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The Zucker Rat as a Model of Obesity-Hypertension

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THE ZUCKER RAT AS A MODEL OF OBESITY-HYPERTENSION

by

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ABSTRACT

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Hypertension is a serious health problem that affects approximately 1 in 4 American adults. Most cases are diagnosed as essential hypertension, meaning that the exact cause is unknown. In most patients, however, excess weight is a major contributory factor to the development of essential hypertension. The role of obesity in promoting hypertension is now well documented and has become the foundation for an entire field of research known alternately as obesity-hypertension, obesity-induced hypertension, or obesity-associated hypertension. In this field, rapid advances are being made in our understanding of how obesity and hypertension are linked. A plethora of related risk factors, mediators, and pathways have now been identified and described through the use of a variety of animal models. Nonetheless, the relationships between these factors and the extents to which they promote obesity-hypertension are often poorly understood. Unscrambling the various cardiovascular and metabolic determinants of obesity-hypertension remains a persistent problem because both obesity and hypertension typically exhibit polygenic etiologies and diet-dependent dynamics in humans. The judicious use of appropriate animal models promises to be an important tool in this quest. In addition, animal models are vital platforms for designing and evaluating approaches to the treatment of obesity-hypertension. One model of particular interest is the obese

Zucker rat, which develops extreme obesity but only develops slight, if any, hypertension. The primary objective of the research in this dissertation was to determine whether obese rats were more sensitive to experimental conditions known to induce hypertension than age- and gender-matched lean controls. Lean and obese Zucker rats were subjected to two experimental protocols that are known to induce hypertension in other rodent strains: (1) the administration of deoxycorticosterone-acetate and salt to uninephrectomized rats and (2) feeding rats a moderately high fat, salt-supplemented (MHF-SS) diet. In each case, the obese Zucker rat was found to be more susceptible to the development of hypertension. A second goal of this research was to ascertain a possible mechanism through which the MHF-SS diet might induce hypertension in obese Zucker rats. In this regard, dysfunction of the nitric oxide system, particularly in the kidney, was implicated in the pathogenesis of the MHF-SS diet in obese Zucker rats. In conclusion, this research suggests that the obese Zucker rat is labile with regard to blood pressure and that renal nitric oxide dysfunction may promote hypertension when obese Zucker rats are fed a moderately high fat, high salt diet.

DEDICATION

To my dearest family, for giving me fortitude:

Barbara, my faithful wife (or soon to be, anyways, I hope)

Cody, my wonderful son

Robby, Patrick, and Paul, my trusty brothers

Robert and Kaye, my devoted parents

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LIST OF SYMBOLS / NOMENCLATURE

1K1C – one kidney, one clip, a model of renin-dependent hypertension

AME – apparent mineralocorticoid excess, a rare monogenic form of human hypertension

DOCA – deoxycorticosterone acetate, a mineralocorticoid

DST – deoxycorticosterone acetate-salt-treated

DST-OZR – deoxycorticosterone acetate-salt-treated obese Zucker rat

fa/fa – genotype of obese Zucker Rats

Fa/? – genotype of lean Zucker rats

GFR – glomerular filtration rate

HandE – hematoxylin and eosin staining

HCl – hydrochloric acid

HF-OZR – obese Zucker rats fed a moderately high fat, salt-supplemented diet

IP – intraperitoneal

IV – intravenous

L-NAME – N-Nitro-L-Arginine Methyl Ester, a nonselective inhibitor of NOS

LC – lean Zucker rats fed a control diet (Purina 5001)

LHF – lean Zucker rats fed a high fat diet (formula D12266B, see table 4.1)

LZR – lean Zucker rat

MHF-SS – moderately high fat, salt-supplemented diet

MSG – monosodium glutamate, which can be injected to induce obesity

NaCl – sodium chloride

NAG – N-acetyl-β-D-glucosaminidase, a lysosomal enzyme and marker of tubular damage

NO – nitric oxide

NOS – nitric oxide synthase

OC – obese Zucker rats fed a control diet (Purina 5001)

OHF – obese Zucker rats fed a high fat diet (formula D12266B, see table 4.1)

OZR – obese Zucker rat

PAS – periodic acid-Schiff staining

RAS – rennin angiotensin system

SBP – systolic blood pressure

SDS-PAGE – sodium dodecyl sulfate polyacrylamide gel electrophoresis

SHR – spontaneously hypertensive rat

SHROB – spontaneously hypertensive obese rat, developed by Richard Koletsky

SNS – sympathetic nervous system

US – United States

PREFACE

The primary research in this dissertation is composed of two published, peer-reviewed manuscripts (chapters 2 and 3) and a manuscript that will be submitted for publication (chapter 4). These manuscripts are summarized and expanded on in the conclusions (chapter 5). In addition, sections of the literature review (chapter 1) may eventually be revised and submitted for publication. The literature review establishes the importance of animal models to the study of obesity-hypertension and briefly highlights the advances being made with popular models. Chapters 2 and 3 describe the development of the deoxycorticosterone acetate-salt treated obese Zucker rat model, while chapter 4 discusses a model using a moderately high fat, salt-supplemented diet in the obese Zucker rat.

