

## ABSTRACT

Title of dissertation: HIGH SUCROSE, FRUCTOSE, AND GLUCOSE DIETS  
AND GLUCOCORTICOID DYSREGULATION IN  
RATS

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Approximately two-thirds of U.S. adults are overweight or obese and the prevalence of overweight in children has tripled since 1980. Intake of added sugars has also increased. The etiology of obesity remains unclear and the role of glucocorticoids in obesity is one area of ambiguity.

The enzyme 11 $\beta$ -hydroxysteroid dehydrogenase-1 (11 $\beta$ -HSD-1) interconverts active and inactive glucocorticoid, thereby regulating intracellular glucocorticoids. Dysregulation of 11 $\beta$ -HSD-1 in liver and adipose is characteristic of human and animal models of obesity. Hexose-6-phosphate dehydrogenase (H6PDH) is colocalized with 11 $\beta$ -HSD-1 and determines the set point for 11 $\beta$ -HSD-1 oxidoreductase activity. In a long-term (10 wk) study, rats given ad libitum access to 16% sucrose solution, chow, and water were fatter than controls, had increased 11 $\beta$ -HSD-1 mRNA in adipose, suppressed 11 $\beta$ -HSD-1 mRNA in liver, and increased H6PDH mRNA in both tissues.

The primary research questions were as follows: Can high sugar diets induce glucocorticoid dysregulation in the absence of excess adiposity? Does sugar type matter? Energy intake, weight gain, and parameters of lipid and carbohydrate metabolism were measured. Rats were randomly assigned to either ad libitum access to chow and water

only (control), or in addition to ad libitum access to either 16% sucrose, fructose, or glucose solution (n=16/gp). After 24h and 1 wk, eight rats per group were randomly selected for sacrifice.

Daily caloric intakes among sugar-fed groups did not differ and were higher than the mean intake of the control group. Within 24h, fructose induced increased 11 $\beta$ -HSD-1 message in mesenteric adipose and liver. Plasma TG and insulin were acutely increased in groups with fructose-containing diets only. All high sugar diets induced suppressed hepatic 11 $\beta$ -HSD-1 mRNA and protein after 1 wk. Upregulation of H6PDH mRNA observed in response to long-term high sucrose diets may result from increased adiposity and not solely diet. High sugar diets, irrespective of sugar type, initiate glucocorticoid dysregulation in the absence of phenotypic changes associated with obesity. Sucrose, fructose, and glucose have distinct metabolic and endocrine responses. Fructose has the unique ability to induce glucocorticoid dysregulation in liver and adipose in 24h.

HIGH SUCROSE, FRUCTOSE, AND GLUCOSE DIETS AND GLUCOCORTICOID  
DYSREGULATION IN RATS

by

Edra London

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## **Foreword**

Included in this dissertation are two previously published papers. In adherence with the policies set forth by the Graduate School of the University of Maryland, College Park, I note here that I am the principal author of both of the aforementioned papers.

## **Dedication**

To my family whose support made my decision to pursue my goals a possibility. I am ever grateful to my husband and best friend, Shaughn, whose understanding and continued love and support enabled me to complete my studies. His companionship made it worlds easier to live with the difficult decision to return to school and give up the luxuries of not being a student (such as free time and money). Without his sacrifices during the hectic times I could not have gotten through the final two years of my graduate program.

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And to my big-hearted sister, Moira, who made me realize that anything is possible.

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## Introduction

The prevalence of obesity in the United States has reached epidemic proportions. The CDC reports that approximately two-thirds of the adult U.S. population is overweight or obese, and statistics indicate that the trend is unabated (CDC 2004, CDC 2009). While it is clear that obesity is a multifactorial disease resulting from the combined effects of genetic, physiological, environmental, social, and psychological factors, it is equally clear that there is currently no cure. One area of ambiguity in the etiology of obesity is the relationship between stress hormones and obesity. The experiments conducted for this dissertation investigated the role of glucocorticoids in promoting adiposity by examining the interaction between diet and its influence on body composition and glucocorticoid metabolism. Changes in two key regulatory enzymes, 11 $\beta$ -hydroxysteroid dehydrogenase-1 (11 $\beta$ -HSD-1) and hexose-6-phosphate dehydrogenase (H6PDH), as well as markers of carbohydrate and lipid metabolism in response to diets proportionally high in simple carbohydrates (sucrose, fructose, glucose) were evaluated in a rat model. High sugar diets have been shown to induce excess energy intake and obesity in rats. 11 $\beta$ -HSD-1 plays a primary role in regulating tissue-specific intracellular glucocorticoid levels, and H6PDH catalyzes the first and rate-limiting step of the oxidative phase of the pentose phosphate pathway and produces NADPH, the cofactor for 11 $\beta$ -HSD-1 oxidoreductase activity. We tested the hypothesis that high sugar diets would increase adipose and decrease hepatic 11 $\beta$ -HSD-1 mRNA and protein levels. This pattern of changes has been reported in obese humans and genetically obese rats (Livingstone 2000a, Rask et al 2001).

The list of regulatory factors identified as influencing food intake, energy expenditure, and body weight has increased dramatically over the past two decades. While these multiple factors interact on both central and peripheral levels (in the brain, gut, liver, etc.), one common finding is that maintenance of normal adrenal glucocorticoid levels is necessary for the normal function of these factors. More recently, there is evidence that elevated intracellular tissue concentrations of glucocorticoids may better predict the promotion and maintenance of obesity than circulating hormone levels which are not consistently higher in obese versus lean animals (Lottenberg et al 1998; Walker et al 2001).

The bidirectional enzyme, 11 $\beta$ -HSD-1, which interconverts the active hormone cortisol (human) or corticosterone (rat) and inert cortisone (human) or 11-dehydrocorticosterone (rat), resides in the intralumenal compartment of the endoplasmic reticulum (ER). 11 $\beta$ -HSD-1 is highly expressed in adipose, liver, and brain in rats (Monder & Lakshmi 1990; Lakshmi, Sakai, McEwen & Monder 1991) and humans (Ricketts et al 1998). 11 $\beta$ -HSD-1 acts primarily as an oxidoreductase which requires the cofactor NADPH to generate active glucocorticoid. The pool of microsomal NADPH that drives the oxidoreductase reaction is thought to be generated by the enzyme H6PDH (Bujalska et al 2005). In both human and animal models of obesity, 11 $\beta$ -HSD-1 has been shown to be upregulated in adipose and downregulated in liver.

Gaining insight into how diet and energy intake affect microsomal pentose phosphate pathway flux as well as H6PDH and 11 $\beta$ -HSD-1 mRNA, expression, and activity will help answer key questions about how glucocorticoid dysregulation is initiated and progresses in diet-induced obesity. Identifying the mechanisms by which

11 $\beta$ -HSD-1 is upregulated and/or reductase activity is increased in adipose, and conversely is downregulated and/or decreased in liver, are crucial to understanding the occurrence of tissue-specific glucocorticoid regulation in different forms of obesity. These experiments investigated the effects of high sucrose, fructose, and glucose diets on mRNA and protein expression of 11 $\beta$ -HSD-1 and H6PDH, and several metabolic parameters essential to energy balance and nutrient partitioning. The measurement of these variables, differences in weight gain and in the long-term study, body composition, at different time points during the high sugar feeding were analyzed in order to better characterize how high sugar diets affect peripheral glucocorticoid metabolism and promote adiposity.

The goal of this research was to determine whether it is exposure to high sugar diets that causes changes in the mRNA and protein levels of two key regulatory enzymes in glucocorticoid metabolism or if it is the result of the increased adiposity associated with the diets that alters these enzymes. Several key markers that represent changes in the regulation of energy balance and metabolism at different time points have been measured to provide insight into the sequential changes that occur in the glucocorticoid dysregulation seen in obesity. The hypotheses tested were:

Hypothesis 1: Sprague-Dawley (S-D) rats given free access to rat chow and water in addition to 16% fructose, sucrose, or glucose solution for 24h or 1 wk will consume more total calories than control rats given free access to rat chow and water only.

Hypothesis 2: Long-term high sucrose, fructose, and glucose diets will induce increased 11 $\beta$ -HSD-1 and H6PDH mRNA in mesenteric adipose and decreased hepatic 11 $\beta$ -HSD-1 and H6PDH mRNA.

Hypothesis 3: High-sugar diets will increase microsomal pentose phosphate pathway flux in mesenteric adipose and liver and therefore increase concentrations of microsomal NADPH compared to a control (low-sugar) diet.

Hypothesis 4: Solutions that containing fructose (i.e., sucrose and fructose, but not glucose) will induce increased plasma triglyceride levels via enhanced de novo lipid biosynthesis.

Hypothesis 5: Changes in 11 $\beta$ -HSD-1 and H6PDH mRNA and protein expression in mesenteric adipose and liver will not occur in response to acute high sugar feeding in rats.

## Chapter 1. Literature Review

### Obesity

Obesity is a multifactorial disorder that involves genetic background, multiple environmental factors including food availability and social interactions, behavioral patterns such as inactivity and emotional eating, underlying illness, and socioeconomic status (Hausman et al 2001). The prevalence of obesity has reached epidemic proportions in the United States and worldwide. The latest data show that approximately 60 million (over 30%) of U.S. adults age 20 and over are obese ( $\text{BMI} > 30 \text{ kg/m}^2$ ), and that the percentage of young people (ages 6–19) that are overweight ( $\text{BMI} > 25 \text{ kg/m}^2$ ) has tripled since 1980 (CDC, 2004). Overweight and obesity are associated with an increased risk of developing several diseases and health conditions: hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea, respiratory problems, and some cancers (CDC, 2006). This increase in obesity and its comorbidities is predicted to have an enormous impact on the U.S. economy that will continue to worsen (King et al 1998; Filozof et al 2001).

Understanding the complexities of obesity requires the application of knowledge and techniques from diverse scientific disciplines. Human obesity, in most cases, is polygenic in nature and is phenotypically expressed when genetic factors interact with key environmental, behavioral and/or social factors. Obesity can be viewed as the result of the malfunctioning of the complex regulatory system that, when functioning normally, works to balance energy intake with expenditure. In healthy individuals, this homeostatic system appears to be involved in maintaining energy balance over long-term periods,

even though day to day energy intake and expenditure are subject to variability caused by multiple factors (Keesey and Powley 1986, Stunkard 1981).

The critical role of the central nervous systems in maintaining this balance has emerged recently as have numerous centrally and peripherally secreted hormones that are critical in regulating this neuronal circuitry (Schwartz et al 2000, Morton et al 2006). Short-term energy intake and expenditure are regulated from meal to meal by numerous physiological signals including neuronal inputs related to circadian rhythms, metabolic signals from the brain and gut in response to the utilization of different energy sources, GI peptides released in response to gastrointestinal distension and nutrient absorption, and also emotional factors such as food palatability (Schwartz et al 1999, Cummings & Schwartz 2003). The long-term regulation of adiposity is thought to be regulated by the hormones leptin (Zhang et al 1994) and insulin (Woods & Porte 1976) which have been shown to exert effects that are slower in onset and offset. It is likely that glucocorticoids play an important role in long-term regulation of energy balance. While glucocorticoids are not direct adiposity-related negative feedback signals like leptin and insulin, glucocorticoids seem to promote positive energy balance by antagonizing the central nervous system (CNS) response to insulin and leptin (Baskin et al 1999, Havel 2001). In just the past several years, the number of peptides and other factors, including a number that act transcriptionally, recognized as regulators of energy homeostasis has increased substantially (Seeley & Moran 2002). Because many of these factors interact with one another, it is increasingly difficult to develop a single theory of obesity. This has led to the identification of a number of different obesities with differing etiologies. For a history of hypotheses of different types of obesity, see Bray (1991) and for a review of the

neuroendocrinology of obesity see Lustig (2001). Three general models that have been used to approach the problem include hypothalamic or neuroendocrine obesity, genetic obesity, and dietary obesity. Each form of obesity has distinct characteristics and key features yet overlap at a common point: each requires the presence of glucocorticoids. The distinctions between the different forms of obesity and improved understanding of the mechanistic underpinnings of the varied characteristics of obesities will likely lead to the identification of obesity type-specific therapeutic approaches.

Over the past century, numerous hypotheses have been developed in an attempt to explain the regulation or in some views, the dysregulation, of energy homeostasis that is partnered with obesity. It has been suggested that obesity is not the effect of lack of body weight regulation, but is instead the result of a regulation of body weight and adiposity that is based on a physiologically predetermined set point. This hypothesis suggests that obese individuals have high set points that their regulatory system defends (Stunkard 1981, Keesey & Hirvonens 1997).

The etiology of human obesity is complex. Molecular technologies available in the recent decades have been useful in identifying some of the rare genetic obesities caused by defects in single genes, also known as monogenic obesity. More common are polygenic obesities, in which polymorphisms in several candidate genes and/or regulatory regions of neighboring genes increase an individual's susceptibility to obesity by interacting with a multitude of additional factors (environmental, psychological, physiological, etc). For a review of advances in the study of the genetics of human obesity see a review by Clément (2006). Studying the role of the hypothalamus in obesity has broadened our knowledge of how signaling in specific nuclei of the hypothalamus

influences intake, satiety, and metabolism. Diet-induced obesity is likely the type of obesity that is most rapidly increasing in prevalence with the societal shift to sedentary lifestyles and the increased availability of inexpensive and palatable foods. Better understanding the interaction between genetics and environmental inputs requires the use of appropriate animal models in order to identify potential interventions and treatments for the growing obese population.

### **Animal Models and the Study of Obesity**

Animal models have become a useful tool in investigating various genetic, environmental, behavioral, and pharmacologic factors that play roles in obesity. Because most obesities involve the interaction of one or more genetic variants with other variables, the introduction of reverse and forward genetics has enabled studying the roles of combinations of variables *in vivo*. These developments have facilitated the investigation of the effects of various mutations at the whole animal level, instead of solely in cell or tissue culture systems. Furthermore, high throughput screening, microarray and proteomics techniques have become accessible to the larger scientific community, and thus large-scale mutagenesis screens have enabled the identification of a growing number of target genes in the pathogenesis of obesity (Challis et al 2002). As a result, the number of animal models available for the study of obesity continues to grow rapidly. For an extensive review of rodent models of obesity including diet-induced models, spontaneous and engineered mutants, and models of hypothalamic obesity, see Tschöp and Heiman (2001). While many of the models available including those that involve mutations of single genes and results may not be readily extended to human obesity, they further our knowledge about specific forms of obesity, signaling pathways,

and roles of specific neuroendocrine factors and their receptors. Models of diet-induced obesity, however, are more closely mimic common forms of human obesity.

### **Rodent Models of Diet-Induced Obesity**

Laboratory animals have helped establish the role of both the quantity and composition of available foods in energy intake and adiposity. For a review of the evidence that implicate cafeteria diets (calorie-dense buffet style diets), high-sugar, and high-fat diets in the induction of hyperphagia and subsequent obesity see Sclafani (2001). Interestingly, the phenomenon of obesity prone and obesity resistant rodents is seen across numerous strains of rat and mice, including Sprague-Dawley, Long-Evans, and Osborne Mendel rats, as well as in AKR/J, C57L/J, A/J, C3H/HeJ, DBA/2J, and C57BL/6J mice (West et al 1992). Dietary manipulations that include forced feeding, changes in nutrient availability and changes in diet composition have led to variable results among individuals as well as different strains of rodent demonstrating a strong interaction between genotype and diet (York and Bray, 1998). Studies using high fat feeding to produce a rat model of dietary obesity have demonstrated that hyperphagia is not a requirement for induction of excess adiposity under these conditions (West and York 1998, Woods et al 2003). When rats are provided with solutions of sucrose, fructose or glucose they typically increase their energy intake by 10–20% above what they would consume if provided with rat chow alone (Kanarek and Orthen-Gambill 1982), and thus high-sugar diets have been used to create models of diet-induced obesity as well.

The role of glucocorticoids in genetic and dietary obesities has been investigated using adrenalectomized (ADX) rats. Obese and lean ADX Zucker rats select diets significantly lower in fat than their obese sham operated counterparts (Castonguay,

Dallman, & Stern 1986). Administration of exogenous corticosterone dose-dependently restored the fat appetites of the lean and obese ADX rats. Obese and lean Zucker rats had attenuated weight gain following adrenalectomy and were resistant to weight gain and increased adiposity on a high fat diet (Bray, Stern, & Castonguay 1992), showing a clear interaction between adrenalectomy and high-fat diet and the development of obesity in both fatty and lean Zucker rats. The role of glucocorticoids in diet-induced obesity is discussed in more depth later in this review.

### **Hypothalamic and Neuroendocrine Obesity**

Several distinct regions of the hypothalamus have been implicated in the regulation of body weight and food intake. Damage to or ablation of the ventromedial hypothalamic nuclei (VMH) via chemical, electrical, or surgical means can cause obesity in humans, nonhuman primates, as well as dogs, mice and rats (York & Hansen 1998). This was first demonstrated by Hetherington and Ranson (1942), who introduced lesions using an electric current in the VMH, thereby inducing obesity in the rat. Although the preliminary studies suggested that hyperphagia was the major cause of VMH lesion obesity, it has become evident that chemically-induced lesions of the VMH can cause obesity without hyperphagia (Bernardis 1985, Parkinson & Weingarten 1990). The obesity caused by VMH lesions seems to result from hyperinsulinemia and changes in autonomic control of thermogenesis (York & Hansen 1998). A second and distinct type of hypothalamic obesity results from lesions of the paraventricular nucleus (PVN). Damage to the PVN has been shown to cause increased food intake and rapidly developing severe obesity (Sclafani & Grossman 1969; Bray et al 1990), but unlike VMH obesity, can be attenuated by restricting food intake.

Manipulation of endocrine function via administration of endogenous hormones and/or the under- or overexpression of regulatory hormones can result in various forms of obesity. Many such manipulations have been explored in animal models. See Tschöp and Heiman for an overview of rodent models (2001).

### **Intake of Dietary Sugars and Prevalence of Obesity and the Metabolic Syndrome**

As obesity and overweight has increased in the adult and child populations in recent decades, dietary sugars, particularly in the form of sweetened beverages, have come under scrutiny for their putative role in this epidemic. Most sweetened beverages are sweetened with high fructose corn syrup (HFCS) which was introduced in two forms, HFCS-42 (42% fructose) and HFCS-55 (55% fructose), in 1967 and 1977, respectively (Bray et al 2004). HFCS-55, the most commonly used form, is also composed of 42% glucose and 3% other sweeteners, thereby resulting in a 1.22 ratio of fructose to glucose, 10% more fructose by weight than sucrose. The Institute of Medicine dietary guidelines recommend consuming from 45 to 65% of total daily energy from carbohydrates, with no more than 25% of total energy from added sugars (IOM 2007). Data from the National Health and Nutrition Survey (NHANES), however, indicate that carbohydrate intake increased by approximately 62g/d and 69g/d in women and men, respectively, between 1971 and 2000 (CDC 2004). Per capita intake of added sugars from all sources has increased by approximately 25% over the past three decades (Havel 2005), and the U.S. Department of Agriculture Continuing Survey of Food Intakes by Individuals (CSFII) conducted from 1994–1996 revealed that about 43% of the energy from added sugars was derived from soft drinks and fruit drinks (Bowman 1999). Most of this added sugar comes in the form of HFCS.

Simple sugars have been shown to stimulate hepatic de novo lipogenesis, making them a likely candidate for promoting the fasting hypertriglyceridemia that has been associated with high sucrose or high fructose diets (Havel 2005). Both fructose and glucose stimulate sterol responsive element binding protein 1c (SREBP-1c), which is linked to de novo lipogenesis, and both SREBP-1c and de novo lipogenesis are more strongly induced by fructose than glucose (Minehira et al 2003).

### **Differential Metabolism of Dietary Sugars**

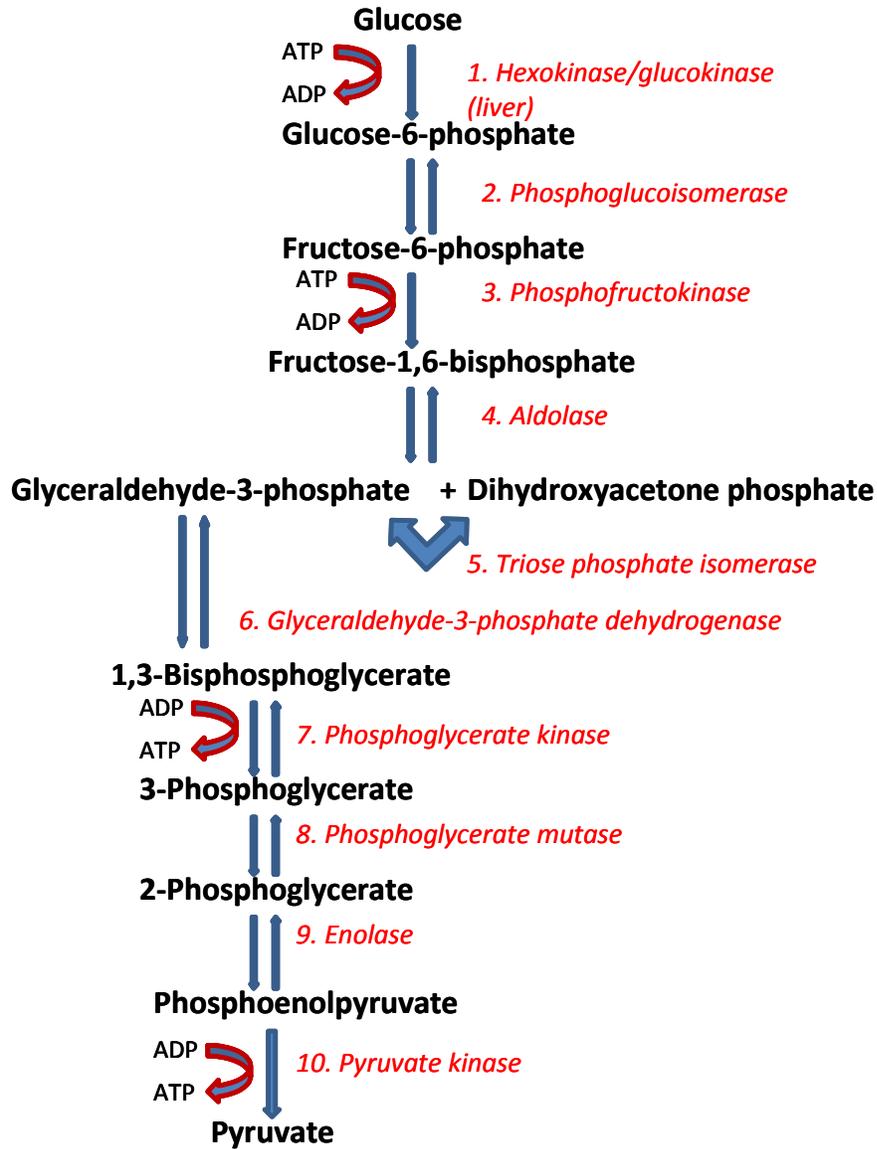
The three monosaccharides that comprise the primary dietary sugars are glucose, fructose, and galactose. The combinations of these three simple sugars form the three primary dietary disaccharides, maltose (glucose-glucose), sucrose (glucose-fructose) and lactose (glucose-galactose), while the polysaccharides that serve as storage are comprised of glucose polymers. Glucose serves as the major fuel source for most organisms, human and rat included, as it can be readily oxidized aerobically or anaerobically to produce ATP. Glucose is both a rich source of energy and a versatile metabolic precursor for biosynthetic reactions. The three major metabolic fates for glucose include: 1) storage, 2) oxidation to pyruvate via glycolysis, and 3) oxidation to pentose via the pentose phosphate pathway. 6-carbon sugars other than glucose are also catabolized in the glycolytic pathway.

### ***Glucose Metabolism***

Glucose is the primary source of fuel in eukaryotes and as such, is also the primary circulating monosaccharide. Glucose is taken up primarily by skeletal muscle cells and hepatocytes, the two major sites of glycolysis. Glycolysis, however, occurs ubiquitously in most cells of the body, adipocytes included. Once glucose is transported

into the cytosol by one of several SGLT or GLUT transporters, glucose can then enter the glycolytic pathway and be converted to glucose-6-phosphate (G6P) by hexokinase and glucokinase, respectively (Fig. 1). Once phosphorylated to G6P, glucose proceeds through the 10-step glycolytic pathway ultimately being converted into two 3-carbon molecules (pyruvate) which then can enter the Krebs Cycle and yield additional energy in the form of ATP. Hepatic glucose metabolism is tightly regulated by the enzyme phosphofructokinase (PFK-1) which is allosterically inhibited by citrate, a Krebs Cycle intermediate, and by ATP. This inhibition, as well as the limited capacity of the liver to store glucose as glycogen, regulates the amount of glucose uptake and flux through the glycolytic pathway.

Glucose is absorbed in the intestinal mucosal cells via the active transport of the glucose-galactose carrier protein sodium-glucose linked transporter 1 (SGLT1) and then enters the portal vein. Glucose transporter 2 (GLUT2) is the transporter for glucose in hepatocytes and while the expression of GLUT4, the major transporter in adipose and skeletal muscle is increased by insulin stimulation, GLUT2 is not.

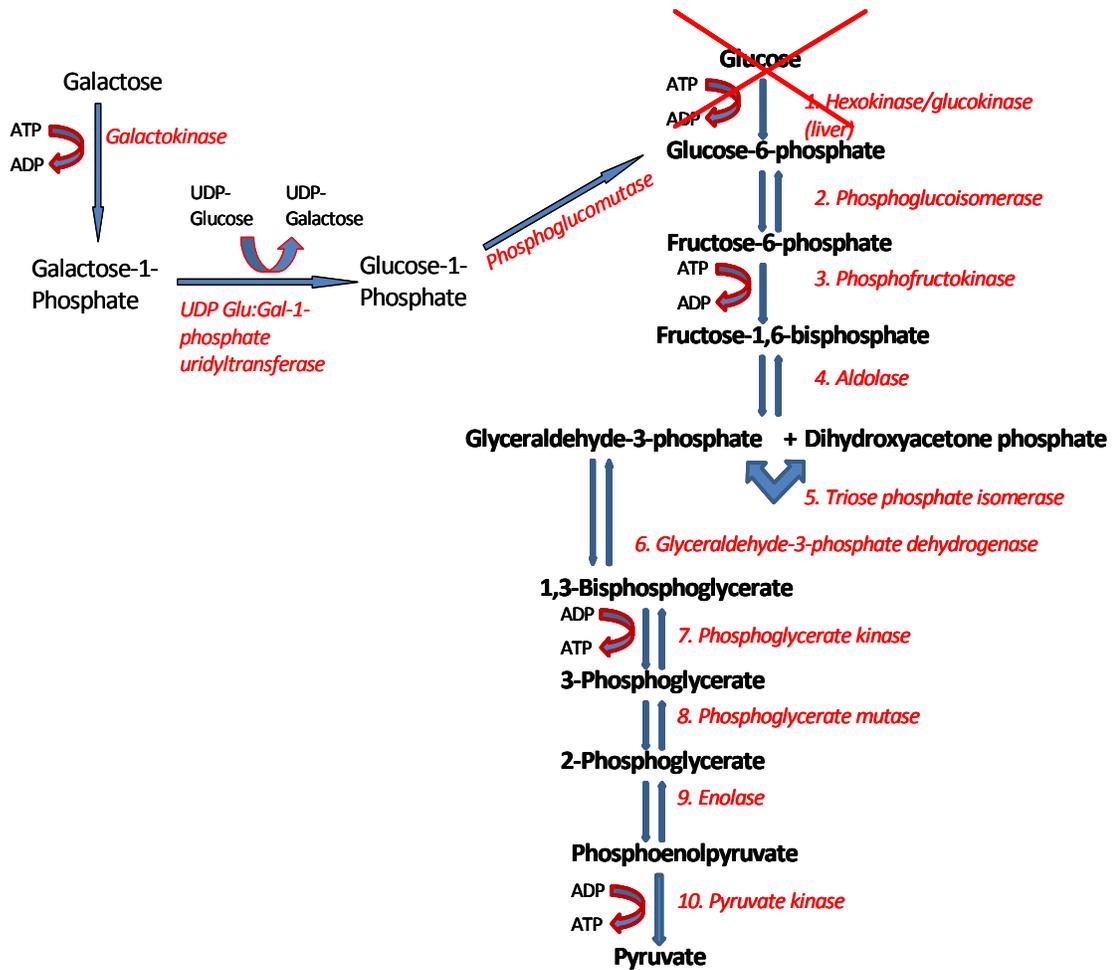


**Figure 1.** The catabolism of glucose to pyruvate via the glycolytic pathway.

### ***Galactose Metabolism***

Like glucose, galactose is absorbed in the intestine by active transport by SGLT1 and then transported into cells by GLUT-2. Galactose can be catabolized by glycolysis once it reaches the liver where it first must undergo several enzymatic reactions beginning with conversion to galactose-1-phosphate via galactokinase (Fig. 2). Galactose-

1-phosphate is then converted to UDP-galactose with concomitant formation of glucose-1-phosphate which can then be converted by phosphoglucomutase into glucose-6-phosphate and can enter the glycolytic pathway.



**Figure 2. The entry of galactose into the glycolytic pathway, and subsequent catabolism**

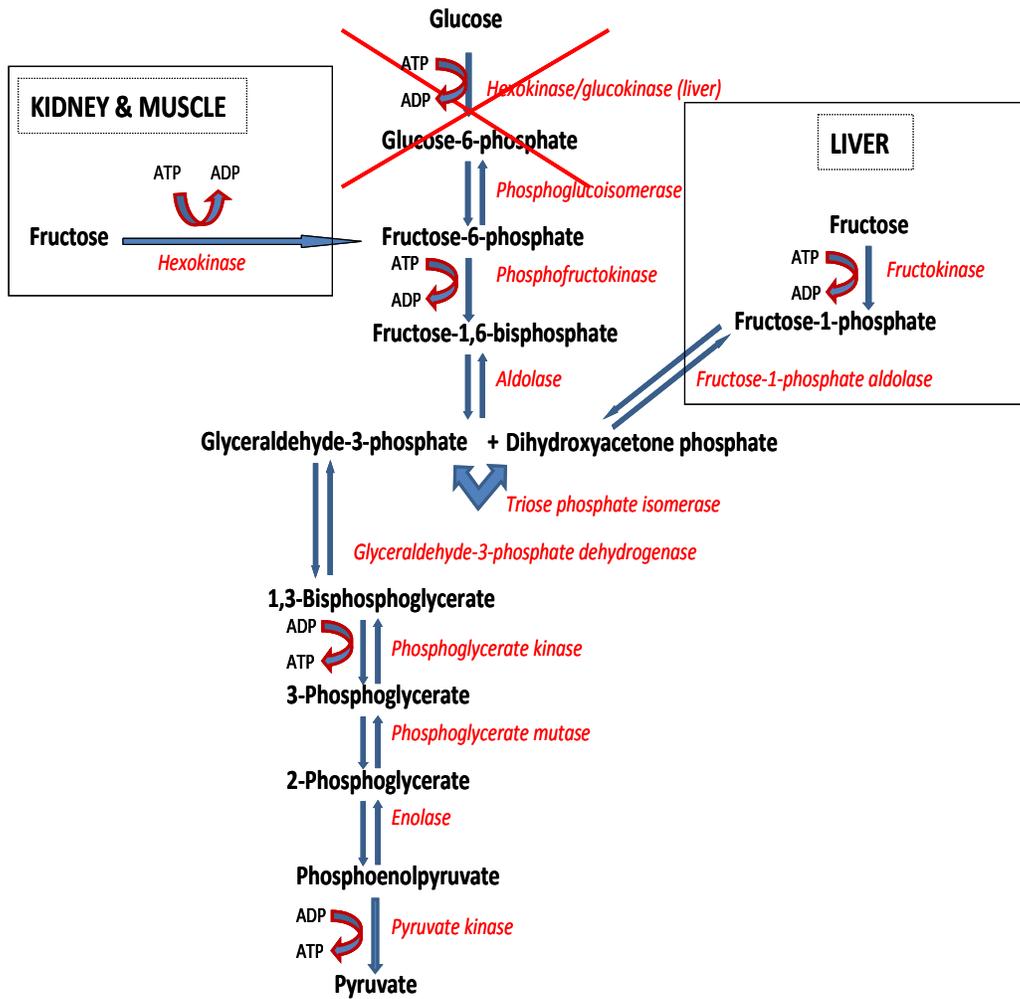
***Fructose Metabolism***

Fructose absorption is less clearly defined than that of glucose or galactose, but active and passive transport are saturable (Groff and Gropper 2000), so consumption of large quantities may lead to malabsorption and intestinal distress. However, when co-

consumed with glucose, fructose absorption becomes more efficient and less symptoms of malabsorption are observed. Once absorbed, fructose has one of two metabolic fates as it is metabolized differentially in muscle and liver after it is transported into cells via the GLUT5 transporter which are expressed primarily in the brush border of the small intestine and jejunum (Levin 1999). In muscle cells, which express only hexokinase, fructose is phosphorylated to fructose-6-phosphate, a direct glycolytic intermediate. In liver, the enzyme glucokinase which has specificity for glucose predominates as the first enzyme of the glycolytic pathway. Therefore, in liver, fructokinase acts on fructose converting it to fructose-1-phosphate which can then feed directly into glycolysis (Fig. 3). In doing so, fructose bypasses three steps of glycolysis and is instead acted upon by aldolase type B, a liver-specific isoform of aldolase that can utilize both fructose-1,6-bisphosphate or fructose-1-phosphate. Because of its entry point in liver, fructose bypasses the most influential regulatory enzyme of glycolysis, phosphofructokinase-1 (PFK-1). This has two important implications: 1) fructose can be readily used as a fuel source for glycolysis, and 2) it is not subject to the same regulation as the catabolism of glucose. Ordinarily, the glycolytic intermediate fructose-1,6-bisphosphate plays an inhibitory role in slowing the action of PFK-1 and slowing the process of glycolysis.

Fructose, which occurs naturally in fruits has become heavily used in prepackaged convenience foods and sweetened beverages as sucrose (50% glucose, 50% fructose) and as high fructose corn syrup (42% or 55% fructose and 53% or 42% glucose, HFCS 42 and HFCS 55, respectively). Another key difference between fructose and glucose metabolism is in cellular uptake. The uptake of fructose by GLUT5 does not rely on insulin-induced relocation of the transporter to the plasma membrane as does the uptake

of glucose by GLUT2 (Levin 1999). Fructose can enter cells via GLUT5 irrespective of energy needs, or circulating glucose levels.



**Figure 3.** The differential entry of fructose into the glycolytic pathway in muscle and kidney, and in liver; and the subsequent catabolism to pyruvate.

### The Association between High Dietary Intake of Fructose and Obesity

The association between HFCS- and sucrose-sweetened beverages and obesity has become an active area for research as the obesity trend of the past few decades coincides with the introduction and widespread usage of HFCS, primarily in sweetened

beverages and grain products in the U.S (Marriott et al 2009). The most common use for HFCS 55 is soft drinks. In 2007, the Center for Food, Nutrition, and Agriculture Policy assembled a panel of experts to review the cumulative literature, based mainly observational data that examined HFCS intake and BMI (Forshee et al 2007). This review, which included new analyses of the existing data to fill in gaps in the available literature, concluded that the fructose:glucose ratio in the U.S. food supply hasn't changed significantly since the introduction of HFCS in the late 1960s. Inconclusive results led to the conclusion that HFCS does not appear to contribute to overweight and obesity any differently than do other sources of energy. However, gaps in this body of research, including the analysis of differences in consumption of sucrose and HFCS, were cited. Another review of the literature based primarily on epidemiological studies similarly found no direct association between sweetened beverages and increased overweight or obesity (Bachman et al 2006). Bachman et al examined four mechanisms for increased adiposity: 1) excess energy intake, 2) glycemic index, 3) decreased satiety from liquid-derived calories, and 4) displacement of milk, and found the strongest support was for excess caloric intake.

Our current knowledge of the metabolism and neuroendocrine signaling associated with the hepatic metabolism of fructose, however, suggests that fructose may induce positive energy balance via enhanced lipogenesis and consequent hyperlipidemia (for a review see Havel 2005). High-fructose diets induce insulin resistance (Fortino et al 2007), weight gain, increased adiposity (Jürgens et al 2005), hyperlipidemia (Reaven et al 1989), and hypertension in animal models that include rats, hamsters, dogs, and certain strains of mice (Havel 2005). The consumption of fructose- and not glucose-sweetened

beverages when provided as 25% of daily energy requirements promoted visceral adipose deposition in overweight and obese men and women (Stanhope et al 2009). Stanhope et al also found that consuming fructose had deleterious effects on lipid metabolism and lipoprotein remodeling while glucose did not. Fructose was shown to induce altered patterns of secretion of several neuroendocrine factors critical to the long-term regulation of energy balance in both obese and normal weight subjects (Teff et al 2004 & 2005). When provided with isocaloric meals (55% carbohydrate, 30% fat, 15% protein) in addition to either a fructose- or glucose-sweetened beverage that comprised 30% of kcals for the meal, mean postprandial insulin excursion was 65% lower in the fructose-fed group in normal weight women (Teff et al 2004). Additionally, plasma leptin was significantly reduced for the duration of the test day and there was a significant decrease in the normal attenuation of plasma ghrelin that was observed in response to glucose.

While dietary fructose does not directly elicit insulin secretion, hyperinsulinemia and/or insulin resistance is characteristic of chronic high fructose feeding (Reaven et al 1990, Lê and Tappy 2006) and may play an important role in fructose-induced obesity. The increased insulin response that occurs in fructose feeding promotes decreased insulin sensitivity in rats in as little as 7 days (Zavaroni et al 1980). Reaven et al (1990) showed that rats fed fructose-enriched diets had significantly elevated plasma insulin and triglyceride levels, but not plasma glucose concentrations. This suggests a mechanism for fructose-induced hyperinsulinemia that is distinct from that induced by excess dietary glucose or increased glucose tolerance. The monosaccharide fructose has also been shown to negatively impact lipid metabolism. A high sugar diet (66% of total energy) composed of fructose but not glucose induced a significant increase in plasma TGs in rats

(Zavaroni et al 1982). This effect was linked to fructose's ability to increase VLDL-TG secretion. Fructose feeding in rats has also been reported to increase lipid deposition in liver and skeletal muscle (Furuhashi et al 2002), a characteristic of the metabolic syndrome. This enhanced fat deposition is thought to contribute to insulin resistance. Nonalcoholic steatohepatitis is an increasingly common disease that is a manifestation of metabolic syndrome and Kawasaki et al have showed that high fructose-feeding produced a better rat model of this disease than a high fat diet did (2009). In comparison to rats fed a control, high fat, high sucrose, or high fructose/high fat diet, rats fed a high fructose diet had higher macrovesicular steatosis grade, liver:body weight ratio, hepatic TG concentration, and higher grade of lobular inflammation than the other four groups (Kawasaki et al 2009).

