ABSTRACT

Title of Thesis: VARIATIONS IN HEPATIC 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 1 MESSAGE IN TWO RAT MODELS OF OBESITY

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Obesity is an epidemic that has been estimated to cost the United States over $117 billion every year. Glucocorticoids have long been implicated in the maintenance of energy homeostasis due to their involvement with the hypothalamic-pituitary-adrenal axis. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) is an enzyme that interconverts the glucocorticoids cortisol and cortisone, and their rat counterparts corticosterone and 11-dehydrocorticosterone. We predicted an association between hepatic levels of this enzyme and either genetic obesity or dietary-induced obesity from any of two fat levels (high fat, low fat) or three fat sources (saturated, polyunsaturated, or monounsaturated). Our results indicated that hepatic 11β-HSD-1 is downregulated in genetic obesity, as has been previously found. High fat diets also caused reductions in hepatic enzyme message, although these were not statistically significant. There did not appear to be any effect of fat type.

VARIATIONS IN DIETARY FAT AND THEIR EFFECTS ON HEPATIC 11BETA-HSD-1 MESSAGE IN TWO RAT MODELS OF OBESITY

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LITERATURE REVIEW

OBESITY

The topic of obesity is such a daunting subject that it is difficult to even pick a direction from which to approach it. Even as you narrow the discussion down to scientific methods of investigation, it expands again as the details inherent in research fields insert their own complications. Consequently, this review will not attempt to address the entire subject, all the associated discoveries made in the history of the field, nor every current theory available in the literature today. Instead, it will be limited to the sub-topics relevant to the experiment presented - glucocorticoids, most specifically corticosterone, 11-β-hydroxysteriod dehydrogenase, the history of study in this field, as well as interactions with the hormone leptin and the rodent models appropriate for examination of these.

The Epidemic

The United States is in the midst of an obesity epidemic, with an estimated 65% of our population overweight or obese. In the past 20 years the incidence of overweight adults has increased by 40%, and the incidence of obese adults by 110% (Hedley, Ogden et al. 2004) (Flegal, Carroll et al. 1998). Even more disturbing, this trend is not limited to adults. The number of overweight children in the US has doubled in the same period, and the number of obese children has tripled (USDHHS 2001). Nor is the United States the only country experiencing this phenomenon. Worldwide, an estimated 315 million people are now defined as obese (James 2004).

Causes

Factors contributing to obesity are varied and overlapping. The steep slope of the current epidemic seems to indicate that the cause in most cases is environmental and behavioral, rather than biological or genetic. Prevalence of obesity has not only an inverse relationship with socioeconomic status/education level, but a direct one with a country's gross national product (Pena and Bacallo 2000) (Bray and Popkin 1998). The result is that poor people in rich countries are becoming fat. This seems to stem not only from increased availability of cheap, fatty foods such as fast food, but also from a lower energy requirement for work and daily living due to advances in technology, mechanization, and transportation. Even entertainment choices contribute, with television watching being associated with increased adolescent onset of obesity leading to lower remission rates and higher adult incidence (Gortmaker, Dietz et al. 1990). In 1996 the US Department of Health and Human Services reported that 60% of the US adult population does no regular physical activity, 25% does no physical activity at all, and almost 50% of young adults between the ages of 12 and 21 are essentially sedentary (USDHHS 2001).

Comorbidities

The consequences of overweight and obesity are even more complex and interrelated than the causes. In 1990 Murray and Lopez claimed that 4% of total disability and life years lost worldwide were attributable to obesity (Murray and Lopez 1994). In the US alone, 300,000 deaths per year can be traced to obesity, making it the country's second leading cause of preventable death (Allison, Fontaine et al. 1999). The most commonly increased disease risk factor is Type 2 diabetes mellitus, with over 80%

of cases caused by low activity levels and increased BMI (Stein and Colditz 2004). Counterintuitively, almost all antidiabetic medications are associated with further weight gain. Up to 39% of various types of cancer are caused by obesity, including breast, colon, and renal cancers (International Agency for Research on Cancer 2002). Dyslipidemia is another common effect, thought to result from increased insulin resistance along with excess hepatic triglyceride synthesis from an overlarge free fatty acid pool (Lewis, Carpentier et al. 2002). This often leads to hypertension, although the condition can also stem from physical pressure on the kidneys, activation of the sympathetic nervous system, and excess sodium reabsorption (Hall, Brands et al. 1993). In all, obesity can increase the risk of cardiovascular disease by 100% (Hubert, Feinleib et al. 1983). Other diseases with increased prevalence in obesity include sleep-disordered breathing, fatty liver, polycystic ovary syndrome, degenerative joint diseases, stroke, gallstones, and more. It has been estimated that obesity costs the United States over $117 billion every year, a figure which does not include complications of merely overweight individuals (USDHHS 2001). Clearly, this is an epidemic worth preventing.

In light of this, it is unsurprising that the search for causes, treatments, and cures for obesity has not only a long history but a thriving current place in the realm of scientific investigation. Behavioral modification, genetic manipulation, and even surgical alteration have been explored and exploited. The quest to understand the mechanisms involved in human obesity, to model them appropriately in rodents and to tease out the roles of various genes, hormones, and exogenous influences, has been complex and far-reaching. This review highlights a few of the paths that have been explored.

LEPTIN

Overview

Leptin is a hormone secreted mainly from adipose cells. In rodent models it is released in direct proportion to fat mass (Considine, Sinha, et al. 1996) and causes a decrease in food intake (Schwartz, Peskind et al. 1996). Circulating leptin crosses the blood-brain barrier (also in proportion to fat mass) where receptors in the arcuate nucleus of the hypothalamus interpret its concentration and alter downstream signaling pathways. For example, some hypothalamic neurons with leptin receptors downregulate orexigenic neuropeptides (Figure 1a) such as agouti-related protein (AGRP) (Shutter, Graham et al. 1997), (Breen, Conwell et al. 2004), neuropeptide Y (NPY) (Schwartz, Seeley et al. 1996), and β-endorphins (Appleyard, Hayward et al. 2003). AGRP and NPY stimulate the paraventricular nucleus of the hypothalamus (PVN) to signal the nucleus tractus solitarius (NTS) in the brainstem, which can inhibit satiety signals such as cholecystokinin (CCK). β-endorphins are opiates released in response to food intake and as such have long been associated with obesity, however some research indicates that they can also have an inhibitory effect on food intake and therefore may play a more complex, regulatory roll than previously believed (Appleyard, Hayward et al. 2003).

Other leptin receptors on proopiomelanocortin (POMC) neurons upregulate downstream anorexigenic peptides (Figure 1b), such as α-melanocyte-stimulating hormone (α-MSH) (Cheung, Clifton et al. 1997) and corticotrophin-releasing factor (CRF) (Rothwell, Hardwick et al. 1991) (Rothwell 1990). α–MSH inhibits centrally mediated feeding activity by stimulating melanocortin-4 receptors, while CRF leaves the brain to act on the pituitary as part of the hypothalamic-pituitary-adrenal (HPA) axis.

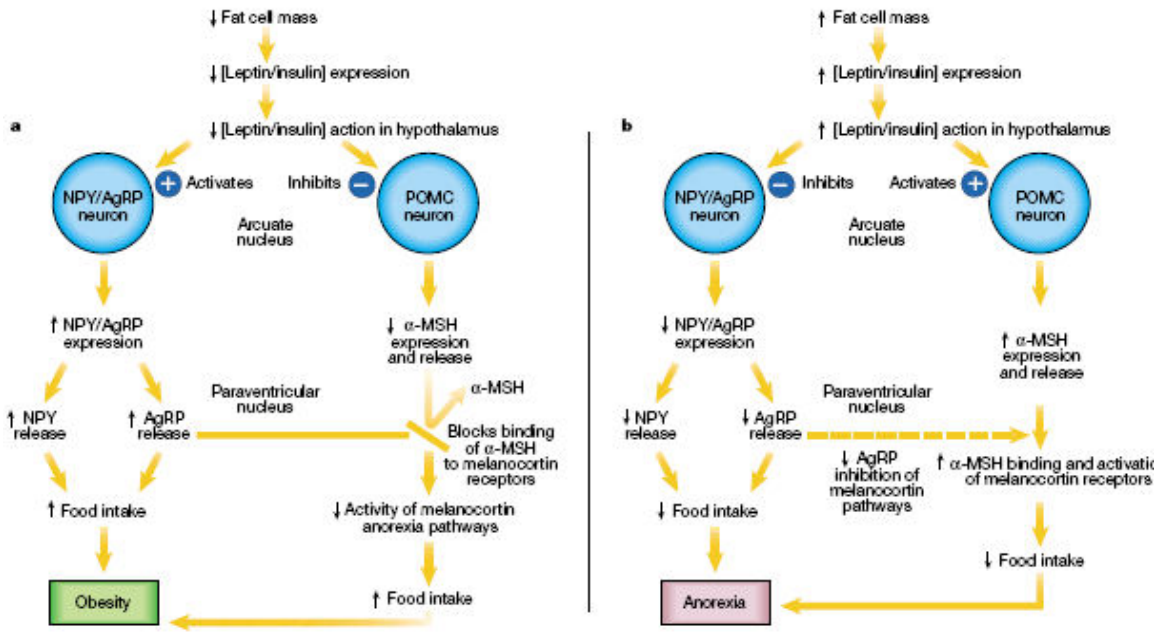


Figure 1 – Leptin pathways in the arcuate nucleus of the hypothalamus (Schwartz, *et al*. "Central nervous system control of food intake" 2000)

There are actually 5 isoforms of the leptin receptor (Zhang, Chen et al. 2005). They all have identical extracellular and transmembrane structures, but differ in the length of their cytoplasmic domains. The second isoform, LRb, has the longest intracellular structure and functions to mediate intracellular signaling (Munzberg and Meyers 2005). The receptor itself has no enzymatic activity but instead forms homodimers capable of activating Jak2, a tyrosine kinase with phosphorylation capabilities (Figure 2).