CHAPTER I

Literature Review

Introduction

Obesity is now recognized by the World Health Organization as a critical global health problem (WHO, 2000). Recently, the prevalence of obesity has skyrocketed in the United States and other industrialized countries. According to the National Health and Nutrition Examination Studies (NHANES), obesity levels in the United States grew from about 14% to nearly 31% from 1980 to 2000 (Flegal et al., 2002). Despite increased awareness, the prevalence of obesity has continued to rise and we now face a worldwide epidemic of obesity-related health problems. To make matters worse, children are being increasingly affected (Sorof et al., 2004) and clinical interventions that promote population-wide weight control have been generally ineffective. Consequently, pressure to develop our understanding of obesity and how it contributes to other disease states is quickly mounting.

Because the prevalence of obesity has risen so rapidly, it seems clear that environmental factors are ushering in this change. In particular, the widespread adoption of high fat diets (Lissner and Heitmann, 1995) and sedentary lifestyles have been implicated

(Williamson et al., 1993). This precipitating effect of lifestyle changes on the increasing incidence of obesity, however, does not rule out the potential of a strong genetic component in the development of obesity. To the contrary, an environment of nutrient excess may allow for the full expression of obesity promoting genes that were previously masked when nutrients were less abundant. It is likely that genes that participate in determining energy balance, metabolism, and some behavioral traits are cooperating with environmental changes to ultimately regulate, or fail to regulate, weight gain. In much the same way that an individual's complex genetic background determines susceptibility to obesity, genetic background determines the susceptibility of obese individuals to a number of obesity-associated comorbidities as well.

Obesity has been defined in various ways. Clinicians in the United States have historically used height-weight based mortality data from the Metropolitan Life Insurance Company to define obesity. Currently, the body mass index (BMI) is the most widely accepted standard (Kushner, 1993). BMI is defined as weight in kg divided by height in meters, squared. Since cardiovascular risks begin to increase substantially when BMI increases above 25, a BMI greater than 25 is typically used to define clinically overweight individuals. Nearly two thirds of the US population (64%) is overweight by this definition (Flegal et al., 2002). Admittedly, this method often inappropriately classifies muscular individuals as clinically overweight. Consequently, methods that assess adipose tissue mass and distribution more accurately than BMI may constitute the future standard. Regardless of how obesity is defined, there is abundant evidence to indicate that excessive body weight is associated with elevated mortality rates. The

magnitude of this obesity-associated risk is further influenced by age, gender, and the pattern of fat distribution (Kushner, 1993). For example, abdominal obesity appears to disproportionately increase the risk of developing certain comorbidities such as cardiovascular disease (Sundquist et al., 2001).

Paralleling the rise in obesity, the prevalence of hypertension has also increased in the last decade. Roughly 29% of the US population over the age of 18 and 65% of the US population over the age of 60 is hypertensive based on the criteria of a blood pressure in excess of 140/90 mmHg or use of hypertensive medications (Hajjar and Kotchen, 2003). The rise in the prevalence of obesity is a chief factor in the increased incidence of hypertension (Care, 2005), which can lead to stroke, heart failure, kidney failure, and other serious health complications.

Obesity and hypertension are common in developed countries, but the rate of co-occurrence of these two disorders in a single individual cannot be explained by mere random coincidence (Hsueh and Buchanan, 1994). Hypertension occurs in 25% to 50% of obese individuals, which is a significantly higher prevalence than in the general population (Bell, 1990). According to risk estimates from the Framingham Heart Study, obesity is linked to about 75% of male and about 65% of female cases of hypertension (Davy and Hall, 2004; Garrison et al., 1987). Even in patients who are not obese, modest changes in body weight have been positively correlated with blood pressure, although individuals vary in their susceptibility to this effect (Davy and Hall, 2004).

Genetic factors such as ethnicity can determine whether obesity will lead to hypertension. Pima Indians have a high rate of obesity, but a low rate of hypertension (Weyer et al., 2000). In addition, hypertension is more prevalent in black men than white men, despite comparable levels of obesity (Abate et al., 2001). Similarly, genetically obese animals vary in their predisposition to hypertension depending on the specific strain being studied. The variation may depend on the specific mechanisms underlying the development of obesity (Mark et al., 1999) or on blood pressure control mechanisms.

Although the connection between obesity and hypertension was recognized nearly a century ago (Antic et al., 2003), we are still at a relatively early stage in achieving the goal of fully understanding the complex neural, cardiovascular, and hormonal pathways that lead from obesity to hypertension. The pace of advance is accelerating, however, in the wake of a growing multitude of manipulations and mutations available in animals for modeling and investigating human obesity-associated hypertension. Even now, existing animal models have provided a foundation of knowledge and hold the potential to unscramble many of the cardiovascular and metabolic determinants of obesity-hypertension. Additionally, novel animal models are rapidly emerging as valuable tools in the quest to discover and evaluate the genetic mutations and pathophysiological pathways that contribute to complex cardiovascular disorders such as obesity-hypertension.