Enhanced stress response has also been suggested as a potential mechanism for the development of fructose-induced insulin resistance. Wei et al (2004) showed that rats fed a high sucrose diet or high fructose diet develop hepatic insulin resistance and that rats administered a single sucrose-enriched meal or dose of fructose infusion had acute increases in c-jun N-terminal kinase (JNK) activity and downstream insulin receptor substrate-1 (IRS-1) serine phosphorylation. JNK activation is a critical part of cellular stress response and proinflammatory cytokines including TNF- $\alpha$  are elevated in fructose-fed rats (Togashi et al 2000). Thus, it has been suggested that fructose may stimulate inflammatory cytokines (Lê & Tappy 2006) and this inflammatory response may contribute to fructose-induced hepatic insulin resistance. The underlying cellular mechanisms for the deleterious metabolic effects of fructose involve the production of reactive oxygen species, initiation of cellular stress, and perhaps increased production of

uric acid (Lê & Tappy 2006). See Rutledge and Adeli (2007) for an in-depth review of the molecular mechanisms involved in fructose-induced metabolic dysregulation.

Few studies have examined the relationship between fructose intake designed to mimic the high intake of soda and sweetened beverages and increased weight gain and adiposity. In one such study, mice given ad libitum access to a 15% fructose, 10% sucrose, or artificially sweetened (zero calories) solution had similar total daily caloric intakes but the fructose-fed mice had increased adiposity, respiratory quotient, and hepatic lipid accumulation (Jürgens et al 2005). Teff et al (2004) reported that plasma ghrelin was suppressed by approximately 35% following three meals that included glucose-sweetened beverages (at 30% total energy requirements), but that this normal suppression in postprandial ghrelin was diminished in subjects who consumed fructose-sweetened isocaloric beverages with the same meals. Another short-term study provided glucose- or fructose-sweetened beverages at 30% of total energy requirements with three daily meals reported a reduction in post-meal insulin secretion and 24h systemic leptin in both overweight and obese men and women (Teff et al 2005). Further, both studies found that fructose-sweetened beverage consumption attenuated the percent change in leptin levels between morning nadir and late night peak (Teff et al 2004 & 2005). Diminished secretory response for ghrelin and leptin as a consequence of fructose intake could promote elevated energy intake, subsequent weight gain, and upset long-term energy balance.

Recently, Stanhope et al (2009) found that dietary fructose, but not glucose, increased de novo lipogenesis and promoted dyslipidemia, decreased insulin sensitivity, and increased visceral adiposity in overweight/obese adults. This 10 wk intervention

study of overweight/obese men and postmenopausal women (n= 32) included 2 wk inpatient metabolic studies both at baseline and the final 2 wk of intervention. Subjects were randomly and blindly assigned to either self-selected ad libitum diets for 8 wk (outpatient) in addition to 1) glucose-sweetened beverages at 25% of energy requirements (n= 15) or 2) fructose-sweetened beverages at 25% of energy requirements (n= 17). Both groups similarly consumed energy in excess of daily needs during the intervention and had comparable weight gain. However, the fructose-consuming subjects had significant increases in both visceral adipose tissue and total abdominal fat while the glucose-consuming subjects had significant increases in subcutaneous adipose tissue. Fasting TG concentrations were increased in glucose- but not fructose-consuming subjects and conversely, postprandial TGs were significantly elevated the fructose-, but not glucose-consuming subjects. Postprandial levels of remnant-like particle lipoprotein (RLP)-TG and RLP-cholesterol and both fasting and postprandial apoB, apoB/apoA1 ratio and total LDL were increased in fructose- but not glucose-consuming subjects, and FFA levels were increased in glucose- but not fructose-consuming subjects. These results demonstrate distinct deleterious effects on lipid metabolism and lipogenesis in humans that result from consuming diets high in fructose. Havel and colleagues have hypothesized that changes in postprandial TG levels and de novo lipogenesis are key in the dyslipidemia and proatherogenic effects of consuming a diet high in fructose and urge further clinical trials including long-term fructose consumption as part of both neutral energy balanced and positive energy balanced diets (Stanhope & Havel 2009).

Clearly there is compelling data that links fructose to primary characteristics of the metabolic syndrome and obesity via the dysregulation of glucose and lipid

metabolism, and several factors critical in maintaining energy homeostasis. The evidence, based primarily on studies of animal models and only very recently on clinical trials, warrants further research.

### **Glucocorticoids**

The adrenal corticosteroids are divided into two classes, glucocorticoids and mineralo-corticoids. The two primary members of the glucocorticoid family of adrenal steroid hormones are cortisol and cortisone in humans, and corticosterone and 11-dehydrocorticosterone in rodents. Cortisol is produced and secreted by the zona fasciculata of the adrenal glands in response to adrenocorticotropin (ACTH). Corticotropin-releasing hormone (CRH) secreted from the hypothalamus stimulates the secretion of ACTH from the anterior pituitary gland. Under normal physiological conditions the hypothalamic-pituitary-adrenal (HPA) axis is regulated by a negative feedback loop in which circulating cortisol inhibits further release of CRH and ACTH from the hypothalamus and pituitary, respectively, and ACTH inhibits further CRH release in a short negative feedback loop in order to maintain relatively constant blood cortisol levels (~30nmol/dL). While this endocrine regulatory system maintains relatively constant circulating levels of glucocorticoids, intracellular glucocorticoid concentrations in the critical metabolic tissues, liver and adipose, are more variable in altered physiologic states such as obesity and are thought to be regulated largely by the autocrine action of 11 $\beta$ -HSD-1 (Tomlinson et al 2004).

Glucocorticoids have many functions in the body including a critical role in blood glucose maintenance (Long et al 1940), while the main roles of the mineralocorticoids are maintenance of blood pressure and electrolyte balance (Stewart et al 1988, Whitworth et

al 1989). Glucocorticoids have also been implicated as a factor in inflammatory disease, not as a part of the body's direct stress-responses (like inflammation), but instead as part of a negative feedback system in response to stress that acts to attenuate direct responses (see Munck, 1984 for a review). Thus, glucocorticoids are widely used as anti-inflammatory agents. Glucocorticoids act by binding to intracellular glucocorticoid receptors. The hormone-receptor complex then translocates to the nucleus where it interacts with glucocorticoid response elements. This leads to the transcriptional activation and/or repression of numerous genes, including activation of the gene for phosphoenolpyruvate carboxykinase (PEPCK), a rate-limiting enzyme in gluconeogenesis in liver and kidney (Sasaki et al 1984).

### **Glucocorticoids and Glucose Homeostasis**

Glucocorticoids influence intermediary metabolism via their action on key metabolic tissues including liver, adipose, skeletal muscle, and brain. Under normal circumstances glucocorticoids are primarily catabolic, except in the liver where they induce gluconeogenesis via the upregulation of PEPCK and perhaps in the CNS where they are thought to increase food intake (Tempel et al 1992). However, while systemic administration of corticosterone in ADX rats was found to stimulate intake and body weight gain, corticosterone administered intra-cerebroventricularly did not, suggesting that glucocorticoids may only secondarily have effects on the central control of appetite (Kamara, Kamara, Castonguay 1992). Glucocorticoids have an antagonistic affect on insulin secretion via their influence on PEPCK activity, which affects pancreatic  $\beta$ -cells by decreasing insulin release (O'Brien et al 1990; Delaunay et al 1997). O'Brien et al further demonstrated, however, that insulin has a dominant negative effect on PEPCK in

the presence of glucocorticoids or cAMP (1990). In peripheral tissues glucocorticoids impair glucose uptake by adipose tissue and skeletal muscle (Rooney et al 1993). Glucocorticoids have also been shown to enhance the breakdown of fat and protein from body stores (Roden et al 1996), thereby increasing circulating concentrations of free fatty acids and impairing insulin's capacity for stimulation of glucose uptake by muscle tissue (Randle et al 1963; Boden et al 1991). Additionally, high levels of active glucocorticoid in liver cause an inappropriate increase in gluconeogenesis via PEPCK which has been associated with the metabolic syndrome and some types of obesity (Sasaki et al 1984).

### **The Role of Glucocorticoids in the Regulation of Food Intake**

Numerous mechanical, chemical, and environmental signals participate in the short-term regulation of food intake that is manifest in behaviors that include meal initiation, meal duration, and meal termination. A complex network of neuropeptides and GI peptides interact to signal hunger and satiety in the regulation of food intake. These signals, however, interact with the long-term regulators insulin, and leptin (Schwartz et al 2000, Havel 2001), whose concentrations are proportional to body fat stores, to manage energy homeostasis. The hypothalamic melanocortin system is thought to act as a central mediator of leptin, insulin and other hormones. Glucocorticoids affect food intake and energy homeostasis by exerting orexigenic effects, opposing the actions of insulin and leptin (see review by Strack et al 1995). It has been suggested that glucocorticoid interactions with leptin and insulin at the level of central effector pathways may play a key role in energy homeostasis (Schwartz et al 1999). Circulating levels of cortisol are regulated by the negative feedback loop of the HPA axis. The affects of glucocorticoids on food intake and food choice under the environmental influences of contemporary

society, such as the abundant supply of inexpensive palatable foods and frequent stress, have been explored in adrenalectomized rats and will be discussed later in this review of the literature. Also, see la Fleur (2006) for a succinct review of these studies.

### **11 $\beta$ -Hydroxysteroid Dehydrogenase**

The metabolic interconversion of 11 $\beta$ -hydroxycorticosteroids and inactive 11-oxo-corticosteroids is catalyzed by the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD), which is expressed ubiquitously in most mammalian organs and tissues (Monder 1991). In humans, the isoform 11 $\beta$ -HSD type 1 is chiefly responsible for converting inactive cortisone (Kendall's compound E) to active cortisol (Kendall's compound F).

11 $\beta$ -HSD was first discovered by Amelung and colleagues in 1953, and then in 1968, a liver enzyme that displayed reductase activity by converting inactive cortisone/11-dehydrocorticosterone to active cortisol/corticosterone (human/rat, respectively) was described (Bush et al 1968). The type 1 "liver" isozyme was characterized first, but its activity could not explain the hypertension associated with mineralocorticoid excess. Selective catheterization studies showed significantly lower circulating cortisol/cortisone ratios in renal venous blood when compared to levels in hepatic venous blood, suggesting that while the liver predominantly converts cortisone to cortisol, the kidney predominantly converts cortisol to cortisone (Walker et al 1992). This provided evidence for the existence of two distinct isoforms. The kidney isoform, 11 $\beta$ -HSD type 2, has been linked to a monogenic form of hypertension, Apparent Mineralocorticoid Excess (AME). AME has been characterized by homozygous mutations of the 11 $\beta$ -HSD-2 gene that causes abnormal mineralocorticoid levels and

subsequent hypertension. For an in-depth review of 11 $\beta$ -HSD-2 and hypertension, see White et al (1997).

### ***Enzymology of 11 $\beta$ -HSD-1***

11 $\beta$ -HSD-1 is a microsomal, low affinity, NADP(H)-dependent bidirectional enzyme (Ozols 1995). While it is capable of both oxidoreductase and dehydrogenase reactions, the enzyme functions predominantly as a reductase in cell cultures from liver (Jamieson et al 1995) and adipose tissue (Bujalski et al 1997). Kinetic studies revealed  $K_m$  constants of  $1.83 \pm 0.06 \mu\text{M}$  for corticosterone, and  $17.3 \pm 2.24 \mu\text{M}$  for cortisol (Lakshmi & Monder 1988). The  $K_m$  value for 11 $\beta$ -HSD-1 dehydrogenase activity initially presented a puzzle in that it is more than two orders of magnitude higher than normal levels of free cortisol in the bloodstream. However, Maser et al (2002, 2003) found that 11 $\beta$ -HSD-1 in the human liver exhibits Michaelis-Menton kinetics toward cortisol and cooperative kinetics with cortisone which explained how it could be operational at both nanomolar and micromolar substrate concentrations.

11 $\beta$ -HSD-1 and 11 $\beta$ -HSD-2 have since been characterized (Tannin et al 1991, Palermo et al 1996) and cloned from human tissues (White et al 1997). 11 $\beta$ -HSD-1 is most highly expressed in the liver, adipose, gonad, and brain, while 11 $\beta$ -HSD-2 is predominantly found in tissues expressing high levels of mineralocorticoid receptors (MR) such as the kidney and colon. In addition to human, cDNA and protein sequences for 11 $\beta$ -HSD-1 have been published for rat (Lakshmi & Monder 1988, 1989), and mouse (Rajan et al 1995), as well as a number of other species, including sheep (Yang et al 1992), squirrel monkey (Moore et al 1993), baboon (Davies et al 1997), and guinea pig (Pu and Yang 2000). The 11 $\beta$ -HSD-1 rat cDNA sequence initially purified from rat liver

and cloned by Lakshmi and Monder (1988, 1989) and has since been updated (Maser et al 2002).

11 $\beta$ -HSD-1 belongs to the short-chain dehydrogenase/reductase (SDR) superfamily of oxidoreductase enzymes. This family is characterized as having 250–300 residues with an N-terminal cofactor binding domain and a central active site (Jörnvall et al 1995). SDRs have distinct sequence motifs that are critical to the nucleotide cofactor-binding region and to the active site (Oppermann et al 2001, Jörnvall et al 1995). Highly conserved within this family is the GXXXGXXG motif of the nucleotide cofactor binding region which confers specificity to NADPH (Oppermann et al 2001). Within the amino acid sequence of 11 $\beta$ -HSD-1, this consensus sequence has been identified from residues 41 to 47 (Ozols 1995). The active site of 11 $\beta$ -HSD-1 contains a conserved catalytic tetrad consisting of invariant tyrosine (Y) and lysine (K) residues, an adjacent serine (S) residue that is highly conserved, and the recently identified Asn111 that was found to be essential and highly conserved among most SDRs (Oppermann et al 2003). Mutagenesis studies of Tyr-179 and Lys-183 demonstrate that these residues are critical to the enzymatic activity of rat 11 $\beta$ -HSD-1 (White et al 1994). Asn-X-Ser glycosylation motifs are also present at three distinct sites. However, contradictory results about the importance of these glycosylation sites to enzyme activity have been reported. Mutations in these sites were found to have no effect in rabbit 11 $\beta$ -HSD-1 (Ozols 1995), yet another study found them to be essential to rat dehydrogenase activity (Agarwal et al 1990). The N-terminal contains a hydrophobic domain that contributes to the transmembrane domain and is likely also responsible for ER retention and enabling glycosylation (Jörnvall et al 1995). While both 11 $\beta$ -HSD-1 and 11 $\beta$ -HSD-2 are members of the SDR family, there is only 21% homology in

the human gene transcripts and it has been determined that they are the products of separate genes (Tannin et al 1994).

### **Glucocorticoids, 11 $\beta$ -HSD-1, and Obesity**

Although glucocorticoid production and secretion are increased in human obesity, plasma cortisol levels in obese individuals are not consistently elevated (Walker 2001). This observation is consistent with the belief that the role of these steroids in obesity is permissive. Therefore, while their presence is necessary, they are not the primary cause of obesity. The effects of glucocorticoids on fat metabolism under normal conditions are mainly catabolic, as they induce mobilization of fatty acids and glycerol from adipose, thereby elevating circulating levels of fatty acids. This increase of free fatty acids provides the liver with substrate for gluconeogenesis. However, the effects of glucocorticoids are not entirely lipolytic. Glucocorticoids also stimulate appetite and deposition of fat in the truncal region. This affect of glucocorticoids is dramatically illustrated by the adipose tissue distribution that is characteristic of Cushing's disease, a disease of glucocorticoid excess. Although the mechanism for the truncal obesity seen in Cushing's is not clear, it has become apparent that the relationship between glucocorticoids and obesity is complex. Based on preliminary observations, Seckl and colleagues have encouraged examination of the role of 11 $\beta$ -HSD-1 in obesity (1997).

### ***Tissue Specific Action and Dysfunction of 11 $\beta$ -HSD-1 in Obesity***

Because of the cumulative evidence for enhanced cortisol secretion and clearance in obesity that is distinct from the elevated circulating cortisol levels observed in Cushing's disease, this abnormality in glucocorticoid levels has been linked to enhanced peripheral metabolism (Lottenberg et al 1998). In support of this, several characteristic

tissue-specific changes in the activity of 11 $\beta$ -HSD-1 have been observed in obesity. Bjorntorp et al (1999) found that obese subjects displayed what they termed a “hypothalamic arousal syndrome” and did not exhibit the normal cyclic secretory pattern for cortisol or feedback control of these levels that was seen in lean subjects. In addition to a flat pattern and overall increase in cortisol secretion, these subjects presented clinical manifestations including insulin resistance, central obesity, dyslipidemia, elevated leptin levels, and more.

Tissue-specific glucocorticoid dysregulation has been reported in obese Zucker rats in which adipose 11 $\beta$ -HSD-1 activity is increased and hepatic 11 $\beta$ -HSD-1 action is decreased (Livingstone et al 2000a, Czegle et al 2008). Livingstone et al expanded on this with the observation of increased deactivation of active glucocorticoids by hepatic 5 $\beta$ -reductase activity and by enhanced 11 $\beta$ -HSD-2 activity in kidney along with enhanced urinary excretion of free corticosterone metabolites (2000a). This group also found that adrenalectomy of obese Zucker rats attenuated weight gain, and reversed this pattern of 11 $\beta$ -HSD-1 dysregulation in liver and mesenteric adipose (2000b). Drake et al hypothesized that a protective mechanism may be the explanation for their observation of decreased liver and adipose 11 $\beta$ -HSD-1 activity and increased hepatic 5 $\beta$ -reductase activity in Wistar rats fed a high fat diet for 3 wk (2005). After 20 wk under the same high fat diet conditions, Wistar rats were hyperglycemic, hyperinsulinemic, and obese, yet none of the changes in glucocorticoid metabolism seen at 3 wk had been maintained. Rask et al (2001) found that adipose tissue taken from obese humans has 3 to 4 times the 11 $\beta$ -HSD-1 activity of adipose taken from lean humans. Also, the reactivation of cortisone to cortisol by 11 $\beta$ -HSD-1 in liver was impaired, leading to increased clearance

of glucocorticoid metabolites in obese individuals. It has been suggested that this enhanced clearance may be the cause of increased secretion of cortisol by the hypothalamic-pituitary-adrenal axis in response to the decreased plasma cortisol levels. Currently, the mechanism(s) for these changes in glucocorticoid metabolism are not known.

### ***The Central Role of Glucocorticoids in Animal Models of Obesity***

Most forms of obesity share a dependence upon the availability of glucocorticoids. One exception is the pro-opiomelanocortin knockout mouse which is obese, but is reported to have no circulating corticosterone or aldosterone (Yaswen et al 1999). Solomon and Mayer (1973) and Yukimura and Bray (1978) were the first to identify glucocorticoids as a necessary factor in genetic obesity, finding that bilateral adrenalectomy (ADX) prevented the development of obesity in leptin deficient ob/ob mice and fatty Zucker rats (Yukimura et al 1978). With subsequent glucocorticoid replacement, obesity ensued in both of these models. Similar findings were reported for ADX rats fed a cafeteria diet (Rothwell et al 1984) and rats with ventromedial hypothalamic (VMH) nuclei lesions (King et al 1985). Glucocorticoid deficiency was also found to prevent the hyperphagic effects of neuropeptide Y (NPY). Stanley et al (1989) found that normal but not ADX rats overate and became obese when NPY was administered into the hypothalamus, and that glucocorticoid replacement effectively restored the hyperphagic effect of NPY. These observations have been replicated and related to changes in hypothalamic serotonin (Habash et al 1999, Yeung & Castonguay 2000).

In lean rats, ADX caused an attenuation of the rate of weight gain and altered body composition favoring a decrease in percentage of total fat mass (Schiffer & Wertheimer 1947). However, the duration of the post-ADX observation period seems to impact how ADX affects changes in caloric intake and/or body weight. Longer-term experiments with ADX rats have shown a significant reduction in the rate of weight gain, in ADX mice both body weight and carcass fat decrease significantly. Glucocorticoid replacement reverses all of these observations. Glucocorticoids also seem to interact with insulin and influence food choice in ADX rats. When provided with a choice of macronutrients, ADX rats maintain their intake of carbohydrate and protein, but reduce their intake of dietary fat (Devenport et al 1991). However, another experiment that provided insulin- and corticosterone-treated ADX rats with access to both lard and chow diet, the rats increased lard, but not chow intake in a dose-dependent manner (la Fleur et al 2004).

Mice that overexpress 11 $\beta$ -HSD-1 in mesenteric adipose develop truncal obesity and have impaired glucose tolerance, hyperphagia, and elevated blood lipids and serum leptin (Masuzaki et al 2001). However, while these mice appear analogous to humans with Cushing's disease, this obesity is dissimilar in that the source of the elevated glucocorticoids is adipose tissue and not the adrenal cortex. This would suggest that obesity in these mice may be the result of enhanced peripheral metabolism, but the mechanism for this change is not clear. 11 $\beta$ -HSD-1 null mice show resistance to diet-induced obesity when provided with a high fat diet (Morton et al 2001 & 2004). Berthiaume et al demonstrated that rats fed a high fat, high sucrose diet in addition to an 11 $\beta$ -HSD-1 inhibitor for 3 wk had similar body weight and food intake as controls fed

the same diet, but had reduced mesenteric adipose and adipose cell size (2007). No differences were observed in epididymal or retroperitoneal depots consistent with previous reports that implicate 11 $\beta$ -HSD-1 and glucocorticoids in the depot-specific accretion of fat in the visceral depot.

### **Hexose-6-Phosphate Dehydrogenase**

NADPH is the cofactor for 11 $\beta$ -HSD-1 oxidoreductase activity and therefore is critical to the ability of 11 $\beta$ -HSD-1 to generate active glucocorticoid. Hexose-6-phosphate dehydrogenase (H6PDH), the microsomal enzyme analogous to cytosolic glucose-6-phosphate dehydrogenase (G6PDH), generates a pool of reduced nicotinamide adenine dinucleotide phosphate [NADP(H)] that determines the set point for 11 $\beta$ -HSD-1 activity (Bujalska et al 2004, Bánhegyi et al 2004). H6PDH catalyzes the first two and rate-limiting steps of the pentose phosphate pathway within the endoplasmic reticulum (ER), while G6PDH serves as the first and rate-limiting enzyme for this pathway within the cytosol. H6PDH, however, has a much broader substrate specificity and can use hexoses other than glucose. Pyridine nucleotides are thought to be unable to cross cellular membranes, suggesting that the two enzymes generate reduced NADPH from separate pools of NADP<sup>+</sup>, although this has been recently challenged by McCormick et al (2006) who found that cytosolic pentose phosphate pathway flux impacts 11 $\beta$ -HSD-1 activity in rat adipocytes and microsomes. Critical steps for steroid hormone metabolism that require NADPH, including the action of 11 $\beta$ -HSD-1, occur in the ER and/or mitochondria, and thus, H6PDH has been implicated in 11 $\beta$ -HSD-1 activity. The initial support for this relationship came from the much earlier observation that H6PDH is localized in steroidogenic cells, liver cells, and renal tissue (Tanahashi et al 1980).

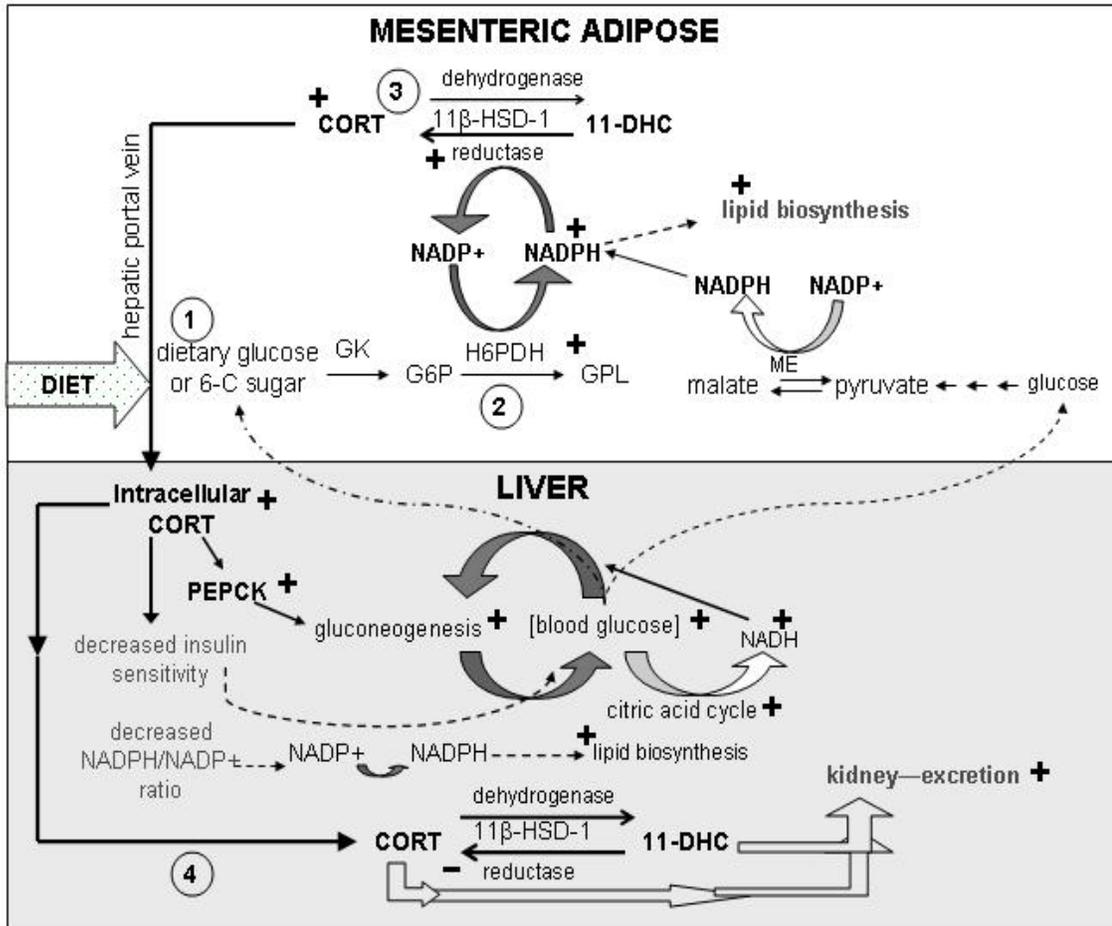
### **Cortisone Reductase Deficiency: The Link between H6PDH and 11 $\beta$ -HSD-1**

Investigations into the underlying genetics of cortisone reductase deficiency (CRD), in which activation of cortisone to cortisol does not occur, led to the discovery of not only a defect in the gene *HSD11B1*, but also mutations in the gene *H6PD* (Draper et al 2003). These mutations in individuals with CRD follow a triallelic digenic model of inheritance and result in low 11 $\beta$ -HSD-1 expression as well as a decrease or complete absence of NADPH generation in the ER. Lavery et al (2006) generated H6PDH knockout mice and showed that the knockouts were unable to convert 11-dehydrocorticosterone to corticosterone, while dehydrogenase activity increased and ultimately led to decreased circulating levels of active corticosterone. Bujalska et al (2005) found the same results in their experiments with CHO cells that were first transfected with 11 $\beta$ -HSD-1 cDNA and then transfected with H6PDH siRNA sequence. This body of research demonstrates that 11 $\beta$ -HSD-1 activity is dependent upon the activity of H6PDH, although it is not clear how diet might interact to change the activity or expression levels of these enzymes.

Recent evidence suggests that within the ER lumen, the glucose-6-phosphate transporter, H6PDH, and 11 $\beta$ -HSD-1 form a triad that can act as a nutrient sensor of not only carbohydrate, but also fatty acids (see review by Bánhegyi et al 2009). In a study of 18 adult non-diabetic men and women, a mixed meal led to increased whole body production of cortisol whereas saline infusion had no effect (Basu et al 2006). In this tracer study, measurement of hepatic venous, femoral artery, and femoral venous cortisol, D<sub>4</sub> cortisol, and D<sub>3</sub> by blood sampling prior to and at various timepoints following ingestion of the meal (or saline infusion) revealed enhanced extra-splanchnic cortisol

production following postprandial blood glucose peak. Dzyakanchuk et al (2009) have recently shown that a high ratio of NADPH/NADP<sup>+</sup> is needed for 11 $\beta$ -HSD-1 and that this reductase activity of the enzyme is stimulated by extracellular glucose in the human HEK-293 cells. Because these three proteins share a common pool of pyridine nucleotides, a cooperative relationship that is affected by the availability of glucose-6-phosphate (G6P) is likely. Hence it is hypothesized that the flux of G6P ultimately impacts the intracellular level of active glucocorticoid. Ambiguities exist about how cytosolic carbohydrate flux influences circulating glucocorticoid levels and feedback mechanisms related to adrenal glucocorticoid production. Additionally, there is no clear mechanism to explain how activity levels of 11 $\beta$ -HSD-1 or H6PDH are increased or decreased in a tissue-specific manner in obesity.

## Novel Hypothesis Regarding Diet, NADPH Availability, 11 $\beta$ -HSD-1, and H6PDH



**Figure 4.** Hypothesized pathway for the dysregulation of glucocorticoid metabolism that occurs in response to dietary obesity induced by a high sugar diet. Circled numbers signify the proposed sequence of events; + = increase in response to high sugar diet. Abbreviations: CORT: corticosterone; 11-DHC: 11-dehydrocorticosterone; G6P: glucose-6-phosphate; GK: glucokinase (or hexokinase); GPL: phosphogluconolactone; ME: malic enzyme; NADPH/NADP<sup>+</sup>: nicotinamide adenine dinucleotide phosphate; PEPCK: phosphoenol-pyruvate carboxykinase.

It seems likely that visceral obesity may be secondary to elevated expression and reductase activity of 11 $\beta$ -HSD-1 in adipose tissue. The initiation of elevated levels of active glucocorticoid locally in adipose likely precedes increased adiposity and then is compounded/perpetuated by increases in adiposity which occur alongside decreased insulin sensitivity and enhanced local glucocorticoid production. We hypothesize that

diets proportionally high in sugar contribute to enhanced pentose phosphate pathway flux in adipose tissue which in turn increase local 11 $\beta$ -HSD-1 expression and reductase activity. Additionally, we propose that the opposite will take place in the liver as evidenced by decreased levels of 11 $\beta$ -HSD-1 reductase activity and increased hepatic glucocorticoid clearance. Local hypercortisolemia increases transcription of PEPCK, decreases insulin sensitivity and increases hepatic gluconeogenic flux (Goldstein et al 2002). It is possible that in the initial stages of glucocorticoid dysregulation increased availability of 6-carbon sugars in adipose tissue leads to increased production of NADPH via the pentose phosphate pathway and subsequent increases in 11 $\beta$ -HSD-1 reductase activity (Fig. 4). The increased corticosterone levels in adipose eventually begin to flow into the hepatic portal vein and elevated local glucocorticoids in liver result. We hypothesize that corticosterone levels then begin to increase in liver which initially increase 11 $\beta$ -HSD-1 dehydrogenase activity. This increase in local hepatic corticosterone causes the upregulation of PEPCK which increases gluconeogenesis (Fig. 4). Additionally, as an adaptive response to the high sucrose and high fructose diets it is likely that both H6PDH and 11 $\beta$ -HSD-1 would be upregulated in adipose tissue, and in that way favor further increases in intracellular corticosterone that then migrates via the circulation back to the liver. The excess of 6-carbon sugars provided by a high sucrose diet may be increasing production of the reducing equivalent NADH via enhanced glycolysis and subsequent citric acid cycle activity in the liver thereby providing more cofactor to fuel the inappropriately increased gluconeogenic pathway.

Our 10 wk study revealed increases in H6PDH mRNA in both liver and mesenteric adipose of high sucrose-fed rats, while 11 $\beta$ -HSD-1 mRNA increased in

mesenteric adipose and decreased in liver of high sucrose-fed rats in comparison to controls (London et al 2007). It is possible that the increased NADPH produced by elevated H6PDH is utilized for de novo fatty acid biosynthesis and not as cofactor for 11 $\beta$ -HSD-1 reductase activity. This hypothesis is supported by evidence suggesting that diets high in fructose can promote the accumulation of lipid, mainly triacylglycerols, as well as other symptoms of nonalcoholic hepatic steatosis (Kawasaki et al 2009). Also, enhanced de novo lipid biosynthesis and subsequent dyslipidemia and increased fat storage may have played a role in the increased adiposity observed in the sucrose-fed rats in the 10 wk pilot study.

## **Chapter 2. The Role of Diet and 11 $\beta$ -hydroxysteroid dehydrogenase-1 on Obesity (London & Castonguay 2009)**

### **Introduction**

11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD-1) is a key regulatory enzyme in glucocorticoid metabolism, specifically in regulating intracellular concentrations of cortisol, the primary glucocorticoid. While the excessive level of circulating cortisol in Cushing's disease is of adrenal origin, it is the intracellular and not systemic level of cortisol that is elevated in obesity. This tissue-specific dysregulation of glucocorticoids observed in obesity results from alterations in 11 $\beta$ -HSD-1 in both liver and mesenteric adipose. While cortisol has been identified as playing a permissive role in obesity, little is known about how diet may regulate message, expression and activity of 11 $\beta$ -HSD-1. In this review, we have integrated three lines of evidence that, taken together, suggest that dietary composition can play a primary role in promoting increased intracellular cortisol, and in that way form the basis of a mechanism that results in excessive adiposity. We review evidence from studies of adrenalectomized rats, as well as studies linking 11 $\beta$ -HSD-1 to the pentose phosphate pathway and other metabolic pathways via the enzyme hexose-6-phosphate dehydrogenase (H6PDH). Emerging evidence from dietary manipulation experiments suggesting that macronutrient composition may elicit changes in 11 $\beta$ -HSD-1 and promote obesity is discussed.

### **The centrality of glucocorticoids in animal models of obesity**

Most forms of obesity share a dependence upon the availability of glucocorticoids, so-named for their role in blood glucose maintenance (Houssay 1964). Solomon and Mayer (1973) and Yukimura and Bray (1978) were first to implicate glucocorticoids as a necessary factor in genetic obesity, observing that obesity was

prevented following bilateral adrenalectomy (ADX) and fully restored by glucocorticoid replacement. Analogous findings were reported for rats fed a cafeteria diet (Rothwell et al 1984) and rats with electrolytic lesions of the ventromedial hypothalamic nuclei (Bruce et al 1982). In lean rats, ADX causes an attenuation of the rate of weight gain and a shift in body composition to lessen carcass fat (Schiffer & Wertheimer 1947). Whether or not ADX elicits a decrease of caloric intake and/or body weight appears to depend upon the duration of the post-ADX observation period. Longer-term experiments typically report a significant reduction in intake and body weight, as well as a significant decrease in carcass fat. Glucocorticoid replacement reverses all of these phenomena (Yukimura et al 1978, Castonguay et al 1986, Freedman et al 1985).

### **Glucocorticoids and Dietary Obesity**

There is far less known about the role of adrenal hormones and dietary obesity. ADX leptin-deficient (*ob/ob*) mice lose carcass fat, have normal glucose tolerance, and maintain normal circulating insulin. However, when ADX *ob/ob* mice are fed a diet that is either high in glucose or fat, they retain their obese phenotype that includes hyperinsulinemia, excess body fat, and abnormal glucose tolerance (Grogan et al 1987). Kang et al reported that a high fat (HF), but not a high glucose diet allowed ADX *ob/ob* mice to maintain high levels of energy retention in the absence of hyperinsulinemia (1992). We have reported that HF diets attenuate the effects of ADX in both obese and lean Zucker rats (Bray et al 1992), and that mice fed a HF diet maintain elevated levels of circulating leptin after ADX (Bai & Castonguay 2000), once again illustrating that HF diets can reduce or reverse some of the effects of ADX. Similarly, ADX rats that are given access to a sucrose solution in addition to their customary chow diet are also

normal with respect to corticotrophin releasing factor and dopamine  $\beta$ -hydroxylase mRNA expression in the brain, food intake, caloric efficiency, fat deposition, and circulating triglyceride, leptin, and insulin (Laugero et al 2001).

### **A Novel Hypothesis about Glucocorticoids and Obesity**

Although glucocorticoid production and secretion are increased in human obesity, plasma cortisol levels in obese people are not consistently elevated (Walker 2001). This observation is congruent with the viewpoint that the steroids play only a permissive role in obesity. However, it has long been recognized that glucocorticoids have a profound influence on adipose tissue distribution and function as evidenced by Cushing's patients. This would imply that the relationship between body fat and glucocorticoids is more than simply permissive. Seckl and colleagues have advocated an examination of the role of 11 $\beta$ -HSD-1 in obesity (1997). 11 $\beta$ -HSD-1 controls intracellular glucocorticoid concentrations via its dehydrogenase and reductase activities. When 11 $\beta$ -HSD-1 acts as a dehydrogenase it inactivates cortisol/corticosterone (human/rat). As a result, cortisol is converted into its inert 11-keto form (cortisone/11 dehydrocorticosterone). When 11 $\beta$ -HSD-1 acts as a reductase the inactive metabolite is converted into active glucocorticoid. Adipose tissue taken from obese humans has 3 to 4 times the 11 $\beta$ -HSD-1 reductase activity of adipose taken from lean humans (Rask et al 2001; refer to Table 1).

Mice that overexpress 11 $\beta$ -HSD-1 develop truncal obesity and display glucose tolerance, hyperphagia, and elevated blood lipids and serum leptin (Masuzaki et al 2001). Hence, these mice appear to be a close model to humans with Cushing's syndrome, a condition characterized by elevated circulating glucocorticoids and visceral obesity. Visceral obesity may be secondary to enhanced local activation of steroid via elevated

levels and activity of 11 $\beta$ -HSD-1 in adipose tissue which result in abnormally elevated levels of cortisol/corticosterone in adipose tissue. This obesity is distinct from that of Cushing's patients in that the source of the elevated glucocorticoids is adipose tissue as opposed to the adrenal cortex.

### **11 $\beta$ -HSD-1 and Tissue-Specific Regulation of Glucocorticoid Action**

Two isozymes of 11 $\beta$ -HSD, 11 $\beta$ -HSD-1 and 11 $\beta$ -HSD-2, interconvert active glucocorticoid and its inactive 11-keto metabolite. 11 $\beta$ -HSD-2 functions primarily as a dehydrogenase *in vivo*, inactivating the hormone by converting the 11-hydroxy group to an 11-keto group. It is mainly expressed in mineralocorticoid target tissues, the kidney and the colon, where it protects the mineralocorticoid receptor from activation by glucocorticoids which are present at much higher concentrations than mineralocorticoids. 11 $\beta$ -HSD-2 is not expressed in the pituitary or most regions of the adult central nervous system. Recently, it has been suggested that the type 2 isoform may play a protective role by decreasing glucocorticoid action in adipocytes as transgenic mice that overexpress 11 $\beta$ -HSD-2 in adipose tissue are resistant to diet-induced obesity (Kershaw et al 2006; refer to Table 1). 11 $\beta$ -HSD-2 mRNA has also been detected in the hypothalamus, suggesting that it may play a role in energy balance (Zhang et al 2005). In contrast, 11 $\beta$ -HSD-1 functions predominantly as an oxidoreductase *in vivo*, generating active hormone from inactive metabolite. It is highly expressed in adipose, liver, pituitary, and brain (Sakai et al 1992).

