in a healthy, young population.
2. To determine whether chronic fructose consumption increases inflammatory markers associated with low-grade inflammation and whether increased physical activity alters such a response. *Hypothesis:* We hypothesized that a high fructose diet over a 2-wk period increased pro-inflammatory markers induced by the elevated lipid production and these effects were be ameliorated with high physical activity.
3. To determine whether chronic fructose ingestion alters glucose tolerance and insulin resistance and to elucidate whether increased physical activity alters such responses. *Hypothesis:* We speculated that chronic fructose consumption induced hyperglycemia and hyperinsulinemia which would be minimized with increased physical activity.

Limitations:

1. Subjects were participating in an ad libitum diet therefore dietary habits were not altered during the intervention period. Although subjects were instructed not to consume any additional sugar aside from their normal dietary consumption, this may pose a problem when substantiating the results as a direct effect of the added HFCS consumption and not other aspects of their diet. To limit such effects, the subjects completed a three-day

dietary log prior at the start of the study and 24-hour food recall periodically throughout the intervention to evaluate their normal dietary habits.

2. There was no way to determine whether the subjects consumed all the HFCS beverage given. To minimize this problem, the subjects were instructed to return their empty bottles to the lab once per week to help ensure subject compliance.

Delimitations:

1. The study was limited to only healthy, normal weight individuals, thus extending the results to other populations (e.g. individuals with type 2 diabetes) would need to be done with caution.
2. Subjects over the age of 35 were not included to minimize potential differences in hormonal levels that may occur with pre-menopause and the potential effect on fat metabolism. This resulted in a more homogenous age of subjects.

Chapter II: Review of Literature

Introduction:

The prevalence of obesity and obesity-related diseases in the United State and worldwide is increasing rapidly ². More than half of the US population is considered to be overweight with nearly one-fourth being clinically obese ³⁵. Moderate obesity can contribute to chronic metabolic abnormalities characteristic of metabolic syndrome which include insulin resistance, dyslipidemia and hypertension ². Increased fructose consumption has paralleled the increased prevalence of metabolic abnormalities and may be a contributing factor to the rise in such disease-related risk factors ².

The addition of fructose in the food supply became popular in the 1970's when fructose was used to produce high fructose corn syrup (HFCS). HFCS can contain up to 90% fructose, however, most of the HFCS that is commercially sold contains 55% fructose and 45% glucose and is produced by the enzymatic isomerization of dextrose to fructose ². HFCS is frequently used as a sweetener in the food industry because it is cheaper to produce, has a long shelf-life, maintains a long-lasting moisterization in industrial bakeries and is sweeter than most other sugars ³⁶. The use of fructose became even more abundant when individuals with type 2 diabetes began replacing glucose with fructose due to the ability of fructose to attenuate the acute increase in insulin levels, therefore being touted as a low-glycemic alternative to glucose for these individuals ³⁶.

In recent years, there has been an increased interest in the potential role of dietary fructose as a contributing factor to metabolic syndrome. When consumed in elevated concentrations, fructose can promote metabolic changes that may contribute to risk

factors associated with metabolic syndrome as well as hyperuricemia, inflammation and alterations in various metabolic hormones ³⁵.

The following review of literature will present evidence that fructose may cause substantial alterations in the risk factors associated with metabolic syndrome. The first section of the review will discuss fructose metabolism and the link between fructose and hyperlipidemia, insulin resistance, glucose intolerance, hypertension and hyperuricemia followed by inflammation and effect of incretin hormones on fructose ingestion. Lastly, section four will discuss the benefits of physical activity and how increasing physical activity may counteract various risk factors associated with fructose consumption.

I. Fructose:

a. Fructose Absorption and Metabolism:

Fructose enters the brush border of the stomach in the form of either pure fructose, high fructose corn syrup (HFCS) or as sucrose, a disaccharide comprised of 50% fructose and 50% glucose ⁶. When fructose is ingested as a disaccharide in the form of sucrose, the sucrose must first be cleaved into one molecule of glucose and one molecule of fructose before being metabolized. Sucrase, an enzyme located in the brush boarder of the small intestine enterocytes is responsible for this reaction. Fructose is then absorbed and transported through the enterocytes to the portal bloodstream by a fructose-specific hexose transporter, glucose transporter GLUT 5. Unlike glucose, the activation of GLUT 5 transporters is sodium-independent and does not require ATP hydrolysis ⁶. Once inside the enterocytes, fructose diffuses across the basolateral pole of the enterocytes and into the portal circulation via GLUT 2 transporters ³⁷.

Unlike glucose, fructose is incompletely absorbed in the enterocytes. The absorption capacity of fructose is limited to approximately 5-50 grams at one time before some individuals suffer from symptoms of diarrhea and flatulence³⁸. Ushijima et al³⁹ showed that 80% of healthy adults experienced incomplete absorption when given 50 grams of fructose yet when fructose is consumed with glucose, the rate of absorption is increased⁴⁰. Thus, when fructose is consumed as sucrose or as HFCS (glucose and fructose combined), more fructose is absorbed through the enterocytes. The improved absorption of fructose in conjunction with glucose may be due to the up-regulation of GLUT 5 receptors which is stimulated by elevated glucose absorption³⁸.

Once within the enterocytes, fructose can be easily converted to triglycerides. This was evident in a study by Haidari et al⁴¹ where they demonstrated that intestinal triglycerides, in the form of chylomicrons, were increased in hamsters after a three-week chronic fructose diet⁴¹. Haidari et al⁴¹ indicated that chronic, not acute fructose feeding was associated with changes such as greater stability of intracellular apoprotein-B48 (apoB-48) and enhanced intestinal enterocyte *de novo* lipogenesis⁴¹. The intestinal overproduction of apoB-48 containing lipoproteins may be an important contributor to the elevation of circulating TG-rich lipoproteins which may potentially lead to atherosclerosis⁴¹.

Although fructose can be lipogenic within the enterocytes, fructose is also readily absorbed and stimulates lipogenesis within the hepatocytes⁶. Once fructose travels through the enterocytes and into the portal vein, it is readily absorbed by the liver via GLUT 2 transporters. Due to the high concentration of GLUT 2 transporters and fructokinase, there is a high affinity for fructose uptake in the liver⁶. Once within the

liver, fructose is rapidly converted to fructose-1-phosphate via fructokinase. Fructokinase has a low affinity for fructose, resulting in rapid metabolism of fructose by the liver cells. Fructose is further metabolized into triose phosphates, glyceraldehyde and dihydroxyacetone phosphate²⁰. The triose phosphate that is produced can then be converted to pyruvate and oxidized into carbon dioxide and water in the citric acid cycle or a portion of the triose phosphate can be converted to lactate and released into the systemic circulation⁶. A portion of the carbon derived from the triose phosphates can also enter the gluconeogenic pathway where it can be stored as glycogen to be later released as glucose²⁰. This gluconeogenic process results in a small, but measurable increase in systemic glucose concentrations⁶.

Within the liver, fructose metabolism differs substantially from glucose metabolism in that entry of glucose into the glycolytic pathway is under the control of glucokinase which has a low affinity for glucose within the hepatocytes and is dependent on the concentration of glucose. Therefore the rate of glucose phosphorylation varies with changes in glucose concentrations⁶. Moreover, downstream, when fructose-6-phosphate is converted to fructose 1,6-bisphosphate, this reaction is catalyzed by phosphofructokinase (PFK), an enzyme regulated by the energy status of the cell. In particular, PFK is inhibited by elevations in ATP and citrate. This inhibition allows for a close regulation of glycolysis based on the energy status of the cell²⁰. On the contrary, fructose is phosphorylated to fructose-1-phosphate by fructokinase, but this rate-limiting enzyme does not have the tight regulation as seen with PFK³⁶. Figure 3 depicts these proposed mechanisms underlying the aforementioned differential effects of fructose

compared to glucose consumption on postprandial lipid metabolism and glucose tolerance/insulin sensitivity⁴².

Figure 3: Metabolic Differences Between Fructose and Glucose

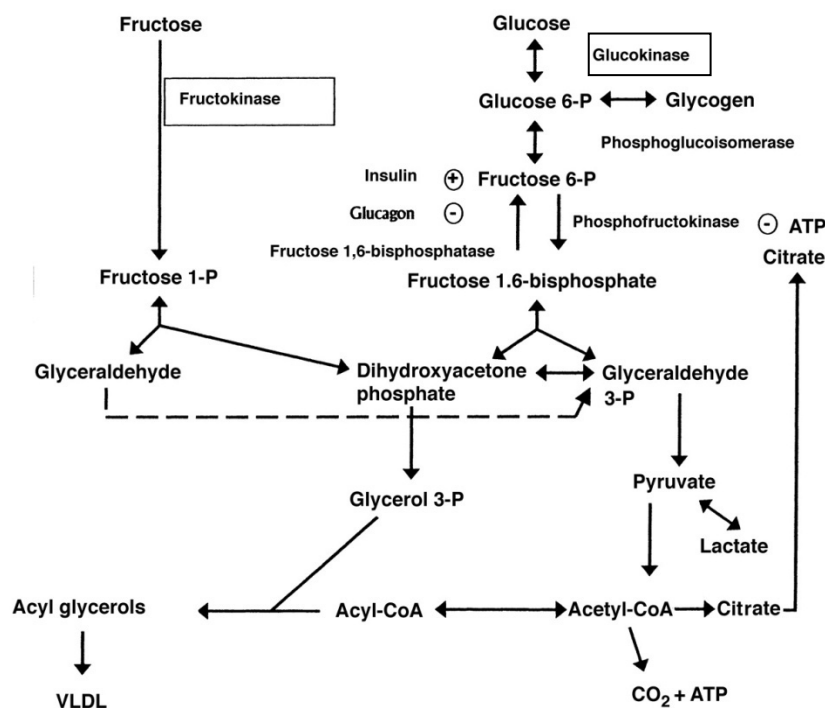


Figure 3: Fructose metabolism differs from glucose (black arrows) due to 1) a nearly complete hepatic extraction and 2) different enzyme and reactions for its initial metabolic steps. Fructose taken up by the liver can be oxidized to CO₂ and then converted into lactate and glucose; glucose and lactate are subsequently either released into the circulation for extrahepatic metabolism or converted into hepatic glycogen or fat. The massive uptake and phosphorylation of fructose in the liver can lead to a large degradation of ATP to AMP and uric acid (Havel, Nutrition Reviews, Vol. 63, No. 5)

b. Fructose-induced Lipogenesis:

The most detrimental aspect of fructose is its ability to be converted to fatty acids within the hepatocytes via DNL, as pictured in figure 3⁶. In rodents, a high-fructose diet (60% fructose) has been shown to increase intra-hepatocellular lipids as well as stimulate hepatic DNL within a few days⁴³. When such diets are sustained over a prolonged period

of time, high fructose or sucrose diets will induce hepatic steatosis and whole-body insulin resistance with a concomitant accumulation of intramyocellular lipids ⁴⁴.

Fructose serves as a preferential source for hepatic lipogenesis. Relative to glucose, the extraction and metabolism of fructose in the liver are exceptionally high due to the extensive amount of fructokinase present in the liver whereas glucose uptake in the liver is specifically dependent on glucokinase ⁶. Glucokinase has a relatively low affinity for glucose, resulting in lower concentrations of glucose being absorbed in the liver. Once glucose is converted to glucose-6 phosphate, phosphofructokinase (PFK) regulates the fate of glucose, whether it is stored as glycogen or catabolized for energy ⁶. On the contrary, the subsequent metabolism of fructose to fructose 1-phosphate at the triose phosphate level bypasses control at PFK ⁴⁵. When large amounts of fructose are consumed, significant quantities of carbon from fructose continue to enter the glycolytic pathway distal of PFK at the level of the triose phosphate (dihydroxyacetone phosphate and glyceraldehydes-3-phosphates), causing an increased production of TG and VLDL production in the liver ²⁰. Once the glycolytic pathway becomes saturated with fructose intermediates, the intermediates can be converted to glycerol-3-phosphate which provides the glycerol moiety of triglyceride synthesis. These intermediates can also be further metabolized to pyruvate, which is then converted to acetyl-CoA. When acetyl-CoA combines with oxaloacetate to form citrate in the mitochondria, the carbon atoms can be used for *de novo* lipogenesis and then form long-chained fatty acids that are eventually esterified into triglycerides ²⁰. This large source of unregulated TG formation is unlike that of glucose metabolism which has a rate-limiting step to regulate it, preventing such effects.

To date there is an abundance of research indicating that acute and/or chronic ingestion of fructose causes hyperlipidemia in rats^{43, 46, 47} and in humans⁴⁸⁻⁵¹. Faeh et al⁵¹ examined whether short-term fructose consumption results in hypertriglyceridemia. The investigators measured fasting *de novo* lipogenesis in seven healthy men who were given 3 grams of fructose/kg of body weight/per day for 6 days⁵¹. At the beginning and end of the study period, the subjects participated in a 13-h metabolic testing day in which hepatic *de novo* lipogenesis was measured. After the six days of fructose loading, subjects' plasma triglyceride concentrations increased by 79% from baseline values. Moreover, fractional *de novo* lipogenesis increased six-fold over the six-day period when compared to baseline and was speculated to contribute to the observed hypertriglyceridemia⁵¹. Caution needs to be taken however, as the fructose load that was given (210 g/day) was an extremely high load and therefore may not be clinically relevant.

Using a more clinically relevant fructose load, Swanson et al⁵² studied 14 healthy male and female subjects on a diet containing 20% of energy from fructose verses an isocaloric starch diet to determine if fructose leads to metabolic disturbances. After twenty-eight days on the high fructose diet, serum total and low-density lipoprotein (LDL) cholesterol levels were 9% and 11% higher, respectively, than the starch diet, resulting in a significant difference between the fructose-only groups and the starch groups ($p < 0.05$)⁵². Furthermore the authors observed a transient and significant increase in serum triglyceride levels in the fructose-fed group after day one of the intervention. The fructose-fed subjects' triglycerides levels increased by 1.68 mmol/L above baseline whereas with the starch diet, triglyceride levels increased by 1.31 mmol/L⁵². Hence, it

was concluded that fructose induced hyperlipidemia within 28 days on a high fructose diet and such alterations occur in as little as 24 hours after the first fructose load.

In a slightly longer intervention, Bantle et al⁵⁴ compared the effects of a diet consisting of either 17% of energy from fructose or 17% glucose for six weeks on 24 adult subjects (12 males/ 12 females). During this randomized, balanced crossover design, subjects underwent a 24 h metabolic profile over a 24 h period on days 7, 14, 21, 28, 35 and 42. The only day in which mean fasting plasma TG concentrations did not significantly increase above that of the glucose diet levels was at 21 days in the male subjects only. Additionally, the plasma TG area under the curve (AUC) on day 42 was 32% greater than on the glucose diet in the male subjects, suggesting that the fructose, per se, was associated with elevations in fasting and postprandial TG concentrations⁵⁴. The fructose diet had no significant effect on fasting or postprandial plasma triacylglycerol concentrations in women.

More recently, Swarbrick et al⁴⁸ investigated the metabolic effects of a high-fructose diet in seven overweight, post-menopausal women while the subjects resided in a supervised setting and consumed standardized, energy-balanced meals for 14 weeks. During the first 4 weeks, the women were instructed to participate in a weight-maintaining, complex carbohydrate diet which was then followed by ten weeks of an iso-caloric, high fructose diet in which subjects consumed a fructose-rich (25% of calories from fructose) drink with each meal⁴⁸. At weeks two and ten of the fructose intervention, subjects had blood samples drawn from 0800 to 2200 h, at 30 min intervals, around meal ingestion. After week two on the diet, TG AUC was unchanged however after week ten, triglyceride AUC values were 141% higher than at baseline. Circulating

TG levels increased more than 1 mmol/L over fasting concentrations for the entire period from lunch until 2200h in the evening. Additionally, fasting apoB concentrations were increased by 19% compared to baseline. The authors speculated that the increases in fasting and postprandial TG concentrations were most likely due to stimulation of TG synthesis ⁴⁸.

Although the research regarding fructose ingestion and fasting and postprandial lipogenesis is apparent in normal weight individuals, research is more limited in the obese population. Stanhope et al ⁴⁹ investigated whether similar results occurred in overweight/obese population (BMI= 25-35kg/m²). The investigators studied 18 postmenopausal women for 12 weeks. For the first two weeks the subjects resided on an inpatient hospital unit where they consumed an energy balanced diet. The next eight weeks was an outpatient intervention in which the subjects consumed either a fructose- or glucose- rich beverage containing 25% daily energy while eating an ad libitum diet. This was then followed by an additional two weeks in the in-patient facility. During the second inpatient phase, subjects continued to consume their fructose or glucose drink but also maintained an energy balanced diet, instead of the ad libitum diet ⁴⁹. The excess fructose intake (~25% of energy requirements) for 10 weeks significantly increased fasting and 24h postprandial triglycerides when compared with the glucose drink of equal caloric content ⁴⁹. Post-intervention, there was a significant increase in fasting apoB (27.2%), LDL (13.9%), small-dense LDL (44%), and oxidized LDL (12%) in the fructose group compared to the glucose group. This study reiterates the fact that long-term consumption of fructose of ≥ 2 weeks negatively alters lipid remodeling in obese, postmenopausal woman ⁴⁹. Stanhope et al ⁴⁹ stated that the mechanism by which fructose

induced lipemia is a result of the carbon atoms from fructose being converted to fatty acids, skipping the rate-limiting step in glycolysis. As stated previously, fructose increases DNL by increasing hepatic TG formation, however, Stanhope et al⁴⁹ also speculated that the fructose-induced hepatic DNL may limit fatty acid oxidation as well. Fructose increases acetyl-CoA concentrations in the liver, subsequently leading to increased production of malonyl CoA which inhibits the entry of fatty acids into the mitochondria³⁶. Taken together, fructose indirectly inhibits fatty acid oxidation by increasing production of malonyl CoA which decreases fatty acid transport into the mitochondria⁵⁵. Malonyl CoA is an important intermediate to fructose-induced lipogenesis because acetyl CoA is added to long-chained fatty acids via malonyl CoA. therefore allowing fructose to provide carbon atoms for both glycerol and the acyl portion of the acylglycerol molecule³⁶.

To better understand the hypothesis of Stanhope et al⁴⁹ that fructose ingestion may also inhibit fat oxidation, Abdel-Sayed et al¹⁹ investigated whether a high-fructose diet (234 g) with an excess energy intake of about 3640 kJ/d (67% carbohydrate, 22% fat and 11% protein) compared to a control diet (55% carbohydrates, 30% fat and 15% protein) impaired lipid metabolism in six healthy males within a seven-day period. Plasma non-esterified fatty acids (NEFA), plasma TG, plasma β -hydroxybutyrate (synthesized from acetyl CoA), lipid oxidation and exogenous lipid oxidation in a basal state, after a lipid infusion as well as during and after a standardized mental stress procedure on days 1 and 7 of the intervention were measured. After seven days on the high fructose diet, basal NEFA concentrations significantly decreased by 19.5%, net lipid oxidation by 21.3% and plasma β -hydroxybutyrate

concentrations by 78.2%. After the lipid loading, the increase in net lipid oxidation and exogenous lipid oxidation were comparable between the control diet and the fructose diet however, after the mental stress, there was a markedly blunted stimulation of plasma NEFA and β -hydroxybutyrate release in the fructose group after seven days. The lower basal plasma NEFA concentrations indicated that an inhibition of adipose tissue lipolysis occurred after the high fructose diet¹⁹. Under those conditions, Abdel-Sayed et al¹⁹ speculated that the decreased NEFA seen with the high-fructose diet was likely related to fructose-induced stimulation of hepatic *de novo* lipogenesis and not secondary to an increased hepatic re-esterification. Additionally, the inhibition of lipolysis may, in turn, be directly responsible for lower whole-body net lipid oxidation following fructose loading since NEFA concentrations are the main determinant in this process¹⁹.

c. Fructose and Postprandial Lipemia

Although research regarding fasting hyperlipidemia and fructose consumption has been well established, high postprandial triglyceride levels have been associated with the risk of coronary artery disease⁵⁶. Hence there is growing evidence linking increased postprandial TG concentrations with a pro-atherogenic state⁵⁶. This link may be due to lipoprotein remodeling induced by increased levels of VLDL's and mediated by cholesteryl ester transfer protein (CETP) and hepatic lipase. Both increased VLDL's and CEPT resulted in increased concentrations of small-dense lipoproteins and remnant-like lipoproteins⁵⁶.

When in the blood, triglycerides can be referred to as “triglyceride-rich lipoproteins” (TRL's) and consist of two main components: VLDL and chylomicrons.

Very low-density lipoproteins are a result of hepatic synthesis and chylomicrons are produced by the gut postprandially in order to transport dietary lipids from the intestines to other locations in the body⁵⁶. Therefore, TRL can be produced exogenously from the diet or endogenously from the liver. Chylomicrons and VLDL's can then form intermediate-density lipoproteins catalyzed by lipoprotein lipase (LPL), an enzyme released from the capillary beds of adipose tissue and skeletal muscle⁵⁶. Lipoprotein lipase, situated in the capillary endothelial, is responsible for hydrolyzing the TG into NEFA²³. This pathway is up-regulated by insulin, which increases rapidly in response to a carbohydrate meal. In a fructose-rich diet, reduced insulin concentrations may contribute to lower postprandial LPL activity⁴⁹. The previously mentioned study by Stanhope et al⁴⁹ stated the importance of LPL on postprandial lipemia. They observed that there was a significantly greater postprandial LPL response in the glucose group than the fructose group, suggesting that reduced TG clearance might also contribute to the fructose-induced postprandial hypertriglyceridemia that was evident in their study⁴⁹.

Chong et al⁵⁷ also observed a lower insulin excursion after a fructose load was given to 14 healthy subjects. In this study, subjects were given a fructose drink (.75 g/kg of BW) on one occasion and a glucose drink (.75 g/kg of BW) on another with an isotope tracer (250 mg D-[U¹³C]fructose or D-[U¹³C] glucose) and then measured for 6 hours to determine the fate of the carbohydrate. The fructose, not glucose drink led to an attenuated LPL response which potentiated postprandial lipidemia as the TG-VLDL and TG-rich chylomicron levels were significantly higher than the glucose group⁵⁷. The lower insulin concentrations seen with fructose load led to a decreased production of LPL, resulting in impaired triacylglycerol clearance⁵⁷.

In the adipose tissue, some fatty acids released by LPL are directed into fat storage and others are released into circulation⁵⁷. Lipoprotein lipase is known to be activated by insulin. In the study by Chong et al⁵⁷, fructose did not stimulate insulin to the same extent as glucose and therefore NEFA levels were lower⁵⁷. As a result of their findings, the researchers suggested that the lower postprandial NEFA concentrations in the plasma in the fructose condition were most likely due to a smaller spillover of LPL-derived fatty acids from the chylomicron-TG⁵⁷.

To determine the postprandial effect of a fructose load after a breakfast and a subsequent lunch, Parks et al⁵⁰ investigated the magnitude of an acute load of fructose on lipogenesis. This two-test meal day represents a more typical Western diet in which a person eats every 4-6 hours. They examined the magnitude of lipogenesis after an acute bolus of fructose in the morning and after a subsequent meal. The six healthy adults were given a carbohydrate meal which consisted of either 100% glucose, 50% glucose:50% fructose or 25% glucose:75% fructose for breakfast followed by a standardized lunch four hours later. After the morning bolus, fractional lipogenesis was stimulated and peaked at 15.9% when the 50% fructose meal was given and at 16.9% when the 75% fructose diet was given. Both of these fructose loads were significantly higher than the glucose only group which only increased by 7.8% above baseline⁵⁰. Post lunch, the TG incremental AUC for 50:50 and 25:75 were significantly higher than the 100:0 bolus, although not significantly different from each other. Over the course of the study day, both fructose doses resulted in significantly higher TG incremental AUC when compared to the glucose only meal. This data implies that fructose acutely and significantly increased lipogenesis (measured by infusion of ¹³C1-acetate and analysis by GC-MS),

which elevated TG concentrations in the morning but also contributed to greater TG concentrations after lunch. Hence, the fructose-induced increase in lipogenesis displaced the use of stored TG for VLDL synthesis and that the stimulation of lipogenesis represents an intracellular signal for liver to esterify fatty acids from any source into TG⁵⁰. These authors concluded that there is an immediate lipogenic effect of fructose in healthy, lean subjects in the morning and after a subsequent meal, suggesting that when fructose is consumed in the fasted state, the subsequent postprandial lipemia is augmented.

In the previously mentioned Stanhope et al⁴⁹ study, postprandial fractional *de novo* lipogenesis after subsequent meals was also measured. Prior to and after a 10-week intervention period of either high fructose (25% energy from fructose) or a high glucose intervention (25% from starch) the obese subjects reported to the lab for a metabolic test day. During the test day, they ingested a buffet meal ad libitum at breakfast, lunch and dinner along with their specific intervention drink. Their results indicated that during a period of steady-state feeding in which subjects were given a meal every four hours during a 12-hour period, an 11.4% increase in *de novo* lipogenesis above fasting values occurred. These values increased to 75.4% when the same metabolic test day was repeated 10 weeks after a fructose-rich meal. In contrast, the glucose intervention group had an increase in *de novo* lipogenesis during their first metabolic test day of 13.4%. This value increased to 27.3% after the 10-week intervention. The researchers stated that both increased *de novo* lipogenesis and decreased lipoprotein lipase-mediated clearance contributed to fructose-induced postprandial hypertriglyceridemia⁴⁹.

Based on the previous review of literature, the following can be concluded: 1) the liver is the main site of fructose metabolism, 2) fructose bypasses the main rate limiting step of glycolysis, thus providing unregulated amounts of lipogenic substrates such as acetyl-CoA and glycerol-3-phosphate and 3) fructose enhanced *de novo* lipogenesis when subsequent meals were ingested. Thus, these factors by which fructose promote lipogenesis are likely to include multiple mechanisms.

d. Fructose and Insulin Resistance:

Type 2 diabetes is a progressive disorder that begins with the development of insulin resistance and potentially ends with pancreatic β -cell failure⁵⁸. A dietary recommendation often proposed for patients suffering from type 2 diabetes is to ingest foods that do not cause an acute rise in insulin levels, therefore preventing over-stimulation of insulin secretion from the pancreas. Initially fructose was a popular macronutrient choice for individuals with type 2 diabetes because fructose does not cause an acute rise in insulin due to the low glycemic index related to fructose. Contrary to no acute rise in insulin, fructose consumption is related to an increase in hepatic VLDL triglyceride secretion, and possibly decreased extra-hepatic clearance of VLDL-TG, both of which are associated with the development of hepatic and adipose tissue insulin resistance.