In humans, both obesity *per se* and hypertension *per se* are typically caused by complex interactions of multiple environmental and genetic factors, resulting in a multiplicity of

etiologies for each disease. Thus, neither obesity-hypertension nor its components (obesity and essential hypertension) can be completely explained by a single pathophysiological mechanism. More likely, a plethora of convoluted pathways with varying levels of significance will need to be elucidated in order to fully comprehend the role of obesity in promoting hypertension.

It has been previously reported that the establishment of cause-and-effect relations in the field of obesity-hypertension is hindered by a shortage of suitable animal models, which, if available, would allow monitoring of the sequence of renal, cardiovascular, and endocrinological functional changes during the development of obesity (Hall et al., 1993). Meaningful advances in the field of obesity-hypertension depend upon the development of an assortment of animal models that have characteristics in common with those observed in obesity-hypertension in humans. These advances may be facilitated by sophisticated techniques that are designed to evaluate complex networks of gene interactions, such as the integrated genomics approach that was proposed by Schadt *et al.* (2005). Alternative statistical models such as factor analysis or structural equation modeling might also be required to unravel the complex network of pathways than link obesity to hypertension (Chan et al., 2002). Meta-analyses, which are performed by synthesizing research results from previously separate, yet related, studies, can now compile past and current research from diverse model-specific studies. As our knowledge of the mechanistic basis of obesity-hypertension advances, new potential targets for treatment may emerge.

Some of the factors that have been implicated in obesity-hypertension are represented in the disorder known alternately as the insulin resistance syndrome, the metabolic (cardiovascular) syndrome (X), and/or the "deadly quartet." Although this syndrome has been defined in various ways, obesity and hypertension are central and persistent elements. Junquero *et al.* (2005) recently summarized the varying syndrome definitions as "the clustering of moderate troubles of glucose, lipid metabolism, body weight, hypertension and vascular inflammation." The most commonly used clinical definition seems to be The National Cholesterol Education Program's Adult Treatment Panel III report (ATP III), which lists the characteristics of the metabolic syndrome as abdominal obesity , atherogenic dyslipidemia, elevated blood pressure, insulin resistance, a proinflammatory state, and a prothrombotic state. This clustering is well documented in humans (Aizawa et al., 2005) and is present in many of the animal models of obesity-hypertension, illustrating both the multifactorial nature of obesity-hypertension and the highly conserved nature of these dysfunctional metabolic relationships in mammals. While obesity may play the most pivotal role in promoting hypertension, it is believed that each of the aforementioned clustering elements can play a role in the advancement of blood pressure dysregulation (Morse et al., 2005). Thus, models that overexpress or lack these clustering elements may be invaluable for discerning how their association with obesity may play a role in promoting hypertension.

Models of Obesity-hypertension: An Overview

An ideal animal model for a disease "closely represents most or all of its pathophysiological characteristics (Tschop and Heiman, 2001)." Although a number of factors have been implicated, the pathophysiological characteristics of obesity-hypertension have not been clearly defined yet. Moreover, no single animal model is universally representative of obesity-hypertension, although some models exhibit many of the aforementioned clustering factors. Hence, nearly any animal that expresses obesity and hypertension may serve as a viable research tool, even if obesity and hypertension are not coupled. At this juncture, it appears that the most practical approach to the study of obesity-hypertension would be to use a wide range of species/models.

Fortunately, experimental obesity has now been produced in a large assortment of rodents using dietary manipulation, chemical and surgical treatments, selective breeding, and/or genetic manipulation (Tschop and Heiman, 2001). Many, but not all, of these models spontaneously develop hypertension as they age and gain weight. Such models are naturally suited for the study of obesity-hypertension. In addition, many of the remaining models of obesity may still be developed into models of obesity-hypertension by applying additional hypertensive influences. For example, results from our laboratory and others indicate that obese rodents may be more vulnerable to the hypertensive influence of salt than their lean counterparts (Dobrian et al., 2003; Morrison et al., 2002; Radin et al., 2003). This association between obesity and salt-sensitivity of blood pressure is also evident in humans and other animals, although the exact mechanism linking these conditions is not fully understood (Rocchini, 2000).

Besides salt, several other environmental stimuli may be useful as tools for developing models of obesity-hypertension. For example, high protein intake has been positively associated with changes in blood pressure in humans (Suter et al., 2002). Alternatively, reducing the metabolic rate by elevating ambient temperatures or increasing caloric intake with palatable diets can directly promote obesity (Tschop and Heiman, 2001), thereby indirectly promoting hypertension. Diets that are high in fat and refined carbohydrates have been shown to promote both obesity and hypertension in inbred rats (Barnard et al., 1993). Moreover, saturated fats and refined carbohydrates have been associated with salt retention and hypertension in humans (Preuss, 1997). Fortunately, researchers now benefit from the increasing commercial availability of standardized and purified diets with adaptable macronutrient compositions.