The search for an ideal selective pharmacological antagonist for 11 $\beta$ -HSD-1 is ongoing. Glycyrrhetic acid does inhibit 11 $\beta$ -HSD-1 activity, but without isoform specificity (Horigome et al 1992). Much of what is known about 11 $\beta$ -HSD-1 therefore

comes from studies of knockout (KO) mice or transgenic mice that overexpress the enzyme. Masuzaki et al. (2001) reported that mice that overexpress 11 $\beta$ -HSD-1 under the control of the enhancer-promoter region of the adipocyte fatty acid-binding protein aP2 gene develop visceral obesity, impaired glucose tolerance, hyperphagia, and elevated blood lipids and leptin. Overexpression of the enzyme is also associated with several other forms of human and rodent obesity (see below). 11 $\beta$ -HSD-1 KO mice have adrenal hyperplasia but attenuated glucocorticoid-induced activation of gluconeogenic enzymes in response to fasting, as well as lower glucose levels in response to stress (Holmes et al 2001). It has been proposed that these mice might compensate for the mutation by increasing adrenal activity so as to maintain homeostasis. Consistent with this, Harris et al (2001) found that these KO mice have elevated plasma corticosterone and ACTH levels at the diurnal nadir as well as a prolonged corticosterone peak. Similar disruptions in glucocorticoid rhythmicity have been reported in human and rodent obesities. Further, the 11 $\beta$ -HSD-1 null mice have exaggerated ACTH and corticosterone responses to restraint stress, as well as impaired stress responsivity. We have observed a similar syndrome in intact Sprague-Dawley rats fed a HF diet (Kamara et al 1998).

Kotolevtsev et al (1997) reported that 11 $\beta$ -HSD-1 KO mice have attenuated activation of the key hepatic gluconeogenic enzymes, glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK). The mice are resistant to hyperglycemia induced by overnight fasting followed by placement in a novel environment. Importantly, giving access to a HF diet significantly lowered fasting plasma glucose in KO mice compared to wild-type controls. Morton et al (2001) found that 11 $\beta$ -HSD-1 KO mice exhibit an improved lipid profile and increased hepatic insulin sensitization. Specifically,

the mice have lower plasma triglyceride levels, increased hepatic expression of the fat-catabolizing enzymes, carnitine palmitoyltransferase-I, acyl-CoA oxidase, and uncoupling protein-2, increased HDL cholesterol and elevated liver mRNA and serum levels of apolipoprotein AI. In summary, these studies collectively imply that the 11 $\beta$ -HSD type 1 isoform is important in energy homeostasis and that the null mutant adopts compensatory responses in systems implicated in several different models of obesity.

Correlational studies also indicate that 11 $\beta$ -HSD-1 is overexpressed in both human and rodent obesities. For example, Aoki et al found that insulin-resistant diabetic C57BL/KsJ-db/db mice have higher blood glucose, plasma insulin, and corticosterone, in addition to increased 11 $\beta$ -HSD-1 activity and elevated hepatic 11 $\beta$ -HSD-1 mRNA levels compared to db/+m heterozygote controls (Aoki et al 2001). They suggested that the increased hepatic corticosterone concentration may antagonize the action of insulin and cause insulin resistance. Livingstone et al (2000a) reported that 11 $\beta$ -HSD-1 activity in omental fat excised from obese male Zucker rats was double that of lean controls. They also found that ADX reversed the difference in 11 $\beta$ -HSD-1 activity observed between obese and lean Zucker rat omental fat. Paulmyer-Lacroix et al (2002) reported that 11 $\beta$ -HSD-1 mRNA expression was increased by more than 2.5-fold in the stromal compartment of visceral adipose tissue compared with subcutaneous adipose tissue. They also reported that 11 $\beta$ -HSD-1 was increased in mature adipocytes taken from abdominal subcutaneous and visceral fat depots of obese patients when compared to lean controls. Similarly, Rask et al found that obese women have elevated 11 $\beta$ -HSD-1 activity in subcutaneous adipose tissue when compared to lean controls, and that the activity of the enzyme positively correlates with body mass index (2001).

11 $\beta$ -HSD-1 obviously plays an important role in normal cell functioning. Bujalska et al compared human omental to subcutaneous adipose stromal cells, and observed that 11 $\beta$ -HSD-1 activity in omental stromal cells increased during differentiation into mature adipocytes (2002). 11 $\beta$ -HSD-1 activity acts primarily as a dehydrogenase (converting the active hormone to inactive metabolite) in preadipocytes, yet the opposite is true in mature adipocytes. They suggested that the oxidoreductase activity of 11 $\beta$ -HSD-1 ensures the local generation of active steroid and in that way further induces adipocyte differentiation. Yau et al (2007) used 11 $\beta$ -HSD-1 KO mice to study aging-related hippocampal changes that are thought to be caused by excess corticosterone. Unlike wild type controls, these KO mice perform significantly better in a Morris water maze, suggesting that age-related learning deficits that are thought to be the result of high levels of corticosterone exposure are ameliorated with deactivation to 11-dehydrocorticosterone.

#### ***The Regulation of 11 $\beta$ -HSD-1 Activity vs. 11 $\beta$ -HSD-1 Message***

Despite rapid advances in our appreciation of the role of this enzyme, there is still little that is known about the factors that regulate its dehydrogenase and reductase activities. Increased 11 $\beta$ -HSD-1 message, protein, and activity in the omental or mesenteric fat of obese patients and genetically obese rats, respectively, are thought to promote elevated intracellular glucocorticoid concentrations in a tissue-specific manner. Genetically engineered mice that overexpress the enzyme in mesenteric adipose become obese. HF diets promote transient increases in circulating corticosterone, increased circulating insulin and insulin resistance, as well as increased circulating leptin (Tannenbaum 1997). Any one of these factors might promote the increased

oxidoreductase activity of 11 $\beta$ -HSD-1 reported in obese humans (Rask et al 2002). Similarly, increased reductase activity (and not just increased message/protein) may be the consequence of any one of these variables that are affected by a HF diet or may result from increased substrate availability for 11 $\beta$ -HSD-1 caused by changes in intermediary metabolism.

### ***Hexose-6-phosphate dehydrogenase and Its Role in Glucocorticoid Metabolism***

Hexose-6-phosphate dehydrogenase (H6PDH) and its putative cooperativity with 11 $\beta$ -HSD-1 within the lumen of the ER has become an active area in the search for a mechanism to explain the tissue-specific regulation of 11 $\beta$ -HSD-1 activity. H6PDH, the microsomal analogue to cytosolic glucose-6-phosphate dehydrogenase (G6PDH), generates a pool of reduced nicotinamide adenine dinucleotide phosphate [NADP(H)] that plays a critical role in determining the set point for 11 $\beta$ -HSD-1 reductase activity (Bujalska et al 2005, Hewitt et al 2005). H6PDH catalyzes the first two (including the rate-limiting) steps of the pentose phosphate pathway within the ER, while G6PDH serves as the first and rate-limiting enzyme of the same pathway in the cytosol. H6PDH also differs from G6PDH in that it has broad substrate specificity and can use various hexose-phosphates. Investigations into the underlying genetics of cortisone reductase deficiency (CRD), in which activation of cortisone to cortisol does not occur, led to the discovery of not only a defect in the gene HSD11B1, but also mutations in the gene H6PDH (Draper et al 2005). These mutations in individuals with CRD follow a triallelic digenic model of inheritance and result in low 11 $\beta$ -HSD-1 expression as well as a decrease or complete absence of NADPH generation in the ER. Preliminary support for this relationship came from the much earlier observation that H6PDH is localized in

steroidogenic cells, liver cells, and renal tissue (Tanahashi and Hori 1980), where 11 $\beta$ -HSD-1 is also highly expressed. Lavery et al (2007) generated H6PDH knockout mice and showed that H6PDH null mice were unable to convert inactive 11-dehydrocorticosterone to corticosterone, while dehydrogenase activity increased, ultimately leading to decreased circulating levels of active glucocorticoid. Bujalska et al (2008) reported the same effects in CHO cells that were first transfected with 11 $\beta$ -HSD-1 cDNA and then transfected with H6PDH siRNA.

It is generally accepted that cellular membranes are relatively impermeable to pyridine nucleotides, which led to the initial suggestion that the two enzymes generate reduced NADPH from separate pools of NADP<sup>+</sup>. This has recently been challenged by McCormick et al (2006) who found that cytosolic pentose phosphate pathway flux can also impact 11 $\beta$ -HSD-1 activity in rat adipocytes and microsomes. In support of the hypothesis that NADPH generated within the lumen of the endoplasmic reticulum is the key source of cofactor for 11 $\beta$ -HSD-1. Bánhegyi et al (Bánhegyi et al 2004) showed cooperativity between 11 $\beta$ -HSD-1 and H6PDH in liver via the inhibition of the glucose-6-phosphate transporter (G6PT) by S3483. Both inhibition of G6PT and the absence of glucose-6-phosphate as substrate led to significantly reduced cortisone activation and H6PDH activity in isolated rat liver microsomes. Enzyme activity studies confirming the presence of distinct intralumenal and cytosolic pools of pyridine nucleotide in the hepatic ER (Czegle et al 2006) and ER of adipocytes (Marcolongo et al 2007) provide additional support for the close relationship between 11 $\beta$ -HSD-1 and H6PDH.

This body of research demonstrates that 11 $\beta$ -HSD-1 activity is dependent upon the activity of H6PDH, which relies upon the availability of its substrate, primarily

glucose-6-phosphate. While there is evidence to suggest that diet and macronutrient composition can influence glucocorticoid metabolism and that carbohydrate intake can impact pentose phosphate pathway flux (London et al 2007), it is not clear how intake is involved in changing the activity or expression levels of these enzymes. Additionally, there is no clear mechanism to explain how 11 $\beta$ -HSD-1 or H6PDH expression and/or activity levels are increased or decreased in a tissue-specific manner in obesity.

### ***Influence of Diet on Message, Expression, and Activity of 11 $\beta$ -HSD-1***

#### ***High Fat Diets***

Obesogenic diets, including high fat diets, may impact glucocorticoid metabolism via their impact on 11 $\beta$ -HSD-1. Rodent studies suggest that inhibiting 11 $\beta$ -HSD-1 may be useful in protecting against high fat diet-induced obesity. 11 $\beta$ -HSD-1 null mice demonstrate resistance to diet-induced obesity when fed a high fat diet (Morton et al 2001 & 2004; refer to Table 1). Morton et al showed that 11 $\beta$ -HSD-1 activity is lower in subcutaneous, epididymal, and visceral fat depots in obesity/metabolic syndrome-resistant (A/J) mice compared to 11 $\beta$ -HSD-1 activity levels in C57BL/6J Lep ob/ob mice, a strain prone to obesity (2004). Transgenic mice that overexpress 11 $\beta$ -HSD-1 in mesenteric adipose display phenotypic and metabolic disturbances that favor obesity, including increased 11 $\beta$ -HSD-1 activity in subcutaneous and epididymal fat (2.4- and 2.7-fold, respectively) (Masuzaki et al 2001). This is comparable to what is observed in obese vs. lean humans. Fat accumulation in the abdominal region of transgenic mice fed a low fat diet (10% fat) was comparable to that of non-transgenic mice fed a high fat diet (45% fat), and this disproportionate accumulation of lipid in visceral depots was even more exaggerated when the transgenic mice were fed the high fat diet. This work

cumulatively supports the notion that 11 $\beta$ -HSD-1 levels and/or activity can predispose rodent models to increased adiposity. There is, however, no evidence to suggest high fat diets in the absence of specific genetic background can impact tissue-specific 11 $\beta$ -HSD-1 message, expression, or activity.

**Table 1.** Summary of data implicating local glucocorticoid metabolism in obesity and obesity-related disturbances

Species	Treatment/condition	Phenotypic and physiological changes	Known dietary influences
Human	Cushing's Syndrome	<ul style="list-style-type: none"> <li>• Adrenal overproduction of cortisol; subsequent elevation of systemic glucocorticoid levels</li> <li>• Visceral obesity, glucose intolerance</li> <li>• Depression, excessive hair growth, irregular periods or amenorrhea in women; infertility in men</li> <li>• Elevated blood pressure</li> </ul>	
Human	Obesity	<ul style="list-style-type: none"> <li>• Elevated 11<math>\beta</math>-HSD-1 activity in subcutaneous tissue in women; activity positively correlated to BMI (Yau et al 2007)</li> </ul>	
Mouse	11 $\beta$ -HSD-1 knockout	<ul style="list-style-type: none"> <li>• Adrenal hyperplasia, attenuated glucocorticoid -induced activation of gluconeogenic enzymes in food-deprived state, decreased glucose levels in response to stress (Kamara et al 1998, Horigome et al 1999);</li> <li>• Elevated plasma corticosterone and ACTH at diurnal nadir; prolonged peak (Holmes et al 2001);</li> <li>• Improved lipid profile, increased hepatic expression of fat catabolizing enzymes (carnitine palmitoyltransferase-I, acyl-CoA oxidase, uncoupling protein-2), increased HDL, elevated hepatic mRNA and serum apolipoprotein AI levels (Kotelevtsev 1997)</li> </ul>	<ul style="list-style-type: none"> <li>• High fat diet lowers fasting plasma glucose levels (Kamara et al 1998)</li> </ul>
Mouse	Over-expression of 11 $\beta$ -HSD-1 in adipose	<ul style="list-style-type: none"> <li>• Truncal obesity, glucose intolerance, hyperphagia, and elevated serum lipids and leptin (Seckl 1997)</li> </ul>	
Mouse	Over-expression of 11 $\beta$ -HSD-2 in adipose	<ul style="list-style-type: none"> <li>• Reduced fat mass accumulation on HF diet; associated with decreased food intake, increased energy expenditure, improved glucose tolerance and insulin sensitivity (Rask et al 2001)</li> <li>• Decreased adipose tissue gene expression of leptin and resistin and increased expression of</li> </ul>	<ul style="list-style-type: none"> <li>• Resistance to diet-induced obesity on high fat diet (Rask et al 2001)</li> </ul>

		adiponectin, peroxisome proliferator-activated receptor gamma, and uncoupling protein 2 (Rask et al 2001)	
Mouse (ob/ob)	ADX	<ul style="list-style-type: none"> <li>Loss of carcass fat; normal glucose tolerance, normal circulating insulin levels (Freedman et al 1985)</li> </ul>	<ul style="list-style-type: none"> <li>High fat diet—obese phenotype maintained including glucose tolerance (Freedman et al 1985, Grogan et al 1987)</li> <li>High—obese phenotype maintained including impaired glucose tolerance (Freedman et al 1985)</li> </ul>
Rat	ADX	<ul style="list-style-type: none"> <li>Decreased weight gain; decreased body fat (Bruce et al 1982)</li> </ul>	<ul style="list-style-type: none"> <li>High fat diet reverses some effects of ADX, including elevated leptin levels (Bray et al 1992);</li> <li>Sucrose access restores CRF and <math>\beta</math>-hydroxylase mRNA levels in brain, food intake, caloric efficiency, fat deposition and systemic TG, leptin, and insulin (Bai &amp; Castonguay 2000)</li> </ul>
Rat (fa/fa)	obesity	<ul style="list-style-type: none"> <li>Increased 11<math>\beta</math>-HSD-1 activity in omental fat; enhanced activity reversed by ADX (Aoki et al 2001)</li> </ul>	

One recent clinical trial suggests that percentage of energy from dietary fat can affect glucocorticoid metabolism independent of fat loss. Stimson et al (2007) showed that healthy obese men fed either ad libitum or isocaloric high fat, low carbohydrate diets (HFLC) (66% and 4%, respectively) had increased cortisol appearance and reduced urinary excretion of cortisol metabolites compared to their counterparts on ad libitum or isocaloric moderate fat, moderate carbohydrate diets (MFMC) (35%:35%). While weight loss was higher among the HFLC diet group, no differences in loss of fat mass were observed between the HFLC and MFMC groups, and changes in cortisol metabolism were deemed independent of weight loss. This suggests differential regulation of cortisol metabolism as a result of macronutrient composition of diet.

Adult male Sprague-Dawley (S-D) rats fed a HF, high sucrose diet in addition to an 11 $\beta$ -HSD-1 inhibitor (a 4-heteroaryl bicyclo[2.2.2]octyl triazole) for 3 wk had similar body weight and food intake as controls fed the same diet, but had reduced mesenteric adipose and adipose cell size (Berthiaume et al 2007). Interestingly, the inhibition of 11 $\beta$ -HSD-1 decreased the message level of enzymes involved in lipid synthesis (fatty acid synthase [FAS], stearoyl-CoA desaturase 1 [SCD1], diacylglycerol acyltransferase 1 [DGAT1]) and fatty acid cycling (adipose triglyceride lipase [ATGL], phosphoenolpyruvate carboxykinase [PEPCK]), while concomitantly increasing the message of fatty acid oxidation-promoting carnitine palmitoyl transferase 1. While these data suggest diet-induced changes in accumulation of fat mass via a glucocorticoid driven mechanism it is not clear how dietary macronutrient composition might play a role in the observed metabolic changes.

Macronutrient composition of diet (specifically carbohydrate content) has also been found to influence glucose homeostasis and lipid metabolism, yet a direct effect of carbohydrate intake on glucocorticoid metabolism has not been elucidated. When provided with access to solutions of sucrose, fructose or glucose, rats increase their energy intake by 10–20% above what they would consume if provided with rat chow alone (Kanarek & Orthen-Gambill 1982). Simple sugars have been shown to stimulate hepatic de-novo lipogenesis, making them a likely candidate for promoting the fasting hypertriglyceridemia that has been associated with high sucrose or high fructose diets (Havel 2005). Minehira et al (2003) examined markers of whole body de novo lipogenesis and lipogenesis in subcutaneous adipose in response to two 4-day dietary interventions in 9 healthy lean subjects (male n= 5, female n= 4): 1) isocaloric feeding

(100% of energy requirement with 50% of total energy as carbohydrate, 35% as lipid, and 15% as protein), or 2) carbohydrate overfeeding (175% of energy requirement with 71% as carbohydrate, 20% as lipid, and 9% as protein). Carbohydrate overfeeding increased basal and post-glucose energy expenditure as well as net carbohydrate oxidation, while whole body net de novo lipogenesis after glucose loading was markedly increased at the expense of glycogen synthesis. mRNA levels for sterol regulatory element-binding protein-1c (SREBP-1c), acetyl-CoA carboxylase (ACC), and FAS were increased significantly by carbohydrate overfeeding. In a similar study of lean vs. overweight individuals, Minehira et al found that carbohydrate overfeeding did not stimulate whole body net de novo lipid biosynthesis nor expression of the lipogenic enzymes ACC and FAS or SREBP-1c in adipose to a greater extent in overweight compared to lean individuals (Minehira et al 2004). While both fructose and glucose stimulate SREBP-1c, which regulates de novo lipogenesis, both SREBP-1c and de novo lipogenesis are more strongly induced by fructose than glucose, providing evidence that different sugars can differentially regulate lipid metabolism (Minehira et al 2003). When S-D rats are given long-term ad libitum access to standard chow as well as 32% sucrose, 32% fructose, or 32% glucose solutions, or granulated sucrose, they exhibited differential responses (Kanarek & Orthen-Gambill 1982). While all sugar-fed rats had decreased glucose tolerance and increased weight gain and retroperitoneal fat, sucrose-fed rats had significantly more brown adipose tissue than either control or fructose-fed rats. Fructose-fed rats had elevated serum triglycerides compared to the others. Importantly, while the mechanism is unclear, these data indicate that different sources of dietary sugars appear to have different metabolic consequences.

Kyoto Wistar (WKY) and spontaneously hypertensive rats (SHR) fed fructose-enriched diets for 14 days exhibited significant increases in plasma insulin and triglyceride concentrations in addition to elevated blood pressure (Reaven et al 1990). Similar results have also been reported in S-D rats fed a high fructose diet (Hwang et al 1987). Reaven et al showed that SHR and WKY rats given ad libitum access to a diet composed of 66% fructose, 12% fat, and 22% protein respond similarly. However, increases in blood pressure and plasma triglycerides were higher in the SHR rats. These data suggest not only a direct influence of dietary fructose on markers of lipid metabolism and blood pressure, but also that underlying genetic background interacts with the carbohydrate composition of diet.

In their summary of recent findings about the short- and long-term metabolic effects of fructose in humans and rodents, Lê and Tappy found that the consumption of large quantities of fructose can lead to the development of a complete metabolic syndrome in rodents including hepatic and extrahepatic insulin resistance, dyslipidemia, and high blood pressure irrespective of the fact that dietary fructose does not directly elicit insulin secretion (2006). Short-term moderate to high fructose intake in humans caused elevated plasma triglycerides and changes in hepatic glucose homeostasis as well as adipose tissue insulin resistance, but did not appear to elicit muscle insulin resistance or elevated blood pressure (Chong et al 2007).

High fructose intake may contribute to postprandial lipemia via changes in the partitioning of fatty acids toward esterification. A randomized crossover study of 14 healthy subjects given test meals of either labeled fructose or glucose in addition to labeled palmitate, showed that respiratory quotient and plasma triacylglycerol and

VLDL-triacylglycerol were significantly higher in those administered the fructose meal. The concentrations of both insulin and labeled palmitate in nonesterified fatty acids were lower after fructose than glucose meals (Bhatnagar et al 2000). This suggests that decreased insulin stimulation after fructose may cause less lipoprotein lipase activation in adipose tissue and, as a result, impaired triacylglycerol clearance.

***Do dietary components, particularly CHO, influence the HPA axis?***

While evidence supports the differential effect of macronutrient composition on both lipid and glucose metabolism, it is not clear what role glucocorticoids and 11 $\beta$ -HSD-1 might play. ADX in a rat model has been shown to have a profound effect on the HPA axis, namely by increasing ACTH secretion and sympathetic activity and decreasing food intake and weight gain (Bhatnagar 2000). Laugero et al found that voluntary consumption of sucrose solution, but not non-nutritive saccharin solution, reversed the effects of ADX in rats by restoring corticotrophin releasing factor (CRF) and dopamine- $\beta$ -hydroxylase mRNA levels in brain (2001). Voluntary sucrose solution intake also restored food intake, weight gain, and circulating triglyceride, leptin, and insulin levels in ADX rats suggesting that dietary sugar can trigger enhanced peripheral glucocorticoid production and/or affect behavior to compensate for the absence of adrenal activity. More recently, we have shown that rats given free access to 16% sucrose solution in addition to chow and water for 10 wk have increased 11 $\beta$  HSD-1 and H6PDH message in mesenteric adipose (London et al 2007). These changes in adipose were accompanied by decreased 11 $\beta$ -HSD-1 message and increased H6PDH message in liver in comparison to controls given ad lib access to chow and water. Further, we showed that while there are no differences in weight gain among the control and sugar-fed groups after 6 wk of sucrose

access, the sucrose-fed rats had a significantly higher mean percentage of body fat. We also found that sucrose, fructose, and glucose have differential effects on the message of 11 $\beta$ -HSD-1 and H6PDH in adipose and liver in as little as 1 wk of access to sugar solutions (previously unpublished data). These data suggest that alterations in mRNA for enzymes related to glucocorticoid metabolism may result from dietary manipulations and not solely changes in body composition or increased adiposity.

### **Conclusion**

It is clear that glucocorticoids are involved in obesity, especially diet-induced obesity. Although elevated circulating corticosterone is not a defining characteristic of all obesities, the steroid nevertheless plays a critical role in its etiology. One plausible hypothesis that links these phenomena is a dysregulation of local levels of active steroid via altered activity of the enzyme 11 $\beta$ -HSD-1. A major tenet of the pathway proposed herein (Fig. 5) is that enzyme activity is induced in specific tissues by consumption of high sugar diets.



Results from our laboratory point to sucrose as a particularly effective dietary component that can alter 11 $\beta$ -HSD-1 message and at the same time promote increased adiposity. Our preliminary inquiries suggest that NADPH is an essential trigger in promoting the oxidoreductase activity of 11 $\beta$ -HSD-1.

It has long been known that sucrose spares ADX induced body weight and body fat loss (Laugero et al 2001). London et al (2007) more recently demonstrated that sucrose promoted a decrease in hepatic 11 $\beta$ -HSD-1 message and simultaneously an increase in 11 $\beta$ -HSD-1 message in mesenteric adipose tissue. This difference in the effect of sucrose on 11 $\beta$ -HSD-1 in liver and in adipose has not been thoroughly explained. Increased adipose 11 $\beta$ -HSD-1 is thought to promote increased intracellular cortisol/corticosterone concentrations. The active hormone is then thought to be transported to the liver, where it can participate in both the upregulation of PEPCCK and subsequent increased gluconeogenesis. Decreased liver 11 $\beta$ -HSD-1 message is likely to be the consequence of downregulation resulting from the influx of cortisol/corticosterone stemming from adipose (Fig. 5). What is interesting is that both adipose and liver have increased H6PDH message in response to sucrose. Work being conducted in our laboratory suggests that acute (24h) access to sucrose fails to induce all of these changes (London & Castonguay, in preparation).

Havel (2005) has suggested a second explanation of why sucrose access results in obesity. He notes that fructose (a component of sucrose) bypasses the key glycolytic enzyme phosphofructokinase, thereby providing unregulated supply of glycerol-3-phosphate and acetyl-CoA so as to favor increased lipogenesis. We have suggested that the sucrose-induced enhanced H6PDH message found in liver may help promote

lipogenesis by increasing available NADPH. Thus, sucrose access favors obesity by having profound effects on both hepatic glucose production and at the same time increased lipogenesis. Perhaps this dual-effect accounts for why access to glucose fails to promote comparable levels of obesity, since dietary glucose is subject to tighter metabolic regulation.

Two sources of evidence have helped to identify the interactions between intracellular glucocorticoid concentrations, diet and body composition (See Table 1). The first set of evidence has been derived from observations of tissue-specific changes in  $11\beta$ -HSD-1 expression that have been observed in human obesity. The second has come from studies manipulating the expression of  $11\beta$ -HSD-1 in animal models. Studies of animal models make it clear that the enzyme has pervasive effects throughout the animal.  $11\beta$ -HSD-1 KO mice show perturbations in the HPA axis including adrenal hyperplasia (Holmes et al 2001, Kotelevtsev 1999) and disruptions in the normal diurnal nadirs and peaks of both corticosterone and ACTH (Harris et al 2001). Additionally, these  $11\beta$ -HSD-1 null mice have improved lipid profiles and enhanced expression of fat catabolized enzymes in liver (Morton et al 2001). Conversely, overexpression of  $11\beta$ -HSD-1 in adipose of mouse results in hyperphagia, truncal obesity, glucose intolerance, and elevated levels of serum lipids and leptin (Masuzaki et al 2001). These observations parallel the enhanced  $11\beta$ -HSD-1 activity in adipose that has been reported in both human obesity (Rask et al 2002) and obese rats (Livingstone et al 2000a). This obesity-linked enhancement in  $11\beta$ -HSD-1 activity in obese rats is effectively reversed by ADX (Livingstone et al 2000b). ADX also decreases body fat and weight gain in ob/ob mice (Gogan et al 1987) and wild type rats (Yukimara et al 1978), and administration of a high

fat diet maintains the obese phenotype and reverses many of the effects of ADX in both a mouse and rat model (Gogan et al 1987, Kang et al 1992, Bai & Castonguay 2000). Laugero et al showed that sucrose access in ADX rats can similarly restore food intake, caloric efficiency, fat deposition, TG, leptin and insulin, and impacts the HPA axis (2001). Interestingly, adipose-specific overexpression of 11 $\beta$ -HSD-2 in mice confers protection against diet-induced obesity in mice fed high fat diet characterized by reduced food intake, reduced fat mass accumulation, increased energy expenditure, improved insulin sensitivity and glucose tolerance (Kershaw et al 2006). These examples illustrate the interplay between diet and glucocorticoid metabolism as well as the potential for obesity intervention by targeting both isoforms of 11 $\beta$ -HSD.

### **Chapter 3. Effect of High Sucrose Diets on Body Composition; Plasma Insulin, Leptin, and Glucose Concentrations; and 11 $\beta$ -HSD-1 and H6PDH mRNA in Liver and Mesenteric Adipose of Adult Male Sprague-Dawley Rats (London et al 2007)**

#### **Introduction**

The prevalence of obesity in the United States has reached epidemic proportions. It has been estimated that two-thirds of the adult U.S. population is overweight or obese. While it is clear that obesity is a multifactorial disease resulting from the combined effects of genetic, physiological, environmental, social, and psychological factors, there is currently no cure. The list of regulatory factors identified as influencing food intake, energy expenditure and body weight has increased considerably over the past few decades. While these multiple factors interact on both central and peripheral levels (in the brain, gut, liver, etc.) (Schwartz et al 2000, Zigman et al 2003), one common finding is that maintenance of normal adrenal glucocorticoid levels is necessary for the normal function of these factors. More recently, there is evidence that elevated tissue-specific concentrations of glucocorticoids may better predict the promotion and maintenance of obesity than circulating hormone levels which are not consistently higher in obese versus lean animals (Lottenberg 1998, Walker 2001). The bidirectional enzyme, 11 $\beta$ -HSD-1, interconverts the active hormone cortisol (human) or corticosterone (rat) and inert cortisone (human) or 11-dehydrocorticosterone (rat). 11 $\beta$ -HSD-1 is highly expressed in adipose tissue, liver, and brain where it acts primarily as an oxidoreductase to generate active glucocorticoid. Adipose tissue taken from obese humans has 3 to 4 times the 11 $\beta$ -HSD-1 oxo-reductase activity compared to adipose taken from lean individuals (Rask et al 2001). This change in 11 $\beta$ -HSD-1 activity in adipose tissue is likely a commonality of different forms of obesity.

Diet-induced obesity in a number of rodent and non-human primate models appears to parallel human diet-induced obesity in its dependence on combined environmental and genetic factors. High fat diets can promote increased weight gain and adiposity in rats (Schemmel et al 1970, West et al 1992), and certain strains are more susceptible to obesity when fed high fat diets demonstrating the critical interaction between genetic and environmental factors (Shier et al 1975). Diets high in sugar have also been used to induce weight gain and obesity experimentally (Sclafani & Xenakis 1984, Castonguay et al 1981). Rats given access to palatable sugar solutions typically consume 60% of their daily intake from the solution, with the remaining 40% of total calories taken from standard chow. Dallman and her associates have more recently reported that sucrose access abates the weight loss and reduced adiposity that takes place after bilateral adrenalectomy (Dallman et al 2003). They have speculated that by consuming sucrose at times when sham operated animals would normally mobilize fat, adrenalectomized (ADX) rats are spared the ADX-induced deficit in gluconeogenesis, an adrenal hormone-mediated regulatory mechanism serving energy balance. Carbohydrate-induced obesity is of particular interest in the context of glucocorticoid dysregulation because of these prior observations in ADX animals and because of the necessity of NADPH to generate active glucocorticoid which is a byproduct of the oxidation of various sugars.

11 $\beta$ -HSD-1 resides in the endoplasmic reticulum lumen (Ozols et al 1995, Odermatt et al 1999) and requires NADPH as a cofactor for its oxidoreductase activity which generates active glucocorticoid. 11 $\beta$ -HSD-1 acts predominantly as an oxidoreductase in vivo and in intact cells (Jamieson et al 1995, Bujalska et al 1997), but

acts as a dehydrogenase in cellular homogenates (Lakshmi & Monder 1988). Reductase activity in cellular homogenates can be initiated upon the addition of NADPH or a system that can generate NADPH such as glucose-6-phosphate dehydrogenase (G6PDH) (Agarwal et al 1990).

H6PDH is the microsomal analog of cytosolic G6PDH, as it catalyzes the first two steps, including the committed step, of the pentose phosphate pathway within the ER lumen (Mason et al 1990, Clarke et al 2002). H6PDH has been linked to 11 $\beta$ -HSD-1 through the pathogenesis of cortisone reductase deficiency (CRD) (Draper et al 2003), in which individuals that carry mutations of both 11 $\beta$  HSD-1 and H6PDH are unable to generate cortisol from inactive cortisone. Spolarics and his colleagues found that increased dietary carbohydrate can promote increased pentose phosphate pathway flux via G6PDH message (1999). The purpose of the present study was to examine the effect of dietary sucrose on body weight, body composition, and other indices of obesity, including plasma glucose, insulin, and leptin, as well as H6PDH and 11 $\beta$ -HSD-1 mRNA in mesenteric adipose and liver in rats.

### **Experimental Design**

Twenty-four male S-D weighing with mean body weights of approximately 250 g were obtained from Charles River Laboratories (Wilmington, MA). Rats were immediately housed in wire-bottom hanging cages and maintained on a 12h-light/dark cycle in a humidity controlled room ( $25 \pm 2^\circ\text{C}$ ). For a 1 wk acclimating period, the rats were given free access to water and the later described commercial rodent diet (Harlan Teklad 7012) (Fig. 12), and were then randomly assigned to one of the following three weight-matched groups (n= 8/group): 1) 16% sucrose (S16), 2) 32 % sucrose (S32), and

3) control. Rats in the S16 group were given free access to the commercial diet, a 16% (W/V) sucrose solution, and water. Rats assigned to the S32 group were given free access to the commercial diet, a 32% sucrose solution, and water. The control group animals were given free access to the commercial diet and water only. Sucrose solutions were composed of commercially available pure cane sugar (Domino brand; Baltimore, MD), and were prepared 24h in advance and kept refrigerated until 1h prior to use. Both water and sugar solutions were provided in 200 mL plastic bottles fitted with standard #7 rubber stoppers and sipper tubes. Food was made available in small glass food cups that were secured to the back wall of each cage with a Plexiglas food cup holder. Food spillage, though minimal, was collected and weighed daily. Total daily food intake was measured by subtracting the amount of food spillage (g) from the amount of diet consumed by each animal as indicated by the difference in weight (g) of the food cup at the beginning and end of each 24h period. Similarly, daily sucrose solution intake was measured by subtracting the weight of each bottle and the remaining sucrose solution from the weight of each bottle with solution when it was prepared on the previous day.

The experimental conditions were in effect for 68 days. On day 69 all rats were weighed and returned to their home cages and given access to water only for 24h. On day 70, each rat was weighed, and then restrained to enable the collection of a 500  $\mu$ L tail blood sample in a Sarsted capillary blood collection tube. The tubes were kept in ice until centrifuged, and plasma was saved (-20°C) for subsequent food-deprived glucose analysis (see below). Each rat was then returned to its home cage and given free access to its appropriate sugar solution as well as chow and water for an additional 2 days.

The rats were killed on day 72 by first administering light anesthesia (CO<sub>2</sub> gas), followed by rapid decapitation and exsanguination. Blood was collected in EDTA treated tubes and kept on ice until centrifuged. The plasma collected was then frozen and stored (-20°C) until subsequent analyses. Small (~1 g) liver from the lobus laterilus sinister and the entire mesenteric adipose depot were dissected quickly and flash frozen. Each carcass was then prepared for body composition analyses and frozen (-20° C). All of the above care and treatment protocols received prior approval by the University of Maryland Institutional Animal Care and Use Committee (protocol: Assessment of Whey Low's Contribution to Diet Energy & Its Effects on Growth & Body Composition in Lab Rats, #R-06-42; approval date: July 27, 2006) (Appendices 1 and 2).

## **Research Methods**

### ***Body Composition***

Rat carcasses were thawed and total body composition of each was measured using an EchoMRI-900™ QNMR analyzer (Echo Medical Systems, Houston, TX). Output included measurement of fat mass, lean tissue mass, and free body fluids.

### ***Measurement of Plasma Insulin and Leptin***

Radioimmunoassay kits purchased from Linco Research (Millipore Corporation, Billerica, MA) were used to measure plasma leptin (kit RL-83K) and insulin (kit RI-13K) of the samples obtained at the time of animal sacrifice. Samples were counted on a Packard Cobra gamma counter for 1 minute each.

### ***Measurement of Plasma Glucose***

Glucose concentrations of plasma samples were determined using a Yellow Springs Instruments glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH).

### ***Rate of Weight Loss and Metabolic Rate***

The percentage of body weight lost was calculated by dividing the amount of weight (g) lost during 24h by body weight at the outset of the fasting period. The mean rate of weight lost by each group was used to estimate differences in basal metabolic rates among the groups (Rixon & Stevens 1957).

### ***mRNA Extraction***

Frozen tissue samples were thawed and 500 mg of each sample homogenized in buffer while kept cold on ice. Total RNA was extracted according to the RNeasy Mini and RNeasy Mini Lipid protocols (QIAGEN), respectively for liver and adipose. Additional centrifugation steps were included in the protocol to enhance sample purity by removing organic solvents and salts from the extracts. Total RNA was then purified to remove contaminating DNA using DNA-free (Ambion). Purified RNA was analyzed spectrophotometrically (NanoDrop) to determine the concentration and quality of product (260/280 ratio).

### ***mRNA Quantification***

cDNA template was created for each sample using 500 ng of purified RNA extract with SuperScript III Reverse Transcriptase (Invitrogen). Finally, each sample was subjected to qRT-PCR (IQ5 cycler, BioRad) using a 40 cycle program and subsequent

melt curve analysis program (55°–95°C). All primers were tested using stock rat cDNA (Table 2). The program for each primer set is detailed in Table 6.

**Table 2. Primer sequences for target genes.**

<b>Gene</b>	<b>Sense primer sequence</b>	<b>Antisense primer sequence</b>
11β-HSD-1	5'-TGCTCTGGATGGGTTCTTTT-3'	5'-GAAGCCGAGGACACACAGAGAG-3'
H6PDH	5'-GGGCTATGTTCCGGATCTTGTTTA-3'	5'-GTTCCGGCACCCAGTGTCT-3'
β-actin	5'-TGGAGTCTACTGGCGTCTT-3'	5'TGTCATATTTCTCGTGGTCA-3'

The cDNA derived from each tissue sample was simultaneously subjected to qRT-PCR using primers for the housekeeper gene β-actin. All reactions were carried out in triplicate.

All primers were tested by performing qRT-PCR and melt curves with stock rat cDNA and the primers. PCR products were then run out on an agarose gel with appropriate DNA reference ladder to confirm the presence of only one product of the expected molecular weight.

Differences in message between treatment and control groups were assessed based on the cycle threshold (CT) values for each treatment group and that of β-actin. First the ΔCT value was calculated by subtracting the CT value of β-actin from that of the gene of interest. Then, the ΔΔCT value for each observation was calculated by subtracting the mean ΔCT value for the control group from ΔCT value of each rat in the treatment groups. Finally, fold change in mRNA of the gene of interest for each rat was calculated as  $2^{-\Delta\Delta CT}$  in message (BioRad, Livak et al 2001).