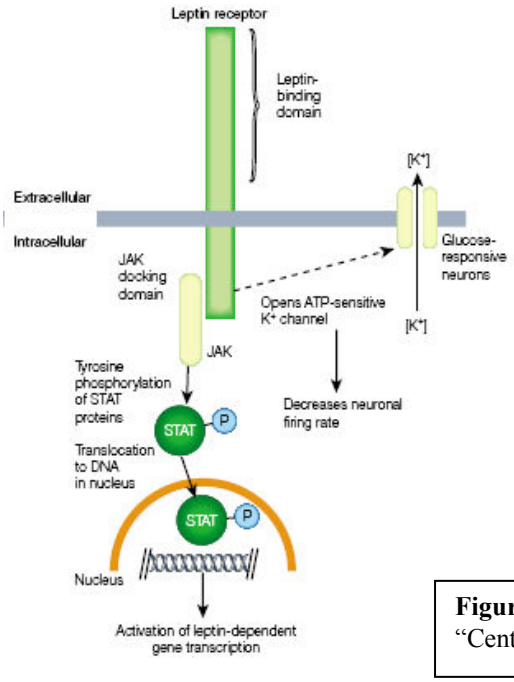


Figure 2 – The leptin receptor. (Schwartz, *et al*. "Central nervous system control of food intake" 2000)

It should also be mentioned that there are a few extra-adipose tissues that secrete leptin as well. Skeletal muscle (Wang, Liu et al. 1998) and the placenta (Masuzaki, Ogawa et al. 1997) have both been identified as sites of leptin synthesis, possibly as a mechanism to increase utilization of available nutrients. Additionally, it is now estimated that up to 25% of circulating leptin originates in the stomach (Bado, Levasseur et al. 1998) (Cinti, Matteis et al. 2000) (Cinti, De Matteis et al. 2000) (Sobhani, Bado et al. 2000). It has been proposed that leptin secretion by gastric epithelial cells is part of a short-term satiety mechanism, possibly through interaction with CCK.

Rodent Models of Obesity

Three of the major rodent models utilized in the study of genetic leptin-related obesity are *ob/ob* mice, *db/db* mice, and *fa/fa* rats. An *ob/ob* mouse, more formally named as Lep^{ob}/Lep^{ob}, has a spontaneous single-base substitution on its fourth chromosome that causes leptin synthesis to be prematurely terminated (Zhang, Proenca et al. 1994). The prevention of mature leptin secretion results in insulin resistance, severe early-onset obesity, reduced energy expenditure, decreased linear growth, and infertility (Nishina, Lowe et al. 1994). Reproductive dysfunction and obesity can be reversed by the administration of exogenous leptin (Campfield, Smith et al. 1995). A *db/db* mouse ($Lepr^{db}/Lepr^{db}$) however, has a mutated leptin receptor (Coleman 1978). The phenotypic results are very similar but cannot be reversed by leptin supplementation.

The Zucker Rat

The *fa/fa* rat is more commonly known as the Zucker rat, named for Lois and Theodore Zucker who discovered it in the early 1960s. They characterized it as having insulin resistance, increased food intake, and decreased energy output, resulting in early-onset obesity (Zucker and Zucker 1961). Later research expanded this description to include abnormal corticosterone release patterns (Gibson, Liotta et al. 1981), increased fat cell number and size (Johnson, Zucker et al. 1971), elevated adipose tissue lipoprotein lipase activity (Greenwood, Cleary et al. 1981), and the tendency to eat extremely large meals (Becker and Grinker 1977) during both stages of its day/night cycle (Castonguay, Upton et al. 1982). The genetic trait stems from a nucleotide missense mutation in the leptin receptor coding region, also on the fourth chromosome, causing the replacement of a single glutamine with proline (Lee, Li et al. 1997). This results in reduced leptin receptor expression on the cell surface, causing intracellular leptin retention, decreased leptin binding, and decreased signal transduction (Yamashita, Murakami et al. 1997). The mutation has an autosomal recessive mode of inheritance, allowing for both lean and obese littermates.

GLUCOCORTICOIDS

Glucocorticoids (GCs) are a family of steroid hormones that play many roles in the body. The principal glucocorticoids involved in energy balance are the human hormones cortisol and cortisone and their rat counterparts corticosterone and 11-dehydrocorticosterone. Their importance in the maintenance of energy balance was first noted in 1940 when Long *et al* noted that adrenal secretions were necessary for regulation

of fasting blood glucose (Long, Katzin et al. 1940). This effect was further localized in the 1970s to the liver where adrenalectomy was shown to abolish gluconeogenetic responses to glucagon, fasting, and diabetes (Friedmann, Exton et al. 1967), (Exton, Mallette et al. 1970). It is now known that the final results of glucocorticoid actions are not simply hepatic but far more widespread. GCs bind to intracellular glucocorticoid receptors (GCCRs) which then translocate into the nucleus and complex with glucocorticoid response elements, resulting in transcriptional activation of numerous enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) (Sasaki, Cripe et al. 1984), a rate-limiting factor in the gluconeogenesis pathway. In the pancreas GCs interfere with β–cells and thereby decreases insulin release (Delaunay, Khan et al. 1997) (O'Brien, Lucas et al. 1990), while in peripheral tissues GCs impair glucose uptake by adipose tissue and skeletal muscles (Rooney, Neely et al. 1993) (Nielson, Caumo et al. 2003). Additionally, GCs have been shown to enhance fat and protein breakdown from storage (Roden, Price et al. 1996), increasing circulating levels of free fatty acids and thereby impairing insulin capacity for stimulation of muscle glucose uptake (Randle, Garland et al. 1963) (Boden, White et al. 1991). They are also well known to have anti-inflammatory effects. This was first demonstrated in the 1940s when doctors began to treat rheumatoid arthritis with cortisone (Hench, Kendall et al. 1950) and led directly to Munck *et al*'s proposal in 1984 that GCs are not, in fact, part of the body's direct stress-responses, such as inflammation, but are instead released during stress as part of a negative feedback system, in order to curtail these responses (Munck, Guyre et al. 1984). This suggestion had a profound impact on the study of GCs, and it has colored the elucidation of their many roles in the body ever since.

Another greatly influential finding occurred when it was found that there are two sub-types of corticoid receptors. In 1986, McEwan *et al*. found that high doses of corticosterone failed to prevent an aldosterone-stimulated increase in salt uptake in rats (McEwan, Lambdin et al. 1986). This indicated the possibility that different corticoid receptors may have preferences for either mineralocorticoids, such as aldosterone, or for glucocorticoids. However, the mechanism behind this specificity remained unknown until two years later when research into the 11β HSD enzymes revealed their role, a discussion of which will be presented. Both receptors are part of a ligand-activated nuclear hormone receptor superfamily that is amazingly well-conserved structurally. Of the six domains members of this family possess, only one varies between GRs and MRs – the initial N-terminus (Krust, Green et al. 1986). The receptor types have since been localized to different tissue types in the body as well – mineralocorticoid receptors (MRs) being more prevalent in the kidneys and colon while glucocorticoid receptors (GRs) are found in the adipose tissue, liver, pituitary and brain. Although there is some receptor co-localization, especially in the brain (Van Eekelen and De Kloet 1992), this tissue specificity parallels functional specificity. While GRs are ubiquitously necessary in most tissues, it is vital that MRs be present wherever aldosterone is active, that is, where the body reclaims water from waste.