Numerous factors determine the most appropriate model selection for an investigator: research scope and objectives, institutional resources, cultural biases regarding species of animals, and individual investigators' preferences. In the US, rodents have been the dominant species adopted for research (FBR, 2006; Jacoby, 1998). They feature low breeding and maintenance costs (Fitzgerald, 1983), easy handling and feeding, short lifespans, wide commercial availability, minimal cultural backlash, and a well-understood physiology. For that reason, this overview focuses primarily on rodents. However, it should be noted that excellent models of obesity-hypertension have been developed using dogs (Rocchini et al., 1987) and rabbits (Carroll et al., 1996) and may be developed from other animals as well.

When using an animal model, careful consideration has to be given to factors such as age and gender when designing experiments and interpreting data. For example, it is known that gender can have a significant effect on susceptibility to blood pressure elevation in rodents; however, the exact nature of the effect varies across strains (Herrera and Ruiz-Opazo, 2005). Susceptibility to hypertension is also influenced by age in rodents, which generally have lifespans of about two years. A good time to initiate many studies may be at about 100 days, when rats cease to gain lean mass, but when fat mass may still be accrued (Tschop and Heiman, 2001) Aging in rats may result in increased susceptibility to hypertension (Ren et al., 1999). This is consistent with human observations, where the prevalence of hypertension and obesity have been shown to increase with age (Bray and Gray, 1988; Kannel et al., 1967). This association between obesity and hypertension in humans, however, tends to weakens with advanced age (Van Itallie, 1985).

Another important general consideration with respect to rodent models is the breeding source and method. Inbred and outbred strains that conform to established standards of breeding are widely available from reputable commercial vendors. Outbred strains have greater genetic diversity, making them well suited as a model for pharmacological studies that attempt to understand how a drug will behave in a diverse population. Inbred animals have a high occurrence rate of potentially harmful and confounding homozygous recessive mutations. Furthermore, inbred animals are subject to genetic homeostasis (Hartl, 2000), a paradoxical effect which leads to a counter intuitive elevated variance with respect to many of the measurable quantitative characteristics in inbred rodents

(Phelan and Austad, 1994). Despite this drawback, inbred animals still have distinct advantages. They allow reliable comparison of data across laboratories because the population is genomically stable and can be repeatedly accessed. The fixed genotypes of inbred animals also allow investigators to isolate the influence of environment from unknown genetic variations that may play a role in determining phenotype. Perhaps most importantly, these models can be rapidly developed and are an invaluable tool for functional genomics, as demonstrated by Svenson *et al.* (2003).

Model Categories

Rodents that develop obesity as a result of a single gene mutation can still be outbred to maintain genetic diversity throughout the rest of their genetic background (e.g. the obese Zucker rat). These outbred monogenic models of obesity provide an excellent source for well-controlled, reproducible analysis of specific pathophysiologic links between obesity and hypertension. In addition, they provide a unique opportunity to study the individual contributions of specific metabolic dysfunctions in obesity-hypertension and are invaluable for testing translational clinical advances that relate to rare monogenic forms of obesity-hypertension.

Rodent models with hypertension resulting from polygenic obesity are in some ways the most relevant for studying human obesity-hypertension. In particular, such models are well-suited for the investigation of generalized therapeutic approaches to obesity-hypertension. As demonstrated by Tsukahara *et al.* (2004), intercrossing strains with specific "clustered" phenotypes that are associated with hypertension is also a useful tool

for understanding polygenic hypertension. Conversely, this study indicated that, in some cases, the development of obesity and the development of hypertension are not genetically associated in some inbred mouse models of obesity-hypertension.

In order to simplify discussion of the strengths and weaknesses of individual models, they are classified in this section according to the following schema, which was loosely based on the system used by Sun and Zhang to review animal models of hypertension *per se* (Sun and Zhang, 2005). The models of obesity-hypertension are categorized as genetic, dietary, or treatment models, depending on the methods used to produce the model and the underlying basis of obesity-hypertension. These categories are given original definitions so that all existing models of obesity-hypertension fall into a specific category. Of course, it may be possible to develop a hybrid model that does not fit neatly into any single category. In addition, models of obesity-hypertension based on environmental factors such as temperature change or stress induction have not been developed, so they are not given a category, although it may be possible to develop such a model.

Genetic Models

Genetic models of obesity-hypertension spontaneously develop both obesity and hypertension on standard laboratory diets as a result of their genetic makeup. Such models can be produced with targeted mutagenesis, transgenics, or selective breeding of spontaneous mutants. These are the most popular and widely used models, probably because they generally require the least technical input, the role of confounding factors is

minimized, and research can be focused on the mechanisms that explain how specific mutations give rise to obesity and hypertension. Currently, the obesity component in most of the genetic models is based on disorders of leptin signaling and transduction. Prominent examples of genetic models with leptin dysfunction include the fa/fa rat, ZSF1 rat, SHROB rat, and ob/ob mouse.