The VLDL-TG formed from fructose-induced hepatic DNL can be released into the systemic circulation, consequently leading to an increase in the levels of fatty acids in the circulation. Signaling abnormalities in adipocytes can also trigger lipolysis of TG stores and efflux of NEFA into the bloodstream, augmenting the problem⁶⁰. NEFA in

the bloodstream as a result of increased fructose-induced lipidemia may be a key mechanistic link between fructose consumption and insulin resistance, type 2 diabetes and metabolic dyslipidemia. These conditions are a result of increased ectopic storage of NEFA by non-adipose tissues such as liver and skeletal muscle where they are stored as TG or diacylglycerol. The exposure of these organs to increased concentrations of NEFA from fructose ingestion may reduce insulin sensitivity by increasing the intramyocellular lipid content⁶¹. Once stored as ectopic lipids, the fatty acids can interfere with the metabolic pathways of that tissue, resulting in fructose-induced insulin resistance⁶⁰.

In a healthy adult, insulin suppresses hepatic gluconeogenesis and glycogenolysis, however, in the insulin resistant state, this suppression no longer occurs, causing a subsequent increase in glucose output from the liver⁵⁸. Insulin resistance in fat cells reduces the normal effects of insulin on lipids and results in reduced uptake of circulating lipids and increased hydrolysis of stored triglycerides. Increased mobilization of stored lipids in these cells elevates free fatty acids in the blood plasma, leading to reduced muscle glucose uptake, and increased liver glucose production, all of which contribute to elevated blood glucose levels⁶². This chronic state of excess fatty acid release into the circulation can induce lipotoxicity, or pancreatic β -cell death. To compensate for the increased peripheral insulin resistance, the pancreatic β -cells increase in mass and secrete more insulin resulting in hyperinsulinemia. Since the β -cells cannot compensate for the resistant state, hyperglycemia occurs. Hyperglycemia further damages the β -cells resulting in glycototoxicity, leading to a progressive loss of the pancreatic islet β -cells manifesting into type 2 diabetes⁵⁸.

The molecular mechanisms underlying fructose-induced insulin resistance are not completely understood but may be similar to that of a high-fat diet. Both high-fructose and high-fat diets interfere with insulin signaling at common steps in skeletal muscle⁶³. In liver cells, both high fructose and high-fat diets elicit hepatic stress responses and activation of pro-inflammatory cascades that lead to insulin resistance. Sucrose-fed rats demonstrate an early alteration of hepatic VLDL-TG secretion, leading to impaired insulin-mediated suppression of glucose production in hepatic tissues after 1-2 weeks, but show no changes in extra-hepatic insulin sensitivity after this time period⁶⁴. After 4-6 weeks, impaired extra-hepatic insulin sensitivity, in conjunction with muscle lipids occurs⁶⁴.

Morino et al⁶⁵ suggested that the mechanism by which interacellular lipid causes insulin resistance in both liver and muscle is through diacylglycerol (DAG)-induced activation of novel protein kinase C (nPKC)⁶⁵. DAG is a known activator of nPKC, and both DAG and nPKC are associated with lipid-induced insulin resistance in humans⁶⁵. Activation of nPKC causes a decrease in insulin receptors or insulin receptor substrate 1 (IRS1) tyrosine phosphorylation⁶³. This IRS-1 inhibition decreases insulin-stimulated glucose transporters (GLUT 4) activity resulting in reduced glucose uptake into the cell⁶⁶. Increases in DAG also activate several other serine/threonine kinases such as inhibitory κ B kinase β (IKK β) and nuclear factor κ B (NF- κ B) which are also activated by inflammatory markers such as tumor necrosis factor α (TNF- α) and interleukin 6 (IL-6) which are both known to down-regulate IRS-1 phosphorylation⁶⁶. This is in contrast to a healthy cell in which case insulin binds to its receptor, and causes auto-phosphorylation of the receptors. The phosphorylated receptor then phosphorylates the insulin receptor

substrate proteins (IRS) on the tyrosine residues. The phosphorylated IRS recruits a variety of second messenger proteins, initiating a complex signaling cascade which involves Akt/PKB stimulation of glucose uptake into the cell. Insulin sensitivity is thus maintained as a result of enhanced glycogen synthesis, suppression of hepatic gluconeogenesis, increased fatty acid and triglyceride synthesis and suppression of lipolysis in adipose tissue ⁶⁷.

Cortright et al ⁶⁸ found in isolated human skeletal muscles strips and adipocytes that activation of PKC reduced insulin-stimulated glucose uptake; whereas, pharmacological inhibition of PKC activity increased insulin-stimulated glucose uptake by twofold. This increase was associated with elevated insulin receptor tyrosine phosphorylation of PI 3-kinase activity. Hence, inappropriate activation of PKC may interfere with insulin action by promoting serine/threonine phosphorylation of IRS-1 resulting in prevention of tyrosine phosphorylation of these proteins that is necessary for adequate function on the insulin-signaling pathway ⁶⁸.

Human research investigating the effects of fructose on insulin sensitivity is limited but the animal literature is more extensive. Thorburn et al ⁶⁹ fed rats a diet containing 35% of energy as fructose for four weeks and found reduced insulin sensitivity associated with impaired hepatic insulin action and whole-body glucose disposal. This study demonstrated that although fructose does not increase insulin acutely, the long-term consumption of fructose results in insulin resistance ². Moreover, Blakely et al ⁷⁰ showed that fasting serum insulin and glucose concentrations were significantly increased in rats that consumed 15% of energy from fructose for 15 months compared to corn-starch ⁷⁰. These results were in conjunction with a more recent animal

study in which mice were fed an isocaloric, standard diet; a 60% glucose diet; or a 60% fructose diet for twelve weeks. Glucose disposal was reduced in the fructose fed animals compared to the other two groups ⁷¹. Moreover, the fructose-fed mice exhibited a 1.3 fold lower glucose-stimulated increase in insulin. From these results, it was suggested that a high-fructose diet results in reduced glucose-stimulated insulin release in comparison to a standard diet and impaired glucose disposal ⁷¹.

In humans, there is limited research confirming the negative effects of fructose on insulin sensitivity and glucose intolerance in adults and adolescents. In a short-term study Sunehag et al ⁷² studied obese adolescents to determine the metabolic effects of dietary fructose on insulin sensitivity, insulin secretion and glucose tolerance ⁷². When six male and female adolescents were put on either a high fructose diet (24% of total energy intake) for seven days or a low-fructose diet (6% of energy intake) there was no change in insulin sensitivity or secretion as well as no change in glucose kinetics, as assessed by the stable labeled intravenous glucose tolerance test ⁷². In addition to having a very small sample size, this study was confounded as the obese adolescents were insulin resistant initially. In order to maintain substrate homeostasis, normal rates of glucose production, gluconeogenesis, lipolysis and appropriate substrate oxidation, the obese subjects' required more than twofold increase in their insulin secretion as compared what would have been needed if lean adolescences were studied ⁷². Similarly, Le et al ⁴⁴ found that moderate fructose (1.5g/kg of body weight) intake for four weeks in seven male subjects induced significant increases in plasma TG, and VLDL-TG with no change in insulin sensitivity or ectopic fat deposition. The authors speculated that the duration of fructose

consumption may need to be longer than 4 weeks in order for the increases in plasma TG and VLDL-TG to affect insulin sensitivity⁴⁴.

In contrast, Dirlewanger et al¹⁸ investigated the effects of an acute fructose infusion on hepatic insulin sensitivity during moderate hyperglycemic conditions in ten healthy adults. These researchers monitored the plasma insulin concentrations required to maintain euglycemia at 8 mmol/l during somatostatin and low physiological glucagon infusions with or without fructose infusions. Subjects underwent three different protocols: 1) hyperglycemic pancreatic clamp without fructose infusion; 2) hyperglycemic pancreatic clamp with fructose infusion ($16.7 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and 3) hyperglycemia-hyperinsulinemic clamp. Glucose rate of disappearance, net endogenous glucose production, total glucose output, and glucose cycling were measured with [6,6-⁽²⁾H₍₂₎]- and [2-⁽²⁾H₍₁₎]glucose. Hepatic glycogen synthesis was estimated from uridine diphosphoglucose kinetics as assessed with [1-⁽¹³⁾C]galactose and acetaminophen. The infusion with fructose resulted in alterations in endogenous glucose production such that insulin requirements increased 2.3 fold above the two other infusions in order to maintain blood glucose levels¹⁸. The increased total glucose output indicated that the absolute rate of glucose-6-phosphate hydrolysis and release of free glucose from the liver cells was increased during fructose infusion. Simultaneously, glucose cycling was increased, indicating enhanced reuptake and phosphorylation of glucose by the liver cells¹⁸. Therefore, an acute fructose infusion induces both extrahepatic and hepatic insulin resistance, with the latter being secondary to an increased intrahepatic glucose 6-phosphate synthesis¹⁸.

In a longer-term, previously mentioned study, Stanhope et al ⁴⁹ proposed that the increased hepatic lipid accumulation resulting from fructose-induced DNL would lead to hepatic insulin resistance by increasing levels of DAG. Increases in both DAG and novel PKC are associated with lipid-induced insulin resistance ⁴⁹. After assessing insulin sensitivity with deuterated glucose disposal prior to and after the 10-week intervention, the fructose group had significantly higher fasting insulin and glucose levels as well as increased insulin excursions and endogenous glucose production as compared to the glucose group. Additionally, DNL was significantly higher in the fructose-fed group than the glucose-fed group after the 10-week intervention. These results indicated that hepatic insulin resistance was most likely due to increased DNL from increased DAG and novel PKC ⁴⁹.

Fructose over-feeding, regardless of total energy intake, exposes the liver to large carbohydrate loads, therefore increasing hepatic glycogen stores. This process may contribute to the enhanced endogenous glucose production seen in the aforementioned study ⁵¹. Feah et al ⁵¹ suggested that the increased endogenous glucose production seen in the high fructose diet can be explained by alterations in hepatic gene expression ⁵¹. High carbohydrate diets stimulate carbohydrate response element-binding protein (ChREBP), a hepatic transcription factor that up-regulates the expression of lipogenic and glycolytic enzymes. ChREBP also regulates the expression of key enzymes of hepatic fructose metabolism. Although ChREBP is primarily regulated by glucose through the increase in intrahepatic glucose metabolites, Feah et al ⁵¹ specify that these enzymatic reactions may also be stimulated by a high fructose diet.

The most commonly proposed mechanism for the fructose-induced insulin resistance in the previous studies appear to be the diminished ability of insulin to suppress hepatic glucose output and decrease insulin receptor density apparent in skeletal muscle and liver ⁷³. Catena et al ⁷³ found that insulin receptor number and mRNA levels were significantly decreased in skeletal muscle and liver of fructose-fed rats (66% fructose) after two-weeks compared to control rats. These findings suggested that a down-regulation of insulin receptor gene expression is a possible molecular mechanism for insulin resistance. Moreover, abnormalities in insulin action at a post-receptor level in muscles and liver during fructose consumption may also occur such as decreased phosphorylation of IRS-1 and decreased associated of IRS-1 with phosphatidylinositol 3-kinase ⁷⁴. This evidence shows that these early steps in insulin signaling are important for insulin's metabolic effect ⁷⁵. Therefore, it is concluded that the mechanisms behind fructose-induced insulin resistance is possibly due to the combination of various factors such as a reduction in the number of insulin receptors in skeletal muscle and liver as well as decreased phosphorylation, both caused by increased fat production.

e. Fructose-induced Hypertension:

The ingestion of fructose has also been associated with alterations in various hemodynamic responses such as increases in heart rate and blood pressure ⁷⁶. Numerous reports of fructose-induced hypertension in rodents are apparent ³¹; however, the link between fructose intake and high blood pressure in humans is mainly base on indirect evidence ⁶. Animal studies have shown that fructose feeding for only two-weeks (66% fructose) can cause systolic blood pressure to increase significantly above baseline values

⁷⁷. Likewise, when rats are fed 60% of energy from fructose for eight weeks, systolic blood pressure also increased significantly compared to the control rats ³¹. Similarly, Nakagawa et al ¹³ reported that rats fed 60% fructose diet for ten weeks had elevated systolic blood pressure compared to the controls study. Four weeks into the intervention, half of the fructose-fed rats were treated with allopurinol, a xanthine oxidase inhibitor, for the remainder of the 6 weeks. Xanthine oxidase is an enzyme that increases uric acid levels leading to increased systolic blood pressure ¹³. The fructose-fed rats treated with allopurinol had a significantly lower systolic blood pressure than their untreated fructose-fed counterparts ¹³. Hence, a large dose of fructose increases blood pressure, possibly from increased production of uric acid and when treated with a substance that inhibits the activity of xanthine oxidase, the effects are reversed ¹³.

Hyperuricemia is considered a component of the metabolic syndrome ²⁴. Lowering uric acid levels has been found to ameliorate a number of features of metabolic syndrome in fructose-fed rats such as hypertension, hypertriglyceridemia, hyperinsulinemia, insulin resistance, renal vasoconstriction, glomerular hypertension and renal microvascular disease ²⁴. In the first step of fructose metabolism, the phosphorylation to fructose 1-phosphate via fructokinase utilizes an ATP and the accumulation of fructose 1-phosphate depletes hepatic ATP and generates adenosine diphosphate (ADP). Metabolism of ADP stimulates adenosine monophosphate (AMP) deaminase and increases the degradation of nucleotides to form uric acid ⁷⁸. Thus ATP depleting effects of fructose can cause a transient arrest of protein synthesis, production of inflammatory proteins, endothelial dysfunction and oxidative stress ²⁴.

Similarly to the above-mentioned study, Sanchez-Lozada et al³¹ also observed a gradual increase in systemic blood pressure and plasma uric acids with increasing doses of fructose consumption after rats were fed either 10% fructose solution, 60% fructose solution or control solution for eight weeks. A direct relationship between increasing fructose concentrations and increased blood pressure was observed³¹. These investigators also found a positive linear relationship between uric acid levels and systolic blood pressure³¹. Fructose ingestion, over a period of time, resulted in elevated uric acid levels as well as increased systolic blood pressure. Thus, higher uric acid levels induced by a 60% fructose diet may be partially responsible for these hemodynamic alterations mentioned³¹. Mazzali et al⁷⁹ also studied rats that were treated with a uric acid supplement for three weeks. The treated rats developed hyperuricemia, resulting in hypertension. These hyperuricemic levels were 7-to 8-fold higher than the control rats not treated with the supplementation⁷⁹.

As early as 1967, serum uric acid levels have been shown to increase rapidly after ingestion of fructose, resulting in increases as high as 2 mg/dl within one hour⁸⁰. Although these increases seem transient, studies in which fructose-rich diets were fed over several weeks exhibited elevated fasting uric acid levels⁸¹. The Third National Health and Nutrition Examination Survey (NHANES), which studied 14,791 participants over the age of 20 years, found that hyperuricemia was associated with increased consumption of sugar-sweetened beverages⁸². The authors cited a study performed by Fox et al⁸³ in 1974 which found that large amounts of fructose in the sugary drinks increase ATP degradation to AMP which results in uric acid production. Fox et al⁸³ also stated that with purine nucleotide depletion, rates of *de novo* purine synthesis is

accelerated, thus potentiating uric acid production⁸³. In contrast, glucose and other simple sugars do not have this effect. Based on the NHANES survey and previous research, Choi et al⁸² suggested that fructose has the ability to indirectly increase serum uric acid levels, leading to an increased risk of gout as a result of increased circulating insulin levels and eventually insulin resistance⁸².

In a study with 4867 adolescents, dietary data gathered from a 24-hour recall led researchers to observe the association between higher sugar-sweetened and elevated serum uric acid levels and systolic blood pressure⁸⁴. In this cohort of subjects, serum uric acid increased by 0.18 mg/dL and systolic blood pressure increased by 2 mmHg from the lowest to the highest category of sugar-sweetened beverage consumption. In a general normotensive adult population, a 2 mm Hg reduction of systolic blood pressure would lower stroke mortality by 10% and ischemic heart disease by 7%. For every 1mg/dL increase in serum uric acid, there is a 7% increase in the development of kidney disease or death after adjusting for multiple variables such as age, sex, race, systolic blood pressure, alcohol, smoking, HDL-cholesterol, and baseline kidney function⁸².

Another link between fructose, hyperuricemia and hypertension are defects in the nitric oxide (NO) pathway leading to subsequent impairments in endothelial-dependent relaxation⁶⁷. A diminished NO synthesis, release, or sensitivity of vascular smooth muscle cells to NO may allow cells to become more susceptible to the effects of vasoconstrictors, leading to enhanced vascular tone and elevated blood pressure⁷⁸. Uric acid infusion in humans has led to impaired endothelial NO release which can be reversed with allopurinol, an uric acid inhibitor⁸⁵. Likewise, when Nakagawa et al¹³ fed male Sprague-Dawley rats a diet containing 60% fructose for ten weeks, they observed

that these rats had a significant increase in uric acid levels and inhibited NO bioavailability compared to the rats fed a control diet. Furthermore, when those fructose-fed rats were treated with allopurinol to lower uric acid levels, NO bioavailability was enhanced and features of metabolic syndrome were lowered¹³. Researchers have speculated that the decreased bioavailability of NO is a result of decreased endothelial nitric oxide synthase (eNOS)⁸⁶.

Although numerous animal studies indicate a relationship between fructose, hyperuricemia and hypertension, there is limited direct human evidence of such effects. One of the few human studies investigating the acute effect of fructose on hemodynamic responses noted that consumption of a 500 ml drink containing 60 g of fructose by young, healthy male and females, resulted in significant increase in blood pressure variability and decreased cardiovagal baroreflex sensitivity up to two hours after the drink was consumed. The increase in blood pressure was in response to the fructose load and was also characterized by gradual increases in heart rate and cardiac output (although lower than the glucose drink) with no compensatory reductions in total peripheral resistance¹⁷.

In another study on healthy young men, an acute load of fructose/glucose (100 g) or a glucose solution (100 g) resulted in no changes in blood pressure or uric acid levels acutely⁸⁷. Other studies have also indicated that fructose doses as high as 30% of energy intake in normal-weight⁴⁴ and overweight⁴⁹ individuals failed to significantly alter blood pressure. Likewise, the Nurses' Health Study reported that fructose was not associated with the risk of developing hypertension⁸⁸. Hence, there is little human evidence as to a cause and effect relationship between fructose and hypertension, but, high fructose intake is linked to high caloric intake, weight gain and insulin resistance, all of which are

independent risk factors for hypertension ⁶. These differences indicate a need for more continued research as to how and if a high fructose diet induces hypertension in humans.

Research has indicated that uric acid dose-dependently blocks acetylcholine-mediated arterial dilation, suggesting that uric acid can impair endothelial function and reduce endothelial NO levels ¹³. This mechanism may be due to a blockade of insulin action, as insulin stimulates glucose uptake in skeletal muscle by increasing blood flow to tissues through the NO-dependent pathway ⁸⁹. Insulin can then cause local vasodilation mediated by endothelium-derived nitric oxide (EDNO) ⁹⁰. The increased vasodilation resulting from nitric oxide release enhances blood flow to the skeletal muscle, stimulating glucose uptake into the cell ⁹¹. However, when uric acid levels are elevated from chronic fructose ingestion, endothelial nitric oxide release is diminished, leading to decreased blood flow. Decreased blood flow to the skeletal muscle will result in a decrease in skeletal muscle glucose uptake, over-secretion of insulin and consequently hyperinsulinemia ⁹¹. This provides supporting evidence that uric acid may have a pathogenic role in risk factors associated with metabolic syndrome.

Although the aforementioned studies indicate that acute and chronic fructose consumption induces hyperuricemia, a recent investigation by Bidwell et al ⁸¹ demonstrated that an acute load of high fructose corn syrup (100 g) does not induce hyperuricemia in healthy, adult males. The researchers speculated that the dose used was not high enough to elicit such changes in uric acid and that a healthy, adult male may be protective against increases in uric acid ⁸¹. The results by Bidwell et al ⁸¹ were similar to results by Wang et al ²⁰⁹ in which the authors researched 21 controlled feeding trials in 425 prediabetic/diabetic and nondiabetic participants and demonstrate that the uric acid

response to fructose feeding differs between isocaloric and hypercaloric feeding conditions. Studies which employed isocaloric fructose diets showed no changes in uric acid levels yet when a hypercaloric fructose diet was employed, uric acid level did increase. The contradiction between studies may be that a threshold for fructose-mediated ATP depletion/AMP production, which is thought to lead to increased uric acid concentrations, must occur. The mean fructose dose in the isocaloric trials (93.4 g/d) was below the level of exposure associated with higher uric acid concentrations in the observational studies, which may also have incompletely adjusted for energy compensation. It was also well below the mean dose used in hypercaloric trials (215 g/d) used to induce higher uric acid concentrations and the animal models (60% Energy as fructose, which is equivalent to 300 g/d on a 2000-kcal diet) used to elucidate the mechanism of fructose-induced uric acid production²⁰⁹. A fructose dose threshold has been observed in clinical trials, below which the effects on other metabolic biomarkers are lost, e.g., ≤ 60 g/d in type 2 diabetes and < 100 g/d across all subject types for TG²¹⁰.

Another possible explanation linking fructose to hypertension is the role of insulin on cardiac contractibility. Although fructose does not increase insulin acutely, as previously mentioned, chronic fructose ingestion leads to hyperinsulinemia which has adverse effects on the sympathetic nervous system². Two potential explanations for this link between hyperinsulinemia and hypertension are: 1) an increase in the sympathetic neural outflow and plasma catecholamine concentrations associated with increased plasma insulin concentrations; and 2) insulin action at the level of the proximal tubule increasing fluid reabsorption². Currently research elucidating these mechanisms is very limited⁹². Considering hypertension is a well-known comorbidity associated with

obesity, insulin resistance, hyperinsulinemia and hyperlipidemia ², it is important to determine the effects of fructose consumption on blood pressure in human subjects.

f. Conclusions:

The above review of literature summarizes the proposed mechanisms associated with the fructose-induced metabolic alterations related to metabolic syndrome. These risk factors, such as postprandial hyperlipidemia, insulin resistance, and hyperuricemia, seem to be exacerbated with fructose ingestion in a dose-dependent manner; hence continued research must be conducted to completely elucidate the importance of decreasing fructose consumption.

II. Inflammation

a. Hyperlipidemia and Endothelial Dysfunction:

Although triglyceride-rich lipoprotein (TRL) levels are typically high in a dyslipidemic condition, they are also present in the normal, postprandial state and are adequately cleared by the liver. In individuals with hyperlipidemia, the TRL remain increased in circulation beyond the usual 4-8 hours ⁵⁶ and this prolonged circulation appears to be linked to accelerated atherogenesis. Triglyceride-rich lipoproteins are pro-inflammatory and can cause endothelial dysfunction, up-regulate expression of endothelial adhesion molecules and promote macrophage chemotaxis. This process occurs as a result of increased lipolysis of postprandial lipoproteins by lipoprotein lipase. The adverse effect of this lipolytic action is the formation of small-dense LDL which increase the permeability of the endothelial tissue, whereas lipolysis by fasting

lipoproteins has no such effect ⁹³. In the postprandial state, small-dense LDL are prone to enter the arterial wall where they are ingested by macrophages and form foam cells. A high prevalence of small-dense LDL are very common in patients with metabolic syndrome, diabetes and insulin resistance ²³.

The pro-inflammatory effects of elevated TRL are intricately linked to higher concentration of small-dense LDL and lower concentrations of high-density lipoprotein cholesterol (HDL-C). These small-dense LDL are unable to be cleared by the LDL receptor, enhancing their potential for binding to proteoglycans, heavily glycosylated proteins, in the vascular wall. Additionally, the small-dense LDL's are more susceptible to oxidation which will further increase their atherogenic potential ⁹⁴. LDL oxidation is mediated by the interaction with endothelial cells and other cells of the vascular wall. Once inside the vascular wall, oxidized LDL's further stimulate endothelial adhesion activation and/or injury, stimulating adhesion molecule expression such as E-selectin, P-selectin, vascular adhesion molecules as well as monocyte chemoattractant protein-1(MCP-1) which promotes activation and attachment of lymphocytes and monocytes ⁹⁵.

Monocytes and leukocytes undergo diapedesis, the migration of blood cells through the intact walls of blood vessels in the surrounding tissue, leading to transmigration into the sub-endothelial space where they are differentiated into scavenger cells ⁹⁵. Oxidized LDL's are ingested by scavenger cells leading to the formation of foam cells. This process triggers the inflammatory process resulting in the immune system releasing cytokines such as interleukin 1, 6, 8, TNF- α and NF-kappaB ⁹⁶.

b) Hyperlipidemia, Inflammation and Insulin Resistance

TNF- α , interleukin-6 (IL-6) and c-reactive protein (CRP), are important pro-inflammatory cytokines induced by elevated triglyceride concentrations which have been linked to insulin resistance⁹⁷. Van Oostrom et al⁹⁸ have shown that increases in postprandial TG and glucose stimulate the activation of neutrophils, leading to an increase in pro-inflammatory cytokines such as IL-6 and TNF- α . IL-6 leads to increased insulin resistance by blocking the IRS-mediated insulin signaling in hepatocytes and muscle cells causing impaired insulin-stimulated glucose uptake into muscle cells⁹⁹. Although the exact mechanism as to how IL-6 affects IRS receptors is not completely understood, it could involve the activation of tyrosine phosphatase or an interaction between suppressor of cytokine signaling (SOCS) proteins and the insulin receptor itself¹⁰⁰. One of the primary effects of IL-6 is to induce the production of hepatic CRP which is a known independent risk factor of cardiovascular disease⁹⁷. CRP is an acute phase reactant inflammatory protein which reflects systemic low-grade inflammation¹⁰¹. In rodent models of glucose metabolism, infusion of IL-6 induced gluconeogenesis, subsequent hyperglycemia, and compensatory hyperinsulinemia¹⁰¹. Moreover, other studies have demonstrated elevated levels of IL-6 and CRP levels among individuals with features of the insulin resistance and type 2 diabetes¹⁰². Given IL-6's position in the cytokine cascade as a key mediator of downstream inflammatory processes including activation of coagulation, hepatic release of acute phase reactant proteins, IL-6 may have a potential causal role in metabolic risk factors associated with type 2 diabetes and cardiovascular disease.

TNF- α has been identified as the first molecular link between obesity, diabetes, and inflammation and is over-expressed in adipose and skeletal muscle of obese mice and

humans¹⁰³. This phenomenon is apparent in experimental models in which neutralization of TNF- α improved insulin sensitivity in obese rats. Moreover, TNF- α deficient mice are protected from obesity-induced insulin resistance¹⁰³. Hotamisligil et al¹⁰⁴ demonstrated that TNF- α disrupts insulin signaling by inducing serine phosphorylation of IRS-1 which in turn causes serine phosphorylation of insulin receptors. This process interferes with the normal tyrosine phosphorylation of the insulin receptor, thereby interfering with normal insulin transduction¹⁰⁴.