The HPA Axis

The hypothalamic-pituitary-adrenal (HPA) axis is a classic example of one of the body's short-term neuroendocrine feedback systems. The hypothalamus releases coricotropin-releasing factor (CRF) from the parvocellular region of the paraventricular nucleus (Suemaru, Hashimoto et al. 1986) in response to obesity (Guillaume-Gentil,

Rohner-Jeanrenaud et al. 1990), changes in energy balance (Timofeeva and Richard 1997), stress, immune challenges, and more. The CRF travels through a venous portal system to receptors in the anterior pituitary (DeSouza, Perrin et al. 1985) (DeSouza, Perrin et al. 1984) which initiate the release of adrenal corticotrophic hormone (ACTH) (Saffran and Schally 1955) into general circulation. There are ACTH receptors are in the adrenal glands (Lefkowitz, Roth et al. 1970) which trigger the secretion of glucocorticoids, usually in diurnal patterns (Kakihana and Moore 1976) (Nicholson, Jin et al. 1985) or, again, in response to challenge stimuli. Glucocorticoids affects almost every cell type in the body, and also have an inhibitory effect on the PVN and the pituitary, creating a negative feedback loop within the HPA axis (Forgacs and Hajdu 1954) (Sakakura, Yoshioka et al. 1981) (Figure 3).

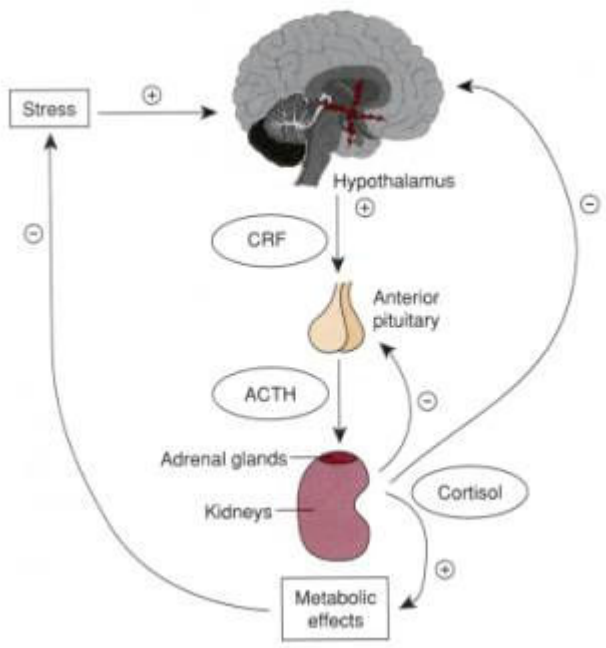


Figure 3 – The hypothalamic-pituitary-adrenal (HPA) axis.
(http://images.google.com/imgres?imgurl=http://www.montana.edu/wwwai/imsd/alcohol/Vanessa/vwhpa_files/image003.jpg&imgrefurl=http://www.montana.edu/wwwai/imsd/alcohol/Vanessa/vwhpa.htm&h=334&w=339&sz=10&tbnid=mAAkW-B7mbDGkM:&tbnh=113&tbnw=115&hl=en&start=5&prev=/images%3Fq%3DHPA%2Baxis%26svnum%3D10%26hl%3Den%26lr%3D%26sa%3DG)

Involvement in Obesity

Glucocorticoids play an interesting and complex role in the regulation of energy balance. They are released at meal times in normal (non-obese) humans, which would seem to indicate an orexigenic effect (Tataranni, Larson et al. 1996). However, they have also been shown to stimulate the release of the anorexigenic hormone leptin independent of food intake (Larsson and Ahren 1996) (Papaspyrou-Rao, Schneider et al. 1997), a response that is upregulated in obesity (Halleux, Servais et al. 1998). So what then is the true role of glucocorticoids in energy balance dysfunction? One clue can be found in Cushing's Syndrome, a disease in which excess glucocorticoids are synthesized and released (Turner & Bagnera 1976) and which is characterized by excessive appetite despite increased circulating leptin levels (Leal-Cerro, Considine et al. 1996). This indication is supported by findings that high leptin levels fail to cause appetite inhibition when they are induced by supplementation of exogenous glucocorticoids (Papaspyrou-Rao, Schneider et al. 1997) (Jacobson 1999). Jacobson observed that calorie restriction impairs appetite control in C57BL/6 mice and hypothesized that this was due to elevated glucocorticoid levels (Jacobson 2002). This idea was supported by the earlier finding that corticosterone replacement can reverse the effects of leptin infusions in adrenalectomized rats (Solano and Jacobson 1999). These clues indicate that the role of glucocorticoids in energy balance pertains to attenuation of satiety signals. However, clinical trials report no significant increases in plasma cortisol levels in obese patients (Lottenberg, Giannella-Neto et al. 1998). How do these seemingly opposite findings not contradict each other? It is important to note that the clinical trials did indicate increased cortisol secretion, but that this was accompanied by greater clearance capabilities as well.

Thus, understanding the whole picture of glucocorticoid contributions in obesity requires investigation into the processes involved in their metabolism.

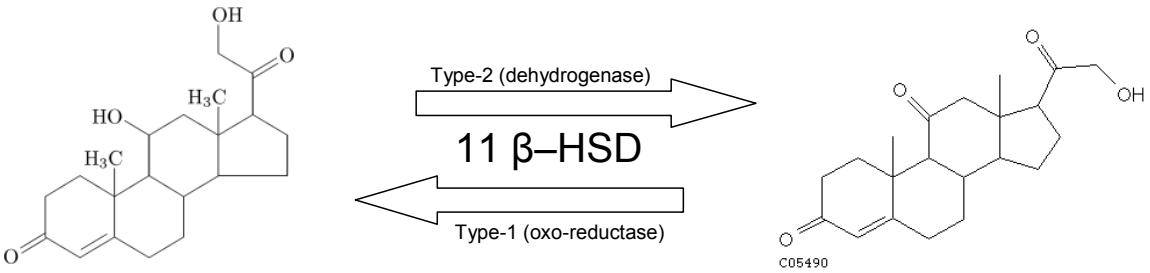
11-β-HYDROXYSTERIOD DEHYDROGENASE

11-β-hydroxysteriod dehydrogenases (11β HSDs) were first characterized in 1953 by Amelung *et al*., who described the interconversion of inert 11-keto glucocorticoids and their active 11-hydroxy forms (Amelung, Hubener et al. 1953). The next major step was taken in the mid 1980s when Monder and associates purified the HSD component from rat liver (Lakshmi and Monder 1988). Still, little notice was taken of the enzyme until a few years later when it solved a long-standing clinical mystery. Adrenal corticosteroids are in fact divided into two major groups – glucocorticoids, such as cortisol and cortisone, and mineralocorticoids, such as aldosterone. Scientists had long been baffled by a syndrome of apparent mineralocorticoid excess characterized by hypokalemia, sodium retention, and high blood pressure. The source of the confusion was that, *in vivo*, mineralocorticoid receptors are aldosterone specific, even in the presence of excess glucocorticoids (Sheppard and Funder 1987). *In vitro*, however, this specificity disappears and mineralo- and gluco-corticoids are bound with equal affinity (Arriza, Weinberger et al. 1987). In 1988 both Edwards *et al*. (Edwards, Stewart et al. 1988) and Funder *et al*. (Funder, Pearce et al. 1988) identified that the enzyme 11β HSD is able to rapidly inactivate glucocorticoids present in any aldosterone-sensitive tissue (kidney, colon, salivary glands, placenta, distal nephron, sweat glands), allowing for preferential binding of the mineralocorticoid.

Once the clinical importance of 11β-HSD had been established, research progressed rapidly. The enzyme was purified and cloned in 1989 by Agarwal et al. (Agarwal, Monder et al. 1989), but it soon became clear that another layer of complication existed. The enzyme they had purified was expressed in many more tissues than had mineralocorticoid receptors (eg., liver, adipose, brain, and gonadal tissue). In 1993-1994 a second 11β-HSD isozyme was identified and dubbed 11β-HSD-2 (Rusvai and Naray-Fejes-Toth 1993). This enzyme adhered much more strictly to the original location limitations applied to the family, and had the dehydrogenase half of the enzymatic capabilities. Its discovery led to the question of the unknown function of the initially purified isozyme, thereafter called 11β HSD-1.

The original experiments characterizing 11β HSD had shown it to have both dehydrogenase and reductase activity (Lakshmi and Monder 1988). The dehydrogenase activity had been individualized mainly to 11β HSD-2 in its protection of mineralocorticoid receptors. Researchers therefore attempted, and succeeded, to assign the reductase activity to 11β HSD-1 (Rajan, Edwards et al. 1996), although they found that it is still capable of low levels of glucocorticoid inactivation. (Figure 4)

Figure 4 – interconversion activity of 11β-HSD



Corticosterone
(active)

11-dehydro-corticosterone
(inactive)

Thus was the interconversion cycle of glucocorticoids and 11β HSDs established. But what regulates these reactions? How is it determined what ratio of active cortisol or corticosterone (in the human and the rat, respectively) to inactive cortisone or 11-dehydrocorticosterone is needed in the body at any given time? And how do these processes relate to tissue-specific variations in GCs that have been observed in obesity? There are some current theories.