The Spontaneously Hypertensive Obese Rat (SHROB)

The spontaneously hypertensive obese rat (SHROB; Koletsky) was developed by Richard Koletsky at Case Western Reserve Medical center after he discovered a spontaneous mutation, designated corpulent (cp), that results in a shortened and completely nonfunctional leptin receptor. The cp mutation was crossed onto the spontaneously hypertensive rat (SHR/N) background, resulting in the SHROB (Greenhouse, 1990). In the SHROB, hypertension and obesity are caused by independent genetic mutations. Studies showing that SHROBs are not any more hypertensive than SHR/Ns suggest that genetically determined hypertension is not exacerbated by genetically determined obesity in this model (Ernsberger et al., 1999b). Since the SHROBs also exhibit severe hyperinsulinemia and insulin resistance, this blood pressure data also suggests insulin resistance does not contribute to hypertension in this model (Friedman et al., 1997). The decoupling of obesity and hypertension in the SHROB may preclude its use as a model for identifying specific metabolic factors that link obesity to hypertension. Nevertheless, the SHROB has proven to be quite useful as a tool for investigating pharmacological approaches for the treatment of obesity-hypertension (Ernsberger et al., 1997; Ernsberger et al., 1999a; Friedman et al., 1998; Koletsky et al., 2003; Mukaddam-Daher et al., 2003)

and is considered by some to be a good model for metabolic syndrome X (Ernsberger et al., 1999a; Velliquette and Ernsberger, 2003).

Obese Zucker Rat (OZR)

The obese Zucker rat (OZR) was discovered by Drs. Theodore and Lois Zucker after a spontaneous mutation, designated fatty (fa), occurred in an outbred stock of their rats (Greenhouse, 1990). The mutation, located in the gene for the leptin receptor (Takaya et al., 1996), is autosomal recessively inherited. Severe obesity results because the rat, which has a diminished ability to respond to leptin, becomes hyperphagic.

Conflicting data exists as to whether the obese Zucker rat is actually hypertensive. Some investigators have reported that the OZR is not hypertensive relative to lean Zucker rats (Bunag and Barringer, 1988; Morgan et al., 1995; Pamidimukkala and Jandhyala, 1996; Pawloski et al., 1992; Reddy and Kotchen, 1992), whereas other investigators have indicated that the OZR is hypertensive, although the degree of hypertension reported is generally mild (Alonso-Galicia et al., 1996; Ambrozy et al., 1991; Carlson et al., 2000; Kurtz et al., 1989; Maher et al., 1995; Turner et al., 1995). The OZR appears to be slightly salt-sensitive (Carlson et al., 2000; Reddy and Kotchen, 1992) and experiments in our laboratory have shown that this sensitivity can be exacerbated by DOCA and uninephrectomy (Morrison et al., 2002) or a high fat diet (not submitted for publication yet). The OZR also develops marked insulin resistance, hyperinsulinemia, hypertriglyceridemia, and hypercholesterolemia. In addition, the renin-angiotensin system may play a role in mediating hypertension in this model (Alonso-Galicia et al., 1996).

Wistar Fatty (WFR)

The fatty gene that spontaneously arose in obese Zucker rats was backcrossed onto outbred Wistar rats by Dr. Hitoshi Ikeda at Takeda Chemical Industries in Osaka, Japan (Greenhouse, 1990). The Wistar fatty rat (WFR) becomes spontaneously hypertensive, hyperglycemic, and hyperinsulinemic in association with increased sympathetic nervous system activity (Suzuki et al., 1999; Yamakawa et al., 1995). In addition, the WFR also exhibits salt sensitivity of blood pressure (Hayashida et al., 2001) and a shifted pressure natriuresis curve (Suzuki et al., 1996). Unfortunately, the American colony of WFRs at Indiana University Medical Center was lost (Greenhouse, 1990) and it seems that research on this strain is limited primarily to Japan.

11-β-Hydroxysteroid Dehydrogenase type 1 (11βHSD1) transgenic mice

In humans, 11-beta-hydroxysteroid dehydrogenase type 1 (11βHSD1) converts inactive cortisone to biologically active cortisol (Dotsch and Rascher, 2002). In rodents, 11βHSD1 converts inactive 11-dehydrocorticosterone to active corticosterone, the major rodent glucocorticoid (Draper and Stewart, 2005). Thus, the main function of 11βHSD1 is to convert specific inactive glucocorticoid metabolites to glucocorticoid metabolites that can activate the glucocorticoid receptor (Draper and Stewart, 2005). Glucocorticoids play an important role in fat metabolism and in the distribution of adipose tissue.

11βHSD1 has been linked to obesity and insulin resistance in humans (Draper and Stewart, 2005). Jeffrey Flier's group in Boston recently developed a strain of transgenic mice that overexpress 11βHSD1. These mice developed visceral obesity and exhibited other symptoms of the metabolic syndrome (Masuzaki et al., 2001). They also developed

salt-sensitive hypertension that may be mediated by the renin-angiotensin system (Masuzaki et al., 2003).