The inhibition of insulin signaling by TNF- α in adipocytes and hepatocytes involves the activation of a kinase utilizing IRS-1 as a substrate¹⁰⁵. Once serine is phosphorylated, IRS-1 becomes a poor substrate for the insulin receptor and preventing phosphorylation of serine restores IRS-1 mediated insulin receptor signaling¹⁰⁵. In the liver, TNF- α infusion leads to decreased tyrosine phosphorylation of the insulin receptor and insulin signaling molecules IRS-1 and Akt, indicating decreased insulin sensitivity¹⁰⁶. Further, there is a strong positive correlation between TNF- α mRNA expression levels in fat tissue and the levels of hyperinsulinemia¹⁰⁶. Likewise, TNF- α infusion decreases tyrosine phosphorylation of insulin receptor IRS-1 and AKT in the intestine, leading to increased production of apoproteinB-48 containing chylomicrons particles. These changes accompany increased production of VLDL-type particles.

TNF- α stimulate I-kappa B (I κ B) kinase- β (I κ k β) to activate NF- κ B which can also alter insulin sensitivity¹⁰⁷. Once NF- κ B translocates to the nucleus, the result is the transcription of genes that promote the development of insulin resistance¹⁰⁷. The induction of targeted genes by NF- κ B leads to the increased expression of inflammatory markers and mediators associated with insulin resistance. This process is associated with

the production of other factors such as inflammatory mediators, chemokines and transcription factors, all stimulating the recruitment of monocytes, eventually leading to the differentiation of macrophages ¹⁰⁷.

Previously, Hotamisligil et al ¹⁰⁸ infused TNF- α or placebo into nine healthy adults for four hours on two separate occasions. Using an euglycemic hyperinsulinemic clamp, insulin resistance in skeletal muscle occurred when the TNF- α was infused; however, endogenous glucose production was not altered. It appeared that TNF- α directly impaired glucose uptake and metabolism by altering insulin signal transduction ¹⁰⁸. The authors reported that the insulin signaling pathway was associated with impaired phosphorylation of Akt substrate 160 which is the most proximal step in the insulin pathway and is responsible for the regulation of GLUT 4 translocation and glucose uptake. Hence, it was concluded that excessive concentrations of TNF- α is linked to negative insulin signaling and decreased whole-body glucose uptake in healthy adults ¹⁰⁸. Furthermore, Kanety et al ¹⁰⁹ studied Fao hepatoma cells in vitro and established that TNF- α caused a reduction in insulin-induced tyrosine phosphorylation of IRS-1 through increased serine phosphorylation of the IRS-1 as a result of inhibition of serine phosphates.

Previous studies have shown that increased consumption of fructose results in hyperlipidemia accompanied by insulin resistance and elevated plasma triglycerides, all leading to increased inflammation ^{51, 110, 111}. Bergheim et al ¹¹¹ found that rats fed a diet with either 30% fructose, glucose, sucrose or water only for eight weeks, experienced increased lipid peroxidation and elevated hepatic TNF- α in the fructose group compared to all other conditions ¹¹¹. The fructose diet increased TNF- α mRNA expression by 6.5

fold. Lipid peroxidation led to induction of iNOS and TNF- α expression in the liver when exposed to high levels of fructose. Moreover, the chronic intake of fructose, and to a lesser extent sucrose, caused significant liver stenosis and increased neutrophil production¹¹¹. Additionally, phosphorylation status of Akt in the liver was altered in mice fed the fructose solution; however, a similar effect of fructose feeding was not found in the TNF- α knockout mice. This implies that TNF- α may be critical in mediating insulin resistance in mice chronically fed fructose¹¹¹. Additionally, it has been suggested that an induction of TNF- α may suppress the activation of AMP-activated protein kinase (AMPK) in the liver¹¹².

Kanuri et al¹¹⁰ found similar results when wild-type mice or TNF- α knockout mice were fed a 30% fructose solution or tap water for eight weeks. The authors were examining whether TNF- α plays a critical role in the development of hepatic stenosis in fructose-fed mice. Hepatic TG levels, markers of inflammation and ATP peroxidation along with indices of insulin resistance were measured in the liver and plasma of the mice¹¹⁰. The fructose-fed, wild-type mice had significantly higher TG accumulation which resulted in a 5-fold increase from baseline values. Moreover, the fructose-fed wild-type mice had significantly higher neutrophil infiltration; whereas, in the fructose-fed TNF- α knockout mice, the neutrophil infiltration was similar to the water-fed controls¹¹⁰. In the fructose-fed TNF- α knockout mice, hepatic stenosis and neutrophil infiltration was attenuated which resulted in increased phosphorylation of AMPK and Akt, similar to the water-fed controls. Since phosphorylation status of Akt in the liver was altered in the fructose-fed mice wild-type mice and not the TNF- α knockout mice, it was concluded that TNF- α and its receptor 1 may be critical in mediating insulin resistance in the mice

chronically fed fructose¹¹⁰. Spruss et al¹¹³ found similar results when they tested mice which were fed 30% fructose for five weeks.

In a longer duration study, Sanchez-Lozada et al¹¹⁴ investigated whether a drink containing 30% glucose with 30% fructose or 60% sucrose induced fatty liver when compared to rats fed a standard show diet for 16 weeks¹¹⁴. Liver inflammation was induced as a result of elevated TNF- α with both the fructose + glucose diet as well as the sucrose diet when compared to the control group (standard chow). The increases in inflammatory markers significantly correlated with increases TG levels as well.

The aforementioned studies have indicated that increases in inflammatory markers such as TNF- α can create changes in insulin signaling which can be exacerbated with fructose ingestion¹¹⁴. Although there is a lack of direct experimental evidence linking fructose and inflammation, the process of lipid accumulation within the liver may induce a sub-acute inflammatory response that is similar to that seen in obesity-related inflammation within adipocytes¹⁰⁷. NF- κ B is activated in the hepatocytes and cytokines such as TNF- α , IL-6 and IL-1 β are overproduced in fatty liver¹⁰⁷. These pro-inflammatory cytokines participate in the development of insulin resistance and activate hepatic macrophages called Kupffer cells. Unlike adipose tissue in which macrophages are relatively sparse in a basal state and increase with increased adiposity, the liver is densely populated with Kupffer cells. Toll-like receptor 4 (TLR4) and CD14, receptors on the Kupffer cell that internalize endotoxins activate the transcription of pro-inflammatory cytokines such as TNF α and interleukins. More research needs to be conducted to fully elucidate the impact that fructose has on inflammation¹⁰⁷.

III. Incretins, Fructose Ingestion and Metabolic Syndrome

Recent evidence has demonstrated that the incretin hormones may play a role in the progression of T2D ¹¹⁵. The “incretin effect” implies that the ingestion of glucose causes a release of substances from the intestines which act to enhance insulin secretion beyond the release of absorbed glucose itself ¹¹⁶. A defective incretin response may cause a severe postprandial hyperglycemic state ¹¹⁵. The hormones conveying this effect are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP); they are highly insulintropic in a glucose dependent fashion ¹¹⁷. Both hormones increase insulin secretion, however, only GLP-1 suppresses glucagon secretion ¹¹⁸. In patients with type 2 diabetes, the secretion and/or action of incretin hormones is greatly impaired, with GLP-1 defects being more pronounced than GIP ¹¹⁹.

Endogenous GLP-1 is a gastrointestinal hormone secreted from the L-cells of the distal aspect of the small intestine after the ingestion of nutrients, specifically glucose, and is highly correlated with the release of insulin ¹¹⁸. GLP-1 is reduced in the fasted state and increases rapidly after a meal. It has potent effects on the β -cell secretion of insulin and is attenuated in T2D patients ¹¹⁸. Previous research has shown that the GLP-1 response to glucose in T2D is reduced by approximately 50% when compared to healthy, glucose tolerant controls ¹¹⁹. Studies have shown that GLP-1 infusion in subjects with T2D results in a near normal insulin response ^{115, 120}. Glucose-dependent insulintropic polypeptide (GIP) is an incretin hormone secreted from the K cells of the duodenum in response to an absorbed carbohydrate or lipid ¹¹⁵. GIP is reduced in the fasted state and increases after food ingestion. The primary action of GIP is to stimulate glucose-dependent insulin secretion ¹¹⁸.

Significantly lower postprandial GLP-1 concentrations have been reported in individuals with T2D. Toft-Nielsen et al ¹²¹ attempted to clarify the diminished incretin effect when they studied 54 individuals with T2D and 33 glucose-tolerant following a mixed meal four hours. They speculated that the decreased rate of gastric emptying seen in the individuals with T2D may explain these occurrences. Delayed gastric emptying rate may hypothetically increase the absorption of the proximal intestine resulting in less food reaching the distal intestine where the L-cells are most abundant ¹²¹, thus, affecting the amount and rate at which GLP-1 is released.

Knop et al ¹²² sought to determine whether the reduced incretin response observed in individuals with T2D is a primary effect in the pathogenesis of the disease or rather a consequence of the diabetic state. To do so they studied four different groups of subjects with the following characteristics: 1) chronic pancreatitis and normal glucose intolerance, 2) chronic pancreatitis and secondary diabetes, 3) chronic diabetes, and 4) normal controls ¹²². On two separate occasions, all groups underwent a four-hour study day where they were administered either a 50g-oral glucose load or an isoglycemic intravenous glucose infusion. The total GLP-1 area under the curve was significantly reduced in patients with chronic pancreatitis and secondary diabetes compared to those with chronic pancreatitis with normal glucose tolerance and healthy controls ¹²². Furthermore, the individuals with T2D group had a 36% lower incretin effect as measured by: $100\% \times [\beta\text{-cell secretory response of insulin and c-peptide to oral glucose tolerance test} - \text{intravenous } \beta\text{-cell secretory response}] / \beta\text{-cell secretory response to oral glucose tolerance test}$, than in the chronic pancreatitis patients with normal glucose tolerance and healthy controls. Additionally, the total GLP-1AUC was significantly

greater during the oral glucose tolerance test than the intravenous infusion in all for groups. Hence, the deficiency of GLP-1 and GIP seems to be more of a consequence of the diabetic state rather than a primary pathogenic trait ¹²². Others ¹²³ have also shown that the GLP-1 response to a mixed meal is reduced in patients with T2D ^{124, 125}, a decreased insulinotropic potency of GLP-1 ¹²⁶, and an almost complete loss of late phase insulin secretion in response to GIP ¹²⁷. Further exogenous GLP-1 administration restores blood glucose regulation to near-normal levels in individuals with T2D as a result of increased insulin secretion, reduced gastric emptying and decreased glucagon production ¹²⁸.

Verdich et al ¹²⁹ compared GLP-1 and GIP concentrations in response to a meal in obese and lean male subjects to determine whether weight loss affected the incretin concentrations. Subjects were given a standardized meal test and plasma hormone levels were measured every 30 min for 180 minutes. The obese subjects were retested after they participated in a 6-month weight loss program in which the average weight loss was 18.8kg. After the 6-month intervention, the obese subjects were retested. Prior to the weight loss intervention, GLP-1 area under the curve was significantly lower in the obese subjects compared to the lean controls. At the end of the intervention, the obese subjects had significantly higher GLP-1 concentrations than prior to the weight loss, however, GLP-1 concentrations were still lower than the lean controls ¹²⁹. These results implied that GLP-1 concentrations begin to normalize gradually as weight is reduced. After the intervention, both total and incremental area under the curve was lower in the weight loss group compared to the lean controls. The impaired postprandial GIP response may reflect a primary dysfunction in the obese state that contributes to hyperglycemia ¹²⁹. From this

study, it was not clear whether the reduction in postprandial GIP response after weight reduction reflects a dysfunction in the GIP secretion in humans prone to obesity or rather from the change in habitual diet ¹²⁹.

Similar results were found in a study by Martins et al ¹³⁰ in which weight loss began to alter late postprandial increases in GLP-1 concentrations. Martins et al ¹³⁰ induced weight loss with exercise training instead of dietary changes. Twenty-two sedentary, overweight individuals exercised five times per week at 75% maximum heart rate for a duration that elicited a 500 kcal/day deficit for 12 weeks ¹³⁰. Although GLP-1 AUC levels were not significantly different prior to and after the 12-week exercise intervention when a test meal was given, there was a tendency toward an increase in the delayed release of GLP-1. During the last 90 minutes after the test meal, GLP-1 concentrations were higher than at the same time point during the pre-intervention ¹³⁰. Martins et al suggested that exercise-induced weight loss may improve satiety consistent with the tendency towards an increase in the late postprandial release of GLP-1 ¹³⁰.

Although the effect of a glucose load on incretin hormones in obese with or without T2D individuals is well elucidated, the response of such hormones to a load of fructose is less studied. Kong et al ¹³¹ proposed that an oral load of fructose would cause differing effects on GLP-1 concentrations than a similar load of glucose. On three separate occasions, eight fasted, healthy controls ingested 75 g of fructose, 75 g of glucose or 75 g of glucose + 75 g of fructose an hour later. Plasma GLP-1 concentrations were measured for 180 minutes post drink consumption. Although all conditions elicited an increase in postprandial GLP-1 concentrations, the largest increase in GLP-1 was seen with the glucose only group ¹³¹. Furthermore, ingesting a fructose load one hour after a

glucose load did not alter the GLP-1 levels more so than when given separately.

Although fructose elicits postprandial increases in GLP-1 levels, they do not increase to the same extent as a similar concentration of glucose¹³¹. Research on fructose ingestion and incretin hormones is lacking and further work is needed

IV. Physical Activity/Inactivity

a) Physical Inactivity

Physical inactivity and poor cardiovascular fitness has been consistently associated with an increased risk of chronic diseases such as type 2 diabetes and cardiovascular disease³⁰. Being physically inactive and/or unfit is associated with many health consequences and is an important component of a comprehensive approach to disease prevention and health promotion¹³². Observational studies have demonstrated that the most unfit individuals are at the greatest risk of chronic diseases and all-cause mortality regardless of their gender, race, ethnic background or weight⁵. Research indicates that maintaining a high level of daily, low-intensity physical activity may be important for preventing metabolic risk factors such as coronary artery disease, type 2 diabetes and hyperlipidemia¹³³. Recommendations are now emphasizing the importance of incorporating moderate activity into a person's daily routine in order to avoid the ill effects of physical inactivity¹³⁴.

In 1953, Morris et al¹³⁵ determined that workers who were seated most of the day such as bus drivers and telephonists were twice as likely to develop cardiovascular disease than workers who stand or are ambulatory most of the work day such as mail carriers. This study was reproduced more recently in 2005¹³⁶ in an epidemiologic study of 73,743 postmenopausal women from the Woman's Health Initiative Study in which

those who were inactive had increased risk of cardiovascular disease and this was reversed with increased activity ¹³⁶.

The Australian Diabetes, Obesity and Lifestyle Study which included 8,299 adults 25 years and older with no known metabolic diseases reported that sitting time and self-reported television viewing was positively correlated with undiagnosed abnormal glucose metabolism ¹³⁷. These results persisted after adjustment for sustained and moderate-intensity leisure-time physical activity. A subsequent study from the same Australian cohort found that individuals (n=4064) who reported having participated in the required dose of weekly physical activity (30min/day, 5x/week) still had detrimental waist circumference, systolic blood pressure, and 2-hour plasma glucose after correcting for such variables with television viewing time ¹³⁸. In this same cohort, 1958 adults, over the age of 60 years who reported high levels of sedentary behavior, had a greater prevalence of developing metabolic syndrome ¹³⁹. This data provides evidence that reducing prolonged overall sitting time may reduce metabolic disturbances and that there is a need for more specific sedentary behavior recommendations and health guidelines for adults in addition to the current recommendations of physical activity ¹³⁸.

Data from the Medical Expenditure Panel Survey indicated that both physical inactivity and obesity are strongly and independently correlated with diabetes and cardiovascular disease ¹⁴⁰. According to the survey, the likelihood of having diabetes increases with physical inactivity regardless of BMI, and at any given BMI classification, it is better to be active than inactive. Hence, both physical inactivity and obesity seem to be independently associated with diabetes and diabetes-related risk factors ¹⁴⁰. Healy et al ¹³³ reiterated these findings when they observed that when 67 men and 106 women

wore accelerometers for seven days, the subjects with the lowest activity patterns had the highest 2-hr plasma glucose levels.

Not only does prolonged inactivity decrease the opportunity for cumulative energy expenditure resulting from numerous muscle contractions⁵, physical inactivity also induces molecular changes. Within six to eight hours of physical inactivity, the suppression of skeletal muscle LPL activity and reduced muscle glucose uptake occurs, resulting in elevated plasma TG and reduced HDL levels⁵. Lipoprotein lipase is an important enzyme involved in the molecular alterations affecting physical inactivity¹⁴¹. It is synthesized by parenchymal cells such as muscle fibers and is secreted into the extracellular fluid at which time it becomes functional on the intimal surface of the vascular endothelium¹⁴². LPL is the main enzyme responsible for the breakdown of VLDL-TG and chylomicrons on the endothelial. LPL also enhances the removal of VLDL by the VLDL receptor and indirectly plays a role in maintaining high levels of plasma HDL-cholesterol. Hence, low LPL is associated with blunted plasma TG uptake as well as reduced HDL levels⁵. Local regulation of LPL provides a means of generating a concentrated source of fatty acids as well as other lipoprotein-derived lipids¹⁴¹. Moreover, LPL is involved in the regulation of gene expression of inflammatory markers which lead to cardiovascular disease¹⁴³.

Physical inactivity and/or muscle regulate LPL activity in the vasculature and skeletal muscle. Reduced physical activity can decrease LPL activity in the microvasculature 10-20 fold¹³⁴; but such decreases can be reversed within several hours of ambulatory contractions implying that a reduction in contractile activity is a potent physiological factor determining LPL activity¹⁴¹.

The model of LPL response to physical inactivity and plasma lipid in the microvasculature of skeletal muscle can best be described by first understanding the mechanisms behind the LPL response during ambulation¹⁴⁴. During ambulation, the vascular endothelial cells are at the interface with plasma TG and fatty acids bound to albumin. During standing or ambulation, there is high LPL activity in the microvasculature of the skeletal muscle. Physically active muscles have greater rates of TG-derived fatty acid uptake, albumin-bound fatty acid transport, fatty acid oxidation, and intracellular TG synthesis. Moreover, there are reduced concentrations of intramuscular fatty acids and fatty acyl-CoA¹⁴⁴. In contrast, during inactivity when normal metabolic processes has slowed down, there is a removal of the local energy demands of physical activity, leading to an elevation in TG and fatty acids. Plasma fatty acids and TG accumulate as a result of a lower rate of LPL-induced fatty acid oxidation. This process can be reversed when pharmacological agents such as nicotinic acid lowers the exposure of the muscle tissue to lipids, causing LPL activity to be maintained¹⁴¹.

Bey et al¹³⁴ examined why regulation of LPL activity may be different during states of inactivity versus activity. They studied rats who participated in low-intensity training for 12 hours (active controls) versus rats that were inactive (hind-limb unloaded). They hypothesized that the normally high activity of LPL in oxidative muscle would significantly decrease with physical inactivity and that increased physical activity, after a period of inactivity, would restore such effects. In this acute study, rats were unloaded for 12 h followed by a loading period in which they performed walking and standing for four hours. The 12 h of physical inactivity significantly decreased LPL activity and within four hours of low-intensity activity, the LPL levels were increased back towards

the active control rats levels¹³⁴. In conjunction with the changes seen with LPL, the uptake of TG and HDL-C decreased. This study demonstrated that the steps involved in muscle LPL regulation, which are sensitive to inactivity can be prevented and even reversed with minimal, non-fatiguing contractions (ex: slow treadmill walking)¹³⁴.

Zderic and Hamilton¹⁴¹ also examined whether decreased physical activity would decrease LPL activity in rats. The hind-limb unloaded (HU) (inactive) rats experienced a 47% decrease in LPL activity after 8-h of inactivity and an additional 13% after 12 h of inactivity. A secondary purpose of the study was to determine whether a high-fat meal (~200 kcal/kg) would decrease LPL activity in the ambulatory controls. Plasma TG concentrations were increased by 83% at the 12-h point which resulted in a 63% decrease in LPL activity¹⁴¹. They too concluded that decreased activity depresses LPL activity. Furthermore, increased fat intake with ambulation also suppresses LPL activity, similar to that of inactivity. These results parallel the previously stated concept that there is an inverse relationship between TG concentrations and LPL activity and that decreased activity amplifies the response.

In the Studies of Targeted Risk Reduction Intervention through Defined Exercise (STRIDDE), the researchers investigated whether the training-induced benefits in serum lipids and lipoproteins are sustained over five and/or fifteen days of exercise detraining. Subjects were randomized into one of four groups; 1) high amount/vigorous intensity (caloric equivalent to approximately 20 miles/week at 65%-80% peak oxygen consumption, 2) low amount/vigorous intensity equivalent to approximately 12 miles per week at 65% to 80% peak oxygen consumption and 3) low amount/moderate intensity with a caloric equivalent of approximately 12 miles per week at 40%-55% peak oxygen

consumption and 4) a control non-exercising group for six months. Plasma was collected at baseline, 24 h, 5 days and 15 days after exercise cessation¹⁴⁵. This study demonstrated that six months of exercise increased HDL-C levels which were sustained at 5 and 15 days after exercise cessation¹⁴⁵. Further, the modest-intensity training group reduced total TG and VLDL-TG at 24 hours post- exercise training by twice the magnitude of the two more vigorous exercise-training groups. In the two vigorous-intensity training groups, total TG and VLDL-TG had returned to baseline after only 5 days, indicating that there was no sustained TG-lowering effect in those two groups. While the mechanisms for the aforementioned effects were unclear, the authors speculated that exercise of different intensities may have tissue-specific effects on the LPL bound to the endothelial cells, resulting in differential effects of exercise of varying intensities on TG, VLDL and HDL metabolism¹⁴⁵.

Endurance athletes are also not protected from inactivity related alterations in LPL changes. Herd et al¹⁴⁶ examined the influence of chronic aerobic training, followed by a short period of detraining, on postprandial lipidemia. Fourteen recreationally active males and females completed a 13-week running program in which the first 4 weeks involved running for 20 minutes at a steady-state 3x/week, the next four weeks involved interval training in conjunction with long, easy runs 5x/week and finished with a maintenance phase for the remaining weeks¹⁴⁶. The following nine days the subjects refrained from running. An oral fat tolerance test was conducted prior to the 13-week protocol and again 15 h, 60 h and 9 days after the cessation of running. Sixty hours after the last training session, the runners' lipidemia response to the fat load was 37% higher than baseline, and 46% higher after 9 days of detraining. These changes correlated with a

reciprocal decrease in LPL activity¹⁴⁶. This data supported the previous hypothesis that hydrolysis at the endothelial surface of capillaries by LPL is the rate-limiting step in TG clearance, and changes in LPL activity with changes in exercise or training status are most likely the cause of the above findings¹⁴⁶.

B) Physical Activity:

Physical activity has been shown to decrease body weight and visceral fat accumulation¹⁴⁷, improve insulin sensitivity¹⁴⁷, decrease plasma TG levels, increase plasma HDL-C¹⁴⁸ and decrease blood pressure, all risk factors for metabolic syndrome³⁰. Research has shown that walking is an effective way to increase the level of physical activity and can be easily measured with the use of pedometers¹⁴⁹. Many public health information sources recommend an average daily step count of 10,000 for adults and 12,000 for children that equal a caloric expenditure of approximately 300-400 kcal¹⁴⁹. More specifically, the American College of Sports Medicine guidelines state that adults should accumulate at least 30 minutes of moderate-intensity activity, preferably all days of the week in order to protect against chronic diseases¹³².

Physical exercise improves insulin sensitivity both acutely and chronically as a result of changes in insulin signaling. This process is not mediated by the insulin-dependent rapid phosphorylation of the insulin receptor, IRS -1/2 on tyrosine residues with a subsequent activation of PI3-K¹⁵⁰. In contrast, exercise stimulates an insulin-independent pathway. With a muscle contraction, glucose uptake is mediated by multiple signaling pathways such as protein kinase-C, Ca⁺²/ calmodulin-dependent protein kinase kinase (CaMKK) and AMPK¹⁵⁰. The translocation of GLUT 4 receptors to the cell membrane occurs as a result of increased Akt activity and phosphorylation within the cell

¹⁵¹. This effect is short-lived, lasting 48-72 hours; therefore, to maximize the benefits of physical exercise on insulin sensitivity exercise should be repeated within this timeframe.

The benefits of physical activity, such as lower serum TG levels, lower blood pressure and increased insulin sensitivity, can be observed after a single bout of moderate to vigorous intensity exercise ¹⁵². Increased physical activity activates LPL, which is responsible for the partitioning of triglyceride-derived fatty acid uptake in various tissues as well as increased cholesterol metabolism and downstream intracellular modifications to lipid availability ¹³⁴. A muscle contraction immediately stimulates an increase in skeletal muscle LPL activity which increases TG clearance leading to an increase in mitochondrial metabolic potential and an improvement in oxidation rates of long and medium chain fatty acids, resulting in a reduction in intracellular ceramide productions. Additionally exercise modulates hepatic lipase activity, hence decreasing TG-rich remnant proteins and TG ²⁵.

The use of pedometers is increasingly being targeted as a means to assess increased physical activity and improve motivational levels for sedentary individuals. An extensive literature review by Tudor-Locke and Bassett ¹⁵³ determined that <5000 steps/day correlated with sedentary lifestyle, 5000-7499 steps/day was low-active, 7500-9999 steps/day was somewhat active, 10,000 and 12,499 steps/day was active and $\geq 12,500$ steps/day was highly active. Achieving 10,000 steps/day has been widely recognized by the media and general public as a target threshold for achieving health benefits ¹⁵³. Subsequently, achieving 10,000 steps/day may be a reasonable alternative for compliance with public health recommendations to be moderately active thirty minutes per day on most days of the week ¹⁵³.

In an attempt to determine whether pedometer usage could improve risk factors associated with type 2 diabetes and cardiovascular disease, Freak-Ploi et al¹⁵⁴ investigated pedometer usage in 762 adults in the workplace¹⁵⁴. Participants were required to walk a minimum of 10,000 steps per day for four months. The four months of increased physical activity significantly decreased sitting time (as assessed via questionnaires), improved blood pressure and decreased waist circumference (by 1.6 cm). At the start of the intervention, 27% were not meeting the recommended physical activity recommendations, 23% were above the recommended waist circumference guidelines, 54% had elevated blood pressure. At the conclusion of the 4-month intervention, all of those participants were meeting the recommended guidelines in all categories, reducing their risk for type 2 diabetes and metabolic syndrome¹⁵⁴. These results are similar to previous workplace interventions in which significant decreases in waist circumference occurs from increased physical activity¹⁵⁵⁻¹⁵⁸.