Interaction with Glucocorticoids

The action of 11β HSDs centers on the C11 site of a glucocorticoid. A hydroxyl group at this position indicates an active hormone, while a keto group conveys inactivity (Cope and Black 1958). The ability of the enzymes to effect this change is cofactor dependent: 11β HSD-1 requires NADPH while 11β HSD-2 needs NAD. This gives us a clue into how the 11β HSD-1 reductase activity is regulated. When the enzymes are synthesized within the cell they complex with the membrane of the ER. 11β HSD-2 faces the cytoplasm, where concentrations of NAD are high, while 11β HSD-1 is oriented so that its catalytic and cofactor-binding domains face the lumen of the ER, where NADPH is more abundant (Odermatt, Arnold et al. 1999) (Figure 5). Hexose-6-phosphate dehydrogenase (H6PDH) is co-localized on the inside of the ER membrane and has been shown to control local NADPH availability, thereby affecting 11β HSD-1 activity. H6PDH in the ER performs the first steps of a pentose phosphate pathway that converts glucose-6-phosphate to 6-phospho-gluconolactone, which in turn generates NADPH (Kimura, Endou et al. 1979). Mutations in the H6PDH gene cause attenuated 11β HSD-1

activity and subsequent over-activation of the HPA axis, resulting in hormone excess (Bujalska, Draper et al. 2005). Without 11β HSD-1's oxo-reductase ability to convert inactive glucocorticoids to active ones, the hypothalamus increases CRF signaling, which in turn increases ACTH secretion, which results in glucocorticoid excess.

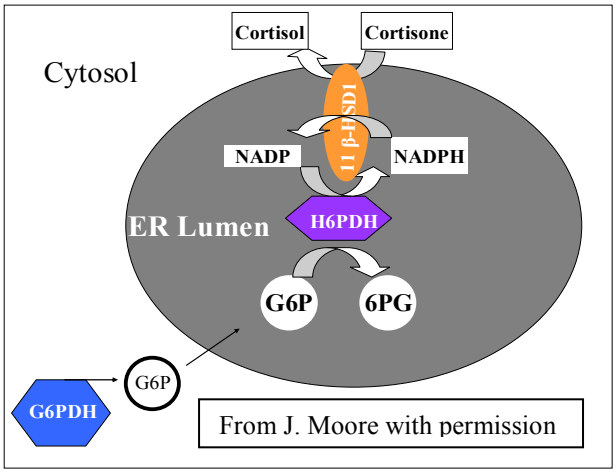


Figure 5 - Interactions among G6PDH, H6PDH, and 11β-HSD1. Cytosolic G6PDH is the major source of intracellular NADPH. However, NAPDH-dependent 11β-HSD1 cannot access this pool because its active site faces the ER lumen. H6PDH is co-localized with 11β-HSD1 to the ER membrane and generates NADPH in the ER by oxidizing glucose-6 phosphate (G6P), which is produced by G6PDH and transported across the ER membrane. H6PDH promotes 11β-HSD1 oxoreductase activity (cortisone to cortisol) by providing an accessible supply of NADPH.

There are many other routes by which 11β HSD-1 activity can be affected. The promoter on the gene contains binding sites for CAAT/enhancer-binding protein (C/EBP), a transcription factor that upregulates gene expression and is in turn regulated by glucocorticoids (Williams, Lyons et al. 2000). On the other hand, chronic treatment with peroxisome proliferator activator receptor-α (PPARα) attenuates gene expression (Hermanowski-Vosatka, Gerhold et al. 2000). Steroids such as progesterone, allopregnanolone, and testosterone have been shown to inhibit both the reductase and the dehydrogenase activity of the enzyme (Latif, Pardo et al. 2005). The list is getting longer every day, and the mechanism of action more complex. Unfortunately, even with our

knowledge of the mechanism growing quickly, the observed results are not much easier to understand.

Hepatic Involvement

Up- and down-regulation of 11β HSD-1, by whatever mechanism, results in the exacerbation or attenuation of a number of phenotypic characteristics. Most of the detrimental ones are commonly grouped under the title The Metabolic Syndrome. This heading usually refers to visceral adiposity, insulin resistance, dyslipidemia, hypertension, type II diabetes mellitus, and increased cardiovascular risk. Cushing's Syndrome patients display a very similar set of symptoms but in the presence of high circulating levels of cortisol, a causal factor often missing in idiopathic obesity (Walker, Soderberg et al. 2000) (Rosmond, Dallman et al. 1998). How then are their similar phenotypes explained? Local variations in GC sensitivity may offer a partial explanation. 11β HSD-1 activity (and therefore cortisol production rate) is upregulated in the adipose tissue of genetically and dietarily obese rats (Masuzaki, Paterson et al. 2001) and humans (Rask, Olsson et al. 2001), causing the Metabolic Syndrome characteristics (Bujalska, Kumar et al. 1997). It should be noted that not all studies agree on this point (Tomlinson, Sinha et al. 2002). However it is downregulated in hepatic tissue, and overall circulating plasma cortisol levels are unchanged from normal weight controls (Stewart, Boulton et al. 1999), (Rask, Walker et al. 2002). Seckl and colleagues have suggested that this downregulation is in response to the high levels of active hormone being sensed in adipose tissue. Blood from visceral adipose tissue drains into the liver via the hepatic portal vein. High levels of active hormone in this blood would trigger a downregulation of the gene/enzyme required to activate more, possibly via nuclear oxysterol receptors

liver X receptor (LXR)-α and β. LXR agonists have been shown to reduce 11β HSD-1 mRNA expression by as much as 50% (Stulnig, Oppermann et al. 2002), resulting in lower levels of hepatic 11β HSD-1. Although this is clearly not a blanket explanation of the complexities of glucocorticoid levels and their involvement in obesity (Paterson, Morton et al. 2004), it is one theory currently under investigation.

PREVIOUS RESEARCH

The issues of glucocorticoid and 11β HSD-1 participation in human and rodent obesity have been approached several different ways over the years. Each method has its advantages and disadvantages and each has been useful for examining particular aspects of HSD involvement. This section will focus on several popular means of manipulating these compounds and the scientific progress that each method has contributed to.

Inhibition of 11β-HSD-1

There has been a significant amount of research done using chemical inhibitors of 11β HSDs. A number of these are naturally occurring compounds such as quercetin, tea polyphenols, furosemide, gossypol, and xenobiotics (Guo and Reidenberg 1998) (Hult, Jornvall et al. 1998). By far the most widely used, however, is glycyrrhetinic acid, the active ingredient in licorice root, and its synthetic derivative succinyl ester carbenoxolone. The first hint of their involvement in glucocorticoid metabolism came in 1953 when Borst *et al* showed a synergistic effect between licorice and cortisone when used to treat Addison's (adrenal insufficiency) and Simmonds' (hypopituitarism) diseases (Borst, Ten Holt et al. 1953). In 1987 Stewart *et al* showed that licorice root, which was

already known to produce symptoms similar to mineralcorticoid excess, also produced changes in cortisol metabolism (Stewart, Wallace et al. 1987). They suggested that this indicated that glycyrrhetinic acid acts by 11β HSD inhibition rather than through interaction with any mineralocorticoids. In 1989 Monder and colleagues provided evidence supporting this theory (Monder, Stewart et al. 1989). They also reported data on carbenoxolone, and both compounds prevented interconversion of glucocorticoids in a dose-dependant manner. Since then glycyrrhetinic acid and its derivatives and analogues have been used to show the effects of 11β HSD inhibition in many different systems and under a variety of conditions. Ulick and colleagues used it to determine the best clinical method of measuring cortisol:cortisone interconversion rates in the urine (Ulick, Wang et al. 1993). In 1995 Walker *et al.* showed that inhibition of 11β HSD by carbenoxolone reduced intrahepatic cortisol concentration, increased hepatic insulin sensitivity, and decreased glucose production, observations with clear clinical implications in the treatment of Type II diabetes (Walker, Connacher et al. 1995). More recently Andrews *et al*. confirmed these results and further specified the decreased glucose production as attributable to reduced glycogenolysis, as opposed to reduced gluconeogenesis (Andrews, Rooyackers et al. 2003). Unfortunately, some data indicate that these possible beneficial effects are attenuated in obese humans (Sandeep, Andrew et al. 2005) and obese Zucker rats (Li, Nakagawa et al. 2004) (Livingstone and Walker 2003), although the rat results could be due to interference by the mutated leptin receptor, and the human data suggests that attenuation could be reversed if the inhibitor could be made to better target adipose tissue.

The major problem with glycyrrhetinic acid and carbenoxolone is that they are non-specific inhibitors of 11β HSDs (Monder, Stewart et al. 1989) (Andrew, Smith et al. 2002). In order to separate out the effects of 11β HSD-1 and -2, other methods were required. A few derivatives of glycyrrhetinic acid, altered at the 11-, 24-, or 30-positions, act differentially by location (renal versus hepatic), but not specifically enough for experimental purposes (Shimoyama, Hirabayashi et al. 2003).