New Zealand Obese Mice (NZO)

New Zealand obese (NZO) mice were developed by selectively breeding obese agouti mice for 5 generations (Giesen et al., 2003). The NZO spontaneously develops obesity and hypertension, as well as insulin resistance, hypertriglyceridemia, and hypercholesterolemia (Ortlepp et al., 2000). Diabetes develops by six to twelve months in the NZO due to a loss of pancreatic β-cells (Plum et al., 2002). Moreover, high fat feeding accelerates or worsens many of the pathophysiological symptoms that are exhibited by the NZO, suggesting that the NZO may be useful in assessing the role of diet in the development of obesity-hypertension (Giesen et al., 2003; Plum et al., 2002).

Dietary Models

There are a number of commercially available diets that can produce obesity in certain susceptible rodents. In turn, these obesity-prone rodents may develop hypertension over a period of time. Dietary models develop obesity as a result of polygenic susceptibility to obesity and hypertension upon exposure to dietary manipulation. Thus, genetics plays a role in determining obesity and hypertension in these models, although this confounding factor may be controlled somewhat by using inbred animals. Animals that gain excessive weight on the special diets are considered to be genetically susceptible, whereas those which are resistant to weight gain are considered to be genetically resistant (Levin et al., 1987). Dietary models are perhaps the most relevant type of model because they closely

resemble the etiology of human obesity-hypertension. In particular, high fat feeding produces obesity that "closely mimics the metabolic, neurohumoral, renal, and cardiovascular changes observed in obese humans" (Hall et al., 2003b). Dietary models have the potential to become a powerful tool for elucidating the complex interplay between genetics and environment that determines whether obesity produces hypertension. In addition, these models can be used to discover previously unconsidered obesity-hypertension genes and markers and to investigate dietary and therapeutic approaches for the treatment of obesity-hypertension. Examples of dietary models of obesity-hypertension include the purified moderately high fat diet used by Dobrian *et al.* (2000) and the high-fat refined carbohydrate diets used by Barnard, Roberts and associates (Barnard et al., 1993). These models have thus far tended to show the importance of oxidative stress (Dobrian et al., 2001; Roberts et al., 2005) and nitric oxide dysfunction (Dobrian et al., 2001; Roberts et al., 2003) as a factor in obesity-hypertension.

Treatment Models

Treatment models use surgical manipulations or chemical injections to produce or exacerbate obesity and/or hypertension. Of course, genetic background still plays an important role in determining susceptibility, just as in dietary models. Hypertension can be experimentally induced using a wide array of surgical and chemical methods, including clipping the renal arteries, wrapping the kidneys, injecting salt retaining agents into uninephrectomized rats (e.g DOCA), and by administering NOS inhibiting agents such (e.g. L-NAME) (Sun and Zhang, 2005).

Animal models of obesity have been developed using a variety of chemical and surgical means. For example, obesity can be produced by radiofrequency, electrolytically, or surgical lesions the paraventricular or the ventromedial nuclei of the hypothalamus. Also, a number of chemical agents, including colchicine, kainic acid, ibotenic acid, monosodium glutamate, piperidyl mustard, goldthioglucose, and monosodium glutamate can be administered to produce obesity (Tschop and Heiman, 2001). These methods have been used primarily to study obesity *per se* but it may be possible to apply these techniques with different strains and diets to develop new models of obesity-hypertension.

DOCA-salt treated obese Zucker rat (DST-OZR)

Our lab has recently used DOCA-salt treatment of obese Zucker rats (DST-OZR) to determine whether obesity increases the sensitivity of rats to experimentally induced hypertension. DST-OZRs become severely hypertensive within a few weeks of initiating treatment, whereas genetically lean Zucker rats were far less affected (Morrison et al., 2002). Furthermore, OZRs developed more extensive glomerulosclerotic kidney damage than LZRs, with or without DOCA-salt treatment (Morrison et al., 2005). This is consistent with reports that classify obesity as a risk factor for nephropathy, especially focal segmental glomerulosclerosis (Cohen, 1999). Additionally, obesity is known to aggravate renal dysfunction in humans (Sasatomi et al., 2001). With respect to this, we have observed significant proteinuria in the DST-OZR, indicating that functional damage to the kidney takes place in these animals (Morrison et al., 2005). It is currently unclear,

however, whether these changes are caused by obesity itself or by the associated hypertension and hyperlipidemia (Sasatomi et al., 2001).

The DST-OZR model is described in chapters 2 and 3. This model effectively illustrates the susceptibility of obese rats to hypertensive pressures. It may also be well suited for studying how obesity affects the rate of end organ damage in malignant hypertensive states (Morrison et al., 2005).