Although there seems to be clear guidelines as to the amount of physical activity needed in order to experience cardiovascular adaptations, there is still a debate as to the amount of physical activity needed for weight loss. Results from the STRIDDE study (previously described) have shown that eight months of exercise resulted in weight loss in a dose-dependent manner with the high-dose group achieving the greatest weight loss and no weight loss in the control group¹⁵⁹. The amount of weight loss is directly related to the amount of exercise performed and calories expended.

c) Physical Activity and Inflammation

Although exercise causes an acute inflammatory response ¹⁶⁰, physical activity and improved cardiovascular fitness decreases low-grade inflammation by decreasing body fat, decreasing production of pro-inflammatory cytokines and increasing production of anti-inflammatory cytokines. Moreover, exercise reduces expression of adhesion molecules, up-regulates antioxidant and other cellular defenses and improves endothelial function ¹⁶¹. Brandt et al ¹⁶² has suggested that cytokines and other peptides that are produced, expressed and released by muscle fibers and exert paracrine or endocrine effects should be classified as “myokines” such as IL-6, IL-8, and IL-15. Although low-grade inflammation characteristic of elevated IL-6 levels has been associated with obesity and insulin resistance, it is markedly produced and released after an acute bout of exercise and may actually help to prevent or reduce risk factors associated with metabolic syndrome and type 2 diabetes ¹⁶².

During exercise, the magnitude of the increase in IL-6 is relative to the duration, intensity of exercise and amount of muscle mass involved. Muscle biopsies from humans and rats have demonstrated increases in IL-6 after exercise up to 100 times that of resting values ¹⁶³. In response to muscle contraction, both type I and type II muscle fibers express the myokine IL-6 which exerts its effects both locally within the muscle by activating AMPK and peripherally in several organs of the body when released into circulation. IL-6 may also work in an endocrine manner to increase hepatic glucose production during exercise or during lipolysis in adipose tissue ¹⁶⁴.

The anti-inflammatory effects of IL-6 have also been demonstrated by the ability of IL-6 to stimulate the release of classical anti-inflammatory cytokines such as IL-1ra and IL-10 ¹⁶⁴. Hence, IL-6 has both pro- and anti-inflammatory properties. When IL-6 is

signaling monocytes or macrophages, the activation of NF- κ B and TNF- α occurs, leading to an inflammatory state but when IL-6 is released from muscle, it creates an anti-inflammatory state¹⁶². Therefore, the possibility exists that the long-term effect of exercise may be a result of the anti-inflammatory process of an acute bout of exercise. For that reason, acute exercise will protect against chronic systemic low-grade inflammation and thereby offer protection against insulin resistance and atherosclerosis.

d) Fructose and Exercise:

For athletes, fructose provides a beneficial aid in training due to its ability to stimulate rapid nutrient absorption in the small intestine and help increase exogenous carbohydrate oxidation during exercise⁶. When fructose is mixed with glucose in sports drinks, carbohydrate oxidation is enhanced by 40%. This dramatic increase in oxidation can be explained by the different transport systems used for intestinal absorption. Moreover, fructose has been shown to reduce the perception of fatigue and stress during exercise and improve exercise performance during cycling exercises¹⁶⁵.

To date, there is only one report of fructose and physical activity. Botzelli et al²⁶ studied 48 Wistar Rats to determine whether aerobic exercise alters markers of fatty liver disease when fed a diet high in fructose. Two groups of rats were studied for 90 days, control rats and fructose-fed rats (60% fructose). After 90 days, the fructose-fed rats had decreased insulin sensitivity and increased total cholesterol and TG, whereas the control rats showed no change²⁶. From days 90-120, the rats exercised one hour a day, five days per week. At the end of the 120 days, the fructose-fed rats had altered metabolic profiles which included elevated plasma TG as a result of the fructose diet, had improved insulin sensitivity and decrease cholesterol levels, resulting from the exercise regimen.

The changes in TG levels were most likely due to the improved lipid oxidation and availability of circulation TG as a result of exercise²⁶. These results indicate that increased physical activity may improve features of metabolic syndrome. Studies on humans still need to be conducted to determine the interaction between fructose consumption and exercise.

IV. Conclusions

Although it seems apparent that increased intake of fructose leads to various risk factors associated with metabolic syndrome such as hypertension, hyperlipidemia, insulin resistance, inflammation and hyperuricemia, there is still numerous contradictory evidence which states that as long as fructose is consumed in moderate doses²¹⁰, fructose may not augment these risk factors. The quantities of fructose administered in many of the studies used concentrations that were well above the average fructose intake of 60-70 g/day, and with increased daily caloric intake, which may results in differing results. Hence, there is a need for research investigating the effects of fructose when using quantities that more closely match that of the average population to determine if fructose is as harmful as research seems to state. Moreover, there is only one study indicating how physical activity may alter such risks. Although fructose consumption cannot be completely to blame for the increased rates of obesity and metabolic syndrome, fructose is often associated with additional detrimental behaviors such as a hypercaloric diet, a diet rich in saturated fats as well as low physical activity⁶. These behaviors lead to risk factors of metabolic syndrome and such could be prevented and/or reduced.

Chapter III

ABSTRACT

High physical activity during fructose loading attenuates the postprandial lipidemic and inflammatory responses to a fructose-rich meal.

Fructose consumption has increased substantially in the past few decades and has been linked to hyperlipidemia. Whether increased physical activity (PA) may confer protection against fructose-induced hyperlipidemia is yet to be determined. **PURPOSE:** The purpose of this study was to determine the interaction between chronic high fructose consumption and PA levels on postprandial lipidemia and inflammation in normal weight, recreationally active individuals.

METHODS: Twenty-two men and women (age: 21.2 ± 0.6 yrs; BMI = 22.5 ± 0.6 kg/m²) consumed an additional 75 g of fructose for 14 days on 2 separate occasions: during the loading, subjects maintained either low PA (~ 4,500 steps/day; FR+Inactive) or high PA (~ 12,500 steps/day; FR+Active). Prior to and immediately following the 14 days of fructose consumption, subjects were given a fructose-rich meal (600 kcal mixed meal (45% carbohydrate [25% fructose], 40% fat, and 15% protein). Blood was sampled at baseline and for 6 h after the meal. Samples were analyzed for triglycerides (TG), very-low density lipoproteins (VLDL), total cholesterol (TC), glucose, insulin, tumor necrosis factor- α (TNF- α), interleukin 6 (IL-6) and c-reactive protein (CRP). Area under the curve (AUC) and absolute change to peak hormone concentrations ((peak-baseline)(Δ peak)) were calculated to quantify the postprandial responses.

RESULTS: Log transformed TG AUC significantly increased from pre (10.1 ± 0.1 mg/dL x min for 6 h) to post (10.3 ± 0.08 mg/dL x min for 6 h; $p = 0.04$) with the FR+Inactive intervention; whereas, TG AUC was unchanged after the FR+Active intervention. Additionally, the FR+Inactive intervention resulted in an 88% increase in Δ peak[TG] ($p=0.009$) and an 84%

increase in $\Delta_{\text{peak}}[\text{VLDL}]$ ($p=0.002$) with no change in either variable after the FR+Active intervention. IL-6 total AUC was not altered in either intervention, however, $\Delta_{\text{peak}}[\text{IL-6}]$ increased by 116% after FR+Inactive intervention but decreased 30% after the FR+Active intervention ($p=0.009$). While there were no changes in glucose concentrations with either intervention, insulin AUC significantly decreased after FR+Active intervention ($p=0.04$) with no change in AUC after the FR+Inactive intervention. No changes were observed in TNF- α and CRP concentrations ($p>0.05$). **CONCLUSIONS:** Low physical activity during a period of high fructose intake augments fructose-induced postprandial lipidemia and inflammation in as little as two weeks of consumption while high PA during fructose consumption appears to minimize these metabolic disturbances. Hence, maintaining high PA may decrease the susceptibility to cardiovascular risk factors associated with high fructose consumption.

Introduction:

There is a growing body of evidence in animal and human models that a high dietary intake of fructose is an important nutritional factor associated with development of insulin resistance¹², fatty liver¹³, hyperlipidemia^{15,35} and hypertension¹⁷; all risk these factors are associated with metabolic syndrome. Fructose overfeeding has also been linked to reduced lipid oxidation and increased triglyceride formation in healthy males¹⁹ and obese adults⁴⁹. This is evident in research conducted by Stanhope et al⁴⁹ who found that 25% of calories from fructose induced hepatic insulin resistance, increased *de novo* lipogenesis and increased visceral adiposity in obese adults within 10 weeks. Similarly, Abdel-Sayed et al¹⁹ found that a high fructose diet (3.0 g/kg of body weight/day) for seven days suppressed lipolysis and lipid oxidation.

Researchers therefore, postulate that increased consumption of fructose might be one of the environmental factors contributing to the development of obesity and abnormalities associated with metabolic syndrome and cardiovascular disease^{2,19,49}. Although previous studies have indicated adverse adaptations to a high fructose diet, these studies often use unrealistically high levels of fructose¹⁹ or study the subjects while they are sedentary⁴⁹, both of which could impact metabolic outcomes related to fructose metabolism.

The metabolic problems associated with high fructose consumption are a result of the unique metabolism of fructose. During fructose metabolism, fructose is rapidly phosphorylated by fructokinase in the liver, but metabolism is not inhibited by the cellular energy state of the cell, causing an influx of carbon atoms into the tricarboxylic acid cycle (TCA) leading to increased acetyl CoA levels¹⁶⁶. Large concentrations of acetyl CoA can increase fatty acid production, resulting in hepatic *de novo* lipogenesis. Simultaneously, fructose inhibits hepatic lipid oxidation which favors fatty acid re-esterification and VLDL-triglyceride synthesis⁷⁶. The

induction of fructose-induced lipogenesis results in an increase of TG to the adipose tissue and ectopic tissues such as muscle and liver, increasing the size of these depots. This leads to impaired insulin signaling, dyslipidemia, and low-grade inflammation ¹⁶⁷.

The main limitations of previous research concerned with fructose consumption are that the high fructose diet resulted in caloric excess ¹⁹ and/or there was no control in the level of physical activity or sedentary behavior of the subjects. Sedentary behavior alone is known to decrease LPL in as little as 8 h ¹³⁴. LPL is a key regulatory enzyme responsible for the partitioning of triglyceride-derived fatty acid uptake in various tissues as well as increased cholesterol metabolism and downstream intracellular modifications to lipid availability associated with fat breakdown ¹³⁴. Low PA and fructose ingestion may interact synergistically to alter LPL activity and/or lipid metabolism. Additionally, physical inactivity is inversely related to low-grade inflammation as a result of increasing pro-inflammatory markers such as IL-6. Fructose consumption may have an additive effect on inflammation as fructose increases low-grade inflammatory markers such as monocyte chemo-attractant monocyte-1 ¹⁶⁸. Hence, the additive effect of physical inactivity, combined with chronic fructose consumption may have a confounding effect on metabolic abnormalities and may increase the risk for cardiovascular disease.

While there is an abundance of research on the importance of increased physical activity to prevent metabolic syndrome and heart disease, there is no evidence investigating the effects of high fructose consumption under conditions of energy balance. Further, no previous research has compared high physical activity and low physical activity on plasma lipid concentrations in a fructose-fed state. Therefore, the purpose of this study was to examine the interaction between high fructose consumption and physical inactivity on postprandial lipidemia and low-grade

inflammation in a young, healthy population. It was hypothesized that a diet rich in fructose would increase postprandial lipidemia more so when coupled with physical inactivity than when accompanied by high physical activity. Moreover, we hypothesized that a high fructose diet over a 2-wk period would increase pro-inflammatory markers associated with elevated lipid production and these effects would be ameliorated with high physical activity.

Methods:

Subjects Characteristics:

Healthy male and females (n=22) between the ages of 18-25 years old were recruited from the Syracuse University community. Subjects had to be recreationally active (3-4 days/week), with a body mass index (BMI) < 27 kg/m² (mean: 22.5 ± 1.6 kg/m²). All study participants completed an informed consent form, approved by the Syracuse University Institutional Review Board prior to participating in this study. Exclusion criteria included the use of lipid and/or glucose-lowering medications or other medications that may affect glucose and lipid metabolism (e.g. antidepressants, oral contraceptives, etc.), chronic NSAID usage (>2 x/week), daily antioxidant supplementation, orthopedic limitations to walking, type 2 diabetes or glucose intolerance, overt cardiovascular disease, hypertension, and/or an abnormal lipid profile. Subjects were excluded if they were currently ingesting more than one high fructose drink/day (>20 grams). Women started the intervention periods within the first 7 days of the menstrual cycle to minimize the potential effects of estrogen on glucose/insulin levels ¹⁶⁹.

Study Design:

Initially, subjects participated in a one-week control period before starting the intervention to determine their normal physical activity and dietary habits. Through this control period, it

was determined that the subjects mean steps/day were equal to $8,754 \pm 173$ steps. The first intervention involved a two-week fructose loading period in which subjects ingested an additional 75 g of fructose per day with an ad libitum diet while increasing physical activity to $>12,500$ steps (FR+Active). This was followed by a two-week washout period. The second intervention involved fructose loading while being physically inactive (<4500 steps) (FR+Inactive). A study day with a fructose -rich meal was given at the beginning and after both 14-day interventions. This was a counter-balanced, cross-over design.

Pre-screening Visit: All subjects completed a medical history, physical activity and sedentary behavior questionnaire prior to the start of the intervention. After a thorough review of the subject's activity level, the potential subjects were disqualified if their activity level included regular exercise more than four times/week. Both the physical activity and sitting questionnaires are in appendix C. Height and weight were measured, and body composition was assessed by air-displacement plethysmography (BODPOD system, Life Measurement, Inc. Concorde, CA)¹⁷⁰. After anthropometric measurements were completed, subjects performed a graded exercise test on the treadmill using a protocol that has been previously published¹⁷¹. Briefly, subjects began exercising at 3.5 mph for two minutes at which time the speed increased by 1.0 mph up to 6.5 mph every two minutes. At that point the grade was increased by 2.5% every two minutes thereafter. Subjects' blood pressure, heart rate and rate of perceived exertion were monitored throughout the test. The test ceased when the subject reached volitional fatigue¹⁷¹.

At the conclusion of the initial visit, the subjects underwent a nutritional consultation with a registered dietician to ensure proper compliance with the dietary intervention and to estimate normal fructose intake. Subjects were instructed to refrain from ingesting any added sugar such as sweetened beverages, fruit juices, pastries, and cookies aside from the study drinks, during the

intervention period. Further, estimates of food intake during the control and intervention period were collected by random 24-hour recall (via telephone) two times per week using the USDA 5-step multiple-pass method¹⁷². The same registered dietician administered the recall to all subjects. Recalls were then analyzed using Diet Analysis Plus (version 7, Thomson Wadsworth, Thomson Corporation, Independence, KY). Following the initial visit, the subjects began a one-week control period at which time they continued with their normal activities of daily living and typical diets. During this period, the subjects monitored their physical activity (steps) with the use of an Accelerometer (Actigraph® GT3X Activity Monitor, Pensacola, FL) which was later uploaded to the computer for further analysis. Subjects also were given pedometers (Accusplit®, Livermore, Ca) to provide visual feedback regarding daily step count. Subjects were instructed to not participate in any additional exercise during the study duration.

Visit 1: At the end of the 7-day control period, the subjects arrived at 0700 h, following a 12-h fast and no exercise 24 h prior to testing. Subjects had a catheter inserted into the antecubital vein by a registered nurse. Subjects then rested in a supine position for 30 minutes before obtaining two baseline blood samples (10 ml each). After baseline blood samples, a test meal was prepared for subjects. The test meal included the following: 600 kcals, 45% Carbohydrate (25% fructose, 20% Complex), 40% Fat, 15% Protein. After the test meal, blood samples were obtained at the following time points: 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 360 minutes. For the duration of the study day, subjects were instructed to sit in a reclining chair and abstain from any strenuous activity.

Intervention: At the end of the study day, the subjects were randomly assigned to either the high or low physical activity intervention. The high physical activity intervention required the subjects to walk at least 12,500 steps per day, while the low physical activity intervention

required minimal activity and ~4,500 steps per day. The subjects consumed a fructose-rich diet containing 75 grams/day of fructose (two 20 oz Lemon Lime WPOP® drinks, Rochester, NY) along with their ad libitum diet during both conditions. The use of an ad libitum diet was based on the fact that sugar-sweetened beverages are usually consumed in conjunction with an ad libitum diet¹⁷³. Previous research has indicated that within 7 days, metabolic abnormalities can occur with a high fructose diet¹⁹, therefore, a 2-week intervention was chosen to ensure changes occur. The subjects obtained their beverage supply twice weekly at the Human Performance Lab. They were required to return their empty drink bottles to the lab once per week to assess drink compliance and to record step counts. During the FR+Inactive intervention, subjects were instructed to reduce their caloric intake by ~400 kcal/day to offset the additional calories from the drink. A dietician assisted in educating the subjects on how to alter their eating habits to achieve caloric balance during the intervention period. Additionally, subjects had their weight measured during these weekly visits to the lab to monitor any weight changes.

Visit 2: On day 15 following the 14-day intervention period, a post intervention meal test was provided. This test followed the same procedures as visit one.

Two-week washout period. Subjects were instructed to maintain their normal activities of daily living and dietary habits. Previous research has indicated that a two-week washout period is adequate to normalize metabolic markers associated with hyperlipidemia¹⁷⁴.

Visit 3 and 4: These visits followed the same procedures as visit 1 and 2. For the second intervention period, the subjects received the opposite intervention that was originally assigned to them.

Metabolic Assays:

Lipids:

A lipid profile (Cholestech LDX, Biosite International, San Diego, CA) was performed on the samples taken at 0, 60, 120, 180, 240, and 360 min and measured triglycerides (TG), very-low density lipoproteins (VLDL), total cholesterol (TC), and glucose concentrations. The use of this equipment has been recently validated ¹⁷⁵.

Insulin, CRP, TNF α , IL-6:

Blood samples obtained at -5, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 360 min were transferred to BD Vacutainer® Plus Plastic EDTA tubes (Franklin Lakes, NJ), separated by centrifugation, divided into two sets of polypropylene tubes and stored at -80°C for subsequent analysis. Insulin, tumor necrosis factor - α (TNF- α) and interleukin 6 (IL-6) were analyzed using Luminex xMap Technology (Linco Research, St. Charles, MO) on a Luminex 100/200 platform (Luminex Corporation, Austin, TX). All procedures followed the manufacturer's instructions (Millipore, Billerica, MA), with quality controls within expected ranges for each assay (*Insulin*: Inter-assay coefficient of variation (CV): 5.0%; Intra-assay CV: 4.0%, *TNF- α* : Inter-assay CV, 9.9%; Intra-assay CV: 10.6%, *IL-6*: Inter-assay CV: 10.3; Intra-assay CV 11.9%). CRP assays were performed using Quantikine assay kit (R&D Systems, Inc., Minneapolis, MN) [Inter-assay CV: 6.5%; Intra-assay CV: 4.2%]. Insulin sensitivity was calculated by the homeostatic model assessment (HOMA) method as previously described by Matthews et al ¹⁷⁶ and the quantitative insulin sensitivity check index (QUICKI) ¹⁷⁷.

Statistical Analysis:

All results were reported as mean \pm SEM using SPSS 19.0 (Chicago, IL, USA). Descriptive variables and dietary analysis were analyzed using a two-way repeated measures

analysis of variance to depict differences in pre and post intervention weight, BMI, % body fat and macronutrient consumption. A between-subjects analysis was performed on all variables to depict differences in genders. A log transformation (Log10) was used for data that was not normally distributed based on visual appearance of skewed data. Transformations were applied so that the data more closely met the assumptions of normality based on parametric analysis statistical procedures²¹⁵. Lipid and inflammatory variables were analyzed using a three-way ANOVA with repeated measures to assess the changes in lipid measures and inflammatory markers over the 6-hour test day: 2 (high vs. low physical activity) x 2 (pre vs. post intervention) x 18 (time points). If a significant interaction was found, differences between timepoints were analyzed using a paired t-test with Bonferroni correction factor. Postprandial responses for all blood variables were determined by calculating area under the curve (AUC) (Excel, Microsoft Corporation, Redmond, WA) and absolute change from peak to baseline concentrations (Δ peak) for all variables. Statistical significance for AUC and Δ peak concentrations was computed using a two-way repeated measures ANOVA (intervention x pre-post). Pearson's correlation coefficient was computed to determine any correlations between fasting TG and inflammatory variables. A priori significance was set at 0.05.

Results:

Subject Characteristics: Table 1 represents the subject characteristics of the study participants. Males were significantly heavier, taller and had a lower body fat percentage than the females ($p < 0.05$). There were no significant differences in weight (Pre: 67.4 ± 9.1 kg, Post: 66.9 ± 10.2 kg), BMI or % body fat after either intervention (data not shown). There were no gender differences in any of the lipid or inflammatory markers; therefore, all subjects were combined for further analysis. Changes in fasting metabolic, inflammatory and glucose

homeostasis markers for pre- and post- intervention are noted in Table 2. No significant differences were evident in fasting measurements for all variables due to either intervention. Moreover, there were no significant correlations between lipid and inflammatory markers ($p > 0.05$).

Table 1: Descriptive Statistics

	Males (n=11)	Females (n=11)
Age (yr)	20.8 ± 0.7	21.5 ± 0.9
Weight (kg)	76.5 ± 4.1*	58.4 ± 1.9
Height (cm)	178.6 ± 2.3	167.3 ± 2.3
BMI (kg/m²)	23.9 ± 0.9*	21.1 ± 0.5
Body fat (%)	13.8 ± 2.5*	23.6 ± 0.6
VO₂ (ml/kg/min)	46.7 ± 1.2	42.0 ± 1.2
Active Intervention (steps/day)	13,959 ± 719 [†]	12,584 ± 413
Inactive Intervention (steps/day)	4291 ± 160	4155 ± 245

Values are mean ± standard deviations. BMI: Body Mass Index; VO_{2max}: Maximum aerobic capacity. * $p < 0.05$ for differences between genders. [†] $p < 0.05$ differences between intervention steps.

Table 2: Fasting Metabolic Characteristics per and post intervention

	Pre-Active	Post-Active	Pre-Inactive	Post-Inactive
Lipid Markers				
Triglycerides (mg/dL)	82.5 ± 9.5	88.1 ± 11.1	81.0 ± 11.1	80.1 ± 7.3
VLDL (mg/dL)	16.3 ± 1.9	17.4 ± 2.2	19.6 ± 4.2	16.0 ± 1.5
Total Cholesterol (mg/dL)	145.2 ± 6.7	150.4 ± 6.3	142.1 ± 5.7	148.4 ± 6.5
Inflammatory Markers				
TNF-α (pg/ml)	4.8 ± 0.4	4.8 ± 0.3	4.6 ± 0.5	4.7 ± 0.4
IL-6 (pg/ml)	6.4 ± 1.3	5.7 ± 1.3	8.0 ± 2.4	7.2 ± 2.1
C-Reactive Protein (ng/ml)	1001.5 ± 307	831.0 ± 234	1228.7 ± 306	972.8 ± 258
Glucose Homeostasis Markers				
Glucose (mg/dL)	79.9 ± 1.5	80.3 ± 2.3	78.5 ± 1.4	78.5 ± 1.4
Insulin (pmol/L)	104.8 ± 9.2	108.5 ± 9.1	124.3 ± 20.0	98.0 ± 5.5
HOMA-IR	2.9 ± 0.5	3.0 ± 0.5	3.5 ± 1.1	2.6 ± 0.3
QUICKI Index	0.56 ± 0.03	0.71 ± .05	0.82 ± .05	0.52 ± 0.04

Mean ± standard error of the mean (SEM). * $p < 0.05$. HOMA-IR: homeostasis model assessment of insulin resistance; VLDL: very low density lipoproteins. TNF- α : Tumor Necrosis Factor- α ; IL-6: Interleukin-6. (n=22)

Dietary Analysis: The caloric intake was not different between the intervention periods and we observed no change in subject body weight. This suggests that the subjects were in energy balance during each intervention. Likewise there was no difference in the macronutrient composition between the interventions (Table 3). The subjects consumed an additional 75 g of fructose per day from the drink provided (500 kcal, 0 g of fat, 135 g of carbohydrates, 74.9 g of fructose). As with most dietary analysis software, the fructose included in most foods was not available in the dietary analysis.

Table 3: Average daily energy and macronutrient intake between the two interventions.

FR+Active Intervention		%-Cals	FR+Inactive Intervention		%-Cals
Calories (kcal)	2,654 ± 205		Calories (kcal)	2404 ± 197	
Fat (g)	65.2 ± 5.2	22%	Fat (g)	57.8 ± 4.1	21%
Saturated	18.9 ± 0.9		Saturated	14.2 ± 1.5	
Monounsaturated	23.5 ± 0.4		Monounsaturated	11.0 ± 0.9	
Polyunsaturated	30.0 ± 0.6		Polyunsaturated	12.6 ± 2.1	
Cholesterol (mg)	595.0 ± 19.9		Cholesterol (mg)	503.0 ± 23.0	
Protein (g)	116.8 ± 10.8	17%	Protein (g)	115.2 ± 14.6	19%
Carbohydrates (g)	339.9 ± 25.1	61%	Carbohydrates (g)	360.6 ± 19.0	60%
Fiber (g)	22.1 ± 0.7		Fiber (g)	19.0 ± 1.7	
Sugar (g)	185.0 ± 5.3		Sugar (g)	199.0 ± 10.3	

Mean ± SEM

Glucose and Insulin: The test meal induced a significant postprandial response in both glucose and insulin levels ($p < 0.05$) (Fig. 4 A-B). Glucose total AUC and postprandial changes in peak concentrations were not different after either intervention but a significant intervention x time interaction occurred in insulin concentrations ($p = 0.04$). Insulin total AUC for FR+Active intervention decreased from pre to post intervention, whereas, there was no change in insulin AUC after the inactive intervention (Fig. 4B). These differences in AUC can be accounted for by a 19% lower peak insulin response after the FR+Active intervention while FR+Inactive condition was 21% higher post-intervention ($p = 0.009$) (Fig. 4C).