One of the first 11β HSD-1-specific chemical inhibitors was chenodeoxycholic acid, an endogenous hormone (Diedrich, Grossmann et al. 2000). Unfortunately, it was shown not to be potent enough for therapeutic use. Then, in 2002, Barf and colleagues identified two compounds that were both selective and potent – arylsulfonamidothiazole 2a and 2b, shortened to BVT.14225 and BVT.2733, respectively (Barf, Vallgarda et al. 2002). Furthermore, the 2a form was specific for human 11β HSD-1 while the 2b form was specific for the rodent enzyme, an unsurprising development in light of the fact that the two enzymes have only 79% amino acid similarity. Barf went on to demonstrate the efficacy of treatment with BVT compounds by curing a diabetic KKA^y mouse colony, reducing blood glucose levels by up to 53%. These results were later replicated by Alberts *et al*, with further specification that hepatic PEPCK and glucose-6-phosphate were also lowered, along with serum insulin concentrations, but that hepatic insulin sensitivity was improved (Alberts, Engblom et al. 2002) (Alberts, Nilsson et al. 2003). On the other hand, body weight, food intake, hepatic 11β HSD-1 mRNA, and liver function marker enzyme expression remained unaffected, further reinforcing the therapeutic viability of the compound.

Overexpression

There are, of course, non-chemical ways to investigate aberrations in 11β HSD-1 function. In 2001 a transgenic mouse model was created that overexpressed the enzyme in adipose tissue (Masuzaki, Paterson et al. 2001). This was done by combining an active 11β HSD-1 fragment with the adipose-specific aP2 promoter in FVB mice. Once the strain had been inbred, male offspring showed 7-fold increases in 11β HSD-1 mRNA expression, and 3-fold increases in enzyme activity levels in adipose tissue, even as compared to obese humans and other obese mice. Accordingly, transgenic mice under non-stressed conditions showed 30% increases in adipose corticosterone concentrations as compared to controls, but completely normal levels of circulating hormone, indicating localized excess of enzyme activity. Phenotypically, aP2-HSD1 mice have increased adipocyte differentiation and lipid accumulation, and they exhibit omental obesity. Glucocorticoids play an inhibitory role in preadipocyte proliferation via cell cycle arrest at the G1 phase (Rogatsky, Trowbridge et al. 1997), and a stimulatory one in mature adipocyte differentiation. Because the aP2 promoter is only expressed in mature adipocytes, and not in preadipocytes, the obesity in this model is due to increased cell size, not cell number. Additionally, the mice are insulin resistant and hypertensive (Masuzaki, Yamamoto et al. 2003).

A transgenic model for overexpression of hepatic 11β HSD-1 has also been created (Paterson, Morton et al. 2004). This was achieved by fusing the active 11β HSD-1 fragment with a human apoE promoter and enhancer in C57BL/6J mice. Two strains, carrying 2-3 or 8-10 times the normal number of target genes, were studied. These strains, labeled 1066 and 1065, respectively, showed 2-fold or 5-fold increased 11β HSD-1 activity, again respectively. They exhibited fatty liver, dyslipidemia, hypertension, and

mild insulin resistance. Interestingly, however, they were not obese, nor were their glucose tolerance or circulating GC levels affected. This situation has been reported in humans, making this mouse model ideal for further therapeutic research (Marchesini, Brizi et al. 2001).

Knockout Mouse

The mirror image of the aP2-HSD1 overexpression mouse has also been created – the knockout mouse. In this model, mice homozygous null for 11β HSD-1 were made by replacing exons 3 and 4 of the gene with useless code (Kotelevtsev, Holmes et al. 1997). The resulting rodents have normal birth weight, growth rates, and reproductive abilities, but undetectable levels of conversion of 11-dehydrocorticosterone to corticosterone, even when implanted with 11-dehydrocorticosterone pellets. This leads to reduced negative feedback on the HPA axis, causing adrenal hyperplasia and increased ACTH-stimulated corticosterone secretion (Harris, Kotelevtsev et al. 2001). Interestingly, no increase in glucocorticoid-sensitive gene expression has been observed.

There is no characteristic adipocyte phenotype for the 11β HSD-1 KO mouse except for resistance to all forms of obesity, including dietary. With no enzyme to enhance GC inhibition of pre-adipocyte proliferation, they do have fat cells, but there is also no enzyme to promote mature adipocyte differentiation and the animals therefore stay lean. The mice are also resistant to fasting hypoglycemia, although their blood sugar levels remain lower than those of control mice under high-fat feeding conditions, and have improved lipid profiles and hepatic insulin sensitivity (Morton, Holmes et al. 2001).

One of the most interesting experimental uses of the 11β HSD-1 KO mouse has been determination of local behaviors of the enzyme. For example, studies have shown

that 11β HSD-1 has both reductase and dehydrogenase activity in the normal rodent hippocampus (Jellinck, Pavlides et al. 1999). The KO model, however, exhibits decreased hippocampal corticosterone levels despite increased levels of circulating glucocorticoids, indicating that the enzyme's main function in this area is as a reductase (Yau, Noble et al. 2001). Findings such as these lead researchers to new areas of experimentation and help elucidate anomalies found in previous work.

Zucker Rat

The Zucker rat has remained an extremely useful experimental model over the years. A series of studies published by Livingstone et al in 2000 elucidated some of the tissue specific behaviors of 11β HSD-1 (Livingstone, Kenyon et al. 2000) (Livingstone, Jones et al. 2000), and went on to spur similar discoveries in human systems (Rask, Olsson et al. 2001) (Rask, Walker et al. 2002). More recently, they have been used to attribute the overactivation of the HPA access during stress to altered levels of mineralocorticoid recepters, as opposed to glucocorticoid receptors, in the hippocampus of the obese (Mattsson, Lai et al. 2003). This finding shed light on the previously unexplained resistance of the HPA to endogenous glucocorticoids. In 2005 Zucker rats were used to test the first liver-selective glucocorticoid receptor antagonist with therapeutic anti-diabetic possibilities (Jacobson, von Geldern et al. 2005). A-348441 reduced hepatic glucose output without affecting peripheral glucose uptake by downregulating hepatic genes normally upregulated by GCs, did not antagonize the HPA

axis, and had synergistic effects on blood glucose when co-administered with an insulin-sensitizer.

Zucker rats are not always used to investigate matters of glucocorticoid behavior. Just this year studies have been published using the model to investigate cardiovascular issues (Sista, O'Connell et al. 2005) (Barbato, Zuckerbraun et al. 2005) (Wang, Lloyd et al. 2005), effects of soy isoflavones (Tovar, Torre-Villalvazo et al. 2005) (Banz, Davis et al. 2004) (Gudbrandsen, Wergedahl et al. 2005), involvement of obesity in breast cancer (Hakkak, Holley et al. 2005), effects of hypobaric hypoxia (commonly used in athletic training) on food intake (Simler, Grosfeld et al. 2006), and exacerbation of hemorrhage by obesity (Frisbee 2006). Clearly, interference with the leptin receptor has far-reaching consequences, and this rodent model will continue to be relevant for a long time.

EXPERIMENT

INTRODUCTION

The United States is in the midst of an obesity epidemic, with an estimated 65% of the population overweight or obese. In the past 20 years the incidence of overweight adults has increased by 40%, and the incidence of obese adults by 110% (Hedley, Ogden et al. 2004) (Flegal, Carroll et al. 1998). It has been estimated that obesity costs the United States over $117 billion every year, a figure which does not include complications of merely overweight individuals. (USDHHS 2001) Animal models of obesity are an excellent window through which various causes of and possible treatments for obesity can be explored.

Leptin is a hormone secreted mainly from adipose cells. In rodent models it is released in direct proportion to fat mass (Considine, Sinha et al. 1996). Endogenous leptin causes a decrease in food intake (Schwartz, Peskind et al. 1996). The *fa/fa* rat, more commonly known as the Zucker rat, has a nucleotide missense mutation resulting in reduced leptin receptor expression on the cell surface, causing intracellular leptin retention, decreased leptin binding, and decreased signal transduction. (Lee, Li et al. 1997) (Yamashita, Murakami et al. 1997). The mutation has an autosomal recessive mode of inheritance, allowing for both lean and obese littermates carrying the *fa/fa* genotype. Animals are insulin resistant, hyperphagic, and have decreased energy output, resulting in early-onset obesity (Zucker and Zucker 1961). They also have abnormal corticosterone release patterns (Gibson, Liotta et al. 1981), increased fat cell number and size (Johnson, Zucker et al. 1971), elevated adipose tissue lipoprotein lipase activity (Greenwood, Cleary et al. 1981), and the tendency to eat extremely large meals during

both stages of its day/night cycle (Becker and Grinker 1977) (Castonguay, Upton et al. 1982).