## **Results**

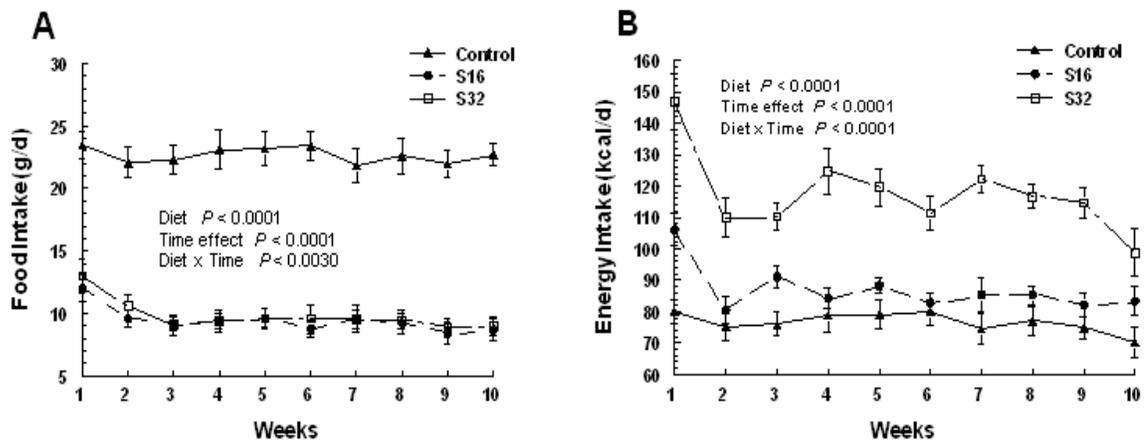
### ***Body Weight and Body Composition***

The mean percentages of body fat for the S16 ( $17.7 \pm 2.3\%$ ) and S32 groups ( $15.6 \pm 0.9\%$ ) did not differ, but both means were greater than that of the control group ( $12.4 \pm 0.9\%$ ;  $P = 0.03$ ). Lean body mass and free body fluid mass did not differ among the three groups (data not shown). The observation that ad libitum access to 16% sucrose solution was enough to elicit differences in the percentage of body fat in comparison to the control group, yet free access to 32% sucrose solution did not elicit further increases was integral in the decision to use only one sucrose-fed group (16%) in the proposed study.

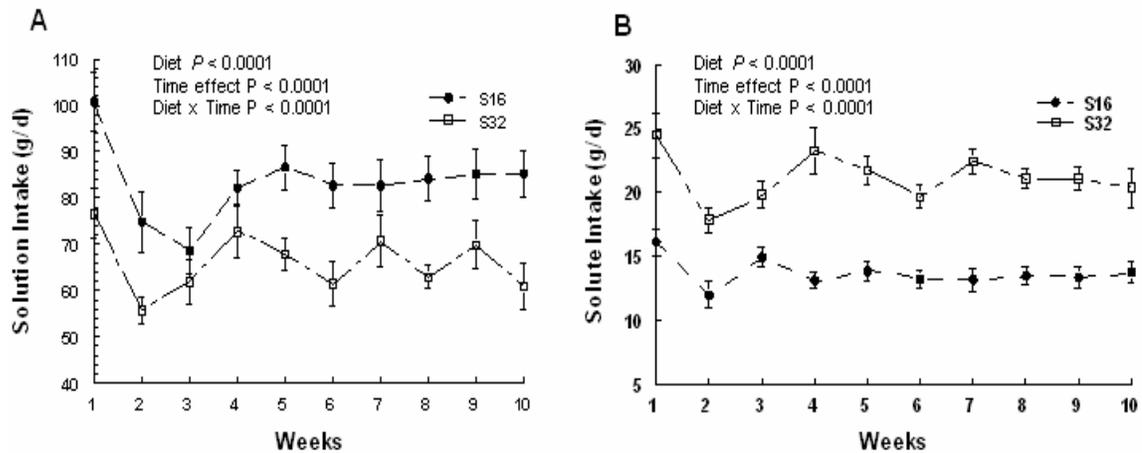
### ***Food, Sucrose, and Total Energy Intake***

As anticipated, the control group ate more chow daily than did the S16 and S32 groups, which did not differ from one another (Fig. 6A). The S32 group did however consume significantly more total energy per day than did the S16 and control groups, which did not differ (Fig. 6B). Additionally, the mean cumulative food intake of the control group was significantly higher than the intakes of the sucrose-fed groups (Table 3,  $P < 0.0001$ ). The mean cumulative food intakes of the S16 and S32 groups did not differ. The mean daily sucrose solution intake of the S16 group ( $86.1 \pm 1.1$  g) was significantly higher than that of the S32 group ( $66.3 \pm 1.4$  g) (Fig. 7A). Mean daily solute intakes for the S16 and S32 groups were  $13.8 \pm 0.2$  and  $21.2 \pm 0.5$  g, respectively, which differed statistically (Fig. 7B,  $P < 0.0001$ ). Interestingly, the mean daily sucrose intake was much less variable from day to day among rats in the S16 group after an initial adjustment period early in the study. This was another contributing factor in selecting a 16% as the concentration for sugar solutions for the subsequent experiments. Cumulative

sucrose intake was also higher for the S32 group compared to that of the S16 group during the 10 wk experimental period ( $P < 0.0001$ ) (Table 3). The percentage of total daily energy intake derived from sucrose was greater for the S32 group than for the S16 group ( $P = 0.006$ ). On average, the S32 group consumed significantly more total calories than the S16 and control groups ( $P < 0.0001$ ) and the S16 group consumed significantly more total calories than did the control group (Table 3,  $P < 0.0001$ ). While total energy intake differed among the groups, interestingly the mean body weights among the rats in the three groups did not differ, nor did the mean percentages of body fat between the S16 and S32 groups to reflect the differences in intakes.



**Figure 6.** (A) Food, and (B) energy intakes of male rats in the control, S16, and S32 groups during the 10 wk study; values are means  $\pm$  SEM;  $P < 0.05$ ;  $n=8/gp$ .



**Figure 7. (A)** Sucrose solution intake, and **(B)** Sucrose intake and of rats in the S16 and S32 groups during the 10 wk study; values are means  $\pm$  SEM;  $P < 0.05$ ;  $n=8/gp$ .

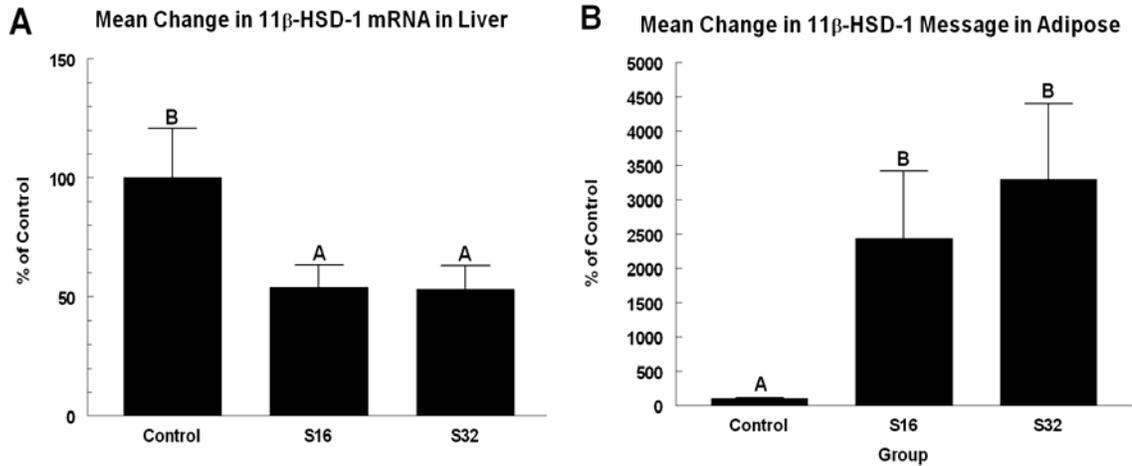
**Table 3.** Initial and final mean body weights; and mean standard chow intake, mean percent of total energy as sucrose, and mean cumulative energy intake for control, S16, and S32 rats.

	Initial Body Weight G	Final Body Weight g	Cumulative Non-purified diet Intake g/10 wk	Cumulative Sucrose Intake g/10 wk	Sucrose Intake % Energy	Cumulative Total Energy Intake <sup>1</sup> kcal/10 wk
Control	309.6 $\pm$ 3.6	407.4 $\pm$ 11.3 <sup>a</sup>	1541.4 $\pm$ 53.6 <sup>a</sup>	0	0	5580.0 $\pm$ 194.2 <sup>c</sup>
S16	306.9 $\pm$ 5.2	413.8 $\pm$ 14.0 <sup>a</sup>	643.2 $\pm$ 27.6 <sup>b</sup>	936 $\pm$ 36 <sup>b</sup>	61.8 $\pm$ 0.02 <sup>b</sup>	6075.4 $\pm$ 130.6 <sup>b</sup>
S32	306.0 $\pm$ 5.2	430.3 $\pm$ 17.7 <sup>a</sup>	667.2 $\pm$ 38. <sup>b</sup>	1443 $\pm$ 31 <sup>a</sup>	70.1 $\pm$ 0.01 <sup>a</sup>	8192.4 $\pm$ 165.1 <sup>a</sup>

Values are expressed as mean  $\pm$  SEM,  $n=8$ ; means in a column with superscripts without a common letter differ;  $P < 0.05$ ; <sup>1</sup>measurement taken at time of kill.

### ***11 $\beta$ -HSD-1 mRNA Levels in Liver and Mesenteric Adipose Tissue***

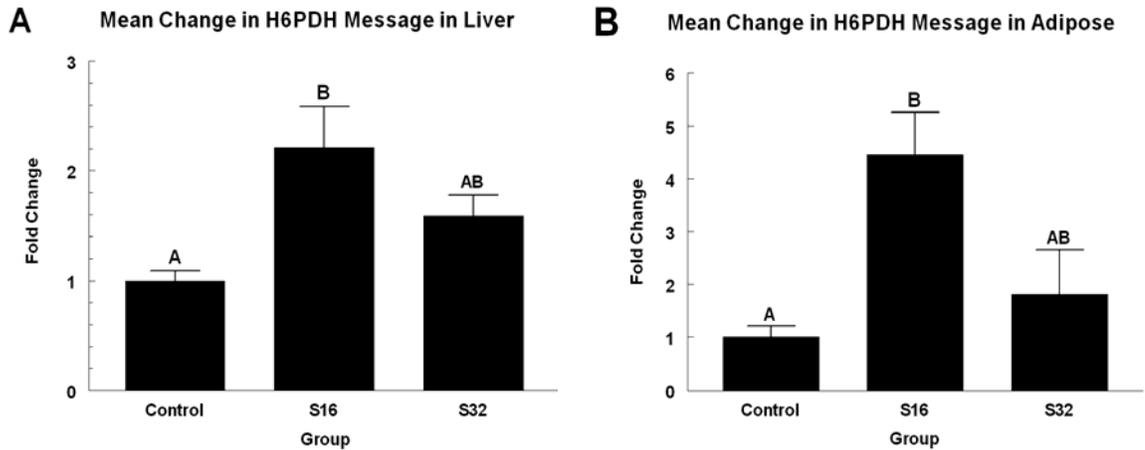
11 $\beta$ -HSD-1 message in liver was suppressed by  $46.1 \pm 0.9\%$  and  $47.2 \pm 1.1\%$ , respectively in the rats in the S16 and S32 groups in comparison to the control group ( $P=0.027$ ; Fig. 8A). Conversely, 11 $\beta$ -HSD-1 message in mesenteric fat was increased by approximately 23-fold and 32-fold, respectively, in the S16 and S32 groups in comparison to the control group ( $P=0.006$ ; Fig. 8B).



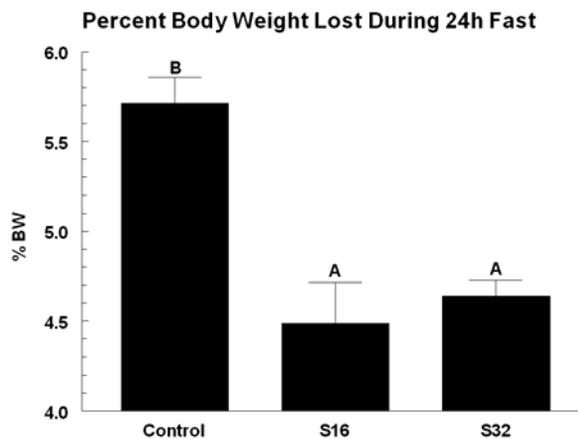
**Figure 8.** Mean 11β-HSD-1 mRNA in (A) Liver, and (B) Mesenteric adipose of rats in the S16, S32, and control groups as measured by qRT-PCR; values are means ± SEM; ( $P < 0.05$ );  $n = 8/\text{gp}$ .

#### ***H6PDH mRNA Levels in Liver and Mesenteric Adipose Tissue***

The H6PDH mRNA level nearly doubled in the liver of the S16 group in comparison to the control group ( $P = 0.009$ ; Fig. 9A). In mesenteric fat, H6PDH mRNA increased by greater than 3-fold in the S16 group compared to both the S32 and the control group ( $P = 0.003$ ; Fig. 8B). A point worth noting is that statistical analysis of the message data for mesenteric fat revealed a positive correlation between H6PDH message and 11β-HSD-1 message ( $r = 0.91$ ;  $P = 0.005$ ).



**Figure 9.** H6PDH message in (A) Liver, and (B) Mesenteric adipose of rats in the S16, S32, and control groups as measured by qRT-PCR; values are means  $\pm$  SEM;  $P < 0.05$ ;  $n = 8/\text{gp}$ .



**Figure 10.** Mean percentage of body weight lost during 24h fast in rats in the S16, S32, and control groups; values are means  $\pm$  SEM; different letters indicate differences among groups; values are means  $\pm$  SEM;  $P < 0.05$ ;  $n = 8/\text{gp}$ .

### *Rate of Body Weight Loss during 24h Fast*

Three days prior to the end of the experiment (day 67), the rats were food deprived overnight (16h) in preparation for a glucose tolerance test. Percent of body weight lost during the fast was calculated by dividing the amount of weight (g) lost during the 24h period between the morning weighings by the body weight on day 67 for each rat. The rats in the control group lost a significantly higher percentage of body

weight ( $5.7 \pm 0.15$  %) than did the rats fed high sucrose diets (S16:  $4.5 \pm 0.23$  %, S32:  $4.6 \pm 0.09$  %) ( $P < 0.0001$ ) (Fig. 10, Table 4). The differences observed between the control and sucrose-fed groups indicate metabolic changes that resulted from the treatments in this 10 wk study and warrant further investigation. It should be noted, however, that the type of body mass loss was not assessed nor were any potential changes in locomotor activity among the rats.

**Table 4.** Rate of weight loss; plasma leptin and insulin; and food-deprived and fed glucose concentrations in control, S16, and S32 rats.

	Rate of Weight Loss** %/d	Plasma Leptin* µg/L	Plasma Insulin* µg/L	Fasting Plasma Glucose** (g/L)	Ad Libitum Plasma Glucose* (g/L)
Control	$5.7 \pm 0.1^a$	$9.9 \pm 0.8^a$	$3.0 \pm 0.4^a$	$0.952 \pm 0.047^a$	$1.589 \pm 0.197^a$
S16	$4.5 \pm 0.2^b$	$11.0 \pm 1.0^a$	$4.5 \pm 0.7^b$	$0.834 \pm 0.048^a$	$1.569 \pm 0.163^a$
S32	$4.6 \pm 0.1^b$	$11.4 \pm 1.3^a$	$5.1 \pm 0.6^b$	$0.761 \pm 0.056^b$	$1.342 \pm 0.182^a$

Different superscript letters in columns represent significant differences;  $P < 0.05$ ; \* Measurements taken at time of sacrifice (day 70); \*\* Measurements taken after 24h food-deprivation.

### ***Plasma Glucose, Insulin and Leptin***

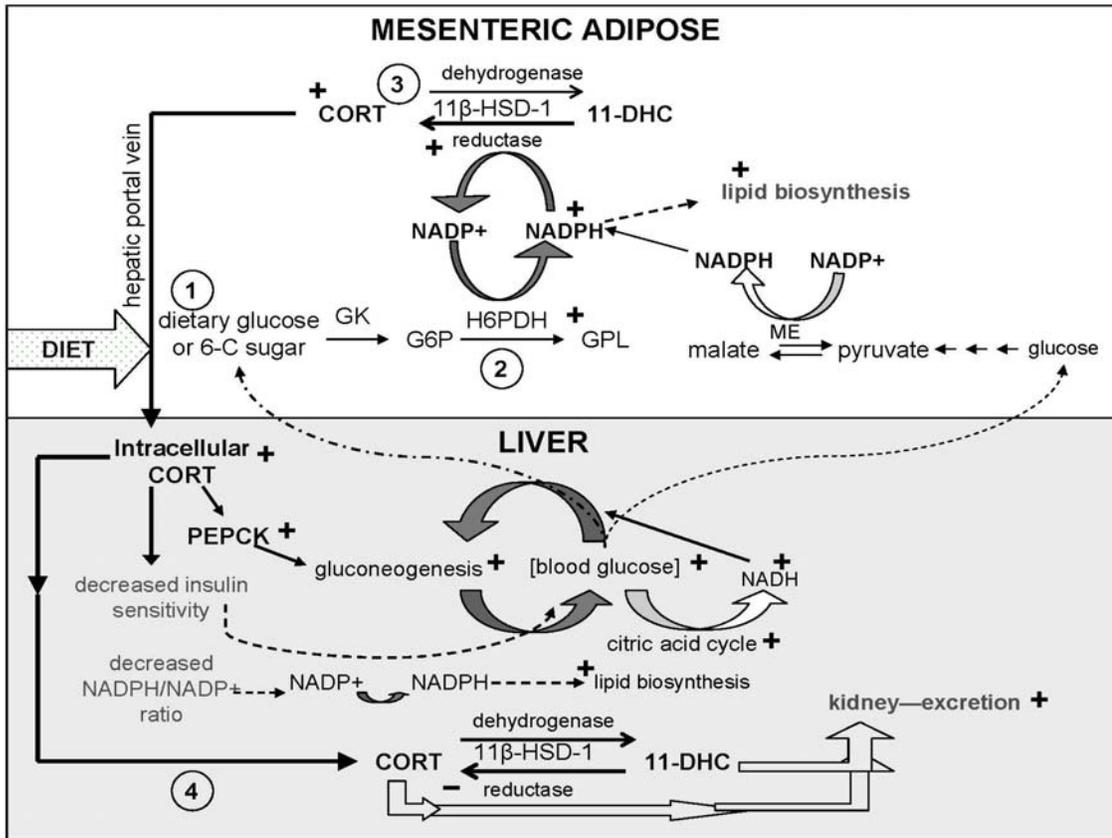
Mean plasma insulin was higher in the S16 and S32 groups compared to the control ( $P < 0.05$ ), and concentrations did not differ between the S16 and S32 groups (Table 4). Plasma insulin concentrations, as determined by radioimmunoassay, correlated positively with percentage of body fat ( $r=0.84$ ;  $P < 0.0001$ ). Mean plasma glucose for food-deprived rats was higher for the S32 group compared to that of the control group ( $P= 0.04$ ), but concentrations did not differ between the S16 and S32 groups or the S16 and control groups (Table 4). Further, no differences were found in rats from the three groups in fed plasma glucose or leptin concentrations.

## Discussion

The aim of the present study was to investigate whether a high sucrose diet would alter the level of message of H6PDH and 11 $\beta$ -HSD-1 in mesenteric adipose and liver in rats. Previous studies have reported obesity-resistance to high fat diets in 11 $\beta$ -HSD-1 null mice (Morton et al 2001, Densmore et al 2006), but the specific effects of diet on the message, expression, or activity of 11 $\beta$ -HSD-1 or H6PDH have not previously been reported. High sucrose diets increase cytosolic pentose pathway flux via G6PDH (Spolarics 1999) so it seems likely that increased microsomal pentose pathway flux could be achieved with a high sucrose diet via increased H6PDH activity. Additionally, Bujalska and colleagues demonstrated that it is H6PDH and not G6PDH activity that impacts the activity of 11 $\beta$ -HSD-1 (Bujalska et al 2005), and Lavery and fellow researchers confirmed the necessity of H6PDH for 11 $\beta$ -HSD-1 oxidoreductase activity in their experiments with H6PDH KO mice (Lavery et al 2006). Here we show that sucrose access leads to increased H6PDH message in both adipose and liver of Sprague-Dawley rats. The importance of this observation is that it now provides a mechanism by which sucrose access leads to conditions favoring the oxidoreductase activity of 11 $\beta$ -HSD-1 in adipose. Dietary sucrose led to an increase in H6PDH message, and presumably the increased NADPH that resulted promoted increased 11 $\beta$ -HSD-1 oxidoreductase activity, resulting in increased intracellular glucocorticoids (Fig. 11).

There are several questions that remain to be addressed regarding these observations. While we do not know when the increase in H6PDH message first occurs or whether these changes are reflected in increased protein levels, the increased H6PDH message is observed after 10 wk access to 16% sucrose solution in which rats consume

an average of 63% of their daily energy intake from sucrose. It should be noted that while statistically significant changes were also observed in mean 11 $\beta$ -HSD-1 message in liver and adipose between the S32 and control groups, the increases in mean H6PDH message in these organs did not reach statistical significance. We suspect that this may have been a result of the increased inter-animal variability that was observed in the S32 group for sucrose and total caloric intake throughout the 10 wk experimental period (Figs. 6 & 7). This same day-to-day variability was not observed in the S16 group, possibly leading to more sustained, robust metabolic changes.



**Figure 11.** Proposed pathway for glucocorticoid dysregulation that results from a high sucrose diet. The sequence of metabolic and glucocorticoid mediated events in liver and mesenteric adipose are shown, beginning with uptake of dietary sugars; circled numbers indicate the proposed chronology of events and arrows identify the flow of the hypothesized pathway. Abbreviations: 11-DHC, 11-dehydrocorticosterone; GK, glucokinase (or hexokinase); GPL, phospho-gluconolactone; G6P, glucose-6-phosphate; ME, malic enzyme; PEPCK, phosphoenolpyruvate carboxykinase.

### *Changes in 11 $\beta$ -HSD-1*

Livingstone et al found that 11 $\beta$ -HSD-1 activity is increased in adipose while 11 $\beta$ -HSD-1 message and activity are decreased in liver of obese Zucker rats in comparison to their lean counterparts (Livingstone et al 2000a). This finding of increased 11 $\beta$ -HSD-1 activity in adipose and decreased activity in liver has since been extended to obese humans (Rask et al 2001). We replicate the finding of decreased 11 $\beta$ -HSD-1 mRNA in rat liver, and extend what is known about the generality that 11 $\beta$ -HSD-1 activity is increased with obesity by showing increased 11 $\beta$ -HSD-1 message in mesenteric adipose of the obese rat. Further, we demonstrate that these changes can be initiated by dietary manipulations. These data provide initial evidence that alterations in the message of both 11 $\beta$ -HSD-1 and H6PDH occur as a result of physiological changes that arise in high sucrose diet-induced obesity. In the present study, obesity was defined among the animals as significantly increased percentage of total body fat. There were no significant differences in mean body weight between the control and sucrose-fed animals. This finding suggests that altered glucose homeostasis and tissue-specific glucocorticoid regulation may be occurring as a function of increased visceral fat depots and altered enzyme action within adipose.

At the conclusion of our 10 wk study, the animals were fasted for 24h prior to blood collection and glucose analysis. Interestingly, we found that during the fast the sucrose-fed animals lost a significantly lower percentage of their initial, ad-libitum body weights than the control animals. This and the differences we observed in 11 $\beta$  HSD-1 and H6PDH message between groups suggests underlying metabolic and physiologic changes occur as a result of high-sucrose diets. Dallman and colleagues found sucrose access

blocked most of the metabolic, behavioral, and neuroendocrine effects of ADX in rats (Dallman et al 2003) including circumventing the loss of gluconeogenesis caused by corticosterone deficit and providing negative feedback input to the hypothalamus to reduce corticotropin releasing factor secretion. From the results of our study we propose that sucrose access is likely causing increased gluconeogenesis in the liver via elevated corticosterone levels resulting from increased substrate availability for H6PDH and subsequent increased 11 $\beta$  HSD-1 activity which affect local glucocorticoid levels and thereby enable increased weight gain.

***Novel Hypothesis Regarding Diet Composition, NADPH Availability, and Message of 11 $\beta$ -HSD-1***

It seems likely that visceral obesity may be promoted by elevated oxidoreductase activity of 11 $\beta$ -HSD-1 in adipose tissue, and the resulting abnormally elevated local (intracellular) levels of active glucocorticoid. We hypothesize that excess dietary sugar contributes to enhanced microsomal pentose phosphate pathway flux in adipose tissue, which in turn increases local 11 $\beta$ -HSD-1 oxidoreductase activity (Fig. 11). Hypercortisolemia increases transcription of PEPCK, decreases insulin sensitivity, and increases hepatic gluconeogenic flux (Livingstone et al 2000a). It is possible that in the initial stages of glucocorticoid dysregulation an increase in the availability of 6-carbon sugars in adipose tissue (Fig. 11) leads to increased production of NADPH via the pentose phosphate pathway. This increased production of NADPH could subsequently increase 11 $\beta$ -HSD-1 reductase activity, and in turn increase adipose corticosterone levels which eventually reach the liver via the hepatic portal vein. We hypothesize that corticosterone levels then begin to increase in the liver which causes the upregulation of

PEPCK, thereby increasing gluconeogenesis and concomitantly decreasing 11 $\beta$ -HSD-1 reductase activity. It would seem likely that both H6PDH and 11 $\beta$ -HSD-1 may be upregulated in adipose tissue as an adaptive response, favoring further increases in intracellular corticosterone that then migrate back to the liver in the bloodstream. The excess of 6-carbon sugars provided by a high sucrose diet might also be increasing production of the reducing equivalent NADH in the liver via enhanced glycolysis and subsequent citric acid cycle activity thereby providing more cofactor to fuel the inappropriately increased gluconeogenic pathway. This might provide an explanation for the onset and maintenance of the improper response of increased gluconeogenesis to hyperglycemia that has been observed in diabetes and obesity, and may also explain the decreased 11 $\beta$ -HSD-1 oxidoreductase activity that has been observed in the obese liver.

## **Chapter 4. Acute Ad Libitum Access to 16% Sucrose, 16% Fructose, and 16% Glucose Solutions on 11 $\beta$ -Hydroxysteroid Dehydrogenase Type 1, Hexose-6-Phosphate Dehydrogenase, and Several Parameters of Carbohydrate and Lipid Metabolism in Rats**

### **Introduction**

Approximately two-thirds of U.S. adults are overweight or obese. The prevalence of overweight among young people has tripled since 1980. Concurrently, the CDC reports an increase in carbohydrate intake of 62 and 69 grams/day, respectively, in women and men between 1971 and 2000 (CDC 2004). To date, there is no experimental data that can link these trends. We have been interested in the role of stress hormones in promoting diet-induced obesity. 11 $\beta$ -hydroxysteroid dehydrogenase-1 (11 $\beta$ -HSD-1) plays a key role in regulating intracellular glucocorticoids. Increased message and/or activity of 11 $\beta$ -HSD-1 in adipose are characteristic of human and animal models of obesity. The purpose of this research was to investigate the acute effects of high sugar diets on glucocorticoid metabolism. We have previously shown that rats given long-term ad libitum access to sucrose solution and chow are significantly fatter, and have suppressed hepatic and increased adipose 11 $\beta$ -HSD-1 message compared to rats given ad libitum access to chow only.

These studies investigated acute (24h and 1 wk) effects of ad libitum access to 16% solutions of different sugars on 11 $\beta$ -HSD-1 message and expression in liver and mesenteric adipose in adult male Sprague-Dawley (S-D) rats. Other analyses included the following: liver and adipose message and expression of hexose-6 phosphate dehydrogenase (H6PDH); message of acetyl CoA carboxylase (ACC) and CCAA/enhancer binding protein- $\alpha$  (C/EBP- $\alpha$ ); intracellular NADPH; and plasma corticosterone, insulin, and triglycerides. CCAAT/enhancer binding proteins have been

recognized as master regulators of adipogenesis (Lefterova & Lazar 2009), and recent genome-wide searches reveal much overlap in their transcriptional targets. Along with sterol regulatory element binding proteins, peroxisome proliferator-activated receptors (PPAR $\alpha$ / $\delta$ / $\gamma$ ), and liver X receptors, C/EBP ( $\alpha$ ,  $\beta$  and  $\delta$ ) have been suggested as potential nutrient sensors that alter gene expression (Al-Hasani & Joost 2005). Little is known about the transcriptional regulation of 11 $\beta$ -HSD-1 generally or in response to diet. C/EBP- $\alpha$  was shown to regulate a number of glucocorticoid-responsive genes, and in fact, C/EBP- $\alpha$  deficient mice had decreased hepatic 11 $\beta$ -HSD-1 expression (Williams et al 2000). Additionally, cortisol stimulation of amnion fibroblasts induced the binding of C/EBP- $\alpha$ , but not C/EBP- $\beta$  to the promoter region of 11 $\beta$ -HSD-1 gene (Yang et al 2007). Ignatova et al recently showed that C/EBP- $\alpha$  and C/EBP- $\beta$  were involved in basal 11 $\beta$ -HSD-1 expression and C/EBP- $\beta$  is involved in TNF- $\alpha$  induced  $\alpha$  11 $\beta$ -HSD-1 expression in HepG2 cells (2009). We decided to quantify C/EBP- $\alpha$  message to explore its potential role in transcriptional regulation of 11 $\beta$ -HSD-1, based on the literature available at the time. However, C/EBP- $\beta$  may have been a better candidate for studying the regulation of 11 $\beta$ -HSD-1 under the influence of high sugar diets because of it is linked to inflammation.

The three treatments included the following: ad libitum access to either 1) 16% sucrose (S16), 2) 16% fructose (F16), or 3) 16% glucose (G16) solution in addition to free access to chow and water. The control group had free access to chow and water only. The primary research question asked was: Is the increased 11 $\beta$ -HSD-1 in adipose and decreased hepatic 11 $\beta$ -HSD-1 that are observed in high sugar diet-induced obesity the result of increased adiposity or diet composition. The experiments outlined were designed

with the goal of identifying acute changes in 11 $\beta$ -HSD-1 and H6PDH message and expression in liver and adipose, and the effects of the experimental diets on several key parameters of carbohydrate and lipid metabolism.

## **Experimental Design**

### ***Animal Model***

Adult male S-D rats (Charles River Laboratories, Wilmington, MA) with a mean weight of approximately 300 grams were used. This strain of rat is susceptible to diet-induced obesity when given free access to a high sugar diet (London et al 2007). Upon arrival, all animals were individually housed and maintained on a 12h light/dark cycle with a room temperature of 22° C  $\pm$  1° C. During the 1 wk acclimation period the rats were given free access to the control diet (Fig. 12) and water. Animals were weighed and 24h food intake and sugar solution intake (when appropriate) were measured daily at 0900h throughout the experiment.

All rats were killed by slow replacement of air in a specialized chamber with pure CO<sub>2</sub> followed by rapid decapitation and exsanguination. This method has been approved for use by the Panel on Euthanasia of the American Veterinary Medical Association as well as the UM IACUC. All procedures described herein are in compliance with the University of Maryland's IACUC guidelines (Appendix 3).

### ***Animal Diets***

The control diet used in these experiments is based upon one previously used in an experiment that manipulated quality and quantity of fat in diet-induced obesity studies with rats (Woods et al 2003) and is the same diet use in the 10 wk experiment previously described. The diet is a nutritionally complete low fat diet [Rodent diet 7012] prepared by

Harlan Teklad (Bethlehem, PA) and provides 3.41 metabolizable kcal/g of diet (Fig. 12). Per gram of chow, 2.14 kcal were derived from carbohydrate, 0.79 kcal was derived from protein, and 0.51 kcal was derived from fat.

The four experimental diets included: 1) ad libitum control diet (Harlan rodent diet 7012), 2) ad libitum control diet plus free access to 16% sucrose (Domino Foods, Baltimore MD) solution (S16), 3) ad libitum control diet plus free

<i>Average Nutrient Composition</i>		
Protein	%	19.92
Fat	%	5.67
Fiber	%	4.37
Ash	%	6.48
Nitrogen-Free Extract	%	53.66
Gross Energy	Kcal/g	4.05
Digestible Energy	Kcal/g	3.75
Metabolizable Energy	Kcal/g	3.41
Linoleic Acid	%	2.90

**Figure 12.** Composition of control diet [7012] (Harlan Teklad LM-485 Mouse/Rat Sterilizable Diet).

access to 16% fructose (Sigma Aldrich, St Louis MO) solution (F16), and 3) ad libitum control diet plus free access to 16% glucose (Sigma Aldrich, St Louis MO) solution (G16). Solutions were prepared 24h in advance and stored at 4°C. All animals were given free access to water. The preliminary data indicated that the experimental diets which provided ad libitum access to sugar solutions achieved both a diet proportionally high in carbohydrate (approximately 85–90% of daily caloric intake with approximately 60–70% of caloric intake as sugar solution) and higher total caloric intake in comparison to control, as well as significant differences in body composition (% body fat) and levels of both 11β-HSD-1 and H6PDH mRNA in mesenteric adipose and liver after long-term exposure (London et al 2007).

### *Study design*

Adult male S-D rats (N=64) were randomly assigned to one of four weight-matched groups (n=16) after an initial 1 wk acclimating period. The four groups included: 1) Control (C)—free access to the standard chow control diet (Fig. 12) and water only; 2) 16% Sucrose (S16)—free access to the control diet, water and 16% sucrose solution; 3) 16% Fructose (F16)—free access to the control diet, water and 16% fructose solution; and 4) 16% Glucose (G16)—free access to the control diet and 16% glucose solution. The rats were maintained on their respective diets for either 24h or 1 wk depending on their random assignment to one of two experimental groups.

Of the initial 64 study animals, one half of the rats in each group were randomly assigned to sacrifice after the initial 24h exposure to the experimental treatments. The remaining 32 animals (n = 8/group) were maintained on the experimental diets for a total of 1 wk and then sacrificed. Mesenteric adipose and liver (lobus lateralis sinister) tissues were dissected at the time of sacrifice and immediately flash frozen and stored at -80°C for subsequent analyses. At this time blood was collected in EDTA treated tubes, kept on ice, centrifuged for plasma separation, and the plasma stored at -20°C for subsequent analyses. Assays performed on mesenteric adipose and liver tissues included: 1) mRNA quantification (qRT-PCR) of H6PDH, 11 $\beta$ -HSD-1, Acetyl CoA Carboxylase (ACC), and CCAAT/enhancer binding protein alpha (C/EBP- $\alpha$ ); 2) Western blot analysis to quantify H6PDH and 11 $\beta$ -HSD-1 protein; and 3) quantification of total cellular NADPH in liver and adipose. Assays performed on insulin collected at the time of sacrifice included: radioimmunoassay quantification of plasma corticosterone and insulin, and 4) quantification of plasma triglycerides with colorimetric assay.

### ***Power Analysis and Calculation of Sample Size***

Power analysis and calculations to determine the minimum required sample size were performed based on the pilot study data collected for male S-D rats (N=24; n=8/group) fed one of the following three diets: 1) control (ad libitum access to Teklad 7012 chow and water), and 2) 16% high sucrose (S16) or 3) 32% high sucrose (S32) (ad libitum access to Teklad [7012] chow, water and 16% sucrose solution or 32% sucrose solution, respectively). Our analyses revealed that after 10 wk exposure to the experimental diets, there was a large difference ( $d = 1.2$ ) between the percentage of body fat in the S16 or S32 groups and the control group. With power ( $1-\beta$ ) set at 0.80 and  $\alpha$  set at 0.05, it was established that a sample size of  $n=8$  would be sufficient to recognize an even smaller effect size ( $ES = 0.80$ ) than what we found in our 10 wk study. Using the same values for power and  $\alpha$ , it was determined that in most instances, a sample size of  $n=8$  was (and often as small as  $n=6$ ) was sufficient to detect a large effect size ( $ES = 0.80$ ) or even a medium effect size ( $ES = 0.50$ ).

### **Research Methods**

#### ***Measurement of Plasma Insulin and Corticosterone***

Radioimmunoassay kits purchased from ICN Pharmaceuticals, Inc. (Costa Mesa CA) were used to measure plasma insulin and corticosterone of the samples obtained at the time of animal sacrifice.

#### ***Measurement of Plasma Triglycerides***

The Dimension® clinical chemistry system Flex® reagent cartridge for measurement of plasma or serum triglycerides (Siemens Healthcare Diagnostics Inc, Newark DE) was used with a Dade Behring Dimension® Xpand™ automated system to

quantify plasma triglyceride levels. The assay is based on an enzymatic procedure using a combination of the following enzymes: lipoprotein lipase, glycerol kinase, glycerol-3-phosphate-oxidase, and peroxidase. Ultimately the changes in absorbance (510, 700 nm) resulting from the formation of quinoneimine from hydrogen peroxide reflect the total amount of glycerol and its precursors. All reactions were run in duplicate.

***Measurement of 11 $\beta$ -HSD-1, H6PDH, ACC, and C/EBP- $\alpha$  mRNA in Liver and Mesenteric Adipose.***

Frozen tissue samples were thawed and 500 mg (when available) of each sample homogenized in buffer while kept cold on ice. Total RNA was extracted according to the RNeasy Mini and RNeasy Mini Lipid protocols (QIAGEN), respectively for liver and adipose. Additionally, centrifugation steps were included in the protocol to enhance sample purity by removing organic solvents and salts from the extracts. Total RNA was then purified to remove contaminating DNA using DNA-free™ (Ambion). Purified RNA was analyzed spectrophotometrically (NanoDrop) to determine the concentration and quality of product (260/280 ratio). cDNA template was created for each sample using 500 ng of purified RNA extract with SuperScript® III Reverse Transcriptase (Invitrogen). Finally, each sample was subjected to qRT-PCR (IQ5 cycler, BioRad) using a 40 cycle program and subsequent melt curve analysis program (55°–95°C). All primers were tested using stock rat cDNA (see Table 5). The program for each primer set is detailed in Table 6.

**Table 5.** Primer sequences for target genes in acute studies.

<b>Gene</b>	<b>Sense primer sequence</b>	<b>Antisense primer sequence</b>
11 $\beta$ -HSD1	5'-TGCTCTGGATGGGTTCTTTT-3'	5'-GAAGCCGAGGACACACAGAGAG-3'
H6PDH	5'-GGGCTATGTTCCGATCTTGTTTA-3'	5'-GTTCCGGCACCCAGTGTCT-3'
$\beta$ -actin	5'-TGGAGTCTACTGGCGTCTT-3'	5'TGTCATATTTCTCGTGGTCA-3'
ACC	5'-GCCATCCGTTTGTGTC-3'	5'-GGATACCTGCAGTTTGAGCCA-3'
C/EBP- $\alpha$	5'-GAATCTCCTAGTCCTGGCTC-3'	5'-GATGAGAACAGCAACGAGTAC-3'

**Table 6.** qRT-PCR programs used for each of the five primer sets.

Target Gene (primers)	Cycle 1	Cycle 2 (40X)		
	Denaturation temp (°C); time (m)	Denaturation temp (°C); time (s)	Annealing temp (°C); time (m)	Elongation temp (°C); time (m)
11 $\beta$ -HSD-1	95; 3	95; 15	60;1	55;1
H6PDH	95; 3	95; 15	60;1	72;1
$\beta$ -actin	95; 3	95; 15	60;1	72;1
ACC	95; 3	95; 15	59.5;1	72;1
C/EBP- $\alpha$	95; 3	95; 15	59.5;1	72;1

The cDNA derived from each tissue sample was simultaneously subjected to qRT-PCR using primers for the housekeeper gene  $\beta$ -actin. All reactions were carried out in duplicate.