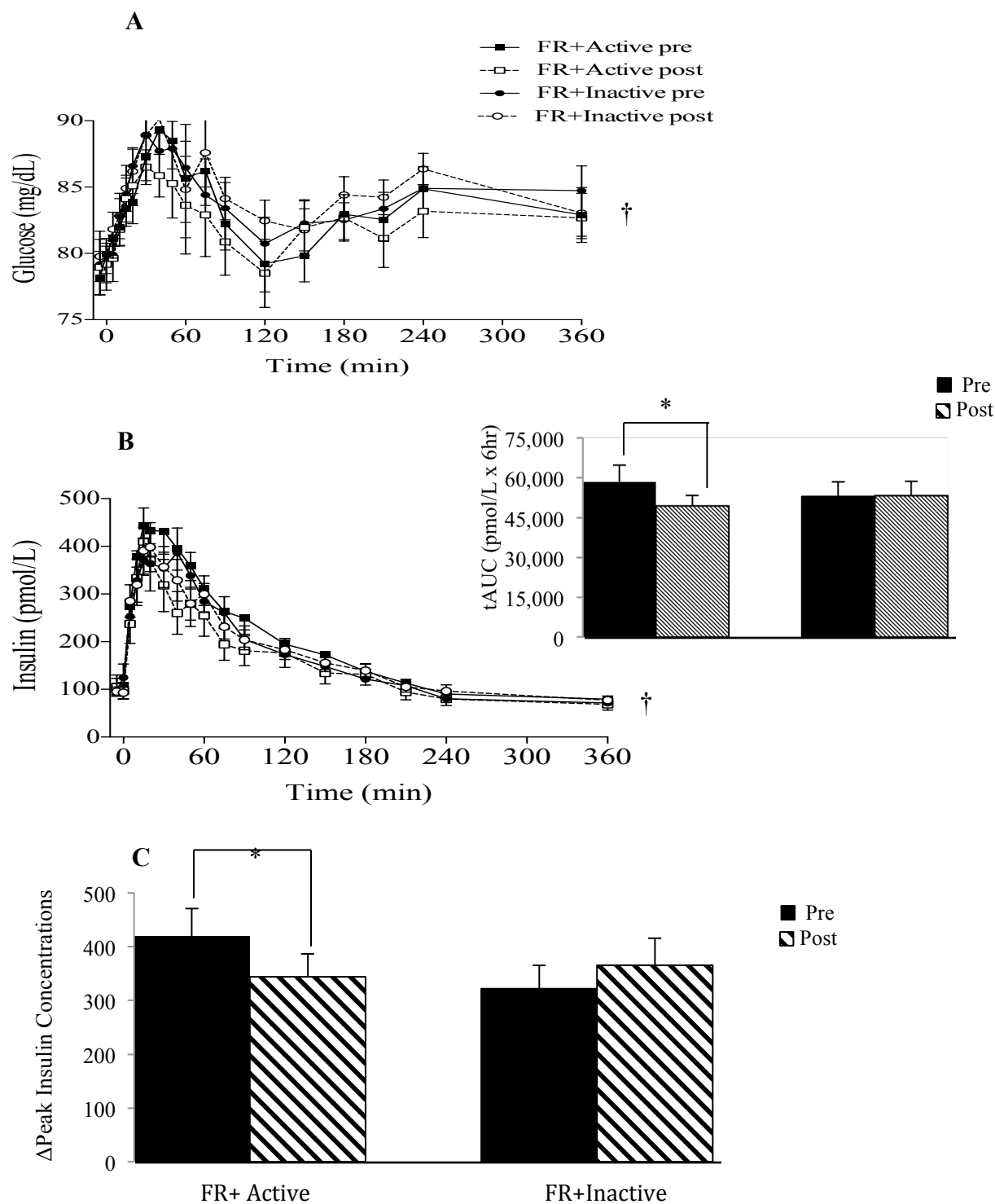


Figure 4: (A) Postprandial response to the test meal on glucose concentrations. (B) Postprandial response to the test meal on insulin concentrations and Insulin total AUC. (C) Δ peak insulin concentrations. Data are expressed as mean \pm SEM. * $p < .05$ significant intervention \times time interaction. † $p < .05$ for main effect of meal.

Triglycerides: Triglyceride concentrations significantly increased in response to the test meal under both interventions ($p= 0.000$) (Fig. 5A). Total AUC for TG concentrations showed a significant intervention x time interaction such that the tAUC increased from pre- to post-FR+Inactive intervention; whereas, there was no change from pre-to-post FR+Active intervention ($p= 0.04$) (Fig. 5B). Similarly, $\Delta_{\text{peak}}[\text{TG}]$ increased by 88% as a result of the FR+Inactive intervention but decreased by 5% ($p= 0.002$) with the FR+Active intervention (Fig. 5C).

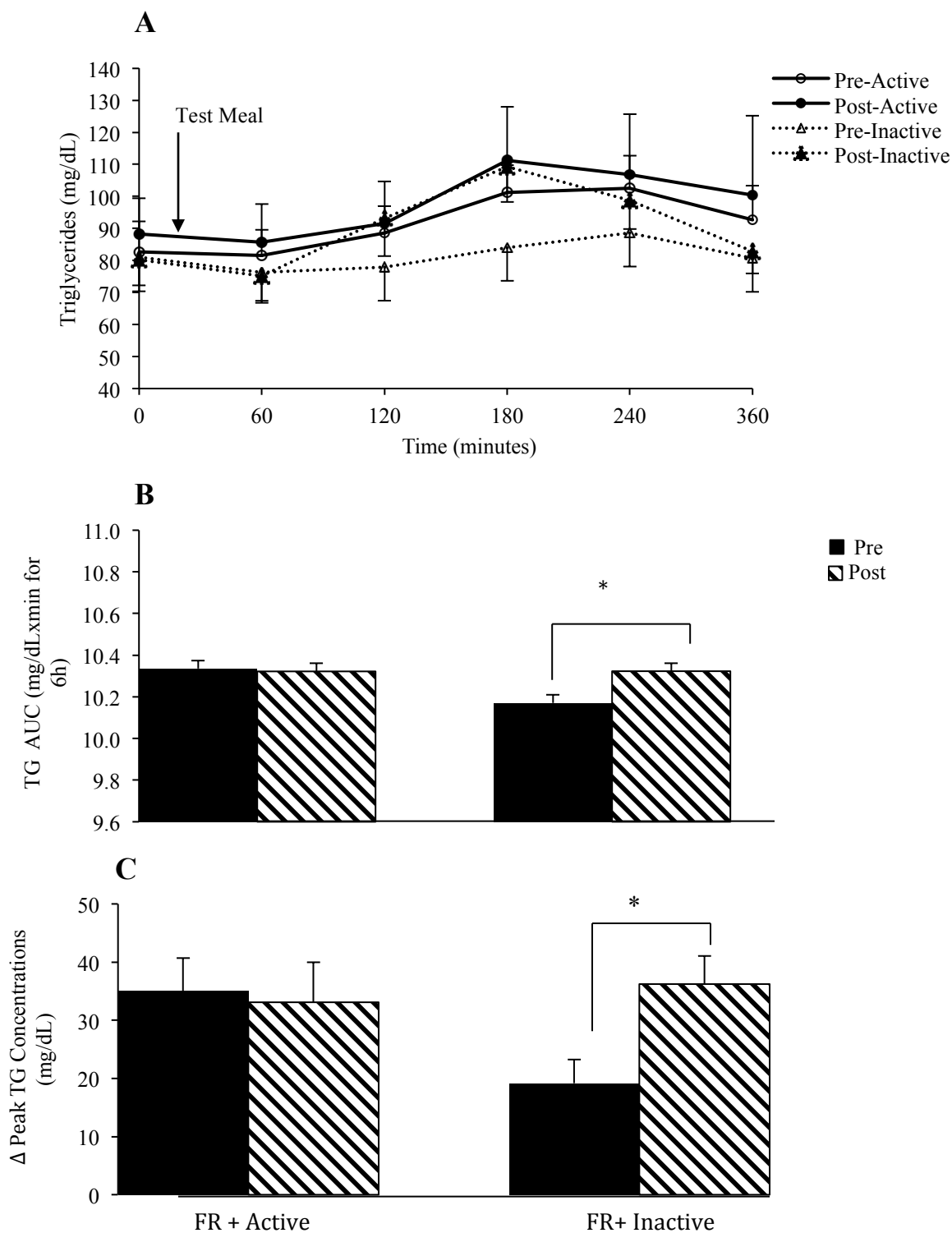


Figure 5: (A) Postprandial effects of the test meal on triglyceride concentrations. (B) Triglyceride total AUC during the 6-hour test visits. (C) Change in triglyceride concentrations from baseline to peak levels. Data are expressed as mean \pm SEM. * $p < 0.05$ intervention \times time interaction. † $p < 0.05$ for main effect of meal.

Very-Low Density Lipoproteins (VLDL): Figure 6a represents the 6 h postprandial response of VLDL concentrations after a test meal. Both pre- and post- FR+Active and FR+Inactive interventions demonstrated a significant main effect across time for VLDL concentrations at which point concentrations peaked at 3 h ($p= 0.029$). Change in [VLDL] induced a significant intervention x time interaction such that the change from pre to post FR+Inactive intervention was significantly larger than the change from pre to post FR+Active intervention. The inactivity induced an 84% increase in Δ [VLDL] whereas only a 33% increase following the FR+Active intervention ($p= 0.009$) (Fig. 6B).

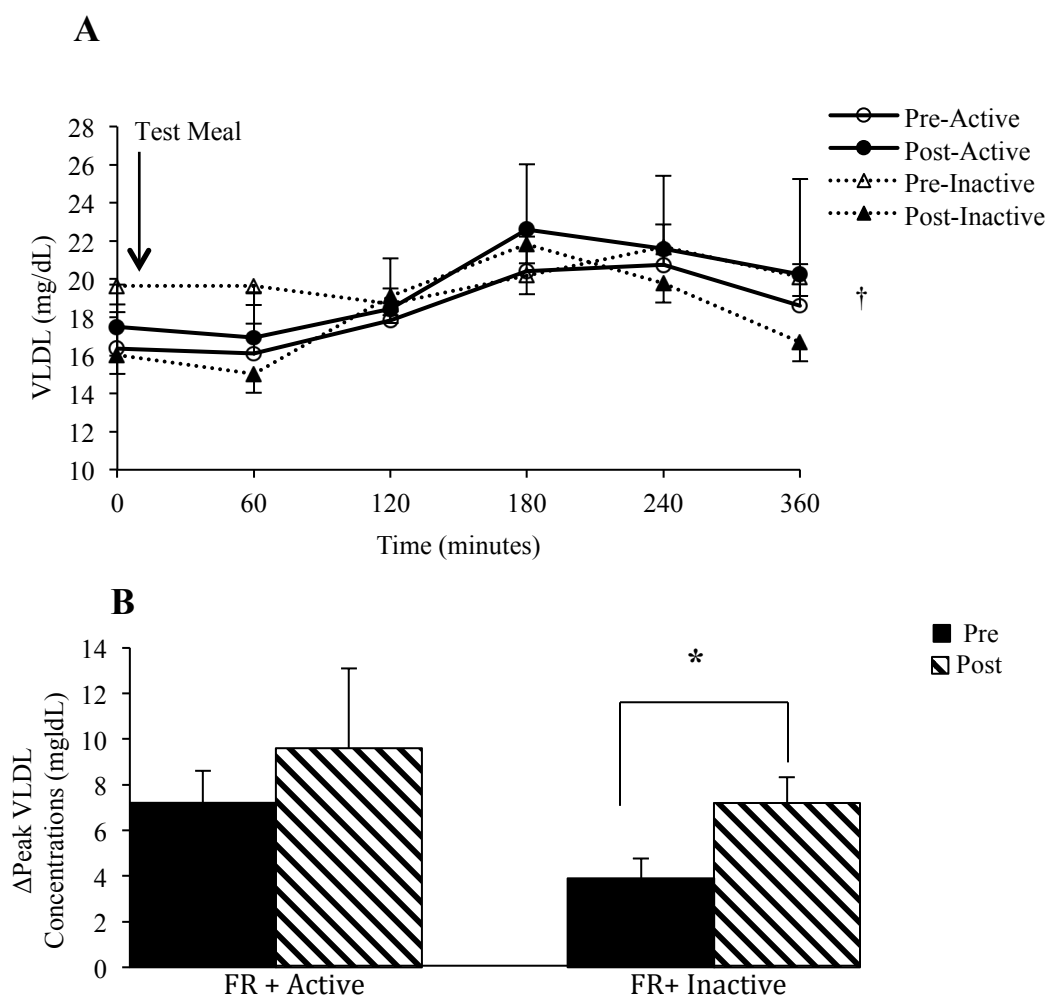


Figure 6: (A) Postprandial effects of the test meal on VLDL concentrations. (B) Change in VLDL concentrations from baseline to peak levels. Data are expressed as mean \pm SEM. * $p<0.05$

intervention x time interaction. † $p < 0.05$ main effect of meal.

Cholesterol: Although subtle, there was a significant main effect across time following the meal for total cholesterol ($p=0.03$) (Fig 7), but total AUC and $\Delta[\text{Cholesterol}]$ was not significantly different between interventions (figure not shown).

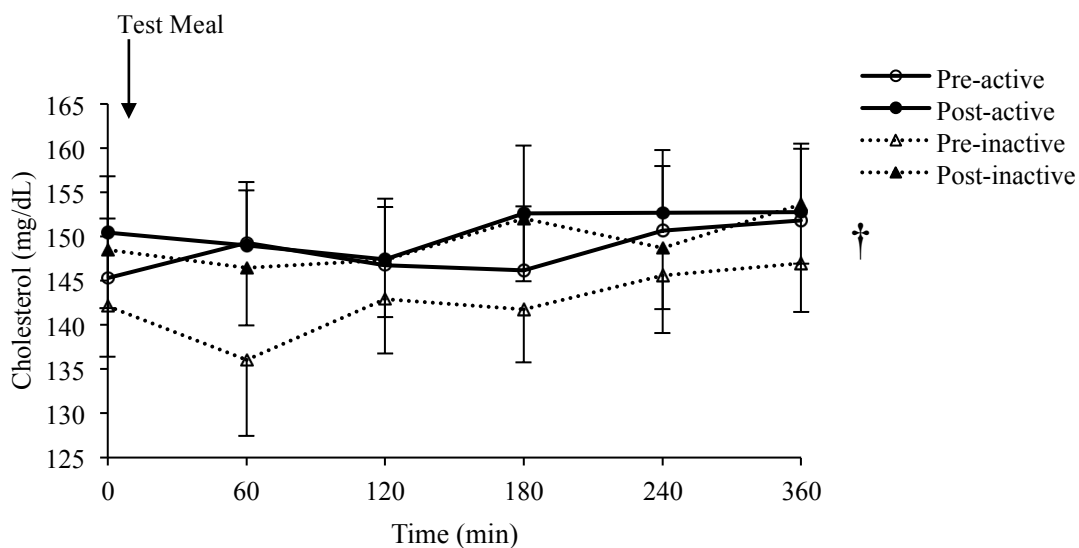


Figure 7: Postprandial response of the test meal on total cholesterol concentrations. Data are expressed as mean \pm SE. † $p < 0.05$ main effect of meal.

TNF- α : In response to the fructose test meal, a significant meal effect ($p=0.02$) was observed for TNF- α concentrations, such that the test meal resulted in an increase in TNF- α concentrations over the course of the 6 h (Fig. 8), however, no changes occurred in CRP concentrations over time (figure not shown). The FR+Active and FR+Inactive interventions did not result in any differences in TNF- α or CRP AUC or change from peak-baseline concentrations ($p > 0.05$).

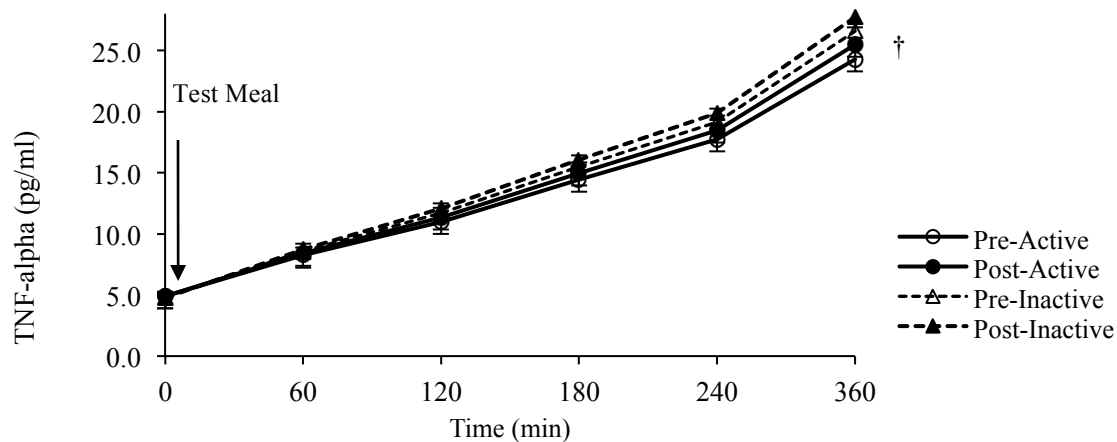


Figure 8: Postprandial response of the test meal on TNF- α concentrations. Data are expressed as mean \pm SEM. † $p < 0.05$ for main effect of meal

In response to the test meal, IL-6 levels showed a significant main effect of time ($p = 0.02$) (Fig. 9A). Change in IL-6 concentrations demonstrated a significant intervention \times time interaction such that IL-6 levels decreased by 30% following the FR + Active intervention while they increased by 116% following the FR + Inactive intervention from pre to post intervention ($p = .048$) (Fig. 9B).

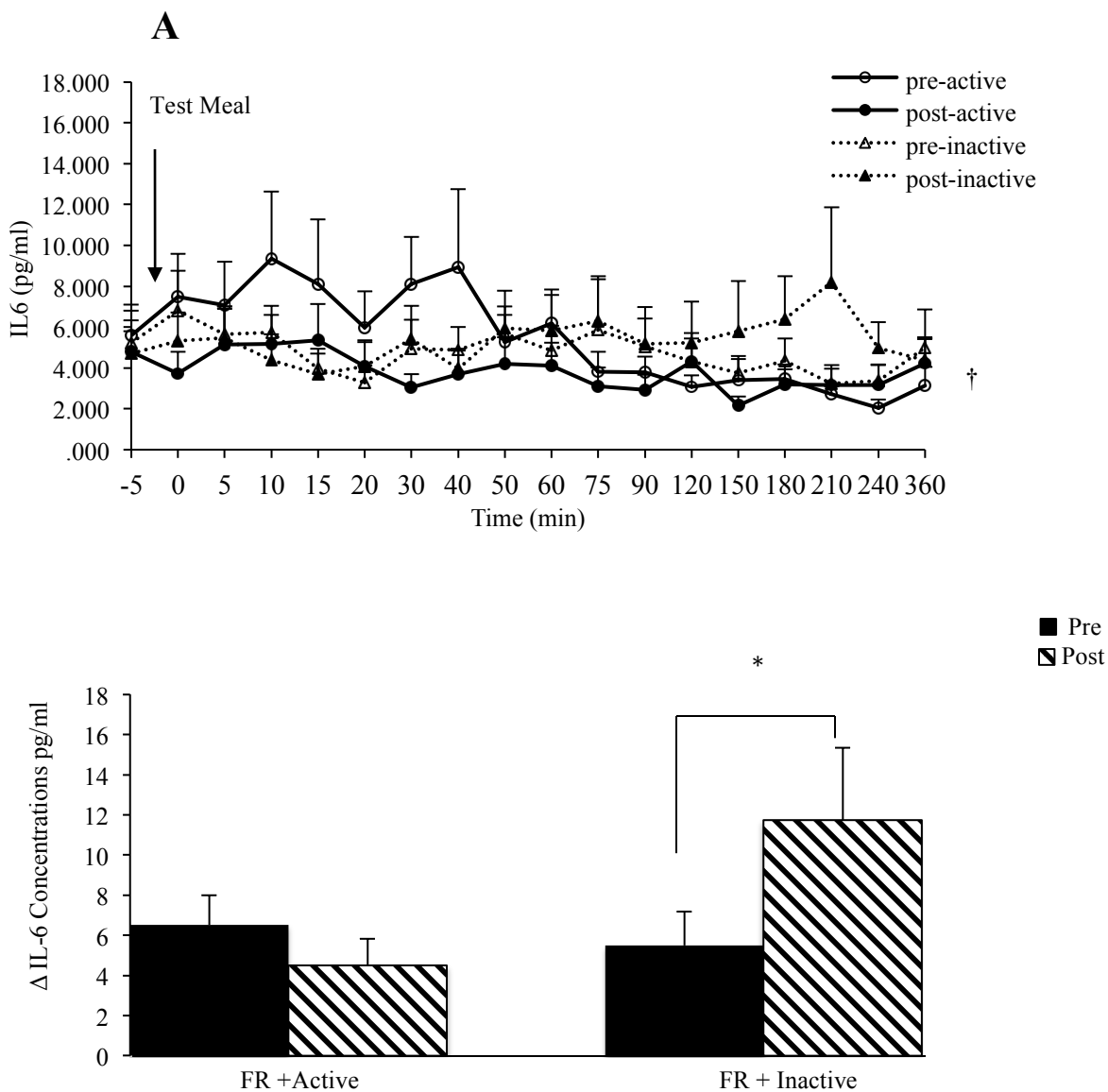


Figure 9: (A) Postprandial effects of the test meal on IL-6 concentrations. (B) Change in IL-6 concentrations from baseline to peak levels. Data are expressed as mean \pm SEM. * $p < 0.05$ intervention \times time interaction. † $p < 0.05$ meal main effect

Discussion:

The purpose of the current study was to determine whether a moderate dose of excess fructose (~75 g/day), ingested for two weeks with an ad libitum diet, would alter the susceptibility for metabolic risk factors associated with metabolic syndrome while simultaneously manipulating physical activity. This study has shown for the first time, that physical inactivity augments the TG and VLDL response to a high fructose meal, as well as enhances the inflammatory response (IL-6 concentrations). These changes were not observed when fructose was consumed while an active lifestyle was maintained, suggesting that the high physical activity offsets the metabolic stress that leads to these hyperlipidemic changes.

Previous research has already suggested that a chronic fructose diet induces hepatic DNL, causing an increase in plasma TG and VLDL concentrations^{19, 49, 178}. For instance, Stanhope et al⁴⁹ found increases in DNL after 10 weeks of fructose loading (25% energy intake) in overweight individuals. Shorter fructose intervention studies (< 3weeks) have discovered similar findings in that increased fructose intake of up to 2 g/kg of body weight/day induced hepatic DNL, leading to increased release of VLDL-TG particles into the systemic circulation^{19, 179}. In agreement with these studies, postprandial TG and VLDL concentrations were elevated in the current study (88% and 84% from baseline, respectively), indicating possible increased hepatic DNL.

During physical inactivity, there is an increased concentration of VLDL-TG's which accumulate most likely as a result of a lower rate of lipoprotein lipase (LPL)-induced fatty acid oxidation¹⁴¹. LPL is responsible for hydrolyzing TG's associated with VLDL and chylomicrons and has a central role in several aspects of lipid metabolism such as the partitioning of triglyceride-derived fatty acid uptake between different tissues, plasma cholesterol metabolism and the subsequent downstream intracellular effects related to lipid availability¹⁴². Levels of

LPL are reduced by 10-20 fold within 12 hours of physical inactivity and may be the contributing factor to increased lipid formation present in the FR + Inactive intervention ¹⁴². During ambulation in which the larger muscles of the body are contracting, there is high LPL activity in the microvasculature of the skeletal muscles. Physically active muscles have greater rates of TG-derived FA uptake, transport and oxidation, which result in reduced FA re-esterification ¹⁴¹. Consequently, during times of inactivity, when energy demands are low, there is an accumulation of TG and fatty acids within the skeletal muscle. In the current study, the elevated postprandial systemic TG and VLDL concentrations during the FR+Inactive intervention could indicate that there was a blunted response of LPL-derived uptake of these fats.

Research by Mamo et al ¹⁸⁰ found that fructose-fed rats had decreased concentrations of LPL activity compared to glucose-fed rats after 10 weeks and this coincided with decreased clearance of VLDL-TG in liver of the fructose-fed rats. Similarly, Roberts et al ¹⁸¹ indicated that TG clearance at the level of the forearm is decreased after a high fructose diet, as a result of decreased LPL activity hydrolysis of VLDL-TG. Although LPL activity was not specifically measured in the current study, the increased VLDL and TG concentrations in the plasma may have been due to suppressed LPL activity resulting from low physical activity. Taken together, the inability of our subjects to hydrolyze VLDL-TG, as a result of decreased LPL activity, as well as increased fructose-induced hepatic DNL, may explain the fructose-induced increases in plasma TG concentrations following FR+Inactive intervention. Therefore, fructose, combined with decreased physical activity may be contributing factors related to the elevated plasma lipids present after periods of physical inactivity.

Although glucose was unaffected by the fructose consumption, insulin levels were 19% lower with increased physical activity. This is consistent with previous research showing

decreased plasma insulin concentrations with physical activity¹⁵⁰. Regular physical activity has beneficial effects on insulin resistance by enhancing insulin signaling, glucose transport and substrate metabolism in muscles¹⁵⁰. Consequently, the increased plasma TG and VLDL concentrations from the FR+Inactive intervention may have caused by an increase in ectopic fats which interfered with second-messengers within the muscle cell responsible for insulin signaling and glucose disposal⁴⁹. Therefore, despite the fact that a high fructose diet increases plasma lipids, which may alter the insulin signaling cascade, increased physical activity seems to offset these deleterious consequences, mostly likely by increasing substrate utilization for energy. Thus this highlights the importance of maintaining high physical activity when the dietary intake is poor.

While it seems apparent that increased physical activity causes a decrease in insulin secretion and plasma insulin concentrations, as is evident in the current study, research has indicated that increased levels of plasma lipids may also interfere with insulin sensitivity by simultaneously stimulating a rise in tissue inflammation^{26, 183}. Our data from the low physical activity arm of the study support this concept as the high fructose diet elicited a 116% increase in peak postprandial IL-6 concentrations, while simultaneously responding to the fructose intake with increased peak postprandial TG and VLDL concentrations. These results are similar to those by Hojbjerg et al¹⁸⁴ who showed that low levels of physical activity were associated with increased levels of CRP, IL-6 and TNF- α . Other studies¹⁸³ also show evidence of increased postprandial lipemia being associated with increased pro-inflammatory cytokines. Though the current study did not find changes in CRP and TNF- α values, the increases in plasma lipids and IL-6 concentrations contribute to the hypothesis that hyperlipidemia and inflammation are linked. Consequently, increased lipid accumulation that occurs with a high fructose diet may be

exacerbating the lipid-induced inflammation that occurs when a low physical activity lifestyle is maintained for an extended period of time. Furthermore, fructose-induced lipid accumulation may play a larger role on inflammation in an obese population, in which low-grade inflammation is often already present ¹⁸⁵.