Monosodium Glutamate Injected Hypertensive Rats

Monosodium glutamate (MSG) has been used to create models of obesity-hypertension from known models of hypertension *per se*, including the SHR (van den Buuse et al., 1985) and the 1-kidney, 1-clip hypertensive (1K1C) rat (Correa and Saavedra, 1992). MSG is injected into the bodies of neonatal rats, where it travels to the hypothalamus and overexcites neurons to the point of cell death, causing an obesity producing lesion. Despite the independence of obesity and hypertension in the MSG injected SHR, this model still displays at least a weak relationship between obesity and hypertension. For example, blood pressure is lower in MSG-treated SHR than in control SHR (van den Buuse et al., 1985), whereas it may not be changed by MSG treatment in 1K1C rats (Correa and Saavedra, 1992). Interestingly, MSG seems to increase susceptibility to weight gain in the SHR, but this effect does not appear to be associated with changes in leptin (Iwase et al., 2000). The vast majority of research with this model has focused on the effects of MSG-induced obesity on the SHR (Hambley et al., 1987; Iwase et al., 2000; Iwase et al., 1998; Mosqueda-Garcia et al., 1986; Ross et al., 1993; van den Buuse et al., 1985).

Proposed Mechanisms of Obesity-hypertension

According to Bray, obesity-hypertension is caused by metabolic changes that are associated with excessive fat cell growth (Bray, 2004). These changes may produce hypertensive pressures directly as in the case of obesity-associated sympathetic nervous system activation or they may reduce the subject's ability to handle other hypertensive pressures (e.g. obesity-associated endothelial dysfunction). The latter case suggests that

models of hypertension generated by making genetically hypertensive rats obese can be useful even when hypertension is not induced by obesity *per se* (e.g. DST-OZR, SHROB).

Studies in animal models have led to the proposal of a wide array of mechanisms that may explain obesity-hypertension. These proposed mechanisms will be briefly reviewed in this section. Since obese hypertensives do not always exhibit all of the clustering factors that are implicated in these mechanisms, the explanatory power of any individual mechanism is limited. Moreover, multiple obesity-associated abnormalities may be required in an individual before hypertension can ultimately develop.

Hyperinsulinemia/Insulin Resistance

Hyperinsulinemia has been repeatedly cited as the primary metabolic derangement underlying the association between obesity and hypertension (Ljutic and Korsic, 1993; Zemel, 1995). Obesity promotes insulin resistance, which is widely believed to cause compensatory hyperinsulinemia in an effort to sustain euglycemia (Olefsky et al., 1982). Studies indicate that insulin resistance and compensatory hyperinsulinemia promote increased sympathetic nervous system activity, renin-angiotensin system activity, and vascular smooth muscle cell proliferation (Imazu, 2002). Both insulin resistance *per se* and hyperinsulinemia have been blamed for increasing peripheral vascular resistance and blood pressure (Zemel, 1995). Insulin resistance *per se* may lead to hypertension because diminished insulin-stimulated Ca^{2+} ATPase activity retards the extrusion of Ca^{2+} from vascular smooth muscle cells (Rocchini, 1991; Zemel et al., 1992). Also, hyperinsulinemia may promote hypervolemia and high blood pressures by directly

inducing renal sodium retention and elevating neural sympathetic output (Ljutic and Korsic, 1993; Rowe et al., 1981; Zemel, 1995). On the other hand, results of other studies suggest a very different role for insulin. For example, there is substantial evidence to indicate insulin is actually a vasodilator (Zemel, 1995). Insulin has been reported to promote NO production and have antihypertensive effects in Zucker diabetic rats (Kawaguchi et al., 2001).

Recently, attention has shifted away from the notion that diminished insulin action on the vascular endothelium can explain hypertension. One reason is that mice lacking insulin receptors in the vascular endothelium have reduced blood pressures, even on a high-salt diet (Nandi et al., 2004). Hall *et al.* (2003) have argued that the kidney is the primary long-term controller of blood pressure in obesity-hypertension, and attention has turned to the direct sodium retaining effects of insulin on the kidney. The mechanism for this direct effect is not known, but insulin appears to increase sodium reabsorption in the distal tubule and decrease it in the proximal tubule (Stenvinkel et al., 1992). Overall, the net effect of insulin is to promote sodium reabsorption and salt-sensitivity of blood pressure (Sechi, 1999). Nevertheless, studies by Hall *et al.* (1993; 1992) suggest that the effects of insulin may be insufficient to produce chronic hypertension.

Hyperleptinemia/Selective Leptin Resistance

The fact that obese Koletsky and Zucker rats become hypertensive, despite a reduced ability or complete inability to respond to leptin, suggests that leptin is not required for obesity and hypertension to associate. Nonetheless, there is growing evidence that leptin may function as a pathophysiological link between obesity and hypertension (Mukherjee

et al., 2006). For example, transgenic mice that overexpress leptin have increased blood pressure relative to wild-type controls, despite having a lower body weight (Aizawa-Abe et al., 2000).