All primers were pre-tested by performing qRT-PCR and melt curve analyses with stock rat cDNA. PCR products were then run out on an agarose gel with appropriate DNA reference ladder to confirm the presence of only one product with the expected molecular weight.

Differences in message between treatment and control groups were assessed based on the cycle threshold (CT) values for each treatment group and that of  $\beta$ -actin. First the  $\Delta$ CT value was calculated by subtracting the CT value of  $\beta$ -actin from that of the gene of interest. Then, the  $\Delta\Delta$ CT value for each observation was calculated by subtracting the  $\Delta$ CT value of each rat from the mean  $\Delta$ CT value of the control group. Finally, fold change in mRNA of the gene of interest for each rat was calculated as  $2^{-\Delta\Delta CT}$  in message (Bio-Rad, Livak et al 2001).

***Measurement of Protein Levels: Western Blot Analysis of 11 $\beta$ -HSD-1 and H6PDH in Liver and Mesenteric Adipose***

***Protein Extraction of Liver Tissue***

Total protein was extracted from 250 mg of tissue with 5 mL T-Per Tissue Protein Extraction Reagent (Pierce Scientific, Rockford, IL). Total protein concentration of the

protein extract was then determined using the Bio-Rad Protein Assay. By comparing the sample extracts to the regression line of the standard curve for bovine gamma globulin, protein concentrations of each sample were determined. The amount of extract needed to obtain 40 µg of protein was then calculated for each sample.

### ***Protein Extraction of Adipose Tissue***

Total protein was extracted from between 15 and 240 mg of tissue (average tissue weight was ~50 mg/rat). A special lysis/extraction buffer was prepared specifically for extracting protein from adipose tissue (Mingrone et al 2008; Monteiro et al 2008; Kim et al 2008). The extraction buffer consisted of: 100 mM Tris-HCl (pH 7.4), 5 mM EDTA, 50 mM NaCl, 50 mM sodium pyrophosphate, 50 mM NaF, 100mM Orthovanadate, 1% Triton X-100, 1 mM phenylmethylsulphonylfluoride, and a cocktail of protease inhibitors added just prior to use that included: 2 µg/mL aprotinin, 1 µg/mL pepstatin A, and 1 µg/mL leupeptin. Homogenization in the extraction buffer was performed at 4°C and then centrifuged (13,000 g) for 20 minutes at 4°C. Total protein concentration of the protein extract was then determined using the Bio-Rad Protein Assay. By comparing the sample extracts to the regression line of the standard curve for bovine gamma globulin, protein concentrations of each sample were determined. The amount of extract needed to provide both 1 µg and 150 ng of protein was calculated for each sample. Due to the lower total amount of tissue available for extraction, increased difficulty of protein extraction, and subsequently lower total concentrations of protein in the adipose tissue extracts, nitrocellulose membranes were prepared separately with two different amounts of total protein per well (1 µg and 150 ng) in hopes that there would be sufficient protein for quantification by Western blot analysis.

### *Nitrocellulose Membrane Preparation*

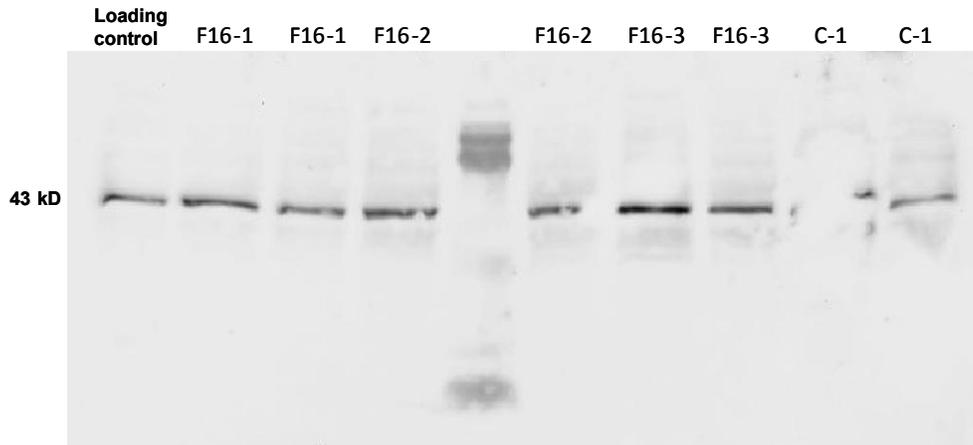
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) was run in duplicate (total volume per well = 15 mL (liver); 25 mL (adipose) on 10 well 12% Tris-HCl precast Ready Gels™ (BioRad). Protein samples then were heated for five minutes to achieve denaturation were loaded, one per well. A Kaleidoscope™ Prestained Ladder (Bio-Rad) was used for qualification of proteins by molecular weight as per the protocol. Additionally, an internal standard (sample #10 for liver tissue and sample #36 for adipose tissue) was used in one well per gel.  $\beta$ -actin is a 43 kD protein that appeared between two markers, Bovine Serum Albumin (66 kD) and Carbonic Anhydrase (31 kD) (Fig. 13). 11 $\beta$ -HSD-1 is 34 kD, which is similar in size to carbonic anhydrase in the reference ladder (Fig. 14). H6PDH is a 96 kD protein that appeared nearest to the marker  $\beta$ -galactosidase (116 kD) (Fig. 15). The gels were run for 3h at a voltage of 60V to produce consistent, high resolution protein bands. Semi-dry protein transfers to nitrocellulose membrane were performed using a BioRad Criterion™ Blotting tank with a 100V current for 30 min. Protein transfer was confirmed by staining Ponceau S solution (0.1% wt/vol with 5% acetic acid). Upon confirmation of successful protein transfer, membranes were destained with multiple washes of dH<sub>2</sub>O and a single one minute wash with 0.1 M NaOH. Membranes were stored in plastic wrap at -20°C for subsequent immunodetection.

Immunoblotting was then performed three times on each of the membranes using the following three primary antibodies: polyclonal rabbit 11 $\beta$ -HSD-1 (generously provided by M. Hardy at Rockefeller University, also Abcam antibody used), polyclonal sheep H6PDH (kindly provided by Elise Gomez-Sanchez, PhD, University of Mississippi

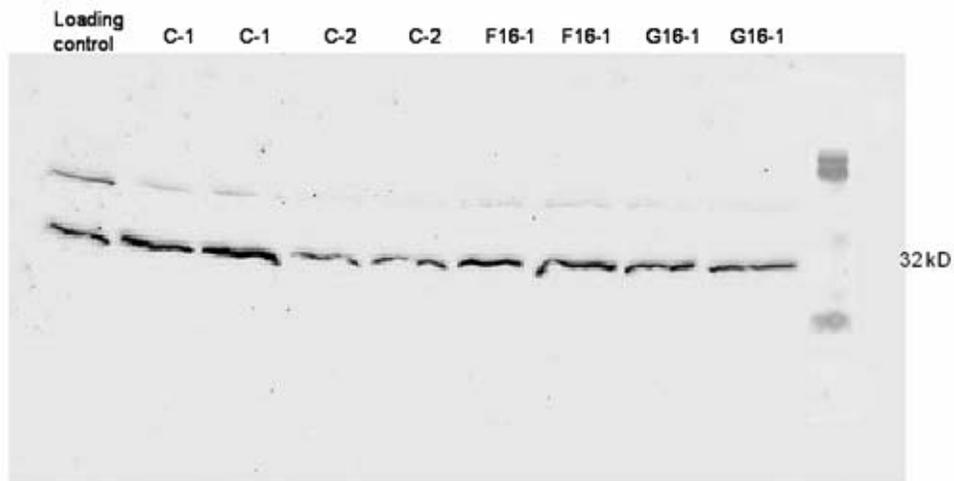
Medical Center), and polyclonal goat  $\beta$ -actin (loading control, Abcam). The membranes were first blocked with a blocking buffer (Tris-HCl, dry milk powder, NaCl, ddH<sub>2</sub>O; pH = 8.0) for at least four hours (4°C). Membranes were then rinsed three times for 5 minutes each rinse with dH<sub>2</sub>O, and incubated with primary antibody solution (concentrations ranged from 1:1,000 to 1:3,000) overnight at 4°C. Membranes were then washed three times for at least 5 minutes each time with wash buffer (1X PBS with 0.01% Tween-20). The membranes were then incubated with their respective secondary antibody solution (sheep anti-rabbit (11 $\beta$ -HSD-1), rabbit anti-sheep (H6PDH), bovine anti-goat ( $\beta$ -actin)). Concentrations of secondary antibodies ranged from 1:20,000 to 1:30,000). The membranes were then incubated for 5 minutes with Pico West Chemiluminescent Substrates (Pierce Scientific) at a 1:1 ratio while covered with aluminum foil to protect from light. Excess substrate was then gently removed and blots were immediately visualized using the BioRad Quantity One® Gel Doc and software system.

The blotting procedures were optimized for each antibody system used to produce the clearest signal with the least background noise. Figures 13, 14, and 15 are representative Western blots that used primary antibodies for  $\beta$ -actin, 11 $\beta$ -HSD-1, and H6PDH, respectively. Each membrane had the loading control that was used for all the liver samples of both 24h and 1 wk experiments. And duplicates of each sample were run side-by side so that each membrane consisted of the loading control (sample # 10), 4 samples (in duplicate), and the ladder. Each protein of interest was quantified using densitometry (BioRad Quantity One software) as follows: the signal densities ((INT/mm<sup>2</sup>) for the duplicates of each sample were averaged and the ratio of the density

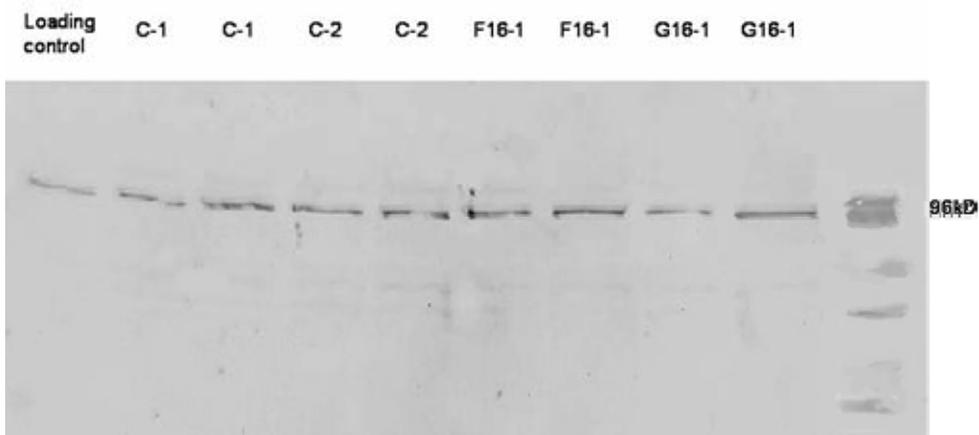
of the signal for the protein of interest to that of the loading control was calculated. Then that ratio was divided by the same density ratio value for  $\beta$ -actin that was calculated for the sample.



**Figure 13.** Representative Western blot of liver samples using a primary antibody for  $\beta$ -actin (43kD) specific to rat. Shown here are 24h liver samples # 25–28.



**Figure 14.** Representative Western blot of liver samples using a primary antibody for 11 $\beta$ -HSD1 (32kD) specific to rat. Shown here are 24h liver samples # 29–32.



**Figure 15.** Representative Western blot of liver samples using a primary antibody for H6PDH (96kD) specific to rat. Shown here are 24h liver samples # 29–32.

### ***Measurement of Intracellular Concentrations of NADPH in Liver and Mesenteric Adipose***

The concentrations of intracellular NADPH in liver and mesenteric adipose homogenates for the two experimental groups were measured spectrophotometrically using a 96-well plate reader (Wallac Victor<sup>2</sup> 1420 Multilabel Counter) with the BioAssay Systems EnzyChrom™ NADP<sup>+</sup>/NADPH colorimetric assay kit according to the manufacturer's protocol. Absorbance was measured at 560nm. All samples were run in duplicate. For each set of samples (i.e., plate), a standard curve was created by preparing eight dilutions of the kit-provided NADPH standard. The R<sup>2</sup> value for all standard curves was  $\geq 0.98$ .

### ***Statistical Analysis and Interpretation***

All statistical analyses for the data acquired were performed using SAS version 9.1 (SAS Institute, Cary, NC). The significance level for all experiments was set at  $P < 0.05$ . Changes in mRNA concentration (message) were determined as fold-change in message as described by Livak et al (2001) using the  $2^{-\Delta\Delta CT}$  method. In short, the mean cycle threshold (CT) for the housekeeper gene,  $\beta$ -actin was subtracted from that of the

gene of interest, to obtain a  $\Delta$ CT value. The mean CT value for the control group was subtracted from that of each sample to give a  $\Delta\Delta$ CT value. Finally, this value was used to calculate the  $2^{-\Delta\Delta$ CT which represents fold change in message. The  $2^{-\Delta\Delta$ CT values were used for statistical analysis; ANOVA was used when data were normally distributed or when assumptions of normality were met after transformation of data. Data that did not meet the assumptions of normality will be discussed below.

Normality tests were performed on all data. Statistics examined to assess normality included: Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling. Data that were not normally distributed were log- and/or square root-transformed, and tested for normality using the “Proc univariate” SAS algorithm. If normality was achieved by log- or square-root transformation parametric statistical tests were performed. Because much of the qRT-PCR data were values between zero and one, one was added to each value prior to transformation. In these instances data was back-transformed by first subtracting one to each value and then back-transforming the data. These back-transformed values were used when graphing the data.

Statistics were performed for the following dependent variables using one-way ANOVA and then Tukey’s Posthoc Test if appropriate:  $11\beta$ -HSD-1, H6PDH, ACC, and C/EBP $\alpha$  message;  $11\beta$ -HSD-1 and H6PDH protein levels; plasma corticosterone, insulin, and triglyceride concentrations; and total cellular NADPH levels. Student’s T-tests were used to compare mean values of control in comparison to the mean values for the treatment group at the two time points. Correlation analyses using Spearman’s Correlation Coefficient were also conducted and included all the above listed variables in

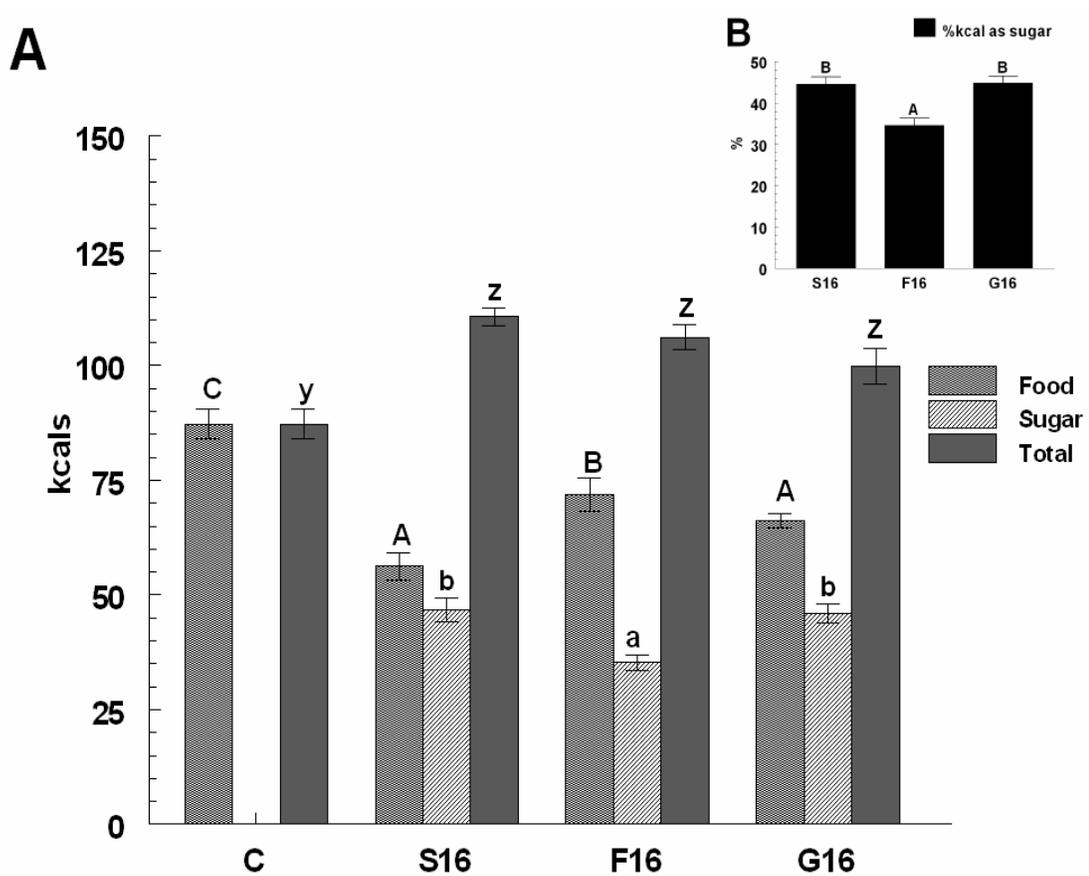
addition to weight gain and intake data to assess the relationships between all combinations of variables.

Because of the variability within groups and perhaps because of small group sizes, some of the data did not achieve normal distribution even when log- or square root-transformed. In these instances non-parametric ranking tests were used to test differences between groups. The Mann-Whitney U non-parametric test was used for all data that did not meet the assumptions of normality.

## Results

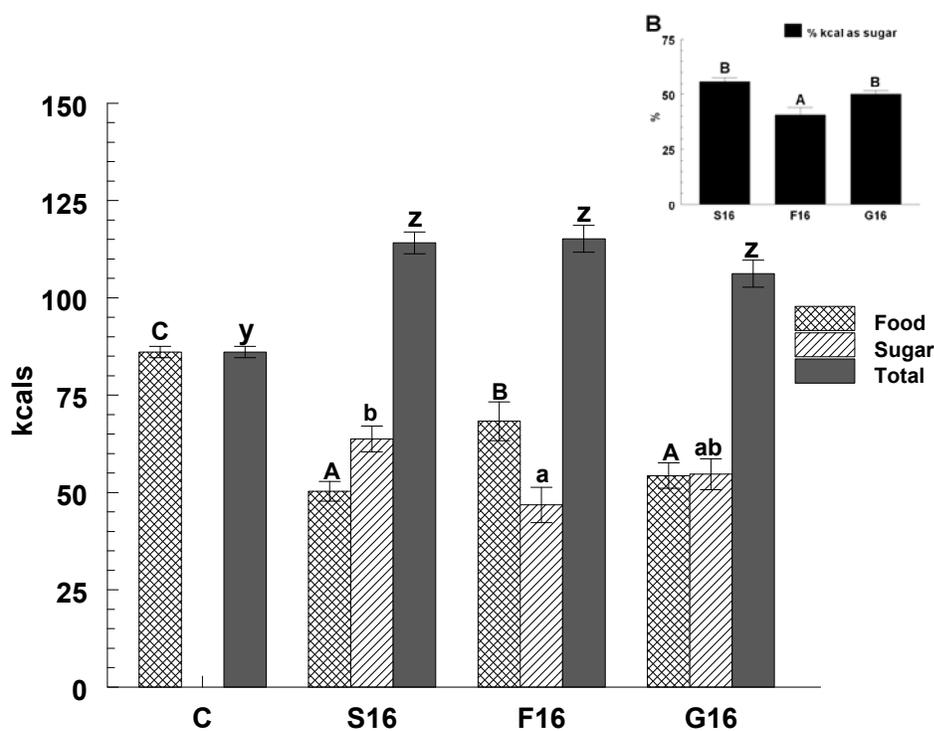
### *Daily Food, Sugar Solution and Macronutrient Intake*

When given ad libitum access to 16% solutions (wt/vol) of sucrose (S16), fructose (F16), or glucose (G16) in addition to ad libitum access to water and standard rat chow for 24h, all sugar-fed groups had higher mean total daily caloric intakes than did control (C) rats with ad libitum access to water and chow only ( $P = 0.005$ , Fig. 16A). The sugar-fed groups on average consumed between



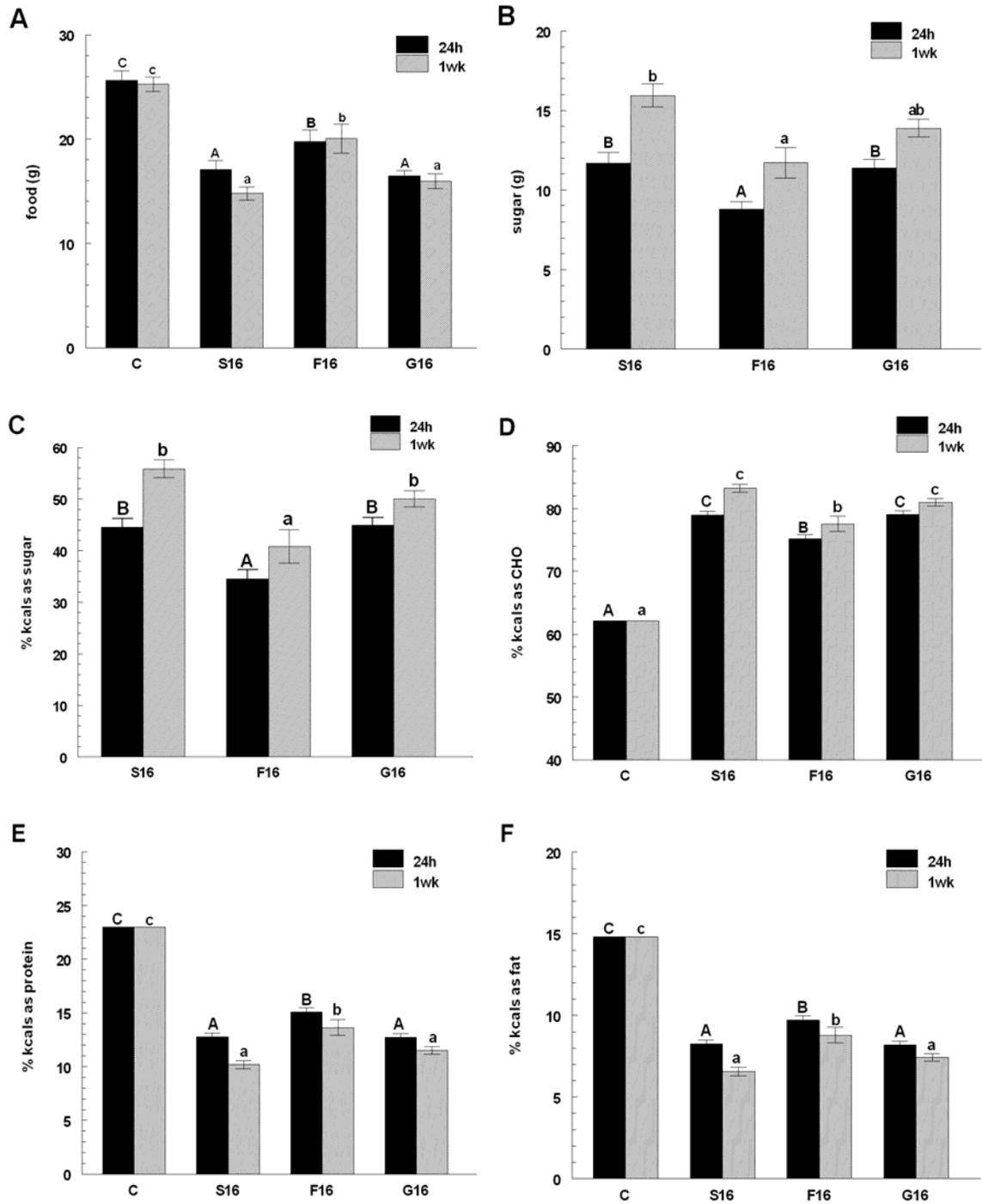
**Figure 16.** 24h mean daily (A) Sugar, food, and total caloric intakes in control, S16, F16, and G16 groups, and (B) Percent of total caloric intake as sugar by rats in S16, F16, and G16 groups; values are means  $\pm$  SEM; different letters indicate significant differences ( $P < 0.05$ ); comparisons were made within time groups only;  $n = 8$ /gp.

34.6 and 44.9 percent of their total daily energy intake as sugar. The S16 and G16 groups selected a higher percentage of their daily caloric intake as sugar solution than did the F16 rats ( $P = 0.0002$ , Fig. 16B). The F16 group, however, compensated for their lower sugar solution intake by consuming more chow than did the G16 and S16 groups ( $P < 0.0001$ , Fig. 16A). As expected, the control group consumed more chow than did the three sugar-fed groups ( $P < 0.0001$ , Fig. 16A). Overall, the mean total caloric intakes did not differ among the sugar-fed groups, and the mean total caloric intake ( $103.3 \pm 3.5$  kcal/d) of the sugar-fed groups was significantly higher than that of the control group ( $87 \pm 3.3$  kcal/d) ( $P < 0.0001$ , Fig. 16A).



**Figure 17.** 1 wk mean daily (A) Sugar, food, and total caloric intakes in control, S16, F16, and G16 groups, and (B) Percent of total caloric intake as sugar by rats in S16, F16, and G16 groups; values are means  $\pm$  SEM; different letters indicate significant differences ( $P < 0.05$ ); comparisons were made within time groups only;  $n = 8$ /gp.

In the 1 wk experiment the control rats consumed more chow per day than did any of the sugar-fed groups ( $P < 0.0001$ , Fig. 17A). Mean daily intake of sugar was higher in the S16 group in comparison to the F16 group, but the mean sugar intake level of the G16 group did not differ from either of the other two sugar-fed groups (Fig. 17A). On average the S16, F16, and G16 groups consumed  $55.89 \pm 1.72$ ,  $40.83 \pm 3.21$ , and  $50.03 \pm 1.58$  percent of their mean daily total caloric intake as sugar, respectively (Fig. 17B). The mean percent of total daily calories consumed as sugar was lower in the F16 group in comparison to that of the S16 and G16 groups ( $P= 0.0005$ , Fig. 17B). The cumulative total caloric intake for the week did not differ among the sugar-fed groups. The total caloric intake for the experimental period was significantly higher in the sugar-fed groups in comparison to that of the control group ( $P < 0.0001$ , Fig. 17A).

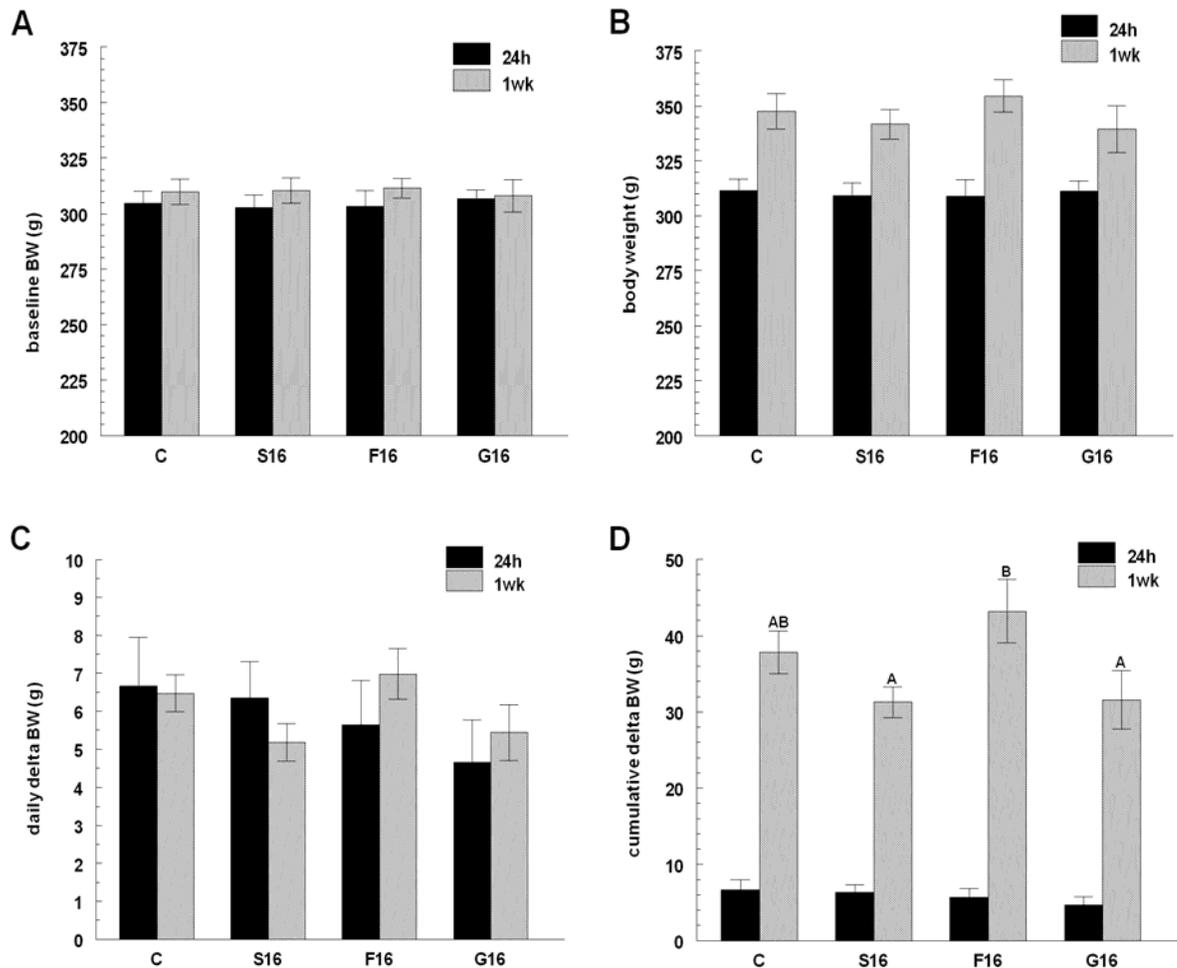


**Figure 18.** 24h and 1wk energy intake data. (A) Mean daily intake of standard rat chow, (B) Mean daily sugar intake, (C) Mean daily percent of caloric intake as sugar, (D) Mean daily percent of caloric intake as carbohydrate, (E) Mean daily percent of caloric intake as protein, and (F) Mean daily percent of caloric intake as fat for rats in the control, S16, F16, and G16 groups; values are means  $\pm$  SEM; different letters indicate significant differences ( $P < 0.05$ ); comparisons were made within time groups only;  $n = 8/gp$ .

In comparing the energy intake patterns 24h and 1wk experiments, few differences were observed (Fig. 18). While the F16 group consumed less sugar (g) per day than either of the other two sugar-fed groups in the 24h study, the F16 group consumed less sugar (g) per day than only the S16 group in the 1 wk study. At 1 wk, sugar intake levels in grams per day did not differ between F16 and G16 groups (Fig. 18B). Also, sugar intake per day by all sugar-fed groups was higher for the 1 wk study when compared to their counterparts in the 24h study (Fig. 18B). As expected by this increase in daily grams of solute intake by the 1 wk sugar-fed groups, the mean daily percentage of caloric intake as sugar solution was higher in the all the sugar-fed groups of the 1 wk study in comparison to their 24h study counterparts ( $P < 0.05$ , Fig. 18D). This increased intake of sugar solution caused differences between 24h and 1 wk studies in macronutrient intake (Figs. 18D, 18E). The percentage of intake as each macronutrient was identical for the control groups of the 24h and the 1wk studies as the same standard chow was used. Percentage of total intake as carbohydrate was higher for each of the sugar-fed groups of the 1 wk study in comparison their 24h wk counterparts ( $P < 0.05$ , Fig. 18D). The percentage of intake as protein was higher in the S16 group of the 24h study in comparison to the S16 group of the 1 wk study ( $P < 0.05$ , Fig. 18E). No differences in percentage of daily caloric intake as protein between the 24h and 1wk counterparts in the other sugar-fed groups were observed. The percentage of daily caloric intake as fat was also higher in the S16 group of the 24h study relative to that of the S16 group of the 1 wk study ( $P < 0.05$ , Fig. 18F). There were no differences in percentage of daily caloric intake as fat between the 24h and 1wk counterparts in the other sugar-fed groups

## Body Weight

In the 24h study, there were no differences in body weight among the four groups either at baseline ( $P= 0.957$ , Fig. 19A), or at the end of the 24h experimental period ( $P= 0.983$ , Fig. 19B) as anticipated. The mean 24h change in body weight also did not differ among the four groups ( $P= 0.606$ , Fig. 19C).

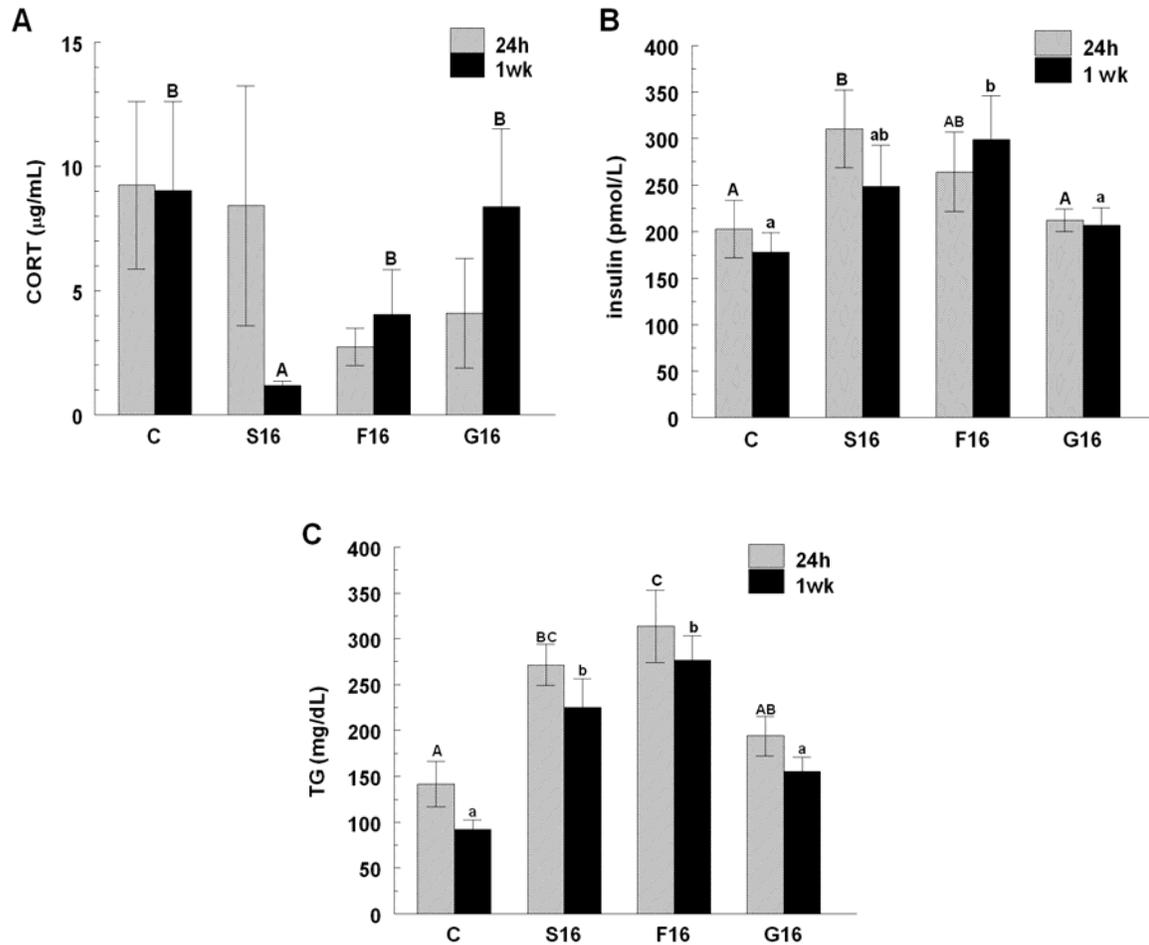


**Figure 19.** Body weight data: (A) Mean body weights at baseline, (B) Mean body weights after 24h or 1 wk exposure to experimental diets, (C) Mean daily change in body weight, and (D) Mean cumulative change in body weight between baseline and 24h or 1wk; values are means  $\pm$  SEM; different letters indicate significant differences;  $P < 0.05$ ; comparisons were made within time groups only;  $n= 8$ /gp.

In the 1 wk study, there were no differences in mean body weight among the four groups at baseline ( $P= 0.98$ , Fig. 19A). At the end of the 1 wk experimental period no differences in body weight were observed ( $P= 0.59$ , Fig. 19B). Also, there were no differences in mean daily change in body weight for the 1 wk experimental period among the control, S16, F16, and G16 groups ( $P= 0.14$ , Fig. 19C). While mean cumulative weight gain for the 1 wk experimental period was not different between the control and sugar-fed groups, mean cumulative weight gain was higher in F16 rats when compared to the G16 and S16 groups ( $P= 0.05$ , Fig. 19D).

### ***Circulating Corticosterone, Insulin, and Triglycerides***

After 24h exposure to the experimental diets, there were no differences in circulating corticosterone concentrations among the groups. As illustrated in Figure 20A, there was considerable intra- and inter-group variability in plasma corticosterone in the 24h study. Corticosterone levels (expressed as mean  $\pm$  SEM) ranged from  $2736.4 \pm 762.5$  pmol/L for the F16 group to  $9254.4 \pm 3366.8$  pmol/L for the control group (Fig. 20A). Mean plasma insulin was higher in the S16 group in comparison to the control group, but mean concentrations among the sugar-fed groups did not differ after 24h exposure to the experimental diets (Fig. 20B). The mean 24h plasma triglyceride (TG) concentration for the F16 group was significantly higher than that of both the control and G16 groups ( $P = 0.001$  and  $P = 0.03$ , respectively; Fig. 20C). The S16 group also had a higher mean TG level than that of the control group ( $P = 0.02$ ). This elevation of plasma TG in the groups with fructose-containing diets is indicative of increased de novo lipid biosynthesis.



**Figure 20.** Plasma concentrations of (A) Corticosterone (CORT), (B) Insulin, and (C) Triglycerides (TG) in the control, S16, F16, and G16 groups after 24h or 1wk exposure to the experimental diets; values are means  $\pm$  SEM; different letters indicate a significant difference;  $P < 0.05$ ; comparisons were made within time groups only;  $n = 8/\text{gp}$ .