The postprandial lipoproteins that are produced with chronic, high fructose, low physically active lifestyle can activate leucocytes in the blood and up-regulate the expression of leucocyte adhesion molecules on the endothelium, facilitating adhesion and migration of inflammatory cells into the sub-endothelial space. One of the known effects of IL-6 is to induce the production of hepatic CRP concentrations which is an independent risk factor of cardiovascular disease ⁹⁷. Earlier studies have found increased fasting CRP concentrations in healthy subjects ingesting chronic levels of fructose for at least three weeks ^{185, 186}. We did not find any changes in CRP levels after either intervention, but our lack of response in CRP levels may be due to the length of the interventions. Our study was only two weeks in duration while the other studies were at least three weeks long ^{185, 187}. Moreover, our study was performed on healthy, young individuals, whereas previous studies indicating changes in inflammatory markers with fructose ingestion have been performed on an obese population ^{48, 168}. Obesity itself can increase the release of various adipokines, leading to systemic low-grade inflammation, therefore making it difficult to determine the extent of fructose-induced inflammation ¹⁸⁸. In addition, previous studies ¹⁸⁷ have shown increases in body weight by as much as 7% of baseline, while we kept energy intake balanced between interventions and the subjects did not experience a weight gain. Thus, the increases in CRP levels in the previous studies may have been linked to the weight gain particularly if it was in the visceral region ¹⁸⁹.

Exercise is a known protector against increases in inflammatory markers ¹⁹⁰ and this may

have accounted for the 30% decrease in IL-6 levels observed with physical activity. The increases in IL-6 levels that occur with exercise are known to stimulate the release of many anti-inflammatory cytokines which may cause a long-term effect of lowering low-grade inflammation and chronic IL-6 release¹⁹¹. On the contrary, physical inactivity has been shown to result in significantly elevated inflammatory markers in as little as 10 days of bed rest. Therefore, not only does physical activity increase the release of anti-inflammatory cytokines, physical inactivity also stimulates the release of pro-inflammatory markers. Although pro-inflammatory markers are often up-regulated with physical inactivity, the addition of physical activity did not cause any change in TNF- α activity. These results were not unexpected as research has suggested that TNF- α is not affected by exercise¹⁸⁹. TNF- α and IL-6 are both known to reduce the expression of insulin substrate receptor-1 and GLUT 4 receptors in adipocytes as well as decrease insulin-stimulated glucose transport. Therefore, low-grade inflammation may be an important risk factor predisposing an individual to insulin resistance and type 2 diabetes¹⁸⁴.

In conclusion, a diet consisting of an additional 75 grams of fructose for two weeks increased postprandial lipidemia and systemic inflammation when combined with physical inactivity. This is the first study to our knowledge to provide evidence that high physical activity can minimize the deleterious effects of high fructose consumption in healthy, recreationally active individuals and thus minimizing their susceptibility to both metabolic and cardiovascular risk factors.

Chapter IV

Abstract:

Increased physical activity alters the fructose-induced glycemic response in healthy individuals

Chronic fructose consumption has been positively associated with insulin resistance and decreased insulin sensitivity while exercise is known to positively impact glycemic control by increasing glucose uptake and decreasing insulin secretion. Whether increased physical activity (PA) attenuates the deleterious effects of chronic fructose consumption on glycemic control (FR+Active) has yet to be studied. **Purpose:** The purpose of this investigation was to determine the effect of high fructose consumption on glucose tolerance and the insulin response when combined with either high or low levels of physical activity. **Methods:** Twenty-two normal weight men and women (age: 21.2 ± 0.6 years; BMI: $22.6 \pm .6$ kg/m²) participated in a randomized, cross-over design study in which they ingested an additional 75 g of fructose for 14 days while either maintaining low PA (FR+Inactive) (<4,500 steps/day) or high PA (FR+Active) (>12,000 steps/day). Prior to and following the 2 wk loading period, a fructose-rich meal challenge was administered (45% carbohydrate [25% fructose], 40% fat, and 15% protein). Blood was sampled at baseline and for 6 h after the meal and analyzed for glucagon-like peptide 1 (GLP-1), glucose-dependent insulintropic peptide (GIP), c-peptide, glucose and insulin concentrations. Area under the curve (AUC) and absolute change from peak to baseline concentrations (Δ peak) were calculated to quantify the postprandial responses. **Results:** Plasma insulin, glucose, c-peptide, GIP and GLP-1 concentrations significantly increased in response to the test meal on all test visits ($p < 0.05$). Insulin total AUC decreased with the FR+Active intervention from pre- ($58,470.2 \pm 6261.0$ pmol/L) to post- ($49,444.3 \pm 3883.0$ pmol/L)

intervention; whereas, there was no change after the FR+Inactive intervention (pre: $53,005.4 \pm 5426$ pmol/L; post: $53,939.6 \pm 5429$ pmol/L), resulting in a significant intervention x time interaction ($p < 0.05$). The alterations in AUC coincided with decreased $\Delta_{\text{peak}}[\text{Insulin}]$ from peak values which also decreased after the FR+Active intervention while FR+Inactive intervention increased in response to the intervention ($P = 0.009$). A significant intervention x time interaction occurred for c-peptide incremental AUC, such that the FR+Active intervention decreased by $10,208 \pm 120$ pmol/L x min for 6 h from pre to post intervention and increased by $16,230 \pm 221$ pmol/L ($p = 0.02$) after the FR+Inactive intervention. GLP-1 total AUC ($n=16$) decreased by 18% after FR+Active intervention and by 6% in the Fr+Inactive intervention ($P = \text{NS}$). Males had a lower total GLP-1 AUC than females after the interventions ($p=0.049$). Following the FR+Active intervention GIP total AUC significantly decreased as a result of the intervention with no changes occurring after the FR+Inactive intervention ($p = 0.005$). There were no sex differences in GIP levels. **Conclusions:** Increased physical activity attenuates the deleterious effects on glycemic control caused by a two-week period of high fructose consumption when compared to low physical activity. These changes in glycemic control with physical activity are associated with decreases in insulin and GIP concentrations.

Introduction:

There is strong epidemiological evidence suggesting a causal role between fructose consumption and metabolic disorders¹⁷⁹. Chronic fructose consumption has been linked to hyperlipidemia, visceral adiposity, hyperglycemia and decreased insulin sensitivity, leading to an increased risk for developing metabolic syndrome and type 2 diabetes^{19, 48, 49, 178}. More specifically, the long-term consumption of fructose in healthy, overweight subjects has resulted in increased levels of glucose, insulin, triglycerides, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)^{7, 178}. Overweight individuals who consumed 28% of energy from sucrose (50% fructose, 50% glucose) for 10 weeks experienced increased energy intake, body weight, fat mass, blood pressure and reduced insulin sensitivity compared to non-nutritive sweetener controls¹⁹². Similarly, Stanhope et al⁴⁹ found that when overweight males and females were fed an ad libitum diet rich in fructose (25% energy intake) compared to glucose (25% energy intake) for 10 weeks experienced reduced insulin sensitivity. Although both of these studies concluded that a chronic high fructose diet contributed to increased hepatic lipid accumulation, increased adiposity and decreased insulin sensitivity^{49, 192}, the subjects did not maintain energy balance throughout the intervention. Thus, it is difficult to determine whether these results occurred as a result of caloric imbalance or the fructose itself. Further, an overabundance of fatty acids, glucose and/or amino acids can also negatively impact insulin sensitivity as well¹⁹³. In order to fully elucidate the metabolic ramifications of a high fructose diet, maintaining energy balance and in turn body weight is necessary.

In addition, most of these previous studies^{10, 16-18, 48, 49} did not measure physical activity during the intervention period, and low physical activity can alter glycemic control. Postprandial glucose levels and insulin sensitivity are improved with increased levels of physical activity¹⁹⁴.

Specifically, when middle-aged women participated in light to moderate physical activity for 2 minutes every 20 minutes for five hours, their glucose response to a glucose bolus prior to the first exercise session was significantly reduced at two hours as compared to a condition of no activity¹⁹⁵. For aerobic exercise training, such as running and swimming, skeletal muscle myofibers take on a slow-twitch phenotype, with an increase in the levels of oxidative enzymes, glycogen, and glucose transporter 4 (GLUT4), allowing for enhanced glucose uptake into the cell¹⁵¹.

Although regular physical activity has undisputed health benefits, such as glucose control, the majority of Americans do not meet the minimum physical activity guidelines²¹¹, and there are recognized deleterious effects of low physical inactivity¹⁴¹. Low levels of physical activity decreases energy expenditure and lowers plasma concentrations of lipoprotein lipase (LPL), a key enzyme responsible for the uptake of chylomicron-rich triglycerides from the blood¹⁴¹. The loss of LPL activity during periods of prolonged physical inactivity can lead to decreased uptake of plasma TG and decreased TG hydrolysis. Consequently, the increased TG that remain in the plasma can increase adipose tissue and skeletal muscle ectopic fats, blunting the sensitivity of insulin and decreasing glucose uptake to the cell⁶⁵. Unpublished research in our lab has shown that increased fructose consumption increased plasma TG concentrations. Hence, physical inactivity combined with a fructose-rich diet may increase the potential for insulin resistance and hyperglycemia, exacerbating a state of positive energy balance by suppressing LPL activity⁶⁵.

To date, there is minimal research investigating the role of chronic fructose consumption and decreased physical activity on glycemic homeostasis. Therefore, the purpose of the current study was to determine whether a high fructose diet combined with differing levels of physical

activity altered glycemic control. It was hypothesized that a diet rich in fructose (75 g/day) for two weeks in conjunction with decreased physical activity would negatively alter glycemic control, while a fructose-rich diet and high physical activity (PA) would maintain glucose control.

Methods:

Subject Characteristics:

Twenty-two male and female subjects between the ages of 18-25 years old were recruited to participate in the study. Inclusion criteria were: being recreationally active (3-4 structured workouts per week), a body mass index (BMI) of $< 27 \text{ kg/m}^2$, and a percent body fat of less than 23% for males and 30% for females. Exclusion criteria included suffering from overt cardiovascular disease, hypertension and/or an abnormal lipid profile; taking lipid and/or glucose-lowering medications, anti-depressants, chronic use of NSAID's ($>2\text{x/week}$), daily anti-oxidants usage and suffering from any orthopedic limitations which may inhibit increased physical activity. Subjects were also excluded if they were currently ingesting more than one high fructose corn syrup drink ($>20 \text{ g}$) per day. The women were studied during the first 10 days of their menstrual cycle. All subjects completed an informed consent approved by the Syracuse University Institutional Review Board.

Study Design:

This study employed a cross-over design where all subjects consumed a high fructose diet (75 g/day) while either increasing physical activity to $>12,000$ steps/day (FR+Active) or decreasing physical activity to less than 4,500 steps/day (FR+Inactive). A two-week washout

period occurred between the two interventions. Both interventions were preceded by and concluded with a six-hour study day in which subjects were given a high fructose meal test prior to six hours of metabolic testing.

Pre-Screening visit: After completing a medical history questionnaire, informed consent, and physical activity questionnaire, subjects had their height, weight and body fat (BODPOD System, Life Measurements, Inc., Concorde, CA) measured. A graded exercise treadmill test was employed using a protocol previously described¹⁷¹. Following this visit, subjects were instructed to participate in a one-week control period in which they were required to wear a pedometer (Accusplit Eagle, Livermore, Ca) and an Accelerometer (ActiGraph, GT3X Activity Monitor, Pensacola, FL) in order to collect baseline physical activity data.

Testing Days: Subjects came to the lab at 0700 h following a 12 h fast and no exercise for the previous 24 h. A catheter was placed in their antecubital vein by a registered nurse and then subjects rested for 30 minutes in a supine position before obtaining two baseline blood samples (10 ml each). After baseline blood samples, a high fructose test meal (600 kcals) was prepared for subjects with a macronutrient composition of 45% Carbohydrate (25% HFCS, 20% Complex), 40% Fat, 15% Protein. Blood samples were obtained at time points -5, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 360 minutes. For the duration of the test visit, subjects were instructed to sit quietly. At the end of visit one, subjects underwent a nutritional consultation with a registered dietician to ensure proper compliance with the dietary intervention and to estimate normal fructose intake. At that time, subjects were instructed to refrain from ingesting any added sugar, particularly from sweetened beverages, aside from the drinks for the intervention. Estimates of food intake during the control period and intervention period were collected periodically via food recall (USDA 5-step multi-pass method)¹⁷² and were

analyzed using Diet Analysis Plus (Thomson Corporation) by a registered dietician.

Physical Activity: At the end of visit one, subjects were randomized into either a physically active condition (FR+Active) which required them to walk at least 12,500 steps/day for two weeks or the physically inactive condition (FR+Inactive) which required subjects to walk <4,500 steps/day. All subjects completed both conditions. To assure compliance to the physical activity intervention protocol, subjects were given an accelerometer to wear throughout the 2-week intervention. Data from the accelerometer was downloaded to the computer using ActiLife 5.0 software (ActiGraph, Pensacola, FL). To allow for visual feedback, subjects were also given a pedometer (Accusplit Eagle, Livermore, CA) to wear during the intervention.

Dietary Intervention: During the intervention period, subjects were required to ingest two 20 oz Lemon Lime (WPOP®, Wegmans Corporation, Rochester, NY) drinks which was equivalent to an additional 75 g of fructose, along with their ad libitum diet. To ensure subjects did not increase body weight with the addition of the intervention drinks, a registered dietician counseled all subjects in making dietary adjustments in order to maintain energy balance throughout the intervention period. Twice weekly, subjects obtained their drink supply from the Human Performance Lab and also returned their empty bottles as a measure of drink compliance. At that time, subjects were weighed to ensure weight maintenance.

Washout Period: At the end of the first intervention period, subjects underwent a second testing visit following the same testing procedures as visit one measuring metabolic and hormonal changes induced by the intervention. Upon completion of this intervention, a two-week wash-out period was required in which subjects were instructed to maintain their normal activities of daily living and dietary habits. Previous research has indicated that a two-week washout period is an adequate duration to normalize any metabolic disturbances that may have

been induced by the intervention period¹⁷⁴. Visit three took place immediately following the washout period and followed the procedures of visit one. After visit three, subjects participated in the two-week intervention (FR+Active or FR+Inactive) that was not initially performed during the first intervention (counter-balanced, cross-over design). At the conclusion of the second intervention, subjects underwent the last testing.

Metabolic Assays:

Glucose: Plasma glucose levels were measured using the YSI 2300 STAT Plus™ Glucose & Lactate Analyzer (YSI Incorporated and Xylem, Inc.).

Hormone Assays: Blood samples were obtained and transferred to BD Vacutainer® Plus Plastic EDTA tubes (Franklin Lakes, NJ), separated by centrifuging for 18 minutes, aliquotted into two sets of polypropylene tubes and stored at -80° C for subsequent analysis. The following metabolites were analyzed: GIP (Inter-assay coefficient of variation (CV): 3.9%; Intra-assay CV: 5.9%), GLP-1(Inter-assay CV: 5%; Intra-assay CV: 12%), insulin (Inter-assay CV: 6.1%; Intra-assay CV: 4%), and c-peptide (Inter-assay CV: 6.5%; Intra-assay CV: 3.2%) using a Luminex xMap Technology (Linco Research, St.Charles, MO) on a Luminex 100/200 platform (Luminex Corporation, Austin, TX). All procedures followed manufacturer's instructions (Millipore, Billerica, MA) with quality controls within expected ranges for each assay.

Statistical Analysis:

Data was analyzed using SPSS 19.0 (IBM® SPSS® Statistics, Chicago, IL, USA) and all results are reported as mean ± SEM. Descriptive statistics were analyzed using a 2 (pre vs. post) x 2 (high vs. low activity) repeated measures analysis of variance (ANOVA) to depict

differences. A paired t-test was performed to measure differences in steps between interventions to ensure adequate alterations in physical activity load between interventions. Hormone concentrations were analyzed using ANOVA with repeated measures to assess the changes in postprandial response: 2 (high vs. low physical activity) x 2 (pre vs. post intervention) x 18 (time points) with a between subjects gender variable. Changes in total and/or incremental area under the curve (AUC) (trapezoidal method) and absolute changes in peak concentrations (peak-baseline) (Δ peak [hormone]) for all variables was determined using a 2 (low PA/high PA) x 2 (pre/post) repeated measures ANOVA). A priori significance was set at 0.05.

Results:

Subject Characteristics: Subject characteristics (weight, height, age, BMI, body fat, aerobic capacity, step count) are presented in Table 4. There were no differences in weight (kg), BMI (kg/m^2), body fat (%) and $\text{VO}_{2\text{max}}$ ($\text{ml}/\text{kg}/\text{min}$) after either intervention ($p>0.05$). Males had a significantly lower BMI and % body fat ($p<0.05$). As planned, the steps per day for the FR+Active intervention (range: 11,567-18,845 steps) were significantly higher than the FR+Inactive intervention (range: 2,774- 5,629 steps) for both males and females ($p= 0.00$).

Table 4: Subject Characteristics.

	Pre-Active	Post-Active	Pre-Inactive	Post-Inactive
Age (yr)	21.15 ± 2.8	n/a	n/a	n/a
Weight (kg)-MALES *	74.6 ± 1.9	74.1 ± 1.8	75.2 ± 1.2	75.5 ± 2.0
Weight (kg)- FEMALES *	59.9 ± 4.1	60.1 ± 4.0	60.4 ± 4.1	59.6 ± 4.1
Height (cm)	172.5 ± 2.0	172.5 ± 2.0	172.5 ± 2.0	172.5 ± 2.0
Body Fat (%)	18.7 ± 1.6	18.6 ± 1.6	18.7 ± 1.9	18.9 ± 1.7
BMI (kg/m²)	22.5 ± 0.6	22.1 ± 0.7	22.9 ± 0.9	22.9 ± 0.8
VO₂ (ml/kg/min)	44.7 ± 4.1	n/a	n/a	n/a
HOMA-IR	2.9 ± 0.5	3.0 ± 0.5	3.5 ± 1.1	2.6 ± 0.3
QUICKI Index	0.56 ± 0.03	0.71 ± .05	0.82 ± .05	0.52 ± 0.04
Physical Activity (steps/day)	n/a	12,586 ± 438 [†]	n/a	4223 ± 143 [†]

Values are mean ± standard error of the mean (SEM). *p<0.05 difference between sex. [†]p=0.00 difference between interventions.

Dietary Analysis: Table 5 depicts the macronutrient composition determined from the dietary logs during both interventions. There were no significant differences between the total calories consumed and the percent of total calories from carbohydrates, proteins and fats between the interventions (p>0.05). The intervention drink consisted of an additional 75 g of fructose per day (500 kcal, 0 g of fat, 135 g of carbohydrates, 74.9 g of fructose).

Table 5: Dietary Analysis (n=20)

FR+Active Intervention	Amount	FR+Inactive Intervention	Amount
Calories (kcal)	2,654 ± 205	Calories (kcal)	2,404 ± 197
Fat	22%	Fat	21%
Protein	17%	Protein	19%
Carbohydrates	61%	Carbohydrates	60%

Values are mean ± SEM *p<0.05

Insulin and Glucose: The fructose-rich test meal stimulated an increase in glucose and insulin concentrations postprandially on all test days (Fig 10A & 10B, respectively) (p<0.05). Glucose concentrations peaked at 40 min post-ingestion, returning to fasting values by 120 min

and then rising slightly again between 120-240 min (Fig. 10A), whereas, insulin concentrations peaked at 15 min post-ingestion and returned to fasting levels at 240 min (Fig. 10B). No changes were apparent in glucose AUC or $\Delta\text{peak}[\text{Glucose}]$ (Figures not shown). A significant intervention x time interaction for insulin total AUC occurred such that the FR+Active intervention decreased from pre- ($58,470 \pm 6267$ pmol/L x min for 6 h) to post- ($49,444 \pm 3883$ pmol/L x min for 6 h) intervention; whereas, there was no change after the FR+Inactive intervention (pre: $53,005 \pm 5426$ pmol/L x min for 6 h; post: $53,292 \pm 5479$ pmol/L x min for 6 h) ($p=0.04$) (Fig.10C). Additionally, the $\Delta\text{peak}[\text{Insulin}]$ decreased after the FR+Active intervention but increased after FR+Inactive intervention ($p<0.05$) (Fig. 10D).

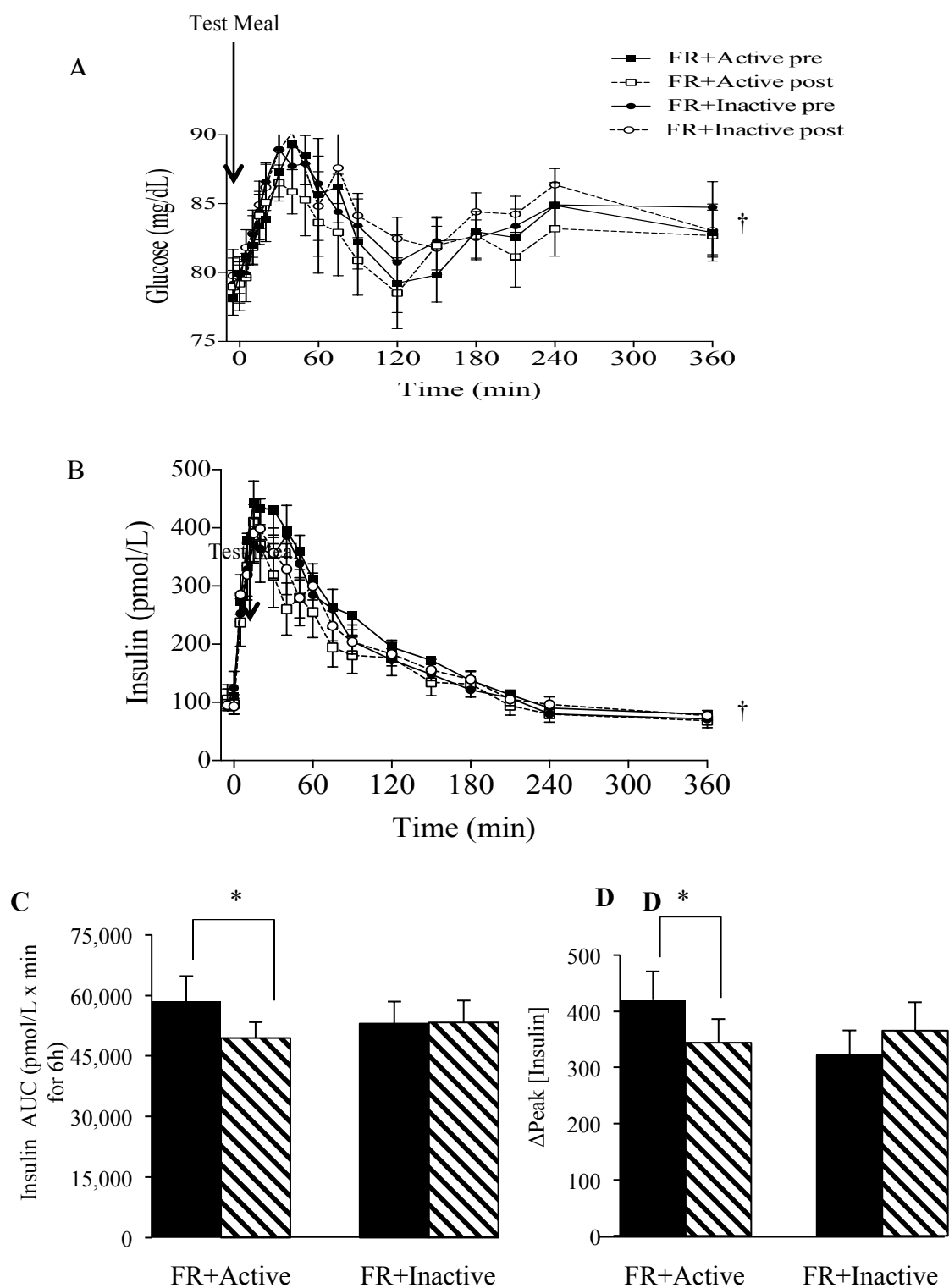


Figure 10: Postprandial response to the test meal on (A) glucose concentrations, (B) insulin concentrations, (C) insulin total AUC, and (D) Δ peak Insulin concentrations. Mean \pm SEM. * $p < 0.05$ significant intervention \times time interaction. ‡ $p < 0.05$ significant main effect for intervention, † $p < 0.05$ significant main effect of meal. (n=22)

C-peptide: Similar to glucose and insulin, c-peptide levels increased significantly in response to the test meal ($p=0.00$) (Fig. 11A). Moreover, the c-peptide incremental AUC for the FR+Active intervention decreased by $10,208 \pm 3300$ pmol/L x min for 6 h from pre to post intervention while c-peptide levels increased by $16,230 \pm 4501$ pmol/L x min for 6 h with the Fr+Inactive intervention ($p=0.04$) (Fig 11B). As a measure of β -cell function, the ratio of Δ c-peptide (30 min-0 min)/ Δ Glucose (30 min-0 min) was calculated. There were no changes in β -cell function with the interventions (data not shown)

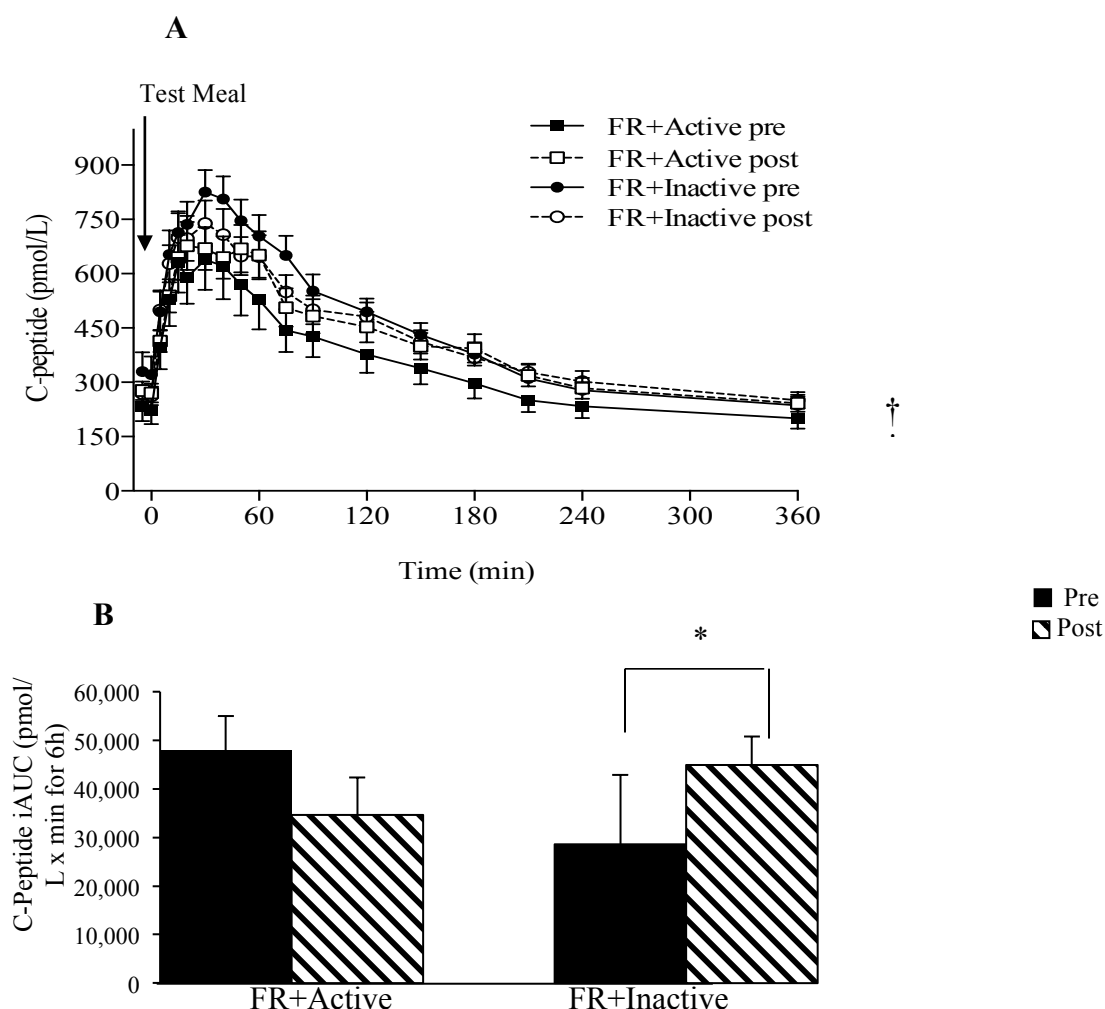


Figure 11 (A): Postprandial c-peptide response to a test meal. (B) incremental AUC c-peptide (pmol/L x min for 6h). † $p=.000$ main effect of test meal. * $p=0.048$ significant intervention x pre/post interaction. Data are expressed as mean \pm SEM. ($n=22$)

GLP-1: There were 2 subjects eliminated from the 22 subjects due to undetectable ranges, therefore GLP-1 and GIP values are based on an N=20. GLP-1 concentrations demonstrated a significant main effect of time in response to the test meal ($p < 0.05$) which was reflected by a decrease during the last three hours (Fig. 12A). There was a significant gender x time (pre/post) interaction such that GLP-1 total AUC in females increased from $13,509 \pm 2488$ pg/ml x min for 6 h to $14,829 \pm 2357$ pg/ml x min for 6 h post intervention yet males decreased from $13,328 \pm 1967$ pg/ml x min for 6 h to $10,877 \pm 1863$ pg/ml x min for 6 h, regardless of the intervention ($p = 0.049$) (Fig. 12B). No changes were evident in peak GLP-1 concentrations.