Glucocorticoids (GCs) are a family of adrenal steroid hormones that have a myriad of functions, including a role in how the HPA axis helps maintain energy balance (Long, Katzin et al. 1940). GCs bind to intracellular glucocorticoid receptors (GCCRs) which then translocate into the nucleus and complex with glucocorticoid response elements, resulting in transcriptional activation of various enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) (Sasaki, Cripe et al. 1984), which is a rate-limiting factor in the gluconeogenesis pathway. GCs have been shown to enhance fat and protein breakdown from storage (Roden, Price et al. 1996), increasing circulating levels of free fatty acids and thereby impairing insulin's capacity for stimulation of muscle glucose uptake (Randle, Garland et al. 1963) (Boden, White et al. 1991). The major human GCs are cortisol and its metabolite cortisone. Their rat counterparts are corticosterone and 11-dehydrocorticosterone.

11-β-hydroxysteriod dehydrogenases (11β HSDs) interconvert inert 11-keto glucocorticoids and their active 11-hydroxy forms (Amelung, Hubener et al. 1953). There are two isoforms – Type 1 and Type 2. Type 2 is mostly active in aldosterone-sensitive tissues and its major function is as a hydrogenase, inactivating nearby GCs so as to avoid competitive binding with aldosterone on mineralocorticoid receptors. Type 1 is much more ubiquitous and its major function is as a reductase, reactivating GCs. 11β HSD-1 activity (and therefore cortisol production rate) is upregulated in the adipose tissue of genetically and dietarily obese rats (Masuzaki, Paterson et al. 2001) and humans (Rask, Olsson et al. 2001), and has been implicated in the Metabolic Syndrome (91). It

should be noted that not all studies agree on this point (Tomlinson, Sinha et al. 2002). However the enzyme is downregulated in hepatic tissue of the obese, and overall circulating plasma cortisol levels are unchanged from normal weight controls (Stewart, Boulton et al. 1999) (Rask, Walker et al. 2002). In this experiment we compare hepatic 11β HSD-1 mRNA levels in two different models of obesity (genetic and dietary). Genetically obese Zucker rats were compared to their lean littermates, while Long-Evans rats fed on different sources (saturated, monounsaturated, and polyunsaturated) and levels (high fat and low fat) of dietary fat were compared to controls fed on standard rat chow. We know the high fat diets to have produced increases in body fat (Woods, D'Alessio et al. 2004) and predicted correlated changes in levels of hepatic 11β HSD. Hepatocytes have been shown capable of gene transcription control in response to dietary-induced obesity and weight loss via a large number of peripheral molecular pathways (Raab, Bullen et al. 2005). Furthermore, dietary obesity has been shown to cause downregulation of 11β HSD (Morton, Ramage et al. 2004) but a comparison has yet to be made of these results with the changes seen in genetic obesity.

METHODS

Experiment 1: genetic obesity

Nine obese and ten lean age-matched 5-6 week old male Zucker rats were purchased from Harland Labs (Indianapolis, IN). The rats were maintained at the University of Cincinnati's AAALAC accredited facility. They were housed in individual tub cages with corncob bedding. A 12 hour light/dark schedule and a 22±1˚C controlled temperature were maintained throughout the experiment. They were allowed water ad

libitum and ate a non-purified Teklad diet (Teklad Sterilizable Mouse/Rat Diet also from Harlan Labs). All treatment and sacrifice procedures complied with the *Guide for the Care and Use of Laboratory Animals* and were approved by the university's Institutional Animal Care and Use Committee. At 5 weeks of age, obese rats are visually distinguishable from their lean littermates, and at sacrifice obese rats weighed 642±19g while leans rats weighed 398±9g. Their livers were extracted, flash frozen in liquid nitrogen, and shipped to the University of Maryland.

Experiment 2: dietary obesity

Twenty-one Long-Evans rats from Harlan Labs (Indianapolis, IN) were housed under the same conditions as the Zucker rats of Experiment 1. They were also fed the same Teklad diet, and allowed *ad libitum* water, for 1 week, after which they were switched to their experimental diets for 10 weeks. Dietary group assignments were done on the basis of body weight matching, although all rats weighed between 250-350g at the start of the experiment. Again, all procedures were approved by the University of Cincinnati Internal Animal Care and Use Committee and complied with the *Guide for the Care and Use of Laboratory Animals*. Sacrifice in both experiments was done by either rapid decapitation or by slow replacement of air in a specialized chamber with pure CO_2. Both methods are approved for use by the Panel on Euthanasia of the American Veterinary Medical Association.

The experimental diets consisted of six test diets and one chow control, with three rats on each diet. Three of the diets were high fat (HF) (20g of fat/100g of diet = 19.34kJ/g of diet = 7.74kJ/g from fat) and three were low fat (LF) (4g of fat/100g of diet = 16.12kJ/g of diet = 1.29kJ/g from fat). The fats used within the HF and LF categories

were saturated (butter), polyunsaturated (corn oil), or monounsaturated (olive oil). The control chow, which consisted of the Teklad diet used during the initial acclimation period, contained 3.5g of fat/100g of diet. All other diets were pelleted, semipurified, and nutritionally complete, based on AIN-93M and prepared by Dyets, Inc. (Bethlehem, PA). In order to emphasize the effects of dietary fat, all diets were equalized per kJ for protein, vitamins, and minerals (see Appendix for Diets Table and final body weights).

RNA, cDNA, RT-PCR

Total RNA was extracted from the livers of all 30 rats with Tri-Reagent (Sigma, Inc., St. Louis, MO), and samples were purified with DNAse (DNA-free™, Ambion Inc., Austin, TX), both per manufacturer's protocol. Double stranded cDNA templates were then synthesized from the purified RNA (SuperScriptTM III First-Strand Synthesis System, Invitrogen Inc., Carlsbad, CA), again per the company's instructions.

Real-Time PCR was performed on an Applied Biosystems Prism 7000. Assay-On-Demand (Applied Biosystems, Foster City, CA) created the probe/primer mix for 11β HSD-1. TaqMan Rodent GAPDH Control Reagents kit provided the GAPDH housekeeper gene. 2.5µl of cDNA template per sample was used, and each sample was run in triplicate along with its GAPDH control. Additionally, a control GAPDH sample was run using rat cDNA provided by the TaqMan kit.

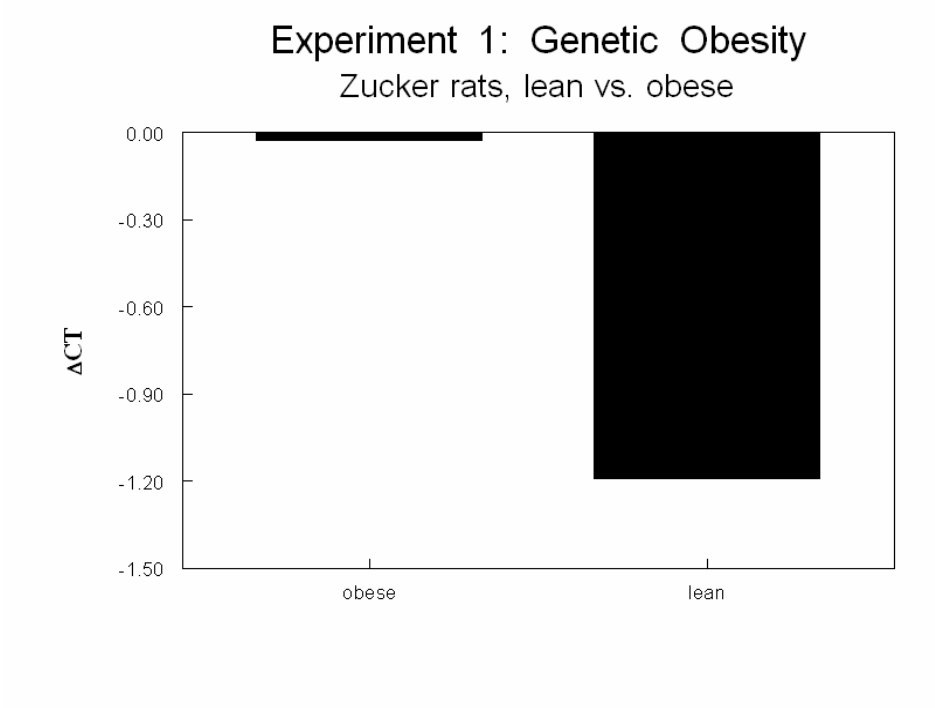
STATISTICS

Statistics were performed per instructions from Applied Biosystems. Threshold cycle (CT) counts for GAPDH were subtracted from those for 11β HSD-1 to give a ΔCT. Then mean CT counts for controls were subtracted from the mean ΔCTs to give ΔΔCTs.