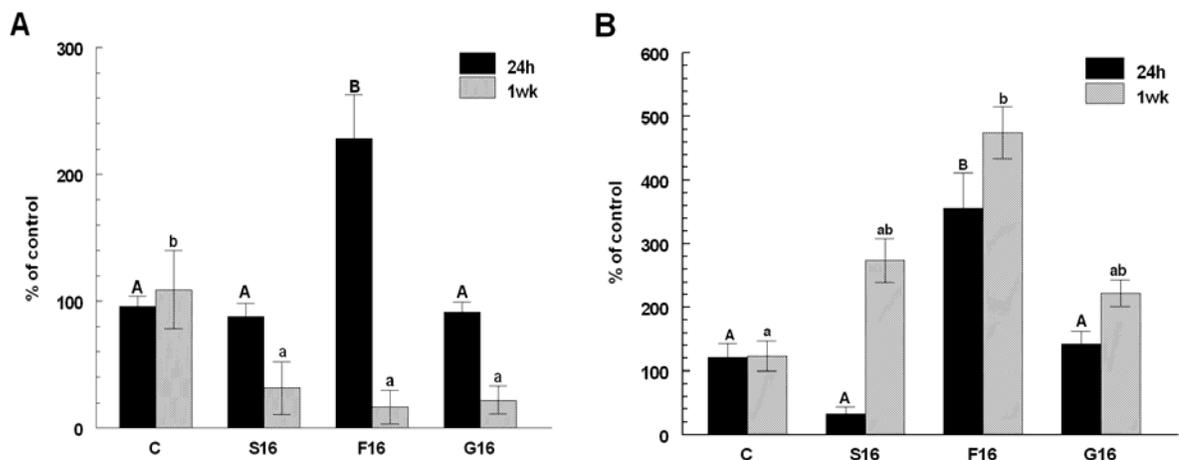
While there were no differences in plasma corticosterone at 24h, the S16 group had decreased plasma corticosterone at 1 wk in reference to the other two sugar-fed groups (Fig. 20A). In both experiments, there was a considerable amount of within group variability. Plasma insulin concentrations were similar between same treatment group counterparts in the two studies. However, at 24h and not 1 wk, the S16 group had a higher mean insulin level than the control group (Fig. 20B). The F16 group had significantly elevated plasma insulin at 1 wk in comparison to the control group, but not

at 24h (Fig. 20B). Mean plasma TGs were elevated at both 24h and 1 wk in the S16 and F16 groups in comparison to the control group (Fig. 20C). Mean plasma TG of the F16 group was also higher than that of the G16 group in both 24h and 1 week studies. The S16 group had higher mean plasma TG than the G16 group only in the 1 wk study (Fig. 20C).

### *mRNA and Protein Quantification in Liver and Mesenteric Adipose*

#### *11 $\beta$ -HSD-1 mRNA*

After 24h exposure to the experimental diets, mean hepatic 11 $\beta$ -HSD-1 mRNA of the F16 group was increased in comparison to that of the control, S16, and G16 groups ( $P < 0.05$ , Fig. 21A). In the F16 group, mean 11 $\beta$ -HSD-1 mRNA was more than double that of the other groups. In mesenteric adipose, the same trend was observed in the 24h study. Mean 11 $\beta$ -HSD-1 mRNA in mesenteric adipose was upregulated by approximately three-fold in comparison to that of the control, S16, and G16 groups ( $P = 0.05$ , Fig. 21B).



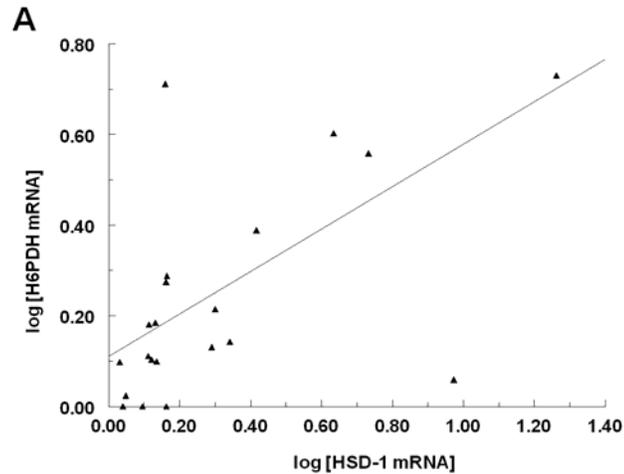
**Figure 21.** 11 $\beta$ -HSD-1 mRNA in (A) Liver, and (B) Mesenteric adipose in the control, S16, F16, and G16 groups after 24h or 1 wk exposure to the experimental diets. All data shown have been log-transformed to achieve normal distribution and graphed as the back-transformed data; values are expressed as mean  $\pm$  SEM; different letters indicate a significant difference;  $P < 0.05$ ; comparisons were made within time groups only;  $n = 8/\text{gp}$ .

After 1 wk exposure to the experimental diets, 11 $\beta$ -HSD-1 mRNA in liver was suppressed by greater than 60% in all the sugar-fed rats ( $P < 0.05$ , Fig. 21A). In mesenteric adipose the increases in 11 $\beta$ -HSD-1 mRNA in the sugar-fed groups at 1 wk ranged from two- to six-fold that of the control group. However, only the increased level of 11 $\beta$ -HSD-1 mRNA of the F16 group reached statistical significance when compared to that of the control ( $P < 0.05$ , Fig. 21B).

In comparing the changes in 11 $\beta$ -HSD-1 mRNA in response to the experimental diets, very different changes in 11 $\beta$ -HSD-1 mRNA were observed in the 24h and the 1 wk studies. Figure 21A and 21B illustrate that only pure fructose solution had an immediate (24h) impact on 11 $\beta$ -HSD-1 mRNA in liver and mesenteric adipose. However, at 1 wk the three experimental diets had the similar effect of suppressing hepatic 11 $\beta$ -HSD-1 mRNA. After 1 wk, the F16 group remained the only group with significantly increased 11 $\beta$ -HSD-1 mRNA in adipose. An intermediate effect was seen in the other two sugar-fed groups after 1 wk as mean 11 $\beta$ -HSD-1 mRNA in adipose had increased relative to levels observed at 24h.

#### ***Correlations with 11 $\beta$ -HSD-1 mRNA***

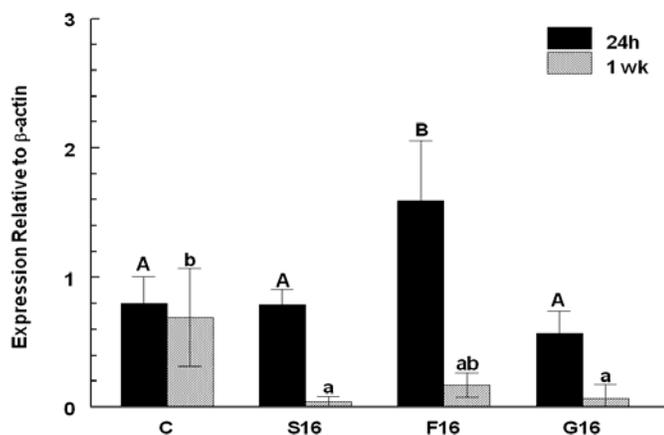
At 24h 11 $\beta$ -HSD-1 mRNA and H6PDH mRNA in mesenteric adipose were positively correlated ( $r = 0.63$  (Spearman),  $P = 0.002$ ; Fig. 22). At 24h, adipose 11 $\beta$ -HSD-1 mRNA was also positively correlated with hepatic 11 $\beta$ -HSD-1 protein ( $r = 0.47$  (Pearson),  $P = 0.02$ ) and hepatic H6PDH mRNA ( $r = 0.38$  (Spearman),  $P = 0.05$ ) (data not shown; See Appendix 5).



**Figure 22.** 24h correlation between 11 $\beta$ -HSD-1 mRNA and H6PDH mRNA in mesenteric adipose for rats in the control, S16, F16, and G16 groups.

### *11 $\beta$ -HSD-1 Protein Expression*

After 24h exposure to the experimental diets, hepatic protein expression of 11 $\beta$ -HSD-1 in the F16 group was increased in comparison to the control, S16, and G16 groups ( $P < 0.05$ , Fig. 23). There were no differences among the control, S16, and G16 groups.



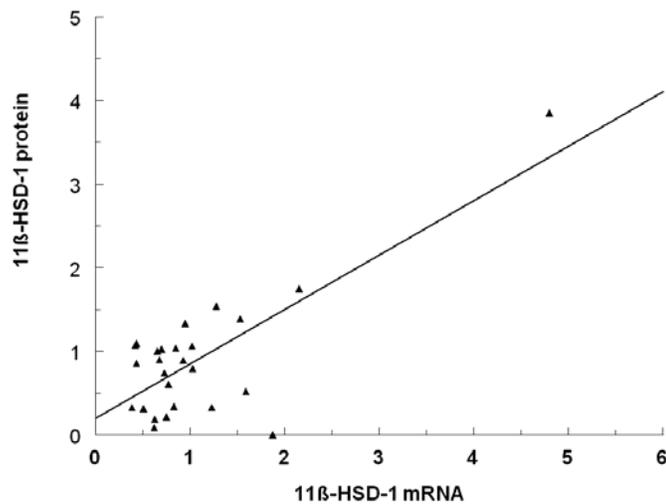
**Figure 23.** Hepatic 11 $\beta$ -HSD-1 protein expression after 24h or 1 wk exposure to experimental diets for rats in the control, S16, F16, and G16 groups. Data have been transformed to achieve normal distribution and graphed as the back-transformed data. Protein was quantified relative to the housekeeper,  $\beta$ -actin and to a loading control; values are means  $\pm$  SEM; different letters indicate a significant difference  $P < 0.05$ ; comparisons were made within time groups only;  $n = 8$ /gp.

After 1 wk, mean hepatic 11 $\beta$ -HSD-1 protein was suppressed in the S16 and G16 groups ( $P < 0.05$ , Fig. 23). Mean hepatic 11 $\beta$ -HSD-1 protein of the F16 group was approximately 25% that of the control, yet this difference did not achieve statistical significance ( $P = 0.06$ ).

Hepatic protein expression data for the 24h and 1 wk studies reveal that distinct changes occur in response to the experimental diets at the two timepoints (Fig. 23). Only fructose-feeding induced an increase in 11 $\beta$ -HSD-1 protein at 24h. After 1 wk all sugar-fed groups follow a trend of suppressed 11 $\beta$ -HSD-1 protein levels, although this suppression only reached significance in the S16 and G16 groups (Fig. 23).

#### ***11 $\beta$ -HSD-1 Protein Correlations***

After 24h, hepatic 11 $\beta$ -HSD-1 mRNA and protein levels were positively correlated ( $r = 0.44$ ,  $P = 0.02$ ; Fig. 24). After removal of the outlier on this line, the positive correlation was still significant. At 1 wk, while means for 11 $\beta$ -HSD-1mRNA and protein in the sugar-fed groups were suppressed, there was no significant correlation between hepatic 11 $\beta$ -HSD-1mRNA and protein levels among the rats collectively, or in any of the four groups individually.



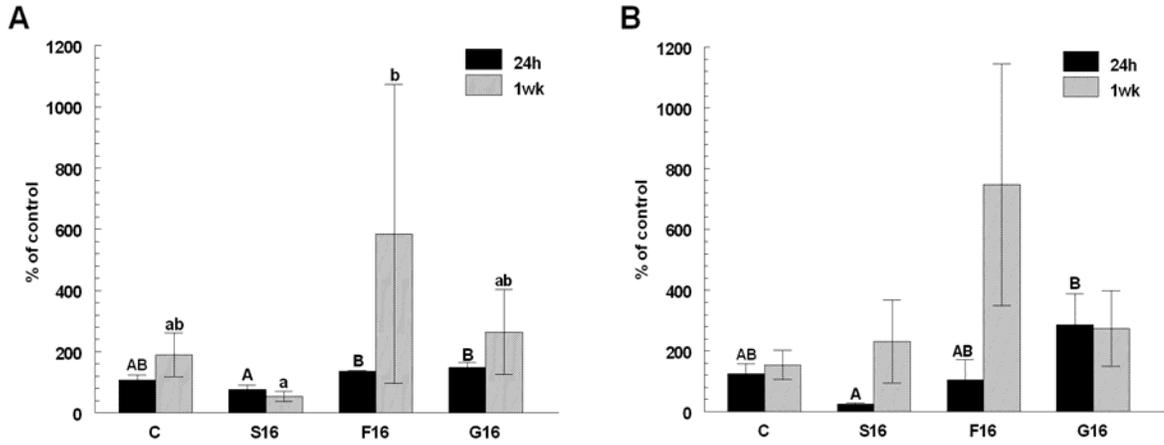
**Figure 24.** 24h correlation between hepatic 11 $\beta$ -HSD-1 mRNA and protein for rats in the control, S16, F16, and G16 groups.

### ***H6PDH mRNA***

After 24h exposure to the experimental diets, mean hepatic H6PDH mRNA was suppressed in the S16 group in comparison to the other sugar-fed groups ( $P = 0.04$ , Fig. 25A), but did not differ from the control group. There were no differences in hepatic H6PDH mRNA among the control, S16, or G16 groups at 24h. Similarly in mesenteric adipose, mean H6PDH mRNA was suppressed in the S16 group at 24h, but this decrease was significant only in comparison to the G16 group ( $P = 0.03$ , Fig. 25B). As observed in liver, there were no differences in H6PDH mRNA in mesenteric adipose among the control, S16, or G16 groups.

After 1 wk exposure to the experimental diets, mean hepatic H6PDH mRNA for the F16 group was increased in comparison to the S16, but not the control group ( $P = 0.05$ , Fig. 25A). While mean H6PDH mRNA in adipose for the F16 group was greater than seven-fold that of the control group, this difference was not significant ( $P = 0.17$ , Fig. 25B). After 1 wk, there were no differences in H6PDH mRNA in mesenteric

adipose. There was a considerable amount of intra-group variability in both organs after 1 wk exposure to the experimental diets as shown in Figures 25A and 25B.



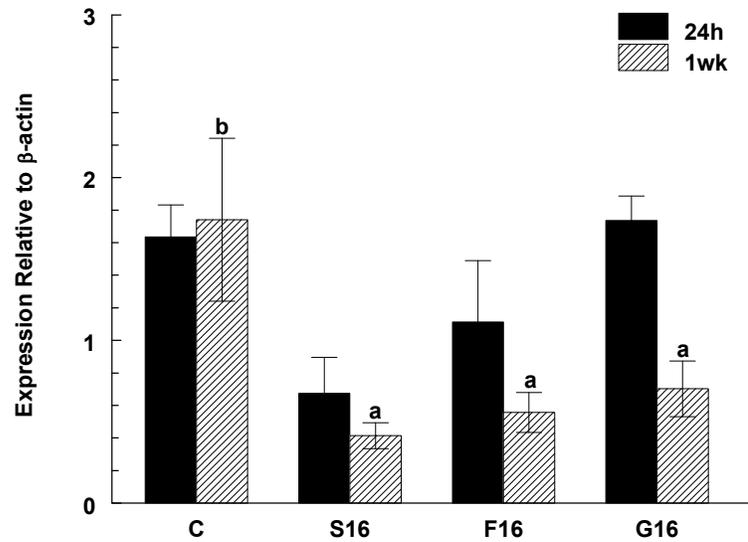
**Figure 25.** H6PDH mRNA in (A) Liver, and (B) Mesenteric adipose in rats of the control, S16, F16, and G16 groups after 24h or 1 wk exposure to experimental diets; values are expressed as mean  $\pm$  SEM; different letters indicate a significant difference;  $P < 0.05$ ; comparisons were made within time groups only;  $n = 8/\text{gp}$ .

In comparing the data from the two studies, there was clearly more intra-group variability in H6PDH mRNA in the 1 wk experiment (Figs. 25A & 25B). After 24h, there were no changes in either tissue in reference to the control group. After 1 wk, the only change in H6PDH mRNA in reference to the control group was an increase in the F16 group in liver (Fig. 25A).

### ***H6PDH Protein***

While mean hepatic H6PDH protein at 24h for the S16 group appears to be approximately half the mean of the control group, this suppression did not reach statistical significance. At 24h there were no differences among the four groups ( $P = 0.39$ ; Fig. 26).

After 1 wk exposure to the experimental diets, mean hepatic H6PDH protein was suppressed in all the sugar-fed groups in comparison to the control group ( $P < 0.05$ , Fig. 26), which was similar to the protein expression trend for 11 $\beta$ -HSD-1.

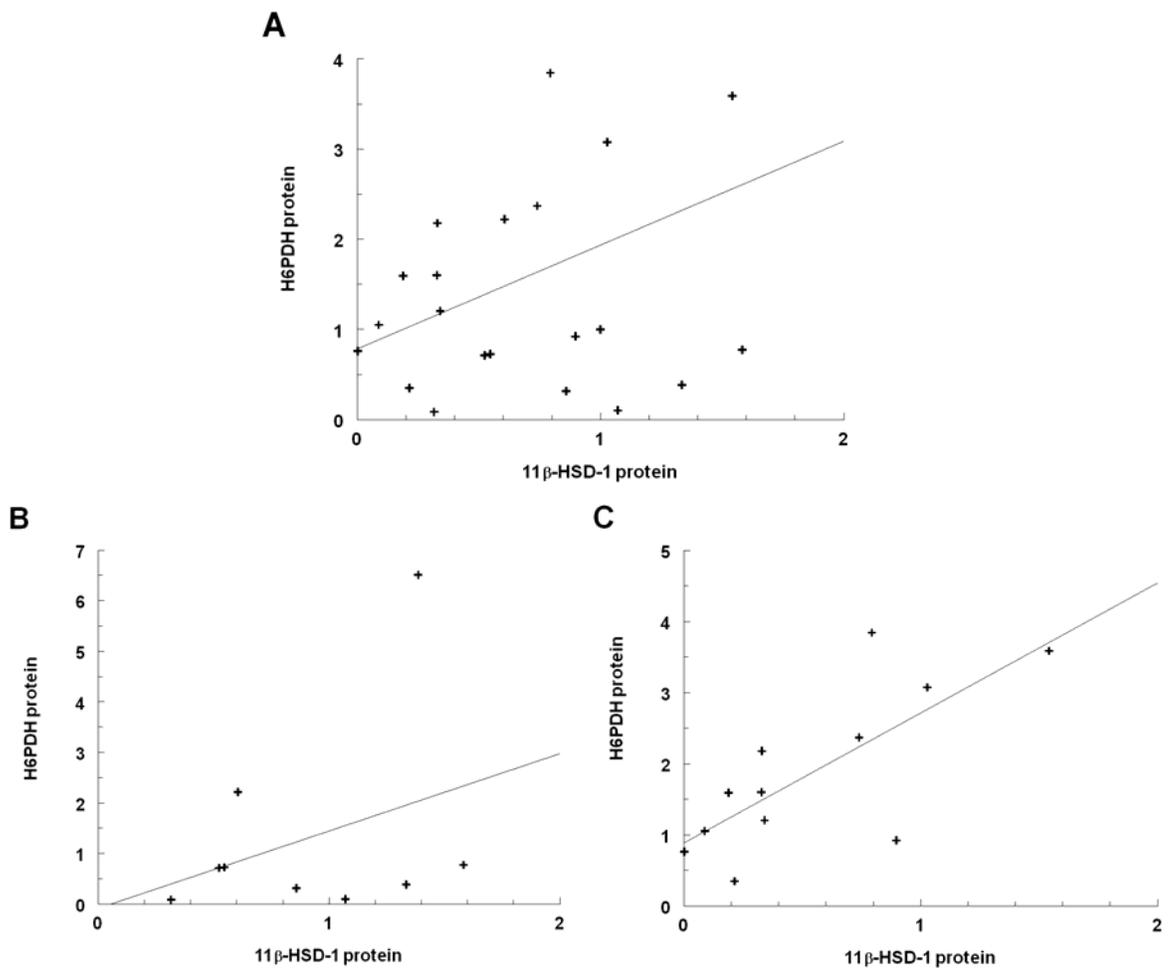


**Figure 26.** Hepatic expression of H6PDH protein after 24h or 1 wk exposure to experimental diets for rats in the control, S16, F16, and G16 groups. Data shown have been transformed to achieve normal distribution and graphed as the back-transformed data. Protein was quantified relative to the housekeeper,  $\beta$ -actin and to a loading control; values are expressed as mean  $\pm$  SEM; different letters indicate a significant difference;  $P < 0.05$ ; comparisons were made within time groups only;  $n = 8/\text{gp}$ .

### ***H6PDH Protein Correlations***

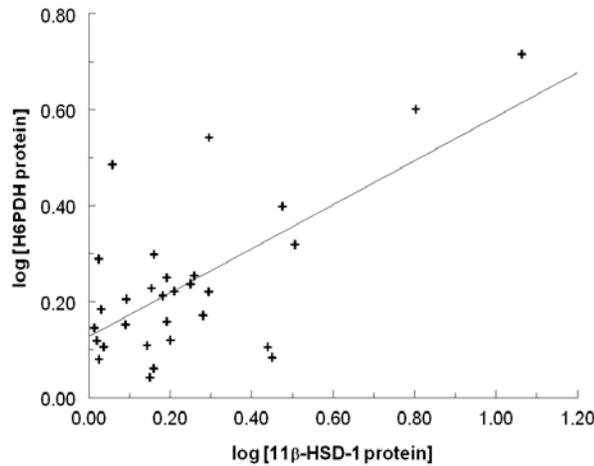
In comparing the H6PDH protein expression data from both studies, a similar trend of suppression among sugar-fed groups is apparent. While there were no significant differences among groups at 24h, the mean level for the S16 group is approximately half that of the control group. By 1wk, suppression of H6PDH protein was statistically significant in all sugar-fed groups.

After 24h exposure to the experimental diets, hepatic 11 $\beta$ -HSD-1 and H6PDH protein levels were not correlated when all four groups were included in the analysis ( $r = 0.19$  (Spearman),  $P = 0.39$ ; Fig. 27A). Similarly, when the same correlation analysis was performed for the rats in the groups with fructose-containing diets (S16, F16), there was no relationship between hepatic 11 $\beta$ -HSD-1 and H6PDH protein levels ( $r = 0.43$  (Spearman),  $P = 0.24$ ; Fig. 27B). However, hepatic 11 $\beta$ -HSD-1 and H6PDH protein at 24h were positively correlated among the groups whose diets did not contain fructose (control, G16) ( $r = 0.66$  (Spearman),  $P = 0.02$ ; Fig. 27C). Significant relationships also existed for each G16 and control group when analyzed individually (data not shown). At 24h, hepatic 11 $\beta$ -HSD-1 and H6PDH mRNA were also positively correlated when data for all four groups were analyzed, yet the P value for this relationship was just outside the range of statistical significance ( $r = 0.54$  (Pearson),  $P = 0.067$ ; Fig. 27C).



**Figure 27.** 24h correlations between hepatic 11β-HSD-1 and H6PDH protein in **(A)** All rats in the control, S16, F16, and G16 groups, **(B)** the F16 and S16 groups only, and **(C)** the control and G16 groups only.

After 1 wk exposure to the experimental diets, the hepatic protein levels for 11β-HSD-1 and H6PDH were positively correlated ( $r= 0.43$  (Spearman);  $P= 0.02$ ; Fig. 28).

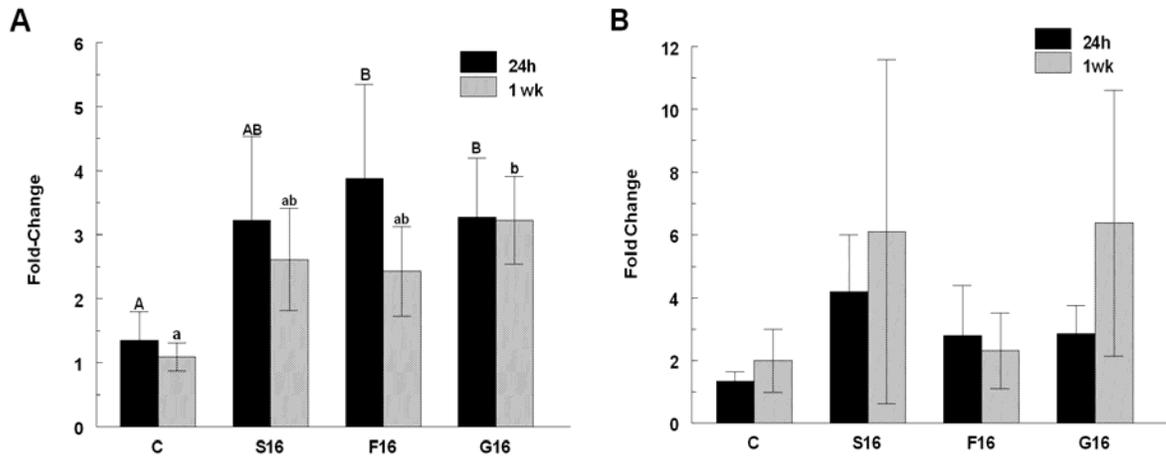


**Figure 28.** 1 wk correlation between hepatic 11β-HSD-1 protein and hepatic H6PDH protein in rats in the control, S16, F16, and G16 groups.

### *Acetyl CoA Carboxylase mRNA*

After 24h exposure to the experimental diets, mean hepatic acetyl CoA Carboxylase (ACC) mRNA was higher in the F16 ( $P= 0.005$ ) and G16 ( $P= 0.05$ ) groups in comparison to that of the control group (Fig. 29A). In mesenteric adipose, there were no significant differences in ACC mRNA after 24h ( $P = 0.59$ , Fig. 29B).

After 1 wk exposure to the experimental diets, hepatic ACC mRNA increased by three-fold in the G16 group in comparison to the control group ( $P= 0.02$ , Fig. 29A). The S16 and F16 groups had elevated mean ACC mRNA in liver that approached, but did not reach statistical significance ( $P = 0.06$  and  $P= 0.08$ , respectively; Fig. 29A). In mesenteric adipose, mean ACC mRNA concentrations were approximately five-fold higher in the S16 and G16 groups relative to the F16 and control groups at 1 wk, yet none of these differences were significant. As illustrated in Figure 29B, there was a high degree of intra-group variability.



**Figure 29.** ACC mRNA in (A) Liver, and (B) Mesenteric adipose of rats in the control, S16, F16, and G16 groups after 24h or 1 wk exposure to the experimental diets. Mesenteric adipose data have been log-transformed to achieve normal distribution and graphed as the back-transformed data; values are expressed as mean  $\pm$  SEM; different letters indicate a significant difference;  $P < 0.05$ ; comparisons were made within time groups only;  $n = 8/\text{gp}$ .

In comparing the effects of the treatments on ACC mRNA at 24h and 1wk, it is clear that in both tissues, there was a considerable amount of intra-group variability in each of the experiments. At both 24h and 1 wk, mean hepatic ACC mRNA for the sugar-fed groups were approximately three-fold that of their respective controls, yet at 24h only the S16 and G16 groups had significantly higher means than that of the control group (Fig. 29A). At 1 wk, only the G16 group had an increase in mean hepatic ACC mRNA that achieved statistical significance. In both experiments, mean ACC mRNA levels in mesenteric adipose spanned a wide range, and no significant differences were observed among the groups (Fig. 29B).

### *ACC Correlations*

After 24h exposure to the experimental diets, hepatic ACC mRNA was positively correlated with hepatic H6PDH mRNA in the rats of the control, S16, F16, and G16 groups ( $r = 0.35$  (Spearman),  $P = 0.05$ ; See Appendix 6). 24h hepatic ACC mRNA was

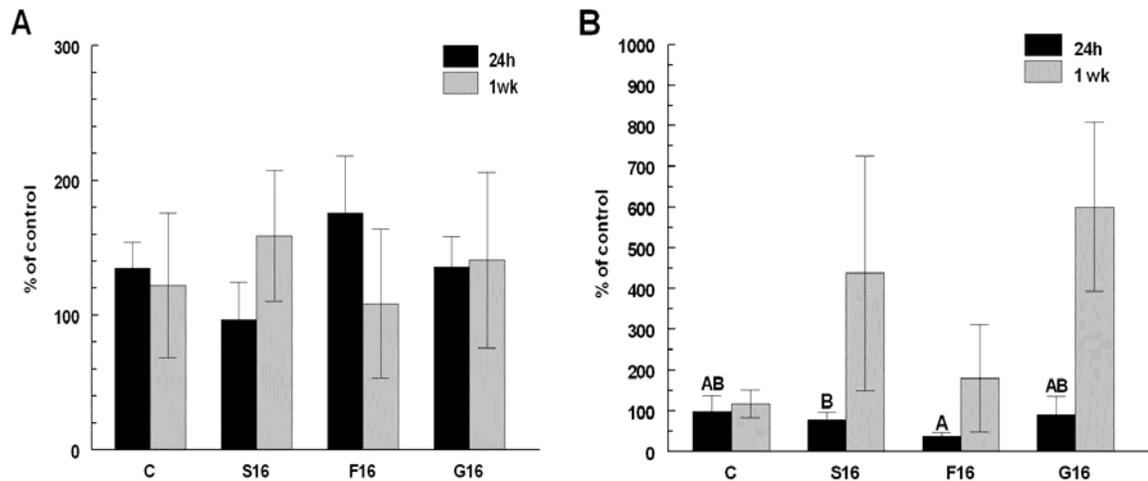
also positively correlated with the percentage of daily intake as fructose ( $r= 0.37$  (Spearman),  $P = 0.04$ ), and with the percentage of intake as sugar ( $r= 0.35$  (Spearman),  $P = 0.05$ ) (See Appendix 6).

After 1 wk, hepatic ACC mRNA was positively correlated with the percentage of daily caloric intake as sugar ( $r= 0.40$  (Spearman),  $P = 0.03$ ), (See Appendix 7). While sugar intake was correlated with ACC mRNA at both time points, there were no correlations at either 24h or 1 wk between hepatic ACC mRNA and total caloric intake.

### ***CCAAT/Enhancer Binding Protein alpha***

After 24h exposure to high sugar diets there were no significant changes in hepatic CCAAT/enhancer binding protein alpha (C/EBP- $\alpha$ ) mRNA among the four groups (Fig. 30A). At 24h, C/EBP- $\alpha$  message was suppressed in mesenteric adipose in the F16 group compared to the S16 group ( $P= 0.05$ , Fig. 30B). No other significant differences were observed. The number of usable data points for adipose C/EBP- $\alpha$  mRNA was reduced due to the difficulty in extracting and purifying RNA and achieving optimal cDNA quality in adipose tissue. This may have contributed to the relatively high degree of variability among rats. While there was only one difference observed in C/EBP- $\alpha$  mRNA at 24h, there were numerous strong correlations between C/EBP- $\alpha$  mRNA and 11 $\beta$ -HSD-1, H6PDH, and ACC in both organs. However, there were considerably more relationships between C/EBP- $\alpha$  and the genes of interest at 24h than at 1 wk (see Appendices 6 and 7). At 1 wk, there were no differences in C/EBP- $\alpha$  mRNA in either liver or mesenteric adipose in response to the experimental diets (Figs. 30A, 30B). As was seen in the 24h study, there was a considerable amount of variability within groups. C/EBP- $\alpha$  mRNA in adipose for F16 and G16 groups appear to be considerably

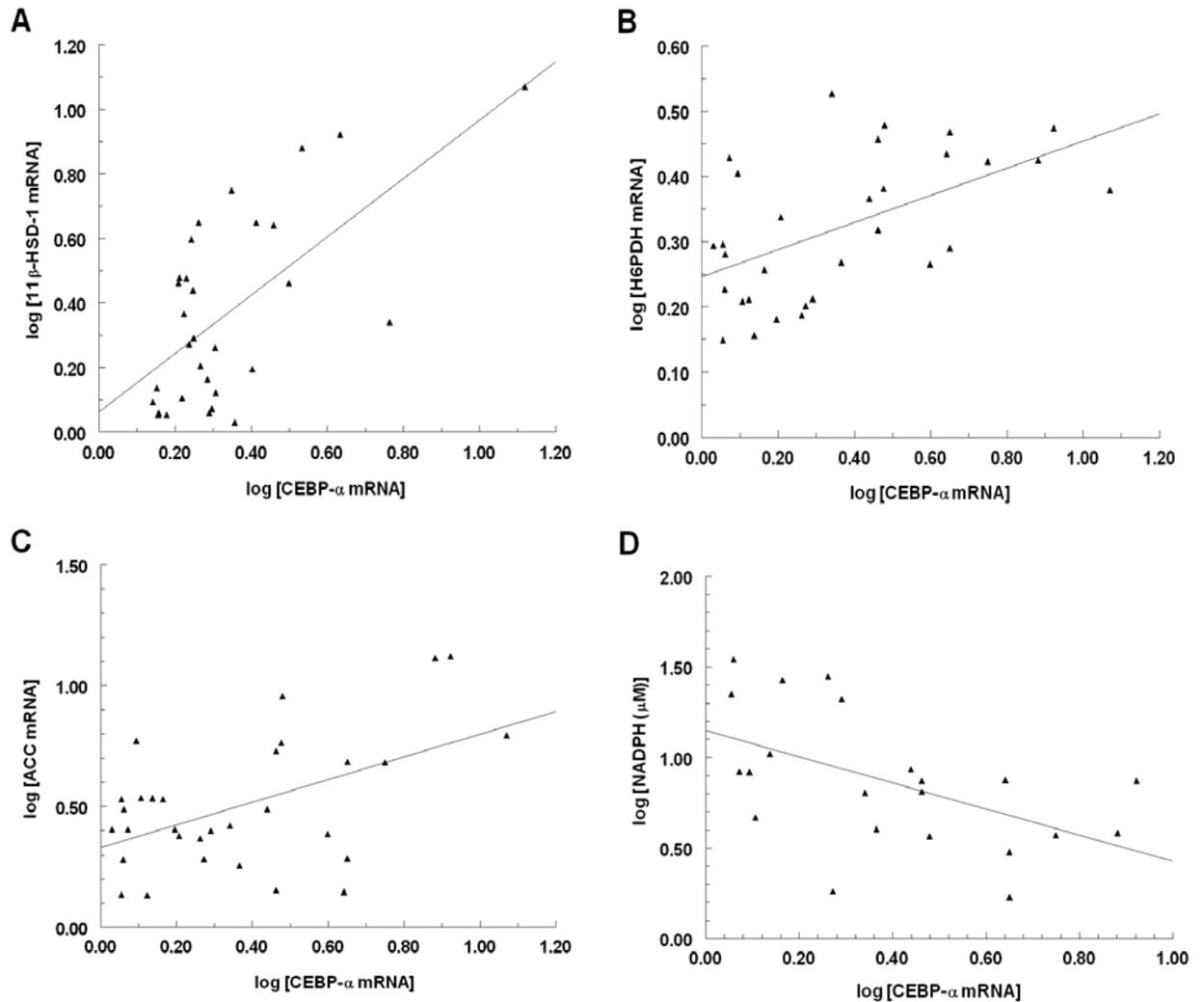
increased relative to the control, such was the case in only a fraction of the animals in those groups. Ultimately, there were no significant differences among the groups.



**Figure 30.** C/EBP- $\alpha$  mRNA in (A) Liver, and (B) Mesenteric adipose in rats in the control, S16, F16, and G16 groups after 24h and 1 wk exposure to experimental diets; values are expressed as mean  $\pm$  SEM; different letters indicate a significant difference ( $P < 0.05$ ); comparisons were made within time groups only;  $n = 8/\text{gp}$ .

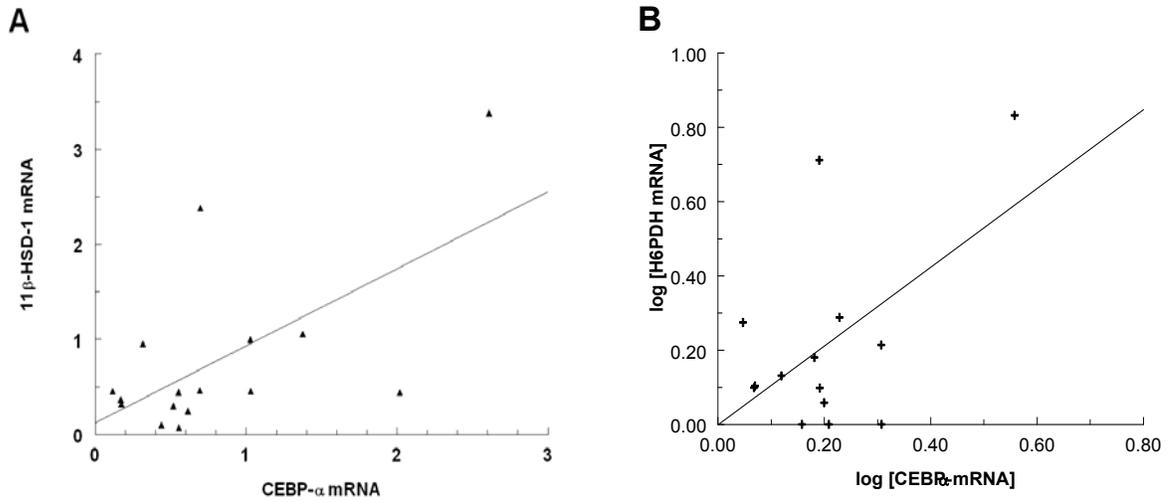
### *C/EBP- $\alpha$ Correlations*

At 24h, hepatic C/EBP- $\alpha$  mRNA was positively correlated with hepatic 11 $\beta$ -HSD-1 mRNA ( $r = 0.48$  (Spearman),  $P = 0.006$ ; Fig. 31A). Additionally, at 24h, hepatic C/EBP- $\alpha$  mRNA was positively correlated with hepatic H6PDH mRNA ( $r = 0.52$  (Spearman),  $P = 0.003$ , Fig. 31B) and hepatic ACC mRNA ( $r = 0.35$  (Spearman),  $P = 0.05$ , Fig. 31C). Also at 24h, there was a negative correlation between hepatic C/EBP- $\alpha$  mRNA and total cellular hepatic NADPH ( $r = -0.626$  (Spearman),  $P = 0.0002$ ; Fig. 31D).



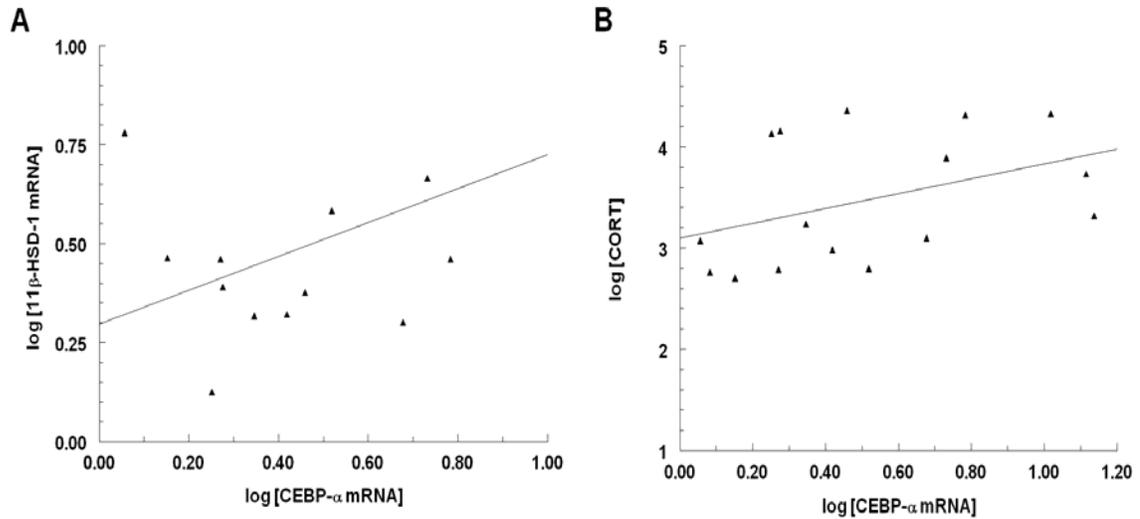
**Figure 31.** 24h correlations between hepatic C/EBP- $\alpha$  mRNA and (A) Hepatic 11 $\beta$ -HSD-1 mRNA, (B) Hepatic H6PDH mRNA, (C) Hepatic ACC mRNA, and (D) Hepatic total cellular NADPH in the control, S16, F16, and G16 groups after 24h exposure to the experimental diets.