Leptin is an appetite-regulating cytokine that is secreted from white adipose tissue in proportion to adipose tissue mass (Considine, 2005). As a result, the plasma concentration of leptin is elevated distinctly in obese humans (Considine et al., 1996) and rats (Tulipano et al., 2004). Substantial evidence from rodents indicates that leptin chronically activates the sympathetic nervous system (SNS) and increases blood pressure (Correia et al., 2001). Chronic hypertension that is induced by leptin can be completely abolished by adrenergic blockade, indicating that the hypertensive actions of leptin are mediated by adrenergic activation (Carlyle et al., 2002). In addition, leptin may also promote hypertension by increasing reactive oxygen species and upregulating endothelin-1 production (Luo et al., 2005). Just as with insulin, though, leptin triggers counteracting hypotensive mechanisms which obfuscate its overall effect on blood pressure. Leptin induces endothelium-dependent vasorelaxation (Kimura et al., 2000; Lembo et al., 2000) and stimulates natriuresis (Jackson and Li, 1997; Villarreal et al., 1998). Leptin also appears to elevate NO metabolites in the plasma, suggesting that its hypotensive actions may be NO dependent (Beltowski et al., 2002; Fruhbeck, 1999).

Selective leptin resistance has been suggested as the causative factor for metabolic and cardiovascular dysfunctions associated with obesity, but the mechanisms that lead to this resistance are still unknown (Ren, 2004). If tissue resistances to leptin are specific for its

hypotensive actions but not its sympathoexcitatory actions, then leptin could potentially induce hypertension. In this regard, Rahmouni *et al.* (2005) have demonstrated that obese mice are resistant to the metabolic effects of leptin, while the stimulatory effect of leptin on renal sympathetic nervous activity is still preserved.

Sympathetic nervous system

It has been hypothesized that obesity may activate the sympathetic nervous system (SNS), a prohypertensive influence, in order to reduce or prevent further weight gain (Landsberg, 1992). This seems like a plausible method by which the body could expend excess energy and thereby diminish fat accumulation. As attractive as this hypothesis is, it has not been consistently supported by reports of SNS activity in obesity (Hsueh and Buchanan, 1994; Young and Macdonald, 1992). Substantial evidence now exists supporting several opposing views: (1) individuals develop obesity as a result of dysfunctionally lowered SNS activity, (2) SNS activity is increased to compensate for obesity (promoting hypertension) and (3) SNS activity is not changed in obese individuals (Young and Macdonald, 1992). The discrepancies may be due to how and where SNS activity is assessed because there is considerable heterogeneity in sympathetic outputs to different organs (Hall et al., 2003a). Hall *et al.* (2003a) believe that renal sympathetic activity, which has been shown to increase in obese subjects, is the primary pathway by which sympathetic nervous activity leads to chronic hypertension. Esler, however, has shown that increased renal sympathetic activity is not sufficient to produce hypertension in obese patients (Esler, 2000). Still yet, increased sympathetic activity may be required in conjunction with other sodium retaining derangements in order for chronic hypertension to develop (Esler, 2000). In conclusion, the effect of

obesity on the SNS varies considerably for reasons that are not clear, even though leptin and insulin, which are both elevated in obesity, apparently stimulate the SNS.

Nitric Oxide (NO)

Nitric oxide (NO) function plays at least two important roles in blood pressure regulation. First, NO has a well known role in endothelial regulation of vascular tone. Disturbance of this physiological role could result in increased peripheral resistance, which is characteristic of all known forms of human and experimental hypertension (Kunes et al., 2004). Secondly, NO helps maintain sodium and water balance by acting as a key signaling molecule in pressure natriuresis, a key long term determinant of blood pressure (Evans et al., 2005). The exact role of nitric oxide in mediating pressure natriuresis, as well as other aspects of pressure natriuresis, is still poorly understood (Evans et al., 2005).

NO metabolism may be dysregulated by metabolic factors associated with obesity, including leptin resistance and insulin resistance. Studies have shown that NO production is increased by both leptin (Beltowski et al., 2002) and insulin (Steinberg et al., 1994) through the activation of phosphatidylinositol 3-kinase (Kim et al., 2000; Zeng et al., 2000). In insulin and leptin resistant states, however, leptin and insulin may be incapable of producing this effect (Jiang et al., 1999). Thus, selective insulin and leptin resistance might increase susceptibility to hypertension by inhibiting NO production but not the sympathoexcitatory actions of insulin and leptin. There is now extensive evidence indicating that the SNS and NO are typically well balanced counterparts of

blood pressure regulation and that this balance is altered in experimental hypertension (Kunes et al., 2004).

Obesity is also associated with oxidative stress, which may be due to extended postprandial hyperlipidemia and/or hyperglycemia (Dandona et al., 2001). Obese humans have increased levels of oxidative stress and this is ameliorated by diet restriction and weight loss (Dandona et al., 2001). Oxidative stress can lead to increased deactivation of NO, which may promote hypertension (Kunes et al., 2004).

Kidney Compression

Another mechanism which may explain obesity-hypertension is obesity-induced kidney compression that results in increased intrarenal pressure (Hall et al., 1998). This compression may be produced by intra-abdominal pressure increases (Bloomfield et al., 2000) and kidney encapsulation by retroperitoneal adipose tissue (Hall et al., 2002). In addition, obesity might also exacerbate kidney compression by promoting extracellular matrix expansion (Henegar et al., 2001; Kasiske et al., 1985). If the loops of Henle and vasa recta