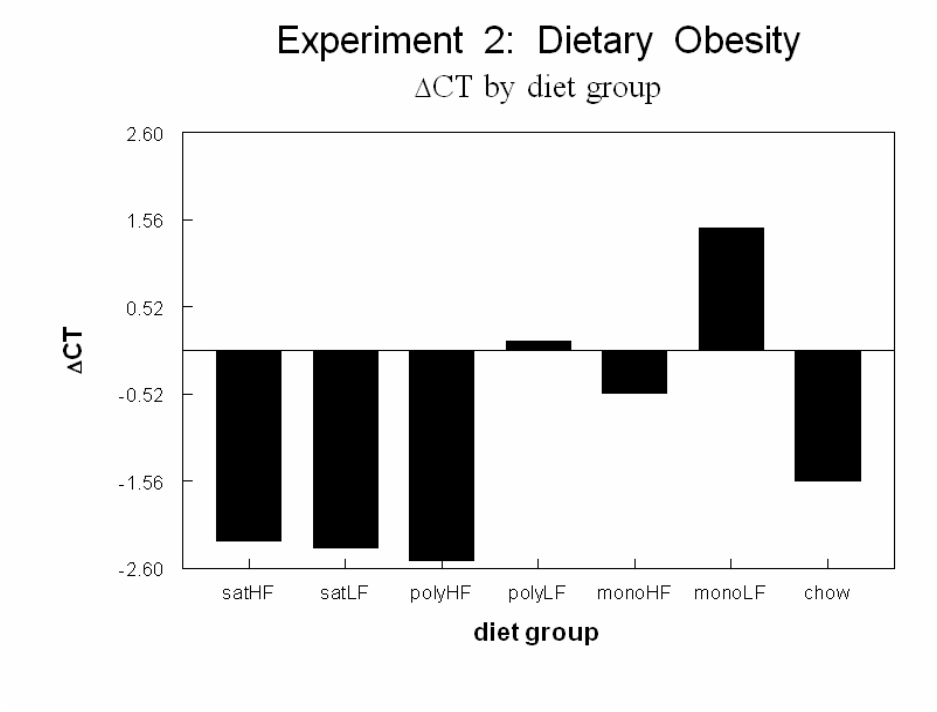
Finally, these results were then used to calculate the $2^{-\Delta\Delta CT}$ equation (see Applied Biosystems Prism 7500 instruction manual) to provide numbers upon which linear statistics could be performed. $2^{-\Delta\Delta CT}$ calculations in Experiment 2 were performed three ways: using chow-fed rats as controls for both HF and LF subjects, using LF subjects as controls for HF subjects of the same fat source (saturated, monounsaturated, and polyunsaturated), and finally using LF subjects pooled across fat sources as controls for HF subjects also pooled across fat sources. Statistical Analysis Software (SAS) was used to run statistical calculations, using both Proc ANOVA and Proc GLM, followed by Duncan comparisons.

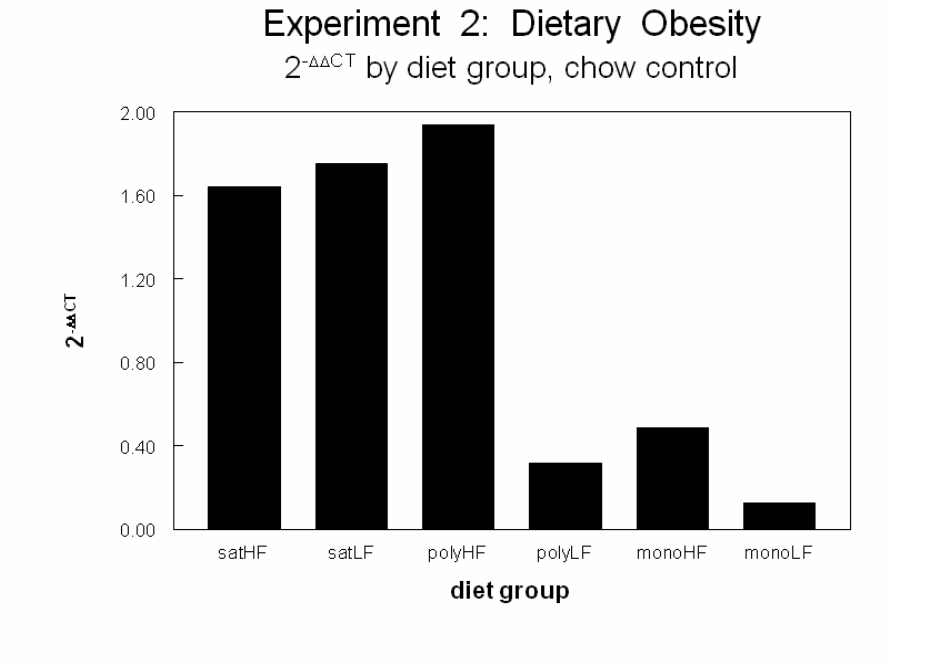
RESULTS

Statistical analysis of the RT-PCR results for Experiment 1 (genetic obesity) revealed that obese Zucker rats had only 45% of the 11β HSD-1 mRNA seen in their lean littermates ($2^{-\Delta\Delta CT} = 0.45$). This is consistent with previous results found in other laboratories (Morton, Ramage et al. 2004).

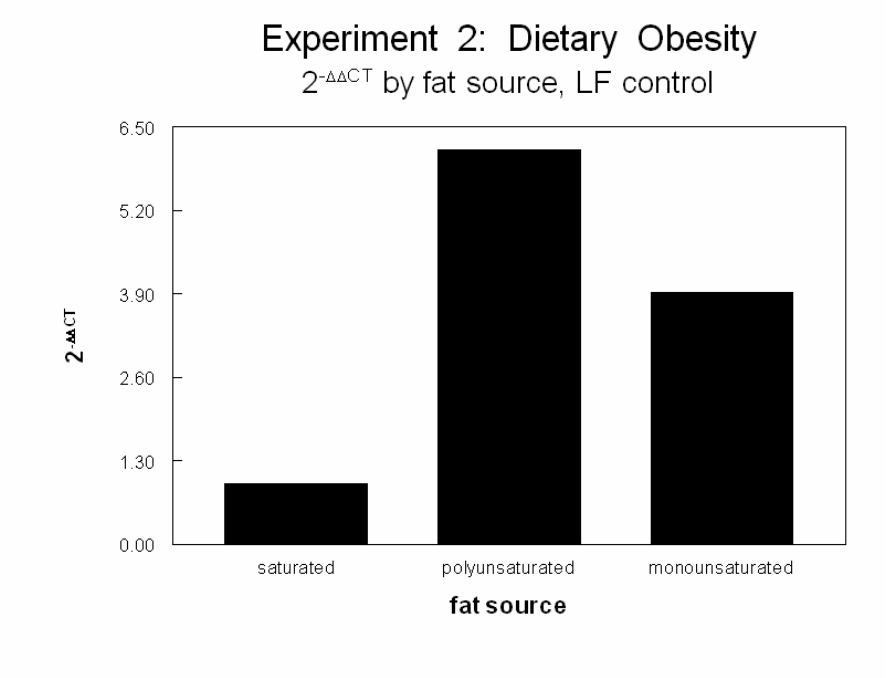


The results for Experiment 2 (dietary obesity) are less clear. The analysis using chow fed rats as controls revealed that only the polyunsaturated fat source appears to show differences in 11β HSD-1 between the high fat (HF) and low fat (LF) diets (HF $2^{-\Delta\Delta CT} = 1.93$, LF $2^{-\Delta\Delta CT} = 0.31$). This data showed that the polyunsaturated HF subjects had almost twice the 11β HSD-1 mRNA seen in chow controls, while the polyunsaturated LF subjects had only 31% of that seen in controls, although we should mention that even these differences did not turn out to be statistically significant. The saturated and monounsaturated fat sources showed no differences between HF and LF 11β HSD levels as compared to chow controls (sat: HF $2^{-\Delta\Delta CT} = 1.64212$, LF $2^{-\Delta\Delta CT} = 1.75816$; mono: HF $2^{-\Delta\Delta CT} = 0.48558$, LF $2^{-\Delta\Delta CT} = 0.124$).

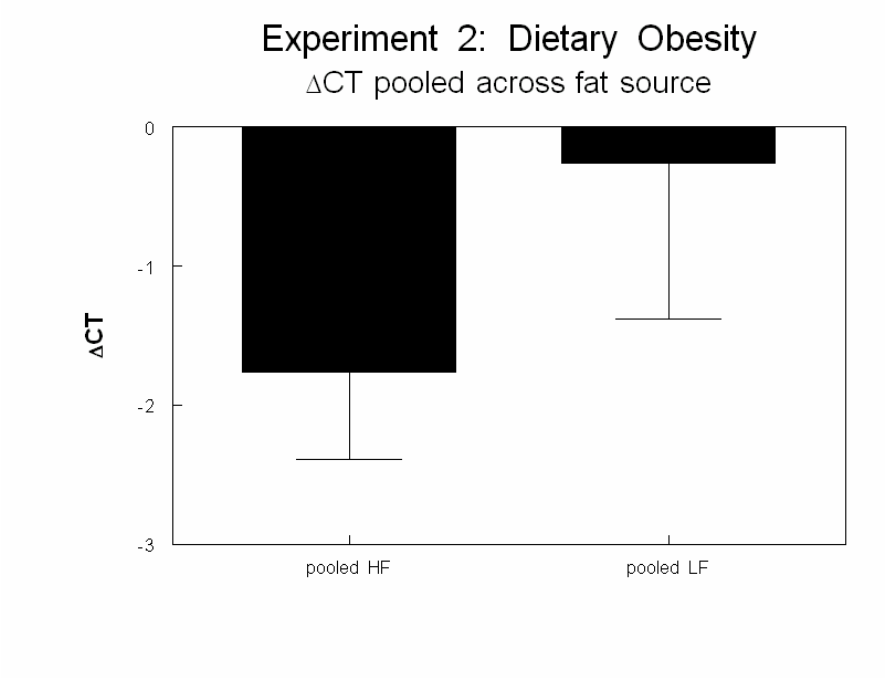




The second analysis of these data used the LF subjects from each fat source category as controls. Results from this approach revealed large but not statistically significant differences in each group: saturated ($2^{-\Delta\Delta CT} = 0.93735$), polyunsaturated ($2^{-\Delta\Delta CT} = 6.15224$), monounsaturated ($2^{-\Delta\Delta CT} = 3.91467$).