In mesenteric adipose, C/EBP- $\alpha$  mRNA and 11 $\beta$ -HSD-1 mRNA were positively correlated in the 24h experiment ( $r = 0.47$  (Spearman),  $P = 0.05$ ; Fig. 32A; Appendix 5). C/EBP- $\alpha$  mRNA and H6PDH mRNA in mesenteric adipose were also positively correlated after 24h ( $r = 0.54$  (Spearman),  $P = 0.03$ , Fig. 32B; Appendix 5). However, these correlations were somewhat weak in that they were reliant on one or two specific points, and the correlations lost significance upon their elimination.



**Figure 32.** 24h correlations between C/EBP- $\alpha$  mRNA in mesenteric adipose and **(A)** 11 $\beta$ -HSD-1 mRNA in mesenteric adipose, and **(B)** H6PDH mRNA in mesenteric adipose in rats in the control, S16, F16, and G16 groups after 24h exposure to the experimental diets.

At 1 wk, C/EBP- $\alpha$  mRNA in mesenteric adipose was positively correlated with adipose 11 $\beta$ -HSD-1 mRNA ( $r= 0.74$  (Pearson),  $P = 0.002$ ; Fig. 33A). At 1 wk, adipose C/EBP- $\alpha$  mRNA and plasma corticosterone were also positively correlated ( $r=0.51$  (Spearman),  $P= 0.04$ ; Fig. 33B).

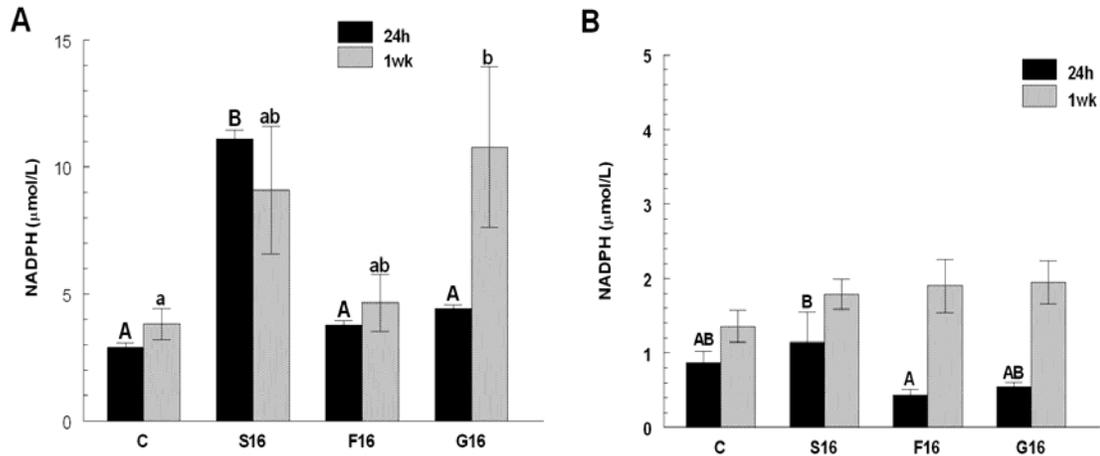


**Figure 33.** 1 wk correlations between C/EBP- $\alpha$  mRNA in mesenteric adipose and (A) 11 $\beta$ -HSD-1 mRNA in mesenteric adipose, and (B) Plasma corticosterone (CORT) in rats in the control, S16, F16, and G16 groups.

#### ***Total Cellular NADPH Concentrations in Liver and Mesenteric Adipose***

After 24h exposure to the experimental diets the S16 group had a higher mean concentration of total cellular hepatic NADPH than did the control, F16, and G16 groups ( $P= 0.002$ ). No other differences in liver at 24h attained statistical significance (Fig. 34A). The S16 group also had a higher mean NADPH concentration in mesenteric adipose in comparison to the F16 group after 24h ( $P< 0.05$ , Fig. 34B). No other differences in mesenteric adipose were observed at 24h.

At 1 wk, the mean total cellular NADPH level for both the S16 and G16 groups was approximately 10  $\mu\text{mol/L}$  compared to just less than 4  $\mu\text{mol/L}$  for the control. Yet, only the difference between the G16 and control groups attained statistical significance ( $P< 0.01$ , Fig. 34A). No other significant differences were observed in liver at 1 wk, and as Figure 34A indicates, intra-group variability was high in liver. In mesenteric adipose, no differences in mean NADPH concentrations were observed among the four groups (Fig. 34B).



**Figure 34.** Mean concentrations of total cellular NADPH in (A) Liver, and (B) Mesenteric adipose after 24h exposure to experimental diets; values are expressed as mean  $\pm$  SEM; different letters indicate a significant difference ( $P < 0.05$ ); comparisons were made within time groups only;  $n = 8/\text{gp}$ .

In comparing liver and adipose NADPH was clearly more abundant in liver than adipose irrespective of time group or treatment. Additionally, liver NADPH was more variable in response to the experimental diets. In liver, differences among the four groups were seen at both 24h and 1 wk (Fig. 34A). In mesenteric adipose, there were no differences relative to the control group (Fig. 34B).

### ***Summary of Results***

Table 7 summarizes changes among groups in the 24h and 1 wk experiments that were observed in the parameters of glucocorticoid, carbohydrate, and lipid metabolism that were quantified in plasma, liver, and adipose.

**Table 7.** Summary of changes in the parameters measured in liver, mesenteric adipose, and plasma in the control, S16, F16, and G16 groups at 24h and 1 wk.

		S16		F16		G16	
		24h	1 wk	24h	1 wk	24h	1 wk
<b>Liver</b>	11 $\beta$ -HSD-1 mRNA	—	↓	↑	↓	—	↓
	11 $\beta$ -HSD-1 protein	> F16 & G16	↓	↑	—	—	↓
	H6PDH mRNA	< F16 & G16	—	> S16	> S16	> S16	—
	H6PDH protein	—	↓	—	↓	—	↓
	ACC mRNA	—	—	↑	—	—	↑
	CEBP- $\alpha$ mRNA	—	—	—	—	—	—
	NADPH	↑	—	—	—	—	↑
<b>Adipose</b>	11 $\beta$ -HSD-1 mRNA	—	—	↑	↑	—	—
	H6PDH mRNA	—	—	—	—	> S16	—
	ACC mRNA	—	—	—	—	—	—
	CEBP- $\alpha$ mRNA	—	—	—	—	—	—
	NADPH	—	—	< S16	—	—	—
<b>Plasma</b>	CORT	—	↓	—	—	—	—
	Insulin	↑	—	—	↑	—	—
	TG	↑	↑	↑	↑	—	—

## **Discussion**

### ***High Sugar Diets and Glucocorticoid Metabolism***

Glucocorticoid dysregulation is a characteristic of different forms of obesity that has been documented in humans and animals. Yet despite its prevalence, little is known about the development of tissue-specific glucocorticoid dysregulation and how diet may interact. This dissertation provides evidence that high intakes of sugar-sweetened solutions can impact glucocorticoid metabolism via the  $11\beta$ -HSD-1 system.  $11\beta$ -HSD-1 dysregulation in obesity has been reported repeatedly. Elevated  $11\beta$ -HSD-1 mRNA and activity in adipose and decreased  $11\beta$ -HSD-1 activity in liver have been reported in obese Zucker rats (Livingstone et al 2000a & 2000b, Czele et al 2008). Rask et al (2001) also reported a three to four-fold increase in  $11\beta$ -HSD-1 activity in adipose of obese versus lean humans accompanied by impaired hepatic reactivation of cortisone to cortisol by  $11\beta$ -HSD-1. The present studies add to these observations, and point to not only a possible link between diet and obesity, but also to a link between diet and glucocorticoid-mediated changes in metabolism that can lead to obesity.

The 24h and 1 wk study data demonstrated that high sugar diets impact peripheral glucocorticoid metabolism in the absence of the phenotypic changes associated with obesity, i.e. excess adiposity. Based on data from a long-term (10 wk) sucrose feeding study, the research question central to these short-term sugar feeding studies asked: Is the glucocorticoid dysregulation observed after long-term exposure to high sugar diets a direct result of diet composition or a consequence of increased adiposity? High glucose, sucrose, and fructose diets all initiated the same dysregulation of hepatic  $11\beta$ -HSD-1 mRNA (and protein) within 1 wk that has been previously reported in obese humans and

rodent models, as well as in the 10 wk study. Although the metabolic and endocrine responses to each of the three sugar solutions were distinct, a common finding among sugar-fed groups was that of suppressed hepatic 11 $\beta$ -HSD-1 mRNA and protein (protein approached significance in F16 group), and decreased hepatic H6PDH protein. To our knowledge these experiments are the first to report the unique acute effects of different high sugar diets on glucocorticoid metabolism.

Changes in glucocorticoid metabolism in response to acute (Drake et al 2005), but not long-term (Parsons 2006) high fat diets have been reported. After 3 wk exposure to high fat diets, Drake et al hypothesized that acutely decreased 11 $\beta$ -HSD-1 activity in liver and adipose constituted a protective mechanism against the metabolic consequences of obesity based on their observation that high fat fed Wistar rats displayed these changes after 3, but not 20 wk, at which point they had become hyperglycemic, hyperinsulinemic, and obese (2005). The suppression of hepatic 11 $\beta$ -HSD-1 mRNA and protein that we saw in response to acute (1 wk) exposure to an obesogenic diet could similarly be hypothesized to be a protective mechanism. It may be that prolonged exposure to enhanced carbohydrate flux and accompanying excesses in energy, inflammation, elevated free fatty acids, ER stress, etc., that occur acutely in response to high sugar diets, may reach a threshold at which the balance tips in favor of glucocorticoid dysregulation. Rats fed an obesogenic (high sucrose, high fat) diet and treated with a specific 11 $\beta$ -HSD-1 inhibitor for 3 wk did not eat less or weigh less than controls fed the same diet, but had significantly reduced mesenteric adipose weight and adipocyte size (Berthiaume et al 2007). Retroperitoneal and epididymal fat depots were unaffected. These data support our

hypothesis that 11 $\beta$ -HSD-1 moderates the effects of macronutrient composition in promoting visceral adiposity.

Each of the three high sugar diets had distinct effects on the parameters of glucocorticoid metabolism, lipid, and glucose metabolism that were measured. Differences in the physiological responses of ingesting sucrose, fructose, or glucose are likely to contribute to the distinct effects observed. These differences include: 1) impact (or lack of) on insulin secretion, 2) regulation of uptake by cells, 3) regulation through the glycolytic pathway, and 4) affect on lipid metabolism.

The effect of each of the three sugar solutions on glucocorticoid metabolism was distinct (see Table 8). Perturbations in 11 $\beta$ -HSD-1 mRNA levels resulted within 24h exposure to high fructose diets and within 1wk in response to high sugar diets more generally. While suppression of hepatic 11 $\beta$ -HSD-1 mRNA and protein, as well as hepatic H6PDH protein was observed within only 1 wk of exposure to high sucrose, fructose and glucose diets, alterations in adipose 11 $\beta$ -HSD-1 mRNA were observed only in response to a high fructose diet. This suggests that the elevation of 11 $\beta$ -HSD-1 mRNA in adipose that we observed in rats fed high sucrose diets for 10 wk likely resulted from increased adiposity that occurred gradually during longer-term exposure to the high sugar diets, and not directly from diet composition. Chronic high sucrose or more generally, high sugar diets likely have broad ranging physiological effects based on the changes we observed within 1 wk exposure, and the obesity that developed within 10 wk exposure. Elevated 11 $\beta$ -HSD-1 mRNA levels in mesenteric adipose developed between 1 and 10 wk of exposure to high sucrose diets during which time a number of changes related to increased adiposity likely developed as well. Alternatively, it is possible that the

cumulative effect of consistent fructose intake at a lower but more chronic level may contribute to the dysregulation of 11 $\beta$ -HSD-1 mRNA seen after 10 wk. We do not know what the dose response is for fructose with respect to glucocorticoid metabolism, inflammation, or other pathways at play in this observed response.

Acute 24h exposure to the high fructose diet we provided appeared to directly impact glucocorticoid metabolism by increasing 11 $\beta$ -HSD-1 mRNA in both adipose and liver. Stanhope et al recently reported that 10 wk high fructose or glucose diets (25% daily energy requirements as sweetened beverages) led to similar body weight gain, but that only the high fructose diet increased visceral adiposity in obese and overweight adults (2009). While 11 $\beta$ -HSD-1 was not measured, only fructose promoted increased visceral adiposity. This finding supports differential metabolic and endocrine effects of different sugars. The acute and short-lived increase in 11 $\beta$ -HSD-1 mRNA in liver in response to fructose is particularly intriguing. It is likely that the decreased hepatic H6PDH mRNA and protein that we observed at 1 wk played a role in decreased 11 $\beta$ -HSD-1 between 24h and 1 wk exposure to the high sugar diets based on earlier findings that H6PDH determines the set point for 11 $\beta$ -HSD-1 reductase activity (Bujalska et al 2004, Bánhegyi et al 2004). Given what we have shown in these acute studies, the phenomenon of increased visceral adiposity in response to fructose intake that Stanhope et al reported may involve tissue-specific dysregulation of glucocorticoid metabolism.

**Table 8.** Comparison of changes in liver and mesenteric adipose and plasma in response to the S16, F16, or G16 diets.

		<b>24h</b>	<b>1 wk</b>
<b>S16</b>		<b>Liver:</b> NADPH↑, H6PDH mRNA > G16 & F16	<b>Liver:</b> 11β-HSD-1 mRNA↓, 11β-HSD-1 protein ↓, H6PDH protein↓
		<b>Adipose:</b> No changes	<b>Adipose:</b> No changes
		<b>Plasma:</b> Insulin ↑, TG ↑	<b>Plasma:</b> CORT↓, TG ↑
<b>F16</b>		<b>Liver:</b> 11β-HSD-1 mRNA ↑, ACC mRNA ↑	<b>Liver :</b> 11β-HSD-1 mRNA↓, 11β-HSD-1 protein ↓, H6PDH protein ↓
		<b>Adipose:</b> 11β-HSD-1 mRNA ↑, NADPH < S16	<b>Adipose:</b> 11β-HSD-1 mRNA ↑
		<b>Plasma:</b> TG↑	<b>Plasma:</b> insulin ↑, TG ↑
<b>G16</b>		<b>Liver:</b> ACC mRNA ↑	<b>Liver:</b> 11β-HSD-1 mRNA↓, 11β-HSD-1 protein↓, H6PDH protein ↓, ACC mRNA ↑, NADPH ↑
		<b>Adipose:</b> No changes	<b>Adipose:</b> No changes
		<b>Plasma:</b> No changes	<b>Plasma:</b> No changes

### *Differential metabolic and endocrine effects of different sugars*

#### *High Sugar Diets and Impact on Lipid and CHO Metabolism*

The presence or absence of fructose in a sweetened beverage appears to make a difference with respect to peripheral glucocorticoid metabolism and also to lipid and glucose metabolism. The differences observed among the groups in the 24h and 1 wk studies illustrated some of the differential metabolic and endocrine consequences of sucrose, fructose, and glucose. The effects of high intakes of dietary fructose that have been cited include decreased expression of genes involved in fatty acid beta-oxidation, increased expression of genes involved in fatty acid biosynthesis (Schwarz et al 1994, Chong et al 2007), and increased mRNA levels of gluconeogenic genes and that of

carbohydrate response element binding protein (Yoo et al 2008). Interestingly, 11 $\beta$ -HSD-1 inhibitors have been found to reverse these deleterious effects on lipid metabolism in liver and visceral adipose in rats fed a high sucrose, high fat diet (Berthiaume et al 2007). Manifestations of a high fructose diet include elevated plasma TG, specifically postprandial levels (not seen in high glucose diets); and increased deposition of fat in liver (Kawasaki et al 2009) and visceral depots (Stanhope et al 2009). Elevated levels of inflammatory cytokines have also been observed in high fructose feeding (Kawasaki et al 2009), which may provide a critical link since TNF- $\alpha$  and IL-1 $\beta$  are known to upregulate transcription of 11 $\beta$ -HSD-1 (Escher et al 1997).

Plasma TG and ACC mRNA in both liver and adipose were measured as parameters of lipid metabolism in the 24h and 1 wk experiments. Within 24h, there were increases in hepatic ACC mRNA and/or plasma TGs in all the sugar-fed groups. At 24h, plasma TGs were significantly elevated in the S16 and F16 groups, but not in the G16 group. Also at 24h, ACC mRNA was increased only in the F16 and G16 groups. It should be noted that the mean hepatic ACC mRNA levels at 24h for all three sugar-fed groups were approximately three-fold that of the control and greater than twice that of control at 1 wk, indicating a trend toward increased ACC mRNA in all sugar-fed groups. Variability in ACC among all sugar-fed rats was considerable in both tissues. A fraction of the rats had very high ACC mRNA levels while the remainder had levels similar to those seen in control rats. Because de novo lipid biosynthesis and TG levels are very sensitive to the fed-state of an animal, it is likely that our lack of control over when the rats ate and drank sugar solutions was a factor in the variability. Finding a way to control for this, such as administration of a fructose, sucrose, or glucose bolus and sampling

blood at set intervals thereafter, might clarify this ambiguity. At 1 wk, the F16 and S16 groups had elevated TG levels in comparison to the control group and the G16 group, yet the G16 group was the only group with significantly elevated hepatic ACC mRNA at that time. The changes observed in plasma TG and hepatic ACC confirm the role of high sugar hypercaloric diets in promoting de novo lipid biosynthesis and/or hypertriglyceridemia. These changes were observed despite differences in the endocrine and metabolic consequences of glucose and fructose. Increased secretion of VLDL-TG has been cited as a contributor to fructose-induced hypertriglyceridemia (Zavaroni et al 1982).

In obesity and diabetes a characteristic metabolic disturbance is one of altered nutrient partitioning such as enhanced lipolysis as well as gluconeogenesis in the fed state. High sugar diets appear to affect nutrient partitioning and this effect is likely exaggerated in the presence of increased adiposity (Rohner-Jeanraud 2006). Measuring the message and expression of other genes involved in lipolysis, fatty acid oxidation, and gluconeogenesis could help to round out this picture. In these studies we reproduced the finding that diets high in fructose induce hypertriglyceridemia (Hwang et al 1987, Havel 2005). We add to this the observation that an acute high sucrose diet induces similar increases in plasma TG in the absence of significant increases in hepatic ACC mRNA. Glucose does not induce hypertriglyceridemia acutely, although hepatic ACC mRNA is upregulated at both 24h and 1 wk suggesting that de novo lipid biosynthesis may be increased nonetheless.

### ***High Sugar Diets and Impact on Carbohydrate Metabolism***

Insulin response, the mechanism for sugar uptake into cells, and glycolytic regulation are important endpoints that reveal differences between the effects of the monosaccharides fructose and glucose. Hyperglycemia can initiate insulin resistance, but hyperglycemia is not a consequence of a high fructose diet. In long-term exposure to high fructose diets, fat accumulation in liver and adipose tissue has been proposed as one potential mechanism of fructose-induced insulin resistance. While glucose uptake by cells is insulin-dependent, the uptake of fructose into cells by GLUT5 is not insulin-dependent. Under the conditions of high fructose intake, the flux of fructose into cells is not regulated. Glucose uptake is limited by negative feedback from high plasma glucose levels and decreased circulating insulin. Fructose bypasses the key regulatory step of glycolysis (PFK-1), so that the production of acetyl CoA from dietary fructose is not restricted by cellular energy needs. It has also been reported that fructose does not stimulate the normal release of leptin that glucose does in a postprandial state, nor does fructose induce the normal attenuation of ghrelin secretion known to occur in response to glucose intake (Teff et al 2004 and 2005). Thus, not only does uptake into and usage by cells differ, but these major satiety signals also appear to be impaired as a consequence of fructose consumption.

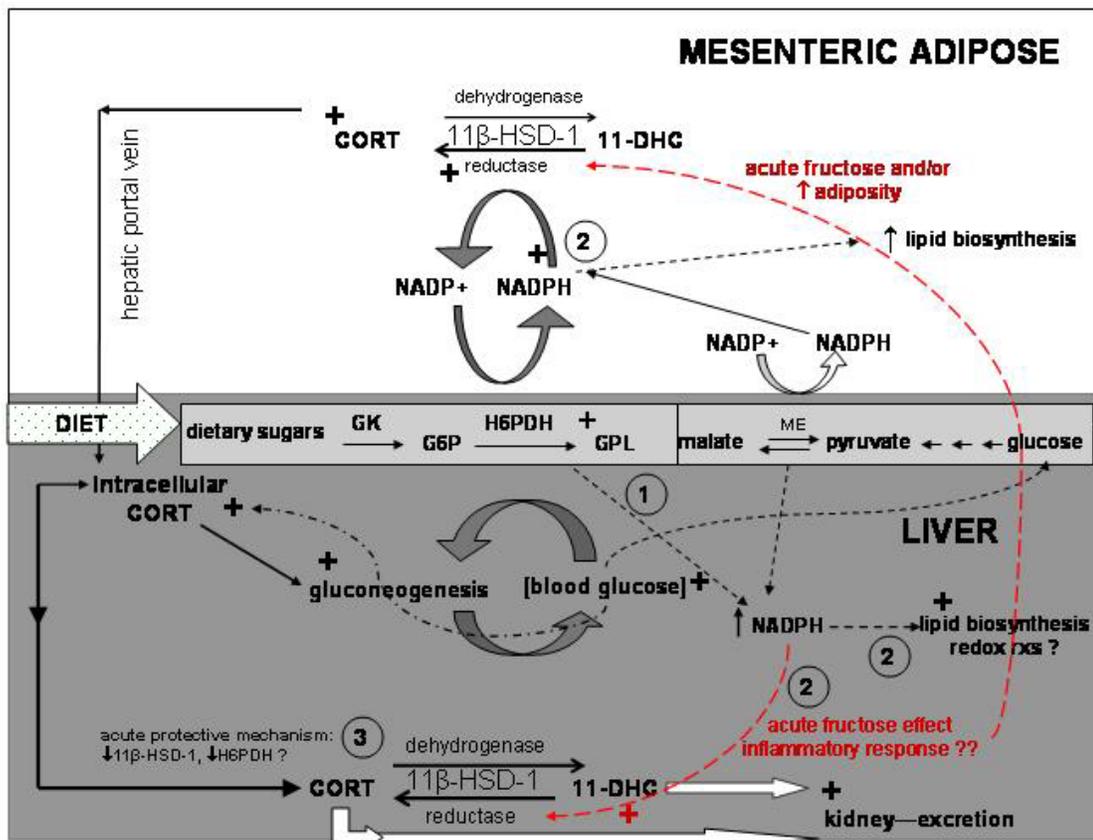
It has been established that high fructose diets induce hyperinsulinemia (Hwang et al 1987, Reaven et al 1990, Havel 2005, Lê & Tappy 2006), but the mechanisms for hyperinsulinemia in high sucrose or glucose, and high fructose feeding appear to be quite different. Insulin levels differed among the sugar-fed groups in both 24h and 1 wk studies. The high sucrose diet was the only treatment that initially caused a significant elevation in plasma insulin (24h), and after 1 wk only the rats fed a high fructose diet had

significantly elevated plasma insulin. However, it should be noted that the F16 group at 24h, and the S16 group at 1 wk had increased mean plasma insulin that approached statistical significance. This is another instance in which the high variability in the parameter measured may have been a result of the lack of control over the patterns of sugar solution intake and the transient nature of the parameter. The high glucose diet did not induce hyperinsulinemia acutely. This difference suggests a difference in mechanism for induction of hyperinsulinemia based on sugar type and indicates that the presence of fructose played a role in the observed elevations in insulin.

The 24h insulin response in conjunction with the elevated hepatic and adipose 11 $\beta$ -HSD-1 may enable an interaction that is critical to initiating a cycle of glucocorticoid dysregulation observed with a high fructose diet. Increased 11 $\beta$ -HSD-1 oxidoreductase activity has been observed in cultured omental adipocytes of obese patients, and stimulation of the cells with cortisol and insulin further increased expression and activity of 11 $\beta$ -HSD-1 (Bujalksa et al 1997). The same type of positive feedback loop could presumably occur with chronic high fructose diets since there is elevated plasma insulin as well as increased active local glucocorticoid. We did not measure enzyme activity, but confirming elevated 11 $\beta$ -HSD-1 activity in this scenario would be helpful to understanding the progression of dysregulation. A positive correlation between fat mass and circulating insulin has been previously established (Gibson et al 1975) and we reproduced this finding in the 10 wk study. Thus, over time increased adiposity also could have an additive effect and contribute to the perpetuation of glucocorticoid dysregulation in adipose in high sugar diet-induced obesity.

### *Carbohydrate Availability and the 11 $\beta$ -HSD-1 Regulatory System*

While the effects of high carbohydrate diets on mRNA and protein expression of H6PDH have not previously been explored, a cooperative relationship between 11 $\beta$ -HSD-1 and H6PDH has been established (Bánhegyi et al 2004). Glucose-6-phosphate is an intermediate in several pathways of carbohydrate metabolism, and is also the primary substrate for H6PDH. Long-term sucrose feeding induced increased 11 $\beta$ -HSD-1 and H6PDH mRNA in adipose. We hypothesized that enhanced H6PDH activity from high carbohydrate availability would upregulate H6PDH mRNA in adipose under the conditions of acute high sugar diets. While we saw increased 11 $\beta$ -HSD-1 mRNA in mesenteric adipose in the F16 group at both time points, there was not a significant increase in H6PDH mRNA in adipose even though mean mRNA was seven-fold higher than the control group at 1 wk. The amount of intra-group variability was substantial. Decreased hepatic 11 $\beta$ -HSD-1 mRNA with concomitant increased H6PDH mRNA was observed as a result of 10 wk high sucrose-feeding. We hypothesized that the increased H6PDH provides NADPH for other uses within the liver such as de novo lipid biosynthesis which would promote further increases in plasma TG and adiposity (See Fig. 35). Hepatic H6PDH mRNA was unexpectedly increased in the fructose-fed group after 24h, and by 1 wk hepatic H6PDH protein and 11 $\beta$ -HSD-1 mRNA were suppressed in all sugar-fed groups. The reason for the lack of significant correlation between mRNA and protein levels of H6PDH in response to fructose is not clear, nor is the impact of elevated hepatic H6PDH mRNA expression in conjunction with suppressed 11 $\beta$ -HSD-1 mRNA that we saw in high sugar diet-induced obesity.



**Figure 35.** Proposed pathway for glucocorticoid dysregulation in liver and mesenteric adipose in response to a high sugar diet, and the acute impact of fructose, specifically on dysregulation these tissues. Circled numbers indicate the chronology of events; lighter large dashed arrows indicate acute effect of fructose; Abbreviations: 11 $\beta$ -HSD-1: 11 $\beta$ -hydroxysteroid dehydrogenase; CORT: corticosterone; 11-DHC: 11-dehydrocorticosterone; GK: glucokinase (or hexokinase); GPL: phosphor-gluconolactone; G6P: glucose-6-phosphate; ME: malic enzyme; PEPCK: phosphoenolpyruvate kinase.

Suppressed levels of hepatic H6PDH were expected to decrease the NADPH/NADP<sup>+</sup> ratio and/or total NADPH. This was not reflected in our total cellular NADPH quantification. Had we been able to quantify NADPH within the ER exclusively, we could have attained specific information about the pool of NADPH of interest in the 11 $\beta$ -HSD-1/H6PDH system. The ratio of NADPH/NADP<sup>+</sup> has been cited as a critical factor in determining 11 $\beta$ -HSD-1 activity and directionality (Dzykanchuk et

al 2009). In addition to these methodological set backs, NADPH/NADP<sup>+</sup> within the cell is a dynamic relationship and likely fluctuates between fed and fasted states, and depends on meal size, composition, fuel source, energy needs, nutrient partitioning, oxidative stress, and more. For these reasons finding meaning in our NADPH measurements was challenging. We measured total cellular NADPH concentrations in adipose and liver after 24h and 1 wk exposure to the high sugar diets. Our assay provided less specific, but nonetheless, interesting information about total carbohydrate flux and generation of total NADPH. However, without specific enzyme activity assays it is not possible to determine how NADPH was diverted for usage in the cell. To the best of our knowledge, an assay that permits the measurement of NADPH and NADP<sup>+</sup> within the ER has yet to be developed.

The distinct acute effects of fructose in comparison to the other sugars have been incorporated added to our earlier hypothesized pathway for high sugar diet-induced glucocorticoid dysregulation (Fig. 35). As indicated by the wide dashed lines and accompanying text (red), fructose initiated immediate increases in 11 $\beta$ -HSD-1 mRNA in liver and mesenteric adipose, as well as enhanced de novo lipid biosynthesis. Our results in combination with already determined effects of fructose in the liver, point to inflammatory cytokines as potential mediators for the deleterious effects observed in liver. We also added to this proposed pathway the finding that acute (1 wk) access to high sugar diets more generally, suppresses hepatic 11 $\beta$ -HSD-1 mRNA and protein in the absence of obesity and prior to increased 11 $\beta$ -HSD-1 in adipose.

### ***Transcriptional Regulation of 11 $\beta$ -HSD-1 under the Influence of High Sugar Diets***

While the message and expression data for 11 $\beta$ -HSD-1 and H6PDH provided some valuable clues into acute glucocorticoid dysregulation, the CCAAT/enhancer binding protein- $\alpha$  (C/EBP- $\alpha$ ) mRNA data were not as clear. C/EBPs have been recognized as master regulators of adipogenesis (Lefterova & Lazar 2009) and recent genome-wide searches reveal much overlap in their transcriptional targets. Along with sterol regulatory element binding proteins, peroxisome proliferator-activated receptors (PPAR $\alpha$ ,  $\delta$ , and  $\gamma$ ), and liver X receptors, C/EBP ( $\alpha$ ,  $\beta$ , and  $\delta$ ) have been suggested as potential nutrient sensors that alter gene expression (Al-Hasani & Joost 2005). Little is known about the transcriptional regulation of 11 $\beta$ -HSD-1 generally or in response to diet. As a result C/EBP- $\alpha$  was measured based on our interest in its possible role as a nutrient sensor involved in the transcriptional regulation of 11 $\beta$ -HSD-1.

There were no significant changes in C/EBP- $\alpha$  mRNA in either liver or mesenteric adipose at 24h or 1 wk, and there was considerable intra-group variability. However, there were several strong correlations in both the 24h and 1 wk studies with our primary genes of interest. At 24h liver C/EBP- $\alpha$  mRNA was positively correlated with hepatic 11 $\beta$ -HSD-1, H6PDH and ACC mRNA. Also at 24h, adipose C/EBP- $\alpha$  mRNA was positively correlated with adipose 11 $\beta$ -HSD-1 mRNA. Adipose C/EBP- $\alpha$  mRNA was still positively correlated with 11 $\beta$ -HSD-1 at 1 wk, but there were no meaningful relationships with hepatic C/EBP- $\alpha$  after 1 wk exposure to the experimental diets. Perhaps this lack of correlation is a byproduct of the glucocorticoid dysregulation that became established over the week. These correlations warrant further, more focused

experiments to investigate possible interaction between *C/EBP- $\alpha$*  and the *HSD11B1* gene under different dietary conditions in liver and adipose.

Perturbations in the liver in response to high fructose diets may be a key to some of the differences observed among the sugars. High fructose and not high sucrose or high fat feeding in Wistar rats has been shown to produce a good model for nonalcoholic hepatic steatosis which is a manifestation of metabolic syndrome (Kawasaki et al 2009). Five wk exposure to high fructose diets caused significantly higher grade of hepatic macrovesicular steatosis, periportal fibrosis, and lobular inflammation. Inflammatory cytokine release in response to fructose may be an important link between glucocorticoid dysregulation, elevated TG levels, and the promotion of adiposity.

Fructose has been shown to induce acute inflammation (Kawasaki et al 2009). We did not measure markers of inflammation, but IL-1 $\beta$  and TNF- $\alpha$  have been shown to upregulate 11 $\beta$ -HSD-1 and increase its oxidoreductase activity (Escher et al 1997). Adding to this scenario of inflammatory response, hepatic inflammatory stress response after 14 day high fructose feeding in Sprague-Dawley rats was also cited as the cause for the cholesterol and lipid dysregulation observed (Kelley et al 2004). These previous findings in concert with our evidence for acute upregulation of 11 $\beta$ -HSD-1 suggest that inflammatory response, primarily in liver where fructose is processed, would be a logical link between diet-induced obesity to tissue-specific glucocorticoid dysregulation. Further studies that examine the interaction between dietary fructose, inflammation and the initiation of glucocorticoid dysregulation in both liver and adipose are warranted. Examination of inflammatory cytokines in high sugar diets and glucocorticoid dysregulation seems to be a logical next step.

Overall, in these acute studies, we showed that fructose has a distinct acute impact on 11 $\beta$ -HSD-1 in adipose and liver. While glucose and sucrose did not affect 11 $\beta$ -HSD-1 mRNA levels in mesenteric adipose after 24h or 1 wk exposure, fructose significantly increased mean mRNA levels at both time points. All high sugar diets had an acute affect on hepatic 11 $\beta$ -HSD-1 and H6PDH within 1 wk of exposure to the experimental diets. These indicate that high sugar diets initiate glucocorticoid dysregulation in the absence of increased adiposity or obesity.

## Chapter 5. General Discussion

The average daily intakes of total energy and carbohydrate in the U.S. have increased by 18% and 41%, respectively, between 1978 and 2004 (Marriott et al 2009). Along with this rise in carbohydrate intake, fructose consumption also increased. Non-alcoholic beverages accounted for the largest portion of the increase derived from fructose. A 2007 review of the literature on HFCS concluded that fructose did not appear to contribute to overweight and obesity more than other energy sources (Forshee et al 2007). However, the documented increase in fructose consumption since the late 1970s when HFCS rapidly infiltrated our food supply in tandem with suspicions that fructose may play a role in obesity has fueled much interest. In the past year there has been a boom in the publication of research investigating the role of fructose in obesity. As an indication of this trend, a search of the PubMed electronic data base for journal papers using the key words “fructose” and “obesity” as the search criteria yields 13 papers for the year 2000 and 40 papers for the year 2009 (as of October 2009).

The wealth of intake data generated by major U.S. surveys has been central to identifying the intake habits of our population. It is important, however, to acknowledge the limitations that are inherent in this these data, including: differences in collection techniques, intake data that is based upon disappearance data (overestimation), and intake data that is based on self-reported diet records (underestimation). Average daily U.S. intake of added sugars by the top tertile and quintile of the population between 1994 and 1996 was estimated at 27% and 36%, respectively (Bowman 1999). The high end of this estimate is in line with our experiments in which approximately 35–50% of total daily calories were derived from added sugar. The estimated U.S. average allows for a subset

of the population who consumes a large amount of calories as sweetened beverages. An individual with a 2000 kcal/d intake level who drinks four 12 oz. cans of cola daily is consuming 25% of his/her daily intake as added sugar. The intake of calorically sweetened drinks does not reduce solid food intake by a comparable amount (Wolf et al 2007).

### **Intake and Body Weight**

In the 24h and 1 wk sugar solution-feeding studies we showed that fructose-fed (F16) rats consumed the same mean amount of calories per day but gained more weight in 1 wk than rats consuming sucrose- or glucose-sweetened solutions. In the 10 wk study, we showed that rats consuming ad libitum 16% or 32% sucrose solutions did not gain more weight than, but were fatter than control rats (London et al 2007). However, Kanarek and Orthen-Gambill showed that when given access to 32% glucose, sucrose or fructose solution, S-D rats fed sucrose had the highest caloric intake followed by fructose, and then glucose (1982). Under these conditions, the sucrose- and fructose-fed rats gained more weight in 50 days than did the control or glucose-fed groups. Yet another study reported increased adiposity in fructose- but not sucrose-fed mice after 10 wk access to 15% fructose or 10% sucrose solutions (Jürgens et al 2005). Results vary among these studies, and results are difficult to compare to our data due to differences in animal model, sugar solution concentrations, and/or intake patterns.

The primary goal of the 24h and 1 wk experiments was to examine the acute effects of different sugars on several parameters of glucocorticoid, lipid, and carbohydrate metabolism. Intake, body composition, and body weight data indicated similar trends to those previously reported in other high-sugar feeding studies. Free

access to sugar solution, water and standard chow induced obesity in S-D rats in 10 wk (Castonguay et al 1980). One important characteristic of this model is significantly increased body fat (London et al 2007). It should be noted that we did not assess body composition in the 24h and 1 wk studies based on the assumption that there would not be significant differences within those time frames. S-D rats appear to be a good animal model of diet-induced, and specifically, high sugar diet-induced obesity. 16% solutions of different sugars were selected for three main reasons: 1) Easy comparison to the literature, 2) the relatively consistent day-to-day intake observed in S-D rats when offered 16% sucrose solutions for 10 wk (see Appendix 4), and 3) the similarity in concentration to commonly consumed soft drinks and other sweetened beverages. The three different sugars were selected to investigate the differences in metabolic and endocrine response associated with high intake levels of the monosaccharides fructose and glucose, and their combination in equal part (sucrose). While we considered using HFCS, limited resources and its similar composition to sucrose guided the decision to use the chosen solutions.

The graphs and tables presented in this dissertation illustrate the consistency of measurements between the control groups of the 24h and 1wk studies. Measurements of plasma insulin and triglycerides, NADPH, as well as those of intake and body weight had low variability thereby enabling the detection of small differences. It should be noted that the ad libitum design of this study allowed the rats to self-select their diets. However, as the data show, the standard error for each of these intake parameters was small, with the exception of the S32 group in the 10 wk study. As previously noted, this was one of the contributing factors in our selection of 16% sugar solutions for the acute studies. We replicated the previously observed phenomenon that rats precisely control both energy

intake levels and macronutrient composition of their diet. This is observed among both the control group and the sugar-fed groups that maintained an elevated daily intake level. This tight “self-regulation” reduced the impact of our lack of control over diet that could presumably occur with such a study design.

Comparable to previous reports, rats given access to sugar solutions ate less food than did rats in the control group (Collier & Bolles, 1968a; Collier & Bolles, 1968b). Interestingly, in both 24h and 1 wk experiments, rats in the F16 group ate more solid food than did the rats in either S16 or G16 groups. The F16 group compensated for this deficit by consuming less sugar than did either the S16 or G16 group in the 24h study, and less than the S16 group did in the 1 wk study. These observations are consistent with Sclafani’s finding that rats accepted more sucrose than glucose and more glucose than fructose (1987). All sugar-fed groups had significantly higher total daily caloric intakes than their respective experimental controls, similar to previous reports that rats given ad libitum access to chow in addition to sugar solutions consumed 10 to 20% more total daily calories (Kanarek et al 1984, Ramirez 1987). In our experiments, groups with ad libitum to sugar solutions for 24h and 1 wk consumed 14.3% to 26.7% and 23.4% to 33.8% more, respectively, than control groups did. Rats composed a diet that varied from 35 to 56% of total calories from sugar solution, consistent with previous reports from our lab (Castonguay et al 1981, London et al 2007) and Kanarek & Orthen-Gambill (1982).