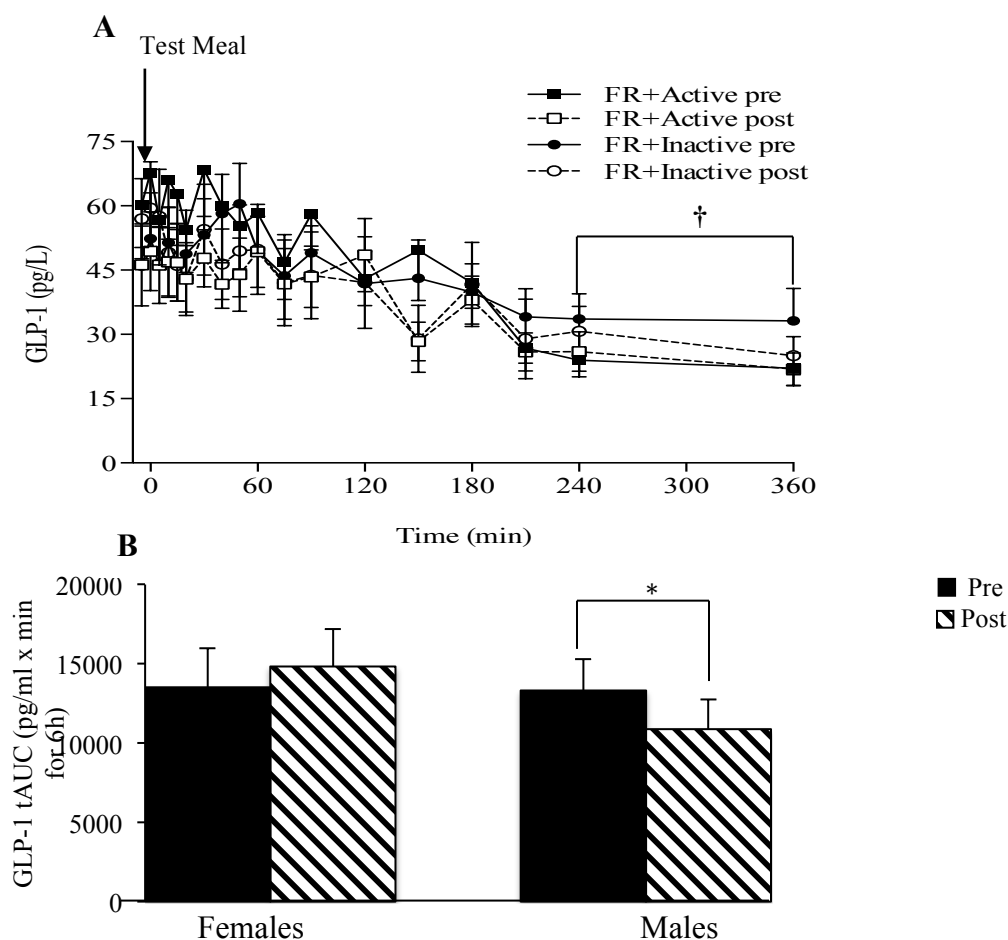


Figure 12 (A): Postprandial GLP-1 (pg/ml) response to a test meal. † $p = 0.000$ main effect of test meal from 180 min to 360 min. (B) total AUC GLP-1 (pg/ml x min for 6 h). * $p = 0.049$ gender x pre/post interaction. Data are expressed as mean \pm SEM. ($n = 20$)

GIP: GIP concentrations demonstrated a significant time effect over the course of the meal ($p=0.00$). GIP increased for the first 75 min postprandially, followed by a decrease over the next 4.5 h in all meal tests (Fig.3A). Following the FR+Active intervention, there was a significant interaction between the intervention and the test meals ($p=0.047$). Incremental GIP AUC decreased by $5,603 \pm 921 \times \text{min for 6 h pg/ml}$ from pre to post FR+Active while GIP AUC increased by $3,231 \pm 657 \times \text{min for 6 h pg/ml}$ following the FR+Inactive intervention (Fig. 3B, $p= 0.047$).

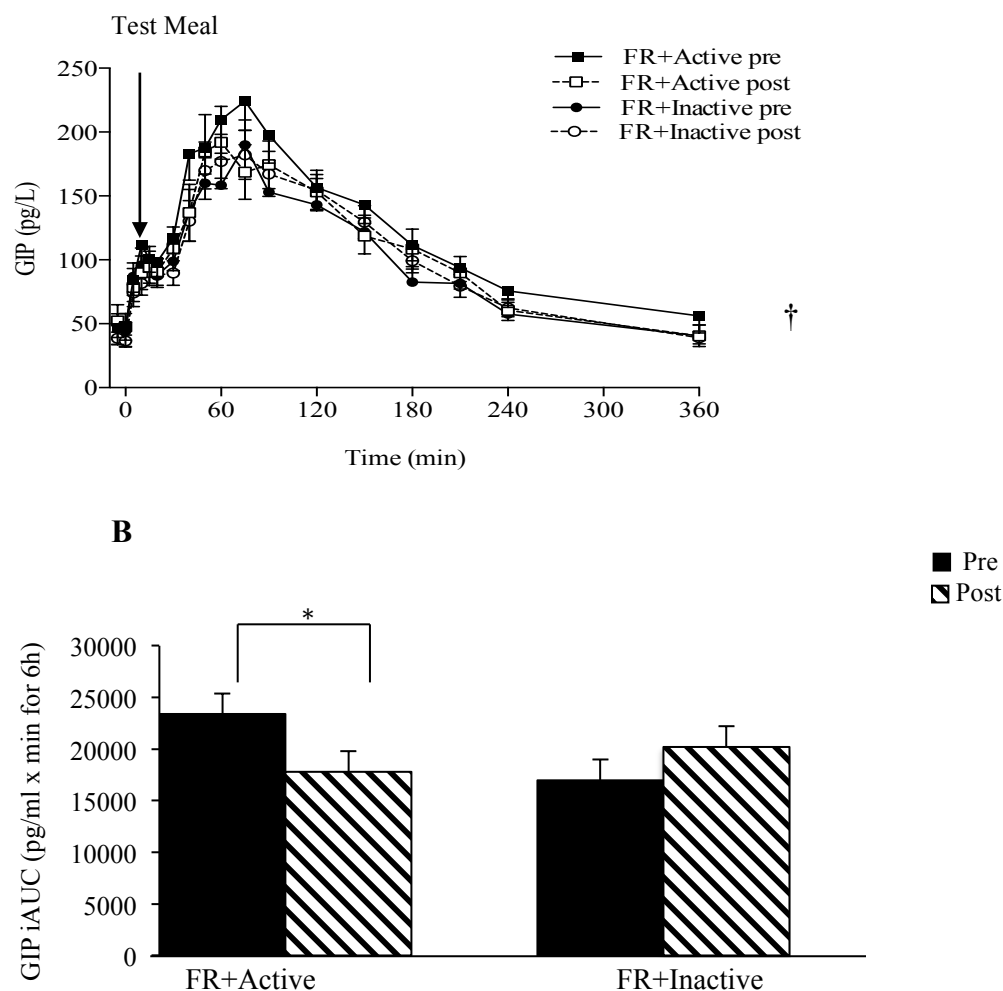


Figure 13 (A): Postprandial GIP response to a test meal. † $p= 0.00$ main effect of test meal. (B) Incremental AUC GIP (pg/ml x min for 6 h). * $p=0.047$ intervention x pre/post interaction. Data are expressed as mean \pm SEM. ($n=20$)

Discussion:

The purpose of the current study was to determine whether decreased physical activity, combined with chronic fructose consumption, altered glycemic control while energy balance was maintained. Although previous studies have already elucidated that chronic fructose consumption negatively alters glycemic control^{49, 64}, this was the first study, to our knowledge, to demonstrate that increased physical activity attenuates the deleterious hormonal changes associated with a fructose-rich diet. Despite no changes in glucose levels, chronic fructose consumption, combined with increased physical activity, elicited decreased insulin secretion (c-peptide levels) and plasma insulin concentrations. Paralleling the changes in insulin secretion was a decrease in GIP levels with increased physical activity, yet no change in GLP-1 concentrations were evident. This study demonstrated that high physical activity conferred protection from poor dietary habits by minimizing the susceptibility to metabolic disturbances.

Modifying physical activity level in the present study did not alter the glucose excursions to the meal test. In contrast however, increased physical activity decreased insulin secretion (c-peptide concentrations) by 27%, leading to a 15% decrease in plasma insulin concentrations after the FR+Active intervention. Although c-peptide and insulin are released from the pancreas in a 1:1 ratio, 50% of insulin secreted is removed on first pass by the liver before reaching circulation²¹⁶ therefore, the lower plasma insulin concentrations, compared to c-peptide levels, were expected. Stephens et al¹⁹³ demonstrated a 19% increase in plasma insulin concentrations with no changes in plasma glucose concentrations after subjects remained sedentary during a 24-hour period. These researchers speculated that changes in insulin action without a corresponding change in glucose concentrations may have been caused by changes in β -cell function. In the current study, we observed no changes in β -cell function but did find increases in peak

postprandial c-peptide concentrations (43%) after the FR+Inactive intervention. It is possible that with a longer period of inactivity, in conjunction with a high fructose diet, elevations in glucose levels may occur via mechanism that were not measured in this study. Consequently, in the face of a high fructose diet and physical inactivity for two weeks, the hormones monitoring glucose control are being modified.

Moreover, peak insulin concentrations occurred sooner than the peak glucose levels. In a healthy, adult population, insulin levels are expected to peak approximately fifteen minutes after peak glucose concentrations, however, in the current study, insulin levels peaked prior to glucose¹⁵. The healthy, active population that was studied may explain this phenomenon as research has demonstrated that active individuals have an increased incretin-stimulated insulin response¹⁹⁸. Therefore, we speculate that the increased peak insulin concentrations, prior to the peak glucose concentrations occurred as a result of insulin being potentiated by the incretin hormones.

Periods of inactivity are known to decrease LPL activity¹⁹⁴, and this suppressed LPL may possibly contribute to changes in insulin concentrations during the FR+Inactive intervention¹⁹⁴. Although LPL activity was not specifically studied in the current investigation, insulin action can be decreased by as much as 18% after one-day of physical inactivity due to decreased LPL activity¹⁹³. The decreased appearance of LPL during periods of inactivity suppresses the uptake and utilization of plasma TG, resulting in increased ectopic fats¹³⁰. In other data from this same project, increased peak postprandial TG concentrations (84%) and very low-density lipoproteins (88%) were observed within two weeks (data not shown). Similarly, Stanhope et al⁴⁹ found that chronic fructose consumption at 25% energy intake increased the average 23-hour postprandial TG concentrations by 38.1 mg/dl after 10 weeks of a high-fructose diet. Increased TG formation is a result of increased fructose-induced hepatic *de novo* lipogenesis⁴⁹, which synthesizes fatty

acids into TG. Thus, the combination of a fructose-rich diet, which increases plasma lipids⁴⁹, and low physical activity, which decreases LPL activity²⁰¹, insulin sensitivity may be modified. Over an extended period of time, ingesting a diet high in fructose while maintaining a sedentary lifestyle, may lead to insulin resistance.

In many of the prior fructose loading studies, caloric intake is not carefully monitored^{43, 47} resulting in the subjects being out of energy balance. A strength of the current study was that energy balance was maintained throughout the intervention period, hence we can attribute the changes observed in insulin action to the decreased physical activity, and not an energy deficit. Although energy balance was maintained, increased plasma TG concentrations from the high fructose diet (data not shown) may have contributed to the elevated insulin concentrations by negatively affecting cell-signaling associated with insulin receptors¹³⁹. The changes observed in insulin secretion (c-peptide levels) may have been to counteract these changes. In the long term, the disruption in insulin cell signaling resulting from elevated TG's may cause decreased glucose uptake into the cell, possibly inducing a hyperinsulinemic state if not balanced with increased physical activity.

One novel finding of the current study was the GIP response to increased physical activity. The decrease in GIP levels mirrors the changes in c-peptide levels such that both hormones decreased with physical activity and increased with physical inactivity. This data suggests that the changes in the insulin response to the meal test after increased physical activity may not only be due to changes in insulin sensitivity¹⁵⁰ but may be mediated by alterations in GIP secretion. Likewise, previous research by Kelly et al¹⁹⁸ and Solomon et al¹⁹⁶ noted that increased long-term physical activity, in conjunction with a hypo-caloric diet, suppressed the GIP-induced insulin secretion from pancreatic cells, suggesting that increased physical activity relieves β -cell stress

by inducing changes in β -cell and k-cells, helping to restore normal incretin function¹⁹⁸. Although Kelly et al¹⁹⁸ suggested that it was the energy deficit induced by the hypo-caloric diet in combination with the exercise that decreased the GIP levels^{196, 198}, in the present study, there were no differences in energy intake or energy expenditure between the two interventions, therefore other mechanisms must be mediating the decreased GIP response in the physically active intervention. Further, other studies^{212, 213} have shown an attenuation of GIP levels after a glucose load in exercise-trained individuals, and reported that this was a result of cellular changes within the β -cells of the pancreas and the K cells of the intestine¹⁹⁸. Interestingly, however, is that fructose seems to augment the GIP release from k-cells. Mazzaferri et al²⁰⁴ has revealed that high levels of sucrose, which is made up of 50% fructose, over-activates the k-cells of the intestine, subsequently leading to increased GIP-induced insulin secretion from the pancreas. Hence, even though a high-fructose diet increases GIP response to a test meal,²⁰⁴ during periods of high physical activity, the increased insulin sensitivity resulting from the exercise decreased the need for GIP-induced insulin secretion, yet the identification of the specific mechanism could not be accomplished in the current study.

Unlike GIP levels, GLP-1 concentrations did not respond to the physical activity loading or unloading. The lack of significance may have occurred due to the smaller sample size used for the GLP-1 analysis or that GLP-1 may not be as sensitive to changes in physical activity as GIP. Furthermore, the gender differences that were detected with GLP-1 concentrations may have altered the findings. Prior research has noted higher GLP-1 AUC in females than males, possibly because of the higher percent body fat in women²⁰⁷. We observed gender differences following the meal test with the males showing an 18% decrease in GLP-1 AUC following the both interventions while females demonstrated an increased GLP-1 AUC (9%) post-interventions.

Gender differences have also been reported in other peptides such as resistin, adiponectin, and leptin and may be related to increased percent body fat but also may be related to differences in sex steroids ²¹⁴.

As with all human research, there were strengths and limitations to this study. A major strength of the current study was that the fructose load consumed by the subjects was more closely aligned with the average fructose intake of the US population ¹⁰. Many of the earlier studies ^{19, 49} used doses that were extremely high, thus these findings have less external validity. Furthermore, although the subjects participated in an ad libitum diet, there were no weight changes indicating subjects were in energy balance. This is in contrast to a previous study ⁴⁹ in which weight gain occurred while on a high fructose, ad libitum diet, confounding the interpretation of their findings. The weight maintenance observed in the present study, allows us to conclude with confidence that the changes observed in glycemic control and insulin secretion were due to the changes in physical activity level and not weight changes. Lastly, the inclusion of both males and females in our study demonstrated that gender appears to play a role in the GLP-1 response to physical activity and fructose consumption, but not with the other metabolic hormones.

In conclusion, our findings demonstrated that increased physical activity conferred protection from the fructose loading. Increased physical activity attenuated fructose-induced GIP release and may be subtly impacting β -cell function with the resultant effect of a decreased insulin secretion, in the face of no change in glucose level. Consequently, chronic long-term use of fructose combined with physical inactivity could cause an increase in deleterious risk factors associated with insulin resistance. Moreover, GLP-1 responses to fructose loading and physical activity seem to be gender dependent and further research is needed in this area.

CHAPTER V:

Conclusions, Strengths/Limitations, and Future Perspectives

I. Conclusions:

Chronic consumption of fructose-sweetened beverages has been associated with hyperlipidemia, hyperinsulinemia, and obesity⁴². Due to the contradictory research^{43,47, 51} available today, the aim of the current investigation was to better elucidate whether a high fructose diet, in which concentrations were more similar to that of the average U.S. daily intake, can result in postprandial lipidemia, low-grade inflammation and altered glucose homeostasis in as little as two weeks. A sedentary lifestyle often accompanies a diet rich in sugar, therefore, we examined whether increased physical activity with a high-fructose diet (75 g) for two weeks would alter postprandial plasma concentrations of TG, VLDL, cholesterol, TNF- α , IL-6, glucose, insulin and GIP and GLP-1 hormones.

This study has determined that a moderate, relevant dose of fructose, in conjunction with physical inactivity, increased postprandial lipidemia and low-grade inflammation, as seen with increases in TG, VLDL and IL-6 levels. Increased physical activity of ~12,500 steps/day ameliorated these effects in as little as two weeks. Additionally, a high fructose diet when consumed during a low physically active lifestyle altered insulin secretion while high physical activity conferred protection by improving insulin secretion. This study also demonstrated that these changes in insulin levels with high physical activity are possibly mediated by changes in the incretin response. Taken together, our investigation supports previous research indicating that chronic consumption of fructose increases postprandial lipidemia, low-grade inflammation and

alters glycemic control. Our study showed that a highly physically active lifestyle alters the susceptibility to cardiovascular risk factors and metabolic syndrome.

II. Strengths

There were several strengths to the current investigation. First, many nutritional intervention studies tightly control the subject's diet, making the results less clinically relevant. In the current investigation, the subjects were instructed to eat their normal diets while only limiting their consumption of sugar-sweetened beverages and sweetened desserts, allowing the results to provide a better understanding of the consequences of the American diet. Additionally, the fact that the subjects maintained body weight throughout the intervention, with little variation in the macronutrient consumption of the diets, allowed us to determine the effects of physical inactivity during a period of fructose loading without the confounding factor of weight gain. In previous studies^{49, 51}, weight confounded the results.

Secondly, numerous studies^{19, 49, 51} have measured the lipidemic effect of excessive doses of fructose up to 200 g of fructose/day. These values do not correspond to the average U.S daily intake of added sugar (~65 g/day), whereas our investigation incorporated a more realistic dose of 75 g/day of added fructose. Lastly, most of the metabolic markers investigated previously were measured under fasting conditions^{51, 205}. Americans spend a majority of their waking time in a postprandial state in which they consume fructose-rich food every 2-4 hours. Examining the metabolic changes following a fructose load allows for the identification of the potential consequences of consuming a high fructose diet.

III. Limitations:

There were a few limitations to this project that must be addressed. First, although the sample size was comparable to previous research^{39, 43, 47}, a larger sample size may offset the large subject variability we observed in the studied variables. Secondly, we studied healthy, recreationally active adults who may have been more resistant to the fructose and activity level. Potentially more deleterious findings may be seen with an older, and/or obese population. Therefore, the ability to provide substantial dietary and lifestyle recommendations across populations is limited. Lastly, insulin sensitivity was not directly measured, which limited the ability to fully establish whether changes seen were due to peripheral glucose uptake or changes in hepatic glucose output.

IV. Future Direction:

Future studies should include quantifying LPL activity to determine if reduced physical activity is associated with changes in LPL activity. This would provide better evidence of whether a reduction in physical activity (<4500 steps/day) altered the enzymatic activity of LPL associated with increased TG uptake. Additional metabolic markers to measure would be the inclusion of various adhesion molecules associated with inflammation such as E-selection, P-selection, monocyte chemo-attractant protein -1 to better understand the extent to which fructose and inactivity alter low-grade inflammation and endothelial dysfunction as both are significant risk factors associated with metabolic syndrome.

Various methodical alterations such as a direct measurement of fractional DNL as well as insulin sensitivity via euglycemic-insulinemic clamp could be employed in order to determine whether an increase in fructose consumption with increased physical activity will directly alter insulin and glucose uptake as well as TG and VLDL formation. In conjunction with the

aforementioned change, the addition of muscle biopsies to clarify changes in insulin cell-signaling pathways would provide better evidence that second messengers associated with insulin signaling are being altered as a result of increased fructose-induced ectopic fats.

Changes in study design in which longer test days are utilized with more test meals, such as those incorporated by Stanhope et al⁴⁷ and Parks et al⁵¹, would provide more specific details of the postprandial effects of a high fructose test meal. Secondly, using an obese population may allow one the determination of whether various populations may be effected differently than the healthy, recreationally active individuals used in the present study. Thirdly, comparing a subject group in which they participated in the required 30-60 min of moderate exercise, 3-5 times/week to a group that was physically active all day (>12,000 steps/day) to determine whether being physically active has the same benefit as exercising most days of the week. Frequently, individuals will exercise for 45 minutes/day but are sedentary the rest of the day. This design would allow for comparison of structured exercise to daily physical activity on these cardiovascular and metabolic risk factors. Lastly, the addition of a control group such as glucose and/or water would allow the researcher to better determine whether the metabolic and hormonal alterations were caused by the fructose ingestion or alterations in physical activity.

Appendix

Items included:

1. Informed Consent
2. Institutional Review Board Approval Letter
3. Physical Activity
4. Health History Questionnaire
5. Dietary Logs



SYRACUSE UNIVERSITY
Institutional Review Board
MEMORANDUM

TO: Stefan Keslacy
DATE: August 10, 2010
SUBJECT: Expedited Protocol Review - Approval of Human Participants
IRB #: 10-203
TITLE: *Metabolic and Cardiovascular Alteration to Fructose Consumption with Physical Activity*

The above referenced protocol, submitted for expedited review, has been evaluated by the Institutional Review Board (IRB) for the following:

1. the rights and welfare of the individual(s) under investigation;
2. appropriate methods to secure informed consent; and
3. risks and potential benefits of the investigation.

Through the University's expedited review process, your protocol was determined to be of no more than minimal risk and has been given **expedited approval**. It is my judgment that your proposal conforms to the University's human participants research policy and its assurance to the Department of Health and Human Services, available at: <http://www.orip.syr.edu/humanresearch.html>.

Your protocol is approved for implementation and operation from **August 10, 2010 until August 9, 2011**. If appropriate, attached is the protocol's approved informed consent document, date-stamped with the expiration date. This document is to be used in your informed consent process. If you are using written consent, Federal regulations require that each participant indicate their willingness to participate by signing the informed consent document and be provided with a copy of the signed consent form. Regulations also require that you keep a copy of this document for a minimum of three years.

CHANGES TO APPROVED PROTOCOL: Proposed changes to this protocol during the period for which IRB approval has already been given, cannot be initiated without IRB review and approval, except when such changes are essential to eliminate apparent immediate harm to the participants. Changes in approved research initiated without IRB review and approval to eliminate apparent immediate hazards to the participant must be reported to the IRB within five days. Protocol changes are requested on an amendment application available on the IRB web site; please reference your IRB number and attach any documents that are being amended.

CONTINUATION BEYOND APPROVAL PERIOD: To continue this research project beyond **August 9, 2011**, you must submit a renewal application for review and approval. A renewal reminder will be sent to you approximately 60 days prior to the expiration date. *(If the researcher will be traveling out of the country when the protocol is due to be renewed, please renew the protocol before leaving the country.)*

UNANTICIPATED PROBLEMS INVOLVING RISKS: You must report any unanticipated problems involving risks to subjects or others within 10 working days of occurrence to the IRB at 315.443.3013 or orip@syr.edu.

Office of Research Integrity and Protections
 121 Bowne Hall, Syracuse, New York 13244-1200
 (Phone) 315.443.3013 ♦ (Fax) 315.443.9889
orip@syr.edu ♦ www.orip.syr.edu

STUDY COMPLETION: The completion of a study must be reported to the IRB within 14 days.