Since the results from the first two analysis methods did not reveal statistically significant differences, we were able to pool all LF subjects across fat sources and use them as a control for all HR subjects also pooled across fat sources. A large decrease in 11β HSD-1 was revealed in HF subjects when compared to LF ($2^{-\Delta\Delta CT} = 0.2456$). However, the difference failed to achieve significance at the p=0.05 level due to appreciable intersubject variability.



DISCUSSION

The goals of these experiments were (1) to see if we could replicate the previous finding that genetically obese Zucker rats have lower levels of hepatic 11β HSD-1 than their lean littermates, (2) to determine if this relationship also exists in rats with dietary obesity as compared to rats fed the same type but lower amounts of various types of fat, and, if so, (3) to compare the levels of hepatic 11β HSD-1 seen in dietary and genetic obesities. We were successful in the first goal, closely replicating previously reported decreases in hepatic 11β HSD-1 gene expression in obese Zucker rats. Unfortunately, while we did see some large differences in mRNA levels for this enzyme in a few fat level and fat source comparisons, none of the results were statistically significant. We feel that the major limitation of the study was the small number of rats available for each dietary fat level/source group. However, even with only three rats per group, we feel that the changes seen in the polyunsaturated high and low fat subjects as compared to chow,

in the HF monounsaturated and polyunsaturated subjects as compared to their LF counterparts, and in the pooled HF subjects as compared to the pooled LF subjects, warrant further investigation with more subjects. If the results of a larger study were to replicate ours, it would be interesting to investigate why some dietary obese (HF) subjects showed increases in hepatic 11β HSD-1 when analyzed by fat source (polyunsaturated $2^{-\Delta\Delta CT} = 6.152$, monounsaturated $2^{-\Delta\Delta CT} = 3.915$), but all showed decreases when pooled across sources ($2^{-\Delta\Delta CT} = 0.2456$). It is possible that the effects of fat source are masked by pooling, or that the increases are false positives due again to the small number of rats per fat source group.

If, however, we consider that, whatever the differences within dietary obesity are, they are definitely different from the results of genetic obesity, then we are still left with some interesting questions. Zucker rats show obesity due to a mutated receptor for the anorexigenic hormone leptin. Because the body is unable to sense the presence of leptin, the HPA axis is upregulated in an attempt to secrete more GCs into adipose tissue in the hopes that it will stimulate leptin release. Plasma GCs then drain from the adipose tissue into the hepatic portal vein, and the presence of excess hormone triggers the downregulation of hepatic 11β HSD-1. This promotes the maintenance of obesity by decreasing circulating GC levels over the body as a whole, which not only decreases the rate of fat breakdown from peripheral muscles but also enhances the release of insulin, causing more glucose uptake. However, in obesity in rat strains with normal leptin receptors, the HPA axis would not be over-stimulated and the resultant levels of hepatic 11β HSD-1 might differ. The phenotypic results would remain the same, however, since the circulating levels of GCs are still reduced.

We predicted differences in the level of hepatic 11β HSD-1 between HF and LF diets because high plasma levels of triglycerides interfere with insulin. Higher circulating triglycerides would result in lower levels of glucose uptake, and consequently less need for activation of GCs which downregulate the release of insulin. We did, in fact, see this result when using pooled LF diets as controls for the pooled HF diets ($2^{-\Delta\Delta CT}$ = 0.2456). Previous research has also showed dietary fat type to affect adiposity and weight gain (Jang, Hwang et al. 2003), possibly via altered neuropeptide signaling in the arcuate nucleus of the hypothalamus (Huang, Xin et al. 2004). We hypothesized therefore that 11β HSD-1 would play a role as well, and that we would see an association between downregulation of the gene and final body weight of the rat, but were disappointed. The results showed no clear trend with which to correlate dietary fat source. It is hoped that such a pattern would emerge out of results from a larger experiment. If the association were to be made, the next step could be to test the theory that regulating levels of 11β HSD-1 would allow us to affect body weight. Knockdown of the enzyme in adipose tissue could decrease plasma levels of active GCs traveling to the liver and thereby avoid the hepatic downregulation associated with obesity. It is important to note, however, that lower levels of 11β HSD-1 message are not necessarily associated with lower intracellular corticosterone. In fact, a similar study of various types of high fat diets over the same period of time showed no effect of fat type on intracellular corticosterone levels (Kamara, Eskay et al. 1998). In order for the theory to hold true, the experiment would also have to test the interconversion activity rates and the levels of hormone produced. Only when we have the whole picture – message, activity,

product, and final body weights – can we fully understand the process and how to affect it.

		LF-SAT		HF-SAT		LF-POLY		HF-POLY		LF-MONO		HF-MONO	
	Product#												
	%	Gm	kcal	gm	*kcal*	gm	*kcal*	gm	*kcal*	gm	*kcal*	gm	kcal
	Protein	14.2	15	16.6	15	14.2	15	16.6	15	14.2	15	16.6	15
	Carbohydrate	72.1	76	52.0	46	72.1	76	52.0	46	72.1	76	52.0	46
	Fat	4.0	9	20.0	40	4.0	9	20.0	40	4.0	9	20.0	40
	Total		100		100		100		100		100		100
	kcal/gm	3.81		4.54		3.81		4.54		3.81			
kcal/gm	**Ingredient**	**Gm**	***kcal***	**gm**	***kcal***	**gm**	***kcal***	**gm**	***kcal***	**gm**	***kcal***	**gm**	kcal
4	Casein, 80 Mesh	140	*560*	164	*656*	140	*560*	164	*656*	140	*560*	164	656
4	L-Cystine	1.8	*7*	2.1	*8*	1.8	*7*	2.1	*8*	1.8	*7*	2.1	8
4	Corn Starch	485.7	*1943*	303.1	*1212*	485.7	*1943*	303.1	*1212*	485.7	*1943*	303.1	1212
4	Maltodextrin 10	125	*500*	115	*460*	125	*500*	115	*460*	125	*500*	115	460
4	Sucrose	100	*400*	89.9	*360*	100	*400*	89.9	*360*	100	*400*	89.9	360
0	Cellulose, BW200	50	*0*	58.6	*0*	50	*0*	58.6	*0*	50	*0*	58.6	0
9	Soybean Oil	10	*90*	10	*90*	10	*90*	10	*90*	10	*90*	10	90
9	Butter, Anhydrous	30	*270*	190	*1710*	0	*0*	0	*0*	0	*0*	0	0
9	Corn Oil	0	*0*	0	*0*	30	*270*	190	*1710*	0	*0*	0	0
9	Olive Oil	0	*0*	0	*0*	0	*0*	0	*0*	30	*270*	190	1710
0	AIN93 G Salts, S10022G	35	*0*	41	*0*	35	*0*	41	*0*	35	*0*	41	0
0	AIN93 M Salts, S10022M	10	*0*	11.7	*0*	10	*0*	11.7	*0*	10	*0*	11.7	0
4	AIN93 Vits, V10037	10	*40*	11.7	*47*	10	*40*	11.7	*47*	10	*40*	11.7	47
0	Choline Bitartrate	2.5	0	2.9	0	2.5	0	2.9	0	2.5	0	2.9	0
0	FD&C Yellow Dye #5	0	0	0.025	0	0.05	0	0	0	0	0	0.025	0
0	FD&C Red Dye #40	0.025	0	0	0	0	0	0.05	0	0	0	0.025	0
0	FD&C Blue Dye #1	0.025	0	0.025	0	0	0	0	0	0.05	0	0	0
	Total	1000	3810	1000	4543	1000	3810	1000	4543	1000	3810	1000	4543
	Vitamin E (IU/kg)	77.1		93.4		85.7		147.9		80.1		112.0	
	Vitamin E (IU/3810 kcal)	77.1		78.3		85.7		124.0		80.1		93.9	

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