One difference among groups and between studies worth noting was in the macronutrient composition of the diet. Percent calories as sugar increased over time in all three groups (Fig. 39A). This phenomenon was also observed in the 10 wk sucrose-feeding study in which the 16% and 32% sucrose solution-fed groups consumed an

average of 61.2% and 71.0% of their daily caloric intake as sugar, respectively. The sugar-fed groups of the 1 wk study compensated for the increased sugar intake by consuming less solid food than their 24h counterparts (See Figs. 17A, 17B, 18A, 18B). As a result of their increased intake of sugar solution, the sugar-fed groups of the 1 wk study had slightly lower mean percentages of daily intake as protein and fat in comparison to the sugar-fed rats of the 24h study. Within each study, there were small differences in macronutrient intake levels among the treatment groups that resulted from the F16 group's lower sugar solution intake and higher solid food intake in comparison to the S16 and G16 groups (see Figs. 19D, 19E, 19F). Though significant, these differences in macronutrient intakes were small and ranged from 1.4% to 3.4 % for fat and protein. The F16 group had greater percentages of daily intake from fat and protein than S16 and G16 groups. Within groups, measurements of the percentages of total calories from different macronutrients were robust measures, as illustrated by the small standard error of means. Despite differences in caloric intake between the control and treatment groups, average daily weight gain did not differ among groups. These findings are similar to those of Sclafani & Mann (1987) and to those of our 10 wk study. After 1 wk, the cumulative weight gain of the F16 group was greater than that of the S16 and G16 groups, but not the control. This difference may be due in part to the higher protein content of the control and the F16 diets that enabled normal growth (Hill et al 1981). The higher mean weight gain of the F16 group may also be due in part to the metabolic and endocrine responses that were observed acutely only among F16 rats. In the long-term study, rats fed high sucrose diets for 67 days and then subjected to a 24h fast lost significantly less body weight than controls fed a standard diet which supports the

hypothesis of metabolic adaptation to a high sugar diet (London et al 2007). The most striking difference observed in the experiments was that tissue-specific changes in glucocorticoid metabolism appeared within 24h only in the fructose-fed group which we conclude to be a function of differences in the diets.

### **High Sugar Diet-Induced Glucocorticoid Dysregulation**

We initially hypothesized that 10 wk chronic feeding of high sucrose diets would result in obesity and tissue-specific glucocorticoid dysregulation in S-D rats as assessed by body composition and mRNA levels, respectively. Once we determined that 10 wk access to 16% or 32% sucrose solutions caused increased adiposity and initiated glucocorticoid dysregulation, we developed a new set of questions in order to investigate the initiation and promotion of glucocorticoid dysregulation in response to short-term high sugar diets using different types of sugar. We hypothesized that changes in 11 $\beta$ -HSD-1 and H6PDH mRNA and protein would not occur acutely in response to short-term access to sugar solutions. Instead we hypothesized that increased carbohydrate flux in hepatocytes and adipocytes would initially lead to elevated NADPH and perhaps enhanced activity of 11 $\beta$ -HSD-1. Further, we predicted that these early changes in response to high sugar diets could initiate some features of the glucocorticoid dysregulation that has been observed in established obesity. It was anticipated that measuring mRNA and protein of 11 $\beta$ -HSD-1 and H6PDH at two early time points, 24h and 1wk, could clarify whether altered message and/or expression of these enzymes occurs in response to a high sugar diet or as a secondary function of increased adiposity that occurs over time. We also hypothesized that there would be differences in NADPH

levels as a result of increased carbohydrate flux, and that acute increases in plasma TG would be specific to the groups fed fructose-containing solutions.

As anticipated in the 10 wk study, long-term high sucrose diets induced obesity in rats, increased 11 $\beta$ -HSD-1 and H6PDH mRNA in adipose, and suppressed hepatic 11 $\beta$ -HSD-1 mRNA. We also found increased H6PDH mRNA in liver which was not expected. This increase was puzzling considering that H6PDH action determines the set point for 11 $\beta$ -HSD-1 reductase activity (Bujalska et al 2004, Bánhegyi et al 2004).

After 24h, fructose increased 11 $\beta$ -HSD-1 in both liver and mesenteric adipose. This effect was not observed with the other high sugar diets. This finding of the acute effect of fructose on tissue-specific glucocorticoid metabolism is novel. We also observed that all high sugar diets suppressed 11 $\beta$ -HSD-1 mRNA and protein within 1 wk. This effect of high sugar diets on 11 $\beta$ -HSD-1 in the absence of obesity has not previously been reported.

The uncorrelated levels of protein and mRNA for 11 $\beta$ -HSD-1 that were observed in the short-term studies are not easily explained. At 24h hepatic 11 $\beta$ -HSD-1 mRNA and protein were positively correlated, but there was no significant correlation at 1 wk. Hepatic H6PDH mRNA and protein levels were not correlated at either 24h or 1wk. We did not, however, measure 11 $\beta$ -HSD-1 activity so it is possible that in liver, while mRNA of 11 $\beta$ -HSD-1 is suppressed, activity may have still been elevated in the presence of elevated H6PDH mRNA and protein. It is also possible that enhanced NADPH production serves to quench reactive oxidation species that occur due to fructose-induced hepatic stress. This scenario would support the hypothesis of a protective role for suppressed hepatic 11 $\beta$ -HSD-1 as well as the acute suppression of hepatic H6PDH

protein that we saw acutely. We found that at 1 wk, hepatic H6PDH protein was suppressed in the absence of changes in mRNA, yet after 10 wk of high sucrose feeding, rats were obese, and had elevated hepatic H6PDH mRNA. This elevation appears to be a function of longer-term developments including increased adiposity, reduced glucose sensitivity, and perhaps accumulation of fat in liver. More studies will be necessary to better understand the acute suppression in hepatic H6PDH protein that was observed in response to high sugar diets.

Correlation analyses of 11 $\beta$ -HSD-1 and H6PDH also provided insight into the sequential events in the initiation and maintenance of glucocorticoid dysregulation that was observed during both short- and long-term sugar solution access. While neither mRNA nor protein levels of 11 $\beta$ -HSD-1 and H6PDH were correlated in liver after 24h exposure to high sugar diets, 11 $\beta$ -HSD-1 and H6PDH protein levels were positively correlated in liver after 1 wk. Conversely, after 24h, 11 $\beta$ -HSD-1 and H6PDH mRNA in mesenteric adipose were not correlated, but were positively correlated after 1 wk. This suggests that dysregulation of the 11 $\beta$ -HSD-1/H6PDH system may occur very acutely (24h) in response to high sugar diets in liver and that equilibrium between 11 $\beta$ -HSD-1 and H6PDH is reestablished within 1 wk but at a much lower level of expression. In adipose it seems likely that dysregulation is also initiated acutely, sometime during the first wk of sugar solution access, and that the relationship between 11 $\beta$ -HSD-1 and H6PDH is reestablished eventually, but at a much higher expression level. We saw that after 10 wk, 11 $\beta$ -HSD-1 and H6PDH mRNA levels were both significantly elevated in adipose.

## **Transcriptional Regulation of 11 $\beta$ -HSD-1 under the Influence of High Sugar Diets**

We found that C/EBP- $\alpha$  was positively correlated with 11 $\beta$ -HSD-1, H6PDH and ACC mRNA in liver, and also with 11 $\beta$ -HSD-1 mRNA in adipose at 24h. The only correlation that remained after 1 wk was that of C/EBP- $\alpha$  and 11 $\beta$ -HSD-1 mRNA in adipose, and this positive correlation, while significant, was weak. Once acute glucocorticoid dysregulation is established, we no longer saw relationships between C/EBP- $\alpha$  and 11 $\beta$ -HSD-1, H6PDH, or ACC. We hypothesized that glucocorticoid dysregulation in liver was initiated in part by hepatic inflammatory response to high sugar intake, particularly high intake of fructose. The correlation data suggest that the high sugar diet-induced changes in mRNA levels of these genes were not mediated by C/EBP- $\alpha$ . Our finding is supported by the recent report that C/EBP- $\alpha$  regulates basal transcription of 11 $\beta$ -HSD-1 while C/EBP- $\beta$  regulates TNF- $\alpha$  stimulated upregulation of 11 $\beta$ -HSD-1 as well as basal transcription of the gene (Ignatova et al 2009). In human amnion fibroblasts C/EBP- $\alpha$  plays a role in feed forward induction of 11 $\beta$ -HSD-1 in response to cortisol stimulation, which was the original impetus to explore the role of C/EBP- $\alpha$  in glucocorticoid dysregulation. We hypothesized that a high sugar diet would initiate increased local glucocorticoid levels, and that C/EBP- $\alpha$  may participate in subsequent cortisol induced glucocorticoid dysregulation in adipose and liver. Instead, it may be that inflammation precedes cortisol excess, and that cortisol induced increases in 11 $\beta$ -HSD-1 occur later. The distinct fructose effects we observed in combination with previous findings about fructose and inflammation in liver suggest that inflammation may be a crucial link between high fructose diets and glucocorticoid dysregulation that promotes visceral adiposity.

## Conclusions

Long-term high sucrose diets increased 11 $\beta$ -HSD-1 mRNA in mesenteric adipose and suppressed hepatic 11 $\beta$ -HSD-1 mRNA similar to what has been observed in both human and rodent models of obesity. Free access to sugar-sweetened solutions, irrespective of sugar type, reduced food intake but not enough to compensate for the increased intake of sugar, a phenomenon similarly observed in humans. The increased adiposity and induced glucocorticoid dysregulation observed after long-term access to sucrose solutions was likely a result of increased total caloric intake as well as the direct metabolic and endocrine consequence of high intakes of sugar. This data provides direct evidence that consuming a significant portion of total caloric intake as added sugar, particularly as sweetened beverages can induce obesity, and indirect evidence that this pattern of intake might play a role in the current obesity epidemic.

The 24h and 1 wk studies establish the unique ability of fructose to induce acute changes in 11 $\beta$ -HSD-1 mRNA, ACC mRNA, and to significantly increase TG levels. Fructose induced increased 11 $\beta$ -HSD-1 mRNA in liver and mesenteric adipose within only 24h, while glucose and sucrose did not. Only fructose-containing sugar solutions increased plasma TG acutely, confirming a unique role for fructose in promoting acute and chronic dyslipidemia. High sugar diets, in general, can initiate hepatic glucocorticoid dysregulation within 1 wk, thereby providing novel evidence that diet can impair tissue-specific glucocorticoid regulation in the absence of phenotypic changes (i.e., excess adiposity). Taken as a whole, the current studies establish the ability of both short-term and chronic high sugar diets to induce glucocorticoid dysregulation similar to what has been reported in obesity. We conclude that high intakes of sweetened beverages cause

perturbations in carbohydrate, lipid, and glucocorticoid metabolism acutely, and these disturbances are likely compounded by excess adiposity, thereby further promoting obesity.

### **Future Directions**

The results from the three experiments discussed in this dissertation indicate links between high sugar diets, obesity, and glucocorticoid dysregulation. Based on our positive and negative findings, there are several experiments that would serve as logical follow-ups to this body of research. First, while we explored differences between sucrose and pure fructose, repeating the studies with a HFCS-55 group is important since it is currently the most prominent sweetener in processed foods and sweetened beverages. While the composition of HFCS approximates that of sucrose, the fructose to glucose ratio of HFCS-55 is 1.22 whereas the ratio for sucrose is 1.0.

One limitation to our acute study was the high degree of intra-group variability in several of the parameters measured. By administering a bolus of sugar and taking tissue samples at several exact time points following the bolus differences in sugar intake and feeding patterns among rats could be eliminated. It was suspected that much of the variability in parameters such as ACC mRNA and H6PDH mRNA was due to the transient nature of the regulation of these proteins in response to intake of sugar, and our lack of control over feeding patterns.

Another related experiment would be a microarray analysis following a sugar bolus and also at the end of a long-term high sugar feeding study to obtain a comprehensive panel of affected, as well as unaffected genes. This could also provide insight into the range of physiological effects of consuming high sugar diets.

One of the most compelling components to the hypothesized pathway for high sugar diet-induced glucocorticoid dysregulation is the possible involvement of acute and chronic inflammation. Fructose intake has been reported to induce increases in inflammatory cytokine release (Kawasaki et al 2009) and, the inflammatory cytokines, TNF- $\alpha$  and IL-1 $\beta$ , are known to upregulate 11 $\beta$ -HSD-1 (Escher et al 1997). Exploring the impact of high sugar diets on inflammatory markers could better explain the acute fructose effect we observed.

Although the S-D rat is a good model of diet-induced obesity, and tissue-specific glucocorticoid metabolism in rats is easily compared to that in humans, a clinical trial is needed in order to extend these results to humans. While obtaining omental fat and liver samples is difficult, obtaining these samples from obese subjects undergoing bariatric surgery would be a good solution to this methodological issue. A long-term high sugar study might not be possible under these conditions, but an acute experiment examining the effects of different sugar-sweetened beverages might be possible.

There is much interest in 11 $\beta$ -HSD-1 as a target for pharmacological treatment of obesity and diabetes. While the combined effects of an obesogenic (high fat, high sucrose diet) and treatment with a specific 11 $\beta$ -HSD-1 inhibitor have been examined in rats (Berthiaume et al 2007), a similar study with high sugar diets in both obese and lean animals would provide valuable information about the 11 $\beta$ -HSD-1 mediated effects of high sugar diets. Specific, small molecule 11 $\beta$ -HSD-1 inhibitors that do not alter food intake have been synthesized within the past few years that would enable such studies (Gu et al 2005, Richards et al 2006). Clearly there is much room for research in the area of high sugar diets and diet-induced glucocorticoid dysregulation. The exciting results of

both the 10 wk and short-term studies warrant further investigation into how high sugar diets, and fructose specifically, can impact glucocorticoid dysregulation and energy balance.

## Appendices

**Appendix 1.** Approval letter from the IACUC of the University of Maryland, College Park for the 10 wk sugar solution feeding study in rats.

	<b>UNIVERSITY OF MARYLAND</b>
GRADUATE STUDIES AND RESEARCH <i>Institutional Animal Care &amp; Use Committee</i>	
<b>James M Dietz</b>	Jmdietz@umd.edu phone: (301) 405-6949
March 26, 2007	
Dr. Thom Castonguay Nutrition & Food Science Marie Mount Hall CAMPUS	
Dear Dr. Castonguay:	
This letter is to inform you that on <b>March 15, 2007</b> , the members of the Institutional Animal Care & Use committee (IACUC) reviewed your addendum to your IACUC approved protocol:	
<b>Assessment of Whey Low's Contribution to Diet Energy &amp; Its Effects on Growth &amp; Body Composition in Lab Rats</b>	
<b>R-06-42</b>	
At that time, the IACUC decided to have your addendum reviewed by designated reviewers. Those individuals have given your addendum approval as presented. The approval date is <b>March 26, 2007</b> .	
Please be advised that addendum approval does not change the period of approval for this protocol. Thus, this protocol is valid until <b>August 2, 2009</b> .	
Sincerely,	
James M. Dietz Professor, Biology Chair, IACUC	

**Appendix 2.** Approval letter from IACUC of University of Maryland, College Park for the Addendum to original protocol # R-06-42

 **UNIVERSITY OF MARYLAND**  
GRADUATE STUDIES AND RESEARCH  
*Institutional Animal Care & Use Committee*

**James M Dietz**

Jmdietz@umd.edu  
phone: (301) 405-6949

March 26, 2007

Dr. Thom Castonguay  
Nutrition & Food Science  
Marie Mount Hall  
CAMPUS

Dear Dr. Castonguay:

This letter is to inform you that on **March 15, 2007**, the members of the Institutional Animal Care & Use committee (IACUC) reviewed your addendum to your IACUC approved protocol:

**Assessment of Whey Low's Contribution to Diet Energy & Its Effects  
on Growth & Body Composition in Lab Rats**

**R-06-42**

At that time, the IACUC decided to have your addendum reviewed by designated reviewers. Those individuals have given your addendum approval as presented. The approval date is **March 26, 2007**.

Please be advised that addendum approval does not change the period of approval for this protocol. Thus, this protocol is valid until **August 2, 2009**.

Sincerely,



James M. Dietz  
Professor, Biology  
Chair, IACUC

**Appendix 3.** Approval letter from IACUC of the University of Maryland, College Park for the protocol for acute (24h and 1 wk) sugar solution feeding experiments in rats.



UNIVERSITY OF  
MARYLAND

GRADUATE STUDIES AND RESEARCH  
*Institutional Animal Care & Use Committee*

James M Dietz

Jmdietz@umd.edu  
phone: (301) 405-6949

January 7, 2008

Dr. Thomas Castonguay  
Nutrition & Food Science  
Marie Mount Hall  
CAMPUS

Dear Dr. Castonguay:

This letter is to inform you that the members of the Institutional Animal Care & Use Committee (IACUC), at their **December 20, 2007** meeting, reviewed your protocol:

**High Sucrose & Fructose Diets & Glucocorticoid Dysregulation in Rats**

**R-07-96**

At that time the Committee requested additional information before your protocol could receive approval. You have now provided this office with that information. The approval date is **January 7, 2008**.

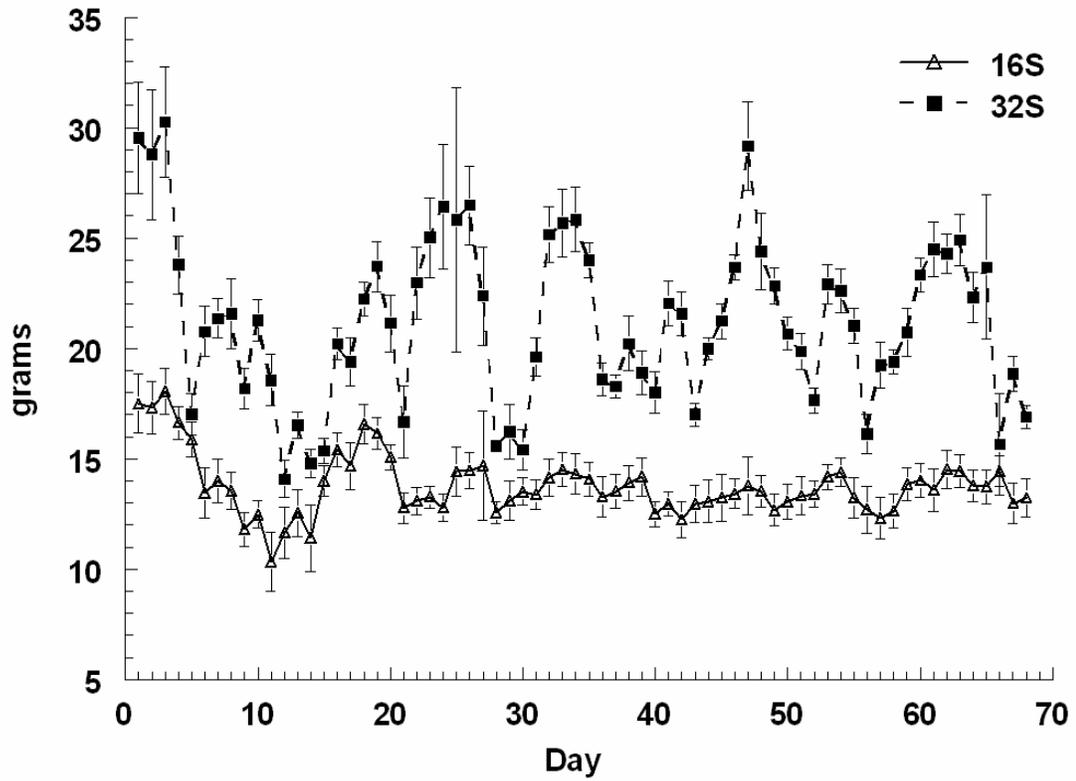
Please note that an approved protocol is valid for three (3) years unless there is a change in the protocol. Thus, this protocol is valid until **January 7, 2011**. Moreover, federal laws indicate that protocols must be reviewed yearly. Thus, in order to keep your approved protocol active you **MUST** submit a protocol renewal/update by the first of the month of the anniversary of your approval (**January 2009 & January 2010**). All subsequent work extending beyond the approval date of the protocol must be submitted to the IACUC as a new protocol.

Sincerely,

James M. Dietz  
Chair, IACUC  
Professor, Biology

A handwritten signature in black ink, appearing to read 'J. Dietz', written over the typed name and title.

**Appendix 4.** Mean daily sucrose intake (g) from either 16% or 32% sucrose solutions by rats in the S16 and S32 groups, respectively, in the long-term (10 wk) sucrose-feeding pilot study.



**Appendix 5.** Correlation analyses data for all rats in the control, S16, F16, and G16 groups in the 24h experiment; P: Pearson correlation, S: Spearman correlation.

Variable 1	Variable 2	Correlation Coefficient (r)	P value
Liver 11 $\beta$ -HSD-1 mRNA	Liver 11 $\beta$ -HSD-1 protein	0.444 (P)	0.02
Liver H6PDH mRNA	Liver ACC mRNA	0.350 (S)	0.05
	Liver [NADPH]	-0.510 (P)	0.02
Liver C/EBP- $\alpha$ mRNA	Liver 11 $\beta$ -HSD-1 mRNA	0.483 (S)	0.006
	Liver H6PDH mRNA	0.523 (S)	0.003
	Liver ACC mRNA	0.348 (S)	0.05
	Liver NADPH	-0.626 (S)	0.002
	Plasma TG	-0.385(S)	0.04
Liver ACC mRNA	% kcal as fructose	0.374 (S)	0.04
	% kcal as sugar	0.350 (S)	0.05
Liver NADPH	% kcal as sugar	0.411 (P)	0.05
	% kcal as CHO	0.436 (P)	0,04
	% kcal as protein	-0.436 (P)	0.04
Percent kcal from fructose	Liver 11 $\beta$ -HSD-1 mRNA	0.389 (P)	0.03
	Liver 11 $\beta$ -HSD-1 protein	0.384 (P)	0.04
	Adipose 11 $\beta$ -HSD-1 mRNA	0.404 (P)	0.03
	Liver ACC mRNA	0.374 (S)	0.04
	Plasma TG	0.629 (P)	0.0002
Percent kcal from sugar	Plasma TG	0.417 (P)	0.02
Adipose 11 $\beta$ -HSD-1 mRNA	Adipose H6PDH mRNA	0.634 (S)	0.002
	Liver 11 $\beta$ -HSD-1 protein	0.468 (P)	0.02
	Liver H6PDH mRNA	0.379 (S)	0.05
Adipose H6PDH mRNA	Liver H6PDH mRNA	0.683 (S)	0.005
	Liver C/EBP- $\alpha$ mRNA	0.438 (S)	0.04
	Liver NADPH	-0.544 (S)	0.02
	% kcal as fructose	-0.488 (S)	0.02
Adipose C/EBP- $\alpha$ mRNA	Adipose 11 $\beta$ -HSD-1 mRNA	0.471 (S)	0.06
	Adipose NADPH	0.487 (S)	0.02
	Liver 11 $\beta$ -HSD-1 protein	-0.842 (S)	.0002
	Liver H6PDH protein	-0.983 (S)	<0.0001

**Appendix 6.** Correlation analyses data for all rats in control, S16, F16, and G16 groups in the 1 wk experiment: P: Pearson correlation, S: Spearman correlation.

Variable 1	Variable 2	Correlation Coefficient (r)	P value
Liver 11 $\beta$ -HSD1 mRNA	Plasma insulin	-0.394 (P)	0.04
	Plasma TG	-0.526 (P)	0.005
	Percent kcal from sugar	-0.511 (P)	0.006
	Percent kcal from CHO	-0.511(P)	0.006
Liver HSD1 protein	Liver H6PDH	0.425 (S)	0.02
Liver H6PDH mRNA	Liver ACC mRNA	0.395 (S)	0.03
	Adipose ACC mRNA	0.483 (S)	0.05
Liver ACC mRNA	Percent kcal from sugar	0.399 (S)	0.03
	Percent kcal from CHO	0.399 (S)	0.03
Liver C/EBP- $\alpha$ mRNA	Adipose H6PDH mRNA	0.542 (P)	0.005
Liver H6PDH protein	Liver ACC mRNA	-0.429 (S)	0.02
	Liver [NADPH]	-0.400 (S)	0.03
	Adipose ACC mRNA	0.483 (S)	0.05
	Adipose total [NADPH]	-0.539 (P)	0.005
	Percent kcal from fructose	-0.382 (P)	0.04
	Percent kcal from CHO	-0.563 (P)	0/001
Liver total [NADPH]	Adipose C/EBP- $\alpha$ message	0.563 (P)	0.02
	Adipose total [NADPH]	0.420 (P)	0.03
	Percent kcal from CHO	0.356 (P)	0.05
	Cumulative weight gain	-0.447 (P)	0.01
Adipose C/EBP- $\alpha$ mRNA	Adipose 11 $\beta$ -HSD-1 mRNA	0.740 (P)	0.002
	Plasma CORT	0.509 (S)	0.04
Adipose NADPH	% kcal from fructose	0.561 (P)	0.002
Plasma CORT	Liver 11 $\beta$ -HSD-1 protein	0.5211 (S)	0.006
	Adipose 11 $\beta$ -HSD-1 mRNA	0.433(P)	0.01
	Plasma insulin	-0.373 (S)	0.04
	Cumulative weight gain	-0.415 (S)	0.02
Plasma Insulin	Cumulative weight gain	0.440 (P)	0.01
	Plasma TG	0.517 (P)	0.003
Plasma TG	Liver 11 $\beta$ -HSD-1 mRNA	0.447 (P)	0.01
	Liver H6PDH protein	-0.365 (P)	0.05
	Adipose 11 $\beta$ -HSD-1 mRNA	0.446 (P)	0.02
	Percent kcal as fructose	0.698 (P)	<0.0001
	Percent kcal as sugar	0.492 (P)	0.005
	Percent kcal as CHO	0.492 (P)	0.02

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## Curriculum Vitae

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### **EDUCATION**

- University of Maryland, College Park, MD  
PhD program in Nutrition (GPA: 3.72) Expected completion date: Fall 2009  
Dissertation: High sucrose, fructose, and glucose diets and glucocorticoid dysregulation in rats
- University of Maryland, College Park, MD  
MS, Nutrition (GPA: 3.58) Spring 2006
- University of Maryland, College Park, MD  
BS, Journalism December 1993

### **RESEARCH EXPERIENCE**

- Graduate Research Asst, Dept of Nutrition, UMDCP  
Supervisor: Thomas W. Castonguay, PhD January-December 2006
- Investigated changes in body composition, 11- $\beta$ -hydroxysteroid-dehydrogenase-1 (11- $\beta$ -HSD-1) and other metabolic markers in response to high-sugar diets.
  - Laboratory techniques: Set-up of animal facility, daily animal care and data acquisition, supervision of technicians for rat feeding studies; culture of 3T3-L1 and GH4C1 cells; transfection of cultured cells with siRNA fragments to knockdown 11- $\beta$ -HSD-1 gene; RNA extraction from tissue and cell samples; RNA purification; qualitative and quantification evaluation of RNA using spectro-photometry; cDNA creation; primer design for real-time PCR (qRT-PCR) amplification using BLAST engine; optimization of qRT-PCR reactions for various primers; qRT-PCR to quantify changes in gene message under different conditions (siRNA transfection, diet, body composition); measurement of 11- $\beta$ -HSD-1 enzyme activity and directionality with  $^3$ H-corticosterone and cortisol reactions in tissue and cell homogenates; quantification of radiolabeled samples [high performance liquid chromatography (HPLC)]; agarose gel electrophoresis; protein extraction from tissue and quantitative protein assays; Western blot; radioimmunoassay (insulin, leptin); plasma glucose quantification; NADPH assay using 96-well plate reader.
- Laboratory Research Asst, Dept of Chemistry & Biochemistry, UMDCP  
Supervisor: Frederick Khachik, PhD February 2004–March 2006
- Research goal was to determine deposition of lutein, zeaxanthin, and their metabolites in plasma, ocular tissues and other major organs. Project involved qualitative and quantitative carotenoid analyses of non-human primate tissues and serum before, during, and after dietary supplementation with high doses of lutein, zeaxanthin and their combination. [NIH grant No. 1RO1EY1265801.]
  - Laboratory techniques: carotenoid extraction from human and non-human primate serum,

ocular and other tissue samples; purification and preparation of samples for HPLC analysis; analysis of extracts quantitatively and qualitatively using HPLC; adjustment, calibration, and maintenance of HPLC systems (normal, reverse, and chiral columns); mass spectrophotometry determination of purity of standards; raw data compilation into tables and graphs; maintenance of chemical and supply inventory; and cataloging of all extracts and samples.

## **TEACHING EXPERIENCE**

Instructor, Nutrition Dept, UMCP  
Fall 2008

- Instructor/Lecturer for NFSC498B: *Dieting and the Science of Weight Loss*.
- Prepared lecture slides based on current literature for two weekly 1.25 hour lectures; prepared exams, assignments; provided individual help for students; graded exams and assignments.

Graduate Teaching Asst, Nutrition Dept, UMCP 2004–2005, 2007–2009

- **NFSC 100: *Introduction to Nutrition***: led three 50 minute weekly discussion sections; assisted with writing and proofreading exams; graded exams, projects, and homework assignments; held regular office hours.
- **NFSC498B: *Dieting and the Science of Weight Loss***: assisted with preparation and grading of exams and assignments; held regular office hours.
- **NFSC250: *The Science of Food***: prepared, instructed, and supervised the lab portion of course; graded weekly lab reports and exams.

Graduate Teaching Asst, Chemistry & Biochemistry Dept, UMCP

Fall 2005–Spring 2006

- General Chemistry I laboratory TA: prepared weekly pre-lab lectures, demonstrated techniques, supervised laboratory exercises; held office hours; graded lab reports and examinations.
- Organic Chemistry II laboratory TA: prepared weekly pre-lab lectures, demonstrations and quizzes; supervised lab exercises; held weekly office hours; graded reports, quizzes, exams.
- Biochemistry I, Biochemistry II, and general biochemistry for non-majors: grader and lecture TA: graded homework assignments, projects, and exams; held regular office hours.

## **AWARDS**

- Jacob K. Goldhaber Travel Award: Fall 2009, University of Maryland, College Park.
- Ann G. Wylie Dissertation Fellowship, Fall 2009, University of Maryland, College Park: Merit-based award of \$10,000 to assist in completion of PhD dissertation.
- Bioscience Day 2008, Poster Contest, University of Maryland, College Park: Winner for the College of Agriculture and Natural Resources.
- Graduate Student Summer Research Fellowship, 2008, University of Maryland, College Park: Merit-based award of \$5,000 stipend to assist in completion of dissertation research.
- Graduate Research Interaction Day 2008, Poster Presentation, University of Maryland, College Park: Third place winner, Health category.
- Bioscience Day 2007, Poster Contest, University of Maryland, College Park: Winner for the College of Agriculture and Natural Resources.

## PUBLICATIONS

**London E**, Castonguay TW. Diet and the role of 11 $\beta$ -hydroxysteroid dehydrogenase-1 on obesity. *J Nutr Biochem*. 20: 485–493, 2009.

**London E**, Lala G, Berger R, Panzenbeck A, Kohli AA, Renner M, Jackson A, Raynor T, Loya K, Castonguay TW. Sucrose access differentially modifies 11 $\beta$ -hydroxysteroid dehydrogenase-1 and hexose-6-phosphate dehydrogenase message in liver and adipose tissue in rats. *J Nutr*. 137: 2616–2621 (2007).

**London E**. Chronic Ingestion of Lutein (3R,3'R,6'R)-Lutein and (3R,3'R)-Zeaxanthin in Rhesus Macaque Primates. Master's Thesis. University of Maryland, College Park, 2006.

Khachik F, **London E**, de Moura F, Johnson M, Steidl S, Detolla L, Shipley S, Sanchez R, Chen X-Q, Flaws J, Luty G, McLeod S, Fowler B. Chronic Ingestion of Lutein (3R,3'R,6'R)-Lutein and (3R,3'R)-Zeaxanthin in Rhesus Macaque Primates. *Invest Ophthalmol Vis Sci*. 47: 5476–5486 (2006).

## ABSTRACTS

**London E**, Castonguay TW. High-sucrose, fructose, and glucose diets and glucocorticoid dysregulation in rats. The Obesity Society (2009).

**London E**, Castonguay TW. Effects of 24h access to sugar solutions on 11 $\beta$ -hydroxysteroid dehydrogenase-1 and hexose-6-phosphate dehydrogenase. Graduate Research Interaction Day, University of Maryland, College Park (2009).

Jiang B, **London E**, Castonguay TW. Differential effects of glucose, sucrose and fructose on hypothalamic neuropeptide expression. Neuroscience and Cognitive Science (NACS)-Fest, University of Maryland, College Park, poster session (2009).

**London E**, Castonguay TW. Effects of 24h access to sugar solutions on 11 $\beta$ -hydroxysteroid dehydrogenase-1 and hexose-6-phosphate dehydrogenase. Neuroscience 2008, Washington, DC, poster session.

**London E**, Castonguay TW. Effects of 24h and 1 week access to sugar solutions on 11 $\beta$ -hydroxysteroid dehydrogenase and hexose-6-phosphate dehydrogenase. Bioscience Day, University of Maryland, College Park, poster (2008).

**London E**, Kohli A, Renner M, Leger J, Castonguay TW. Effects of acute access to sucrose, fructose and glucose solutions on hepatic 11 $\beta$ -hydroxysteroid dehydrogenase-1 and hexose-6-phosphate dehydrogenase message in rats. Graduate Research Interaction Day, University of Maryland, College Park (2008).

**London E**, Lala G, Berger R, Panzenbeck A, Kohli A, Renner M, Jackson A, Raynor T, Loya K, Castonguay TW. Sucrose access and 11 $\beta$ -hydroxysteroid dehydrogenase-1 message in liver and adipose tissue in rats. Bioscience Day, University of Maryland, College Park, poster (2007).

**London E**, Lala G, Berger R, Kohli A, Renner M, Jackson A, Rayner T, Castonguay TW. Sucrose access differentially modifies 11 $\beta$ -hydroxysteroid dehydrogenase-1 and hexose-6-phosphate dehydrogenase message in liver and adipose tissue in rats. Neuroscience 2007, San Diego, CA, poster session.

**London E**, Lala G, Berger R, Panzenbeck A, Kohli A, Renner M, Jackson A, Raynor T, Loya K, Castonguay TW. Sucrose access and 11 $\beta$ -hydroxysteroid dehydrogenase-1 message in liver and adipose tissue in rats. Society for the Study of Ingestive Behaviors, poster (2007).

**London E**, Jackson A, Panzenbeck A, Castonguay TW. RNAi knockdown of 11 $\beta$ -hydroxysteroid dehydrogenase-1 in GH4C1 rat pituitary cells. Annual Nutrition Department Poster Competition, University of Maryland, College Park, poster (2007).

**London E**, Jackson A, Panzenbeck A, Castonguay TW. RNAi knockdown of 11 $\beta$ -hydroxysteroid dehydrogenase-1 in GH4C1 rat pituitary cells. Experimental Biology, poster (2007).

Parsons EZ, **London E**, Moore J, Mengesha D, Castonguay TW. 11 $\beta$ -hydroxysteroid dehydrogenase-1 and obesity. Society for the Study of Ingestive Behaviors, poster and presentation (2006).

Castonguay TW, **London E**, Parsons E, Mengesha D, Jackson A. Glucocorticoids and different models of obesity. Society for Neuroscience, poster (2006).

**London E**, Khachik F, de Moura F, Johnson M, Steidl S, Detolla L, Shipley S, Sanchez R, Chen X-Q, Flaws J, Luttly G, McLeod S, Fowler B. Chronic ingestion of (3R,3'R,6'R)-lutein and (3R,3'R)-zeaxanthin in female rhesus macaque primates. Annual Nutrition Department Poster Competition, University of Maryland, College Park, poster (2006).

**London E**, Castonguay TW, Parsons E, Mengesha D, Jackson A. 11- $\beta$ -hydroxysteroid dehydrogenase-1 and obesity. 10th Annual Shorb Lecture and Symposium, University of Maryland, College Park, poster (2006).

Khachik F, **London E**, de Moura F, Johnson M, Steidl S, Detolla L, Shipley S, Sanchez R, Chen X-Q, Flaws J, Luttly G, McLeod S, Fowler B. Chronic ingestion of (3R,3'R,6'R)-lutein and (3R,3'R)-zeaxanthin in female rhesus macaque primates. International Carotenoid Society, 14th International Symposium on Carotenoid, Edinburgh, Scotland, poster and presentation (2005).

## ADMINISTRATIVE AND OTHER PROFESSIONAL EXPERIENCE

**Publications Production Asst & Production Editor, American Society of International Law (ASIL)**

**Supervisor: Charlotte Ku, ASIL Executive Director**

**September 2000–January 2004**

- Production editor for annual *ASIL Proceedings*, and ASIL monograph series. Facilitated publication process from manuscript submission through printing. *Proceedings* involved working with approximately 100 contributing authors.
- Production and managerial duties: general correspondence with authors, editors, printers, and freelancers; creating and implementing production schedules; conversion of all manuscripts; developing book templates; editing for style consistency; proofreading; typesetting and formatting all text; coordinating advertising; hiring freelancers; preparing files for printer; managing printer contracts and offprint orders. ASIL style follows *The Chicago Manual of Style* and *The Bluebook: A Uniform System of Citation*.
- Managed requests to reprint/translate ASIL publications; assisted in development of permissions policies, i.e., Internet. Obtained copyrights through U.S. Copyright Office.

**Public Relations Intern/Project Assistant, Hager Sharp Inc, Washington DC**

**Supervisor: Rachel Greenberg**

**October 1996–June 1997**

- Compiled/maintained databases using MS Access; created charts, presentations and graphics using MS Office and Adobe Photoshop for National Diabetes Education and Outreach Program.

- Maintained daily log of all project-related correspondence, charted editorial calendars from national magazines and tracked publications for national breast cancer awareness campaign
- Produced *Diabetes Educational Packets* for high-risk population and *Foot Kits* for diabetics.

### VOLUNTEER EXPERIENCE

Guest lecturer at Hardee Middle School, Washington, DC: June 2006

- Gave one-hour lecture on obesity epidemic, reading nutrition labels and making healthy choices.
- Lecture was tailored specifically to the needs of the audience: inner-city eighth-grade females.

American Diabetes Association (Washington, DC chapter): Walk for Diabetes October, 2005

- Provided event support for event intended to raise diabetes awareness and improve education.
- Food and Nutrition Graduate Students (FANGS) Organization Spring 2005
- Assisted with organization, set-up and clean-up for annual poster event and FANGS meetings.

### PROFESSIONAL AFFILIATIONS

American Society for Nutrition, 2006–present; Society for the Study of Ingestive Behaviors, 2006–present; The Obesity Society, 2008–present; Society for Neuroscience, 2008–present.