Thank you for your cooperation in our shared efforts to assure that the rights and welfare of people participating in research are protected.



Kathleen King, Ph.D.
IRB Chair

Note to Faculty Advisor: This notice is only mailed to faculty. If a student is conducting this study, please forward this information to the student researcher.

DEPT: Exercise Science, 201 Women's Bldg.

STUDENT: Amy Bidwell



**Syracuse University
Department of Exercise Science**

**Impact of Physical Activity on Postprandial Hyperlipidemia after
Chronic Fructose Ingestion**
IRB#10-203

You are being asked to participate in a study examining the impact of physical activity on fat levels after a meal following two weeks of fructose ingestion. This study is recruiting 24 non-obese healthy young males and females (18-35 yr). Involvement in the study is voluntary, so you may choose to participate or not. This form will explain the study to you. Please feel free to ask questions about the research if you have any. I will be happy to explain anything in greater detail if you wish.

Over the past decade, the consumption of high fructose corn syrup has increased dramatically in response to the effort of manufactures to market foods that are sweet yet do not cause a dramatic rise in blood sugars. . As a result, the consumption of fructose has increased to a point where it now promotes chronic metabolic abnormalities such as high blood pressure, obesity, elevated cholesterol and high blood glucose levels. The proposed research study will investigate the effects of two weeks of fructose on blood fats in conjunction with a increased physical activity. Physical activity is often a lifestyle intervention used to prevent risk factors associated with metabolic abnormalities. Therefore, we will research whether increased physical activity will alter any metabolic abnormalities associated with fructose ingestion. Additionally the function of your vessels are often altered with increased fat levels, however, whether these changes will occur after chronic fructose ingestion is yet to be determined.

The primary aim of the study will be to assess whether 2 weeks of fructose ingestion will increase postprandial fats and whether increased physical activity will alter such responses. A secondary aim will do to assess whether cardiovascular function will be altered with fructose ingestion and if increased physical activity will improve such responses.

Initial Visit- Pre-screening:

You will have your body composition assessed by measuring height and weight (BMI; kg/m²), and through the use of air-displacement plethysmography (BODPOD system, Life Measurement, Inc. Concorde, CA) which is explained further on page 4. You will then be asked to answer various general health, physical activity, and sedentary behavior questionnaires. Your blood pressure, resting heart rate and cholesterol will be measured.

Once questionnaires are filled out, you will be asked to perform a graded exercise test on a treadmill. During the graded exercise test on a motorized treadmill, peak aerobic capacity (VO₂peak) will be determined by oxygen consumption and CO₂ production using a calibrated Cosmed Quark b2 metabolic breath-by-breath system (Parvomedics). Heart rate will be

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monitored and recorded using a heart rate monitor (Polar USA). Rating of perceived exertion and blood pressure will be collected at the end of each 2-min stage. Treadmill speed will begin at 2.5 mph/0% grade, and will increase every two minutes 0.5 mph until 3.5 mph. Treadmill speed will then be maintained at 3.5 mph and percent grade increased by 2% every 2 min until volitional fatigue is reached. This visit will last approximately 1.5 hours. Starting immediately following this visit, you will begin your one-week control period at which time you will continue with your normal activities of daily living and typical diet.

Visit 1 – Test Day: (Occurring immediately following the one-week control period.) Upon arrival to the lab on the testing days (0700h), following a 12-hour fast and no exercise 24 hours prior to testing, you will have a catheter inserted in the antecubital vein by a registered nurse. You will then rest in a supine position for 30 minutes before obtaining baseline blood samples. Baseline blood samples will be taken prior to the ingestion of the test meal and at 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 360 minutes for measurements of triglycerides (TG), free fatty acids (FFA), oxidized low density lipoproteins (oxLDL), high density lipoproteins (HDL), insulin, glucose, fructose, uric acid and inflammatory markers such as tumor necrosis factor α (TNF- α) and interleukin 6. Test meal will include the following: 600 kcals, 45% Carbohydrate (25% HFCS, 20% Complex), 40% Fat, 15% Protein

Study Day Ingredients and Quantity

Solid Food	Quantity (g)	
Sunny Meadows Large Eggs	139.5	2.79 Whole Eggs
Thomas Better Start Light Multi Grain English Muffins	65.55	1.15 English Muffins
I Can't Believe It's Not Butter Mediterranean Blend	22.54	1.61 Tbsp

HFCS Drink		
Swanson Fructose	20.6	1.58 Tbsp
NOW Sports Glucose (Dextrose)	16.9	1.69 Tbsp
Great Value Artificial Sweetener (Acesulfame K, Aspartame)	1.1	½ Packet
Vintage Sodium Free Carbonated Water	236.588	8 oz

Endothelial Function Measurements: Vascular function will be measured at baseline and at 1,2,3,4,5, and 6 hours post test meals. One inflatable cuff will be placed around your upper arm, while another inflatable cuff will be placed around your wrist to occlude hand circulation. FBF will be determined by inflating the upper arm cuff to 50 mmHg, to allow for arterial inflow without venous outflow, for seven seconds, followed by an eight second deflation, creating fifteen second cycles for 1.5 minutes. To obtain measures of vasodilatory capacity, reactive hyperemia (RH) will be induced by occluding arm blood flow with an additional cuff placed over the existing upper arm cuff and inflated to 100 mmHg above resting systolic blood pressure (SBP) for a period of five minutes. After rapid release of the inflated upper arm cuff, FBF will be measured as described above.

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At the conclusion of visit 1, you will undergo a nutritional consultation with a registered dietician to ensure proper application and compliance with the dietary intervention. Further, estimates of food intake during the control and intervention period will be collected by a periodic, random 24-hour recall (via telephone). The same researcher will administer the recall to all subjects. This visit will last approximately 6.5 hours. You will be instructed to refrain from ingesting any sugar-containing beverages such as fruit juices during the control and intervention period. You will also not be allowed to participate in any exercise during the study duration other than what is prescribed to you. At this time you will begin your first 2-week intervention period during which time you will consume a fructose-rich diet containing an additional 75grams/day of fructose (2 20oz Sprite® drinks) along with your normal diet. You will be instructed to drink 2 servings per day with a meal for two-weeks. You will obtain their beverage supply twice weekly at the Human Performance Lab. At this point you will be randomized to either a physically active group (>12, 000 steps) or an inactive group (< 3,000 steps). You will be instructed to wear an Actigraph ® accelerometers to calculate physical activity as well as a basic step pedometer to provide visual feedback with a step counter.

Visit 2- Test Day: This visit will take place at the conclusion of the first intervention period. At this time the subjects you will repeat the same procedures as visit 1. After visit 2, you will begin the washout period

After visit 2, the two-week wash-out period will begin. You will be instructed to return to your normal dietary habits and again refrain from consuming any additional sugar-containing substances.

Visit 3-Test Day: This will take place immediately after the washout period and will be the same as visit 1 and 2. After visit 3, you will begin the second intervention period in which you will drink the fructose containing beverage for two weeks and perform the physical activity protocol (cross-over design).

Visit 4- Test Day: You will come in 24 hours after the end of the second intervention period for your final study day. The procedures for this visit will be the same as the previous test visits. The total time commitment to participate in this study from start to finish is 7 weeks (1-week control period, 2 week intervention period, 2-week washout period, followed by the last 2-week intervention period).

On one of these visits, you will also have your percent fat measured in the BodPod. You will need to bring a bathing suit. You will be asked to get into a large machine and sit quietly for 10 minutes so we can determine how much air you displace while in this machine. You will be asked to put a mouthpiece into your mouth (like a snorkel), and breathe normally, then give 5

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small puffs of air. This allows us to measure of how much air was in your lungs. This can be done as part of your first visit or can be done at another time. This will take about 20 minutes of your time.

We do not expect this testing will cause you any undue soreness or discomfort. The risks to you are minimal and listed below:

1. The risks associated with the exercise test include increased blood pressure, and heart arrhythmias (abnormal heart beat). There is a very small risk of a heart attack during the exercise. To minimize this risk we will do the initial exercise test to screen for any abnormalities.
2. Bod Pod is safe, although you may feel some discomfort if you do not feel comfortable in small space. A technician well trained with the procedure will conduct this test and can minimize this anxiety.
3. Blood sampling. Blood sampling may cause fainting, and some discomfort and/or bruising at the site on your arm where the blood was taken, and rarely an infection. Using a trained Registered Nurse to place the line will minimize any risks.
4. Forearm blood flow - the brief occlusion with the blood pressure cuff may result in a slight discomfort during the testing. Rarely this may result in bruising.
5. Fructose drink- The participants will be drinking a carbonated beverage containing 75grams of fructose everyday for a total of 4 weeks. Risks associated with the drink may be slight weight gain (minimal) and stomach discomfort. There is a 2 week washout period between interventions to minimize any side effects associated with the fructose drink.

The benefit of this research will help us to better understand how physical activity may impact metabolic abnormalities associated with chronic fructose ingestion

Your participation in this study is entirely voluntary and you may refuse to participate or discontinue participation at any time without penalty or loss of benefits to which you would normally be entitled. Your decision about whether or not to participate in the study will not affect your relationship with Syracuse University. There are no costs to you and/or your insurance carrier for participating in this study. You will be paid \$150.00 on completion of your last visit. If you do not complete the study, this amount will be prorated.

All information will be kept confidential; this means that your name will not appear anywhere and no one will know about your specific test results except me and the other investigators working on this research project.

In the event of illness or physical injury resulting from taking part in this research study, we have not set aside money to pay for related injuries. Signing this form does not waive any legal rights.

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If you have any questions about the research or in the event of a research-related injury, please contact Amy Bidwell at (315) 569-3543 or ajsauve@syr.edu. If you have any questions about your rights as a participant, or if you have questions, concerns or complaints that you wish to address to someone other than the investigator or if you cannot reach the investigator, please contact the Syracuse University Institutional Review Board Office at (315)-443-3013.

All of my questions have been answered, I am over 18 yr of age and I wish to participate in this research study. I also acknowledge that I will be given a copy of this consent form.

_____ Signature of participant	_____ Date
_____ Printed name of participant	
_____ Signature of witness	_____ Date
_____ Signature of investigator	_____ Date

Appendix 3
Physical Activity Questionnaire

NAME: _____

DATE: _____

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling,?

Think about only those physical activities that you did for at least 10 minutes at a time.
_____ days per week or _____ None

1b. How much time in total did you usually spend on one of those days doing vigorous physical activities?
_____ hours _____ minutes

2. Again, think only about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ days per week or _____ none

2b. How much time in total did you usually spend on one of those days doing moderate physical activities?
_____ hours _____ minutes

3. During the last 7 days, on how many days did you walk for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.

_____ Days

3a. How much time in total did you usually spend walking on one of those _____ days?
_____ hours _____ minutes

Health History Questionnaire

Please answer the following questions to the best of your ability. For the following questions, unless otherwise indicated, circle the single best choice for each question. As is customary, all of your responses are completely confidential and may only be used in group summaries and/or reports. All information collected is subject to the Privacy Act of 1974. If you have any physical handicaps or limitations that would require special assistance with this questionnaire, please let your trainer know. This form is in accordance with the American College of Sports Medicine guidelines for risk stratification when followed correctly by your trainer. Your trainer should be certified with a national organization in order to use these forms correctly.

Name: _____ Ht.: _____ Wt.: _____

Gender: _____ Age: _____ Birthdate: _____

Address: _____

City: _____ State: _____ ZIP: _____ Phone: _____

Emergency Contact: _____ Phone: _____

Personal Physician: _____ Phone: _____

E-mail: _____

1. Have you ever had a definite or suspected heart attack or stroke?Yes No
2. Have you ever had coronary bypass surgery or any other type of heart surgery?Yes No
3. Do you have any other cardiovascular or pulmonary (lung) disease
(*other than* asthma, allergies, or mitral valve prolapse)?Yes No
4. Do you have a history of: diabetes, thyroid, kidney, liver disease.Yes No
(**circle all that apply**)
5. Have you ever been told by a health professional that you have had
an abnormal resting or exercise (treadmill) electrocardiogram (EKG)?Yes No

6. If you answered YES to any of Questions 1 through 5, please describe:

7. Do you currently have any of the following:
- a. pain or discomfort in the chest or surrounding areas that occurs when you engage in physical activity?Yes No
 - b. shortness of breathYes No
 - c. unexplained dizziness or faintingYes No
 - d. difficulty breathing at night except in upright positionYes No
 - e. swelling of the ankles (recurrent and unrelated to injury)Yes No
 - f. heart palpitations (irregularity or racing of the heart on more than one occasion)Yes No
 - g. pain in the legs that causes you to stop walking (claudication)Yes No
 - h. known heart murmurYes No
- Have you discussed any of the above with your personal physician?Yes No

8. Are you pregnant or is it likely that you could be pregnant at this time?Yes No
 If yes, what is your expected due date? _____

9. Have you had surgery or been diagnosed with any disease in the past 3 months?Yes No
 If yes, please list date _____ and surgery/disease _____

10. Have you had high blood cholesterol or abnormal lipids within the past 12 months or are you taking medication to control your lipids?Yes No

11. Do you currently smoke cigarettes or have quit within the past 6 months?Yes No

12. Have your father or brother(s) had heart disease prior to age 55 OR mother or sister(s) had heart disease prior to age 65?Yes No

13. Within the past 12 months, has a health professional told you that you have high blood pressure (systolic \geq 140 OR diastolic \geq 90)?Yes No

14. Currently, do you have high blood pressure or within the past 12 months, have you taken any medicines to control your blood pressure?Yes No

15. Have you ever been told by a health professional that you have a fasting blood glucose greater than or equal to 110 mg/dl?Yes No

16. Describe your regular physical activity or exercise program:
 type: _____
 frequency: _____ days per week
 duration: _____ minutes
 intensity: *low* *moderate* *high* (circle one)
 BMI: _____

17. If you have answered YES to any of questions 7-16, please describe:

- 18. Are you currently under any treatment for any blood clots?Yes No
- 19. Do you have problems with bones, joints, or muscles that may be aggravated with exercise?Yes No
- 20. Do you have any back/neck problems?Yes No
- 21. Have you been told by a health professional that you should not exercise?Yes No
- 22. Are you currently being treated for any other medical condition by a physician?Yes No
- 23. Are there any other conditions (mitral valve prolapse, epilepsy, history of rheumatic fever, asthma, cancer, anemia, hepatitis, etc.) that may **hinder** your ability to exercise?Yes No
- 24. During the past six months, have you experienced any **unexplained** weight loss or gain (greater than ten pounds for no known reason)?Yes No

25. If you have answered YES to any of questions 18-24, please describe:

26. Please list below all prescription and over-the-counter medications you are currently taking:

Medicine:	Reason for taking:	Dosage:	Amount/Frequency:
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

27. Are there any medicines that your physician has prescribed to you in the past 12 months which you are currently not taking?Yes No

If so, please list:

I have answered the Health History Questionnaire questions accurately and completely. I understand that my medical history is a very important factor in the development of my fitness/wellness program. I understand that certain medical or physical conditions which are known to me, but that I do not disclose to my trainer, may result in serious injury to me. If any of the above conditions change, I will immediately inform my trainer of those changes. I, knowingly and willingly, assume all risks of injury resulting from my failure to disclose accurate, complete, and updated information in accordance with the attached questionnaire. I also understand that in order to properly risk stratify my Health History Questionnaire, my trainer should have a minimum of a national certification as a personal trainer. My trainer also verbally explained this statement to me to my understanding.

Client's Signature: _____ Date: _____

Trainer's Signature: _____ Date: _____

Appendix 5
Dietary Food Record
Department of Exercise Science

Instructions:

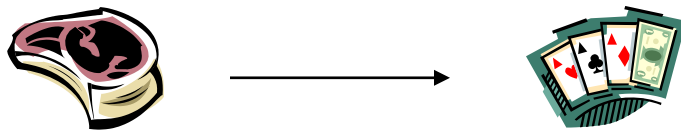
- Fill in as much detail as you can regarding the food and drink that you consumed on these particular days
- The dietary record table is divided into three sessions: Morning, Afternoon and Evening
- Please record all the food and drinks you consume, including any snacks that you had between meals.
- The table has a column where you can write the quantity of food and the food description
- The quantity of food:
 - In this section please provide as much information as possible regarding the quantity of food
 - You do not need to weigh the food necessarily, but if you can provide some sort of measure (1 tablespoon of oil; 1 medium sized apple; 1 handful of nuts)
 - If packaged, the serving information will be available on the package
- Food Description:
 - In this section please provide information regarding the food type
 - Try to be as specific as possible, for instance:
 - Brown Rice; White Rice; long grain rice
 - Potato Crisps-include the manufacturer and flavor
 - Normal spaghetti noodles; Whole-meal spaghetti noodles
 - Low-fat Ice cream (manufacturer)
 - Yoghurt (manufacturer)
 - Juice (manufacturer); high calcium; low sugar

These are just some examples related to the completion of the dietary record. If you have any questions related to completing this sheet please do not hesitate to contact Amy on:

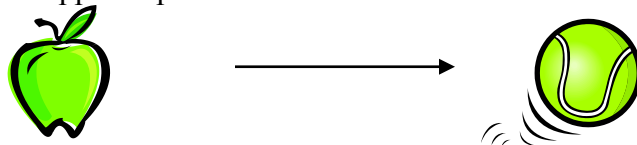
Email: ajsauve@syr.edu

Some helpful serving size comparisons:

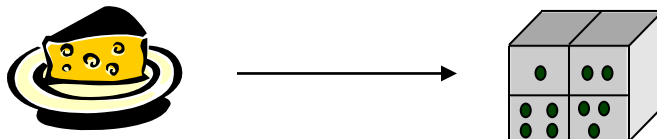
1. 3 ounces of meat is about the size and thickness of a deck of playing cards or a cassette tape.



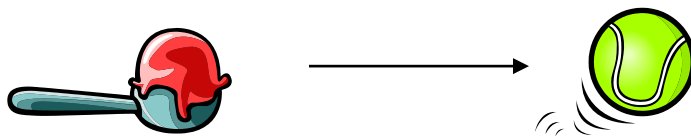
2. A medium apple or peach is about the size of a tennis ball.



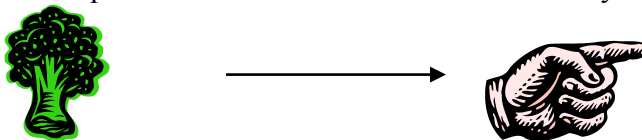
3. 1 ounce of cheese is about the size of 4 stacked dice.



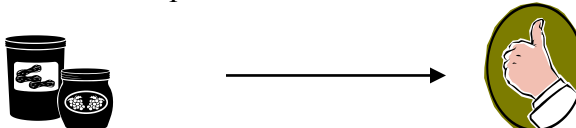
4. ½ cup of ice cream is about the size of a tennis ball.



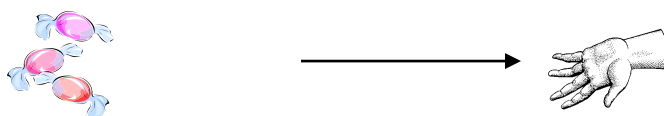
5. 1 cup of mashed potatoes or broccoli is about the size of your fist.



6. 1 teaspoon of butter or peanut butter is about the size of the tip of your thumb.



7. 1 ounce of nuts or small candies equals one handful.



Dietary Food Record
Department of Exercise Science

	Day One:	
	Quantity	Food Description
Morning	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____
Afternoon	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____
Evening	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____
Other	_____	_____
	_____	_____
	_____	_____

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Curriculum Vitae

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Academic Preparation:

Degree	Institution	Years
Ph.D.	Science Education Syracuse University Syracuse, NY	2008-present
M.S.	Exercise Science Syracuse University Syracuse, NY	2005-2007
B.S.	Physical Education SUNY Brockport Brockport, NY	1996-1999

Teaching Experience:

Assistant Professor	<i>SUNY Oswego</i> HSC 315: Nutritional Concepts HSC 332: Scientific Principles of Fitness HSC 386: Contemporary Issues in Wellness	Fall 2011-Present
Adjunct Instructor	<i>New York Chiropractic College:</i> <i>School of Applied Clinical Nutrition</i> NTR 5501: Exercise Physiology and Sports Nutrition NTR 5101:Macronutrients	January 2010-present Sept 2010-present
	<i>Onondaga Community College</i> Personal Training Certification Course	March 2009-Fall 2010

Course Instructor	<i>Syracuse University</i> PPE 483: Principles of Conditioning PPE 295: Introduction to Exercise Science PPE 200: Becoming a Personal Trainer PED 295: Advanced Weight Training	Spring 2010 - 2011 Spring 2011 Spring 2009-2011 Fall '06 – Spring '08
Lab Instructor	<i>Syracuse University</i> PPE 497: Physiology of Exercise PPE 515: Graded Exercise Testing	Fall '06 - present Spring '07
Lab Coordinator	<i>Syracuse University</i> Human Performance Lab	Fall '08 - present
Guest Lecturer:	Invited Lecturer – Syracuse University Department of Nutrition Invited Lecturer- Hamilton College “The Real Truth Behind Fructose” Graduate Seminar Series: Metabolic, Hormonal and Cardiovascular Effects of Fructose Ingestion, Syracuse University,	Fall '10 & Fall '11 October 2010 Fall 2008
Professional Experience:		
Research Assistant <i>Joslin Diabetes Center</i>	Upstate Medical Center, Syracuse, NY - Assisted in patient evaluations for the SHINE study - Observed diabetes nutritional counseling	Summer 2009
Personal Trainer	Mid-Town Athletic Club, Rochester, NY - Taught monthly health and wellness seminars <i>(nutrition, functional training, etc)</i> - Responsible for conducting fitness evaluation for all new members - Performed 30 - 35 personal training sessions per week	May '99 -Aug '05
Fitness Supervisor	Mid-Town Athletic Club, Rochester, NY - Supervised staff of 20 Fitness Specialists - Mentored exercise physiology interns from area colleges - Developed and implemented numerous member retention programs - Responsible for interviewing, hiring and training of all Fitness Specialists - Assisted in preparing a yearly fitness budget of \$750,000.00	Aug '01 – Aug '05

Presentations

“High physical activity attenuates the hyperlipidemic effect of a fructose-rich meal. American College of Sports Medicine Annual Meeting. Poster Presentation. San Francisco, Ca. May 2012.

“Effect of Diet and/or Exercise on GLP-1 Levels in Type 2 Diabetic Women”. FEATURED PRESENTATION at American College of Sports Medicine Annual Conference. Baltimore, Md. 2010

“Growth Hormone Responses to Fructose Ingestion During and Post-Exercise”
Mid-Atlantic Regional Chapter (MARC) of American College of Sports Medicine (ACSM) Annual Meeting – November 2009

“The Effect of Yoga Therapy on Improving Quality of Life in Asthmatics”. American College of Sports Medicine Annual Meeting. Seattle, WA. May 2009.

“Metabolic and Cardiovascular Responses to the Ingestion of Fructose”
APS Intersociety Meeting: The Integrative Biology of Exercise-September 2008

“Metabolic and Cardiovascular Responses to the Ingestion of Fructose”
Mid-Atlantic Regional Chapter (MARC) of American College of Sports Medicine (ACSM) Annual Meeting – November 2008

Guest speaker at Mid-Atlantic Regional meeting of American College of Sports Medicine – November 2008. “Careers in Exercise and Meet the Expert”

Graduate Seminar Series: Metabolic, Hormonal and Cardiovascular Effects of Fructose Ingestion, Syracuse University, Fall 2008

Peer-reviewed Manuscripts:

“Yoga Training Improves Quality of Life in Women with Asthma”.

Amy J. Bidwell, Beth Yazel, RN, David Davin, Timothy J. Fairchild, Jill A. Kanaley. THE Journal of Alternative and Complementary Medicine. Volume 18, Number 8, 2012, pp. 1–7.

“Metabolic and Cardiovascular Responses to the Ingestion of Fructose”

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Body image does not predict caloric estimation accuracy

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Published Abstracts:

Amy J Bidwell, Timothy Fairchild, Jill A. Kanaley. “High Physical Activity Attenuates The Hyperlipidemic Effect Of A Fructose-rich Meal”. *Medicine & Science in Sports & Exercise*: May 2012 - Volume 44 - Issue 5 - pp S171.

Jill A. Kanaley, **Amy J. Bidwell**, Timothy Fairchild. “High Physical Activity Counteracts the Altered Incretin Response to High Fructose Consumption.” *Medicine & Science in Sports & Exercise*: May 2012 - Volume 44 - Issue 5. pp S419.

Amy J. Bidwell, Beth Yazel, David Davin, Timothy J Fairchild, Jill A Kanaley, FACSM. The Effect of Yoga Therapy on Improving Quality of Life in Asthmatics”. *Medicine & Science in Sports & Exercise*: May 2009 - Volume 41 - Issue 5 - pp 43-44.

Lara R DeRuisseau, Daniel M Recca, **Amy J Bidwell**, Nadia J Orosz, Kimberly M Pompo, and Keith C DeRuisseau. Impact of metallothionein deficiency on soleus muscle function following acute spinal cord injury. *FASEB J.* 2008 22:962.25

Professional Committees:

SUNY Oswego School of Education Faculty Council –Secretary

Department of Health Promotion and Wellness Student Organization Faculty Advisor

Search committee member for Associate Professor in the Department of Exercise Science at Syracuse University

Professional Organizations/Certifications:

Future Professoriate Member-Syracuse University

Obesity Society - Member

American College of Sports Medicine(ACSM) – Member

ACSM-Nutrition Task Force Member

Mid-Atlantic Regional Chapter of American College of Sports Medicine – Member

National Academy of Sports Medicine – Certified Personal Trainer (2003)

YogaFit – Certified Yoga Instructor (2007)

American Red Cross – CPR/AED Certified

Funding and Academic Awards

2012 SUNY Oswego Curriculum Innovation Grant (\$2400). Funded.

2012 SUNY Oswego Early Start Faculty Research Grant (\$5000.00). Funded.

2010 Syracuse University School of Education Travel Grant. (\$400). “Effect of Diet and/or Exercise on GLP-1 Levels in Type 2 Diabetic Women”. FEATURED PRESENTATION at American College of Sports Medicine Annual Conference. Baltimore, Md. 2010. Funded

2009 Syracuse University School of Education Travel Grant. (\$400) “The Effect of Yoga Therapy on Improving Quality of Life in Asthmatics”. American College of Sports Medicine Annual Meeting. Seattle, WA. May 2009. Funded.

2009 Syracuse University School of Education Research and Creative Grant (\$1,000)

“Growth Hormone Responses to Fructose Ingestion During and Post-Exercise”. Funded.

2008 Syracuse University School of Education Research and Creative Grant (\$1,000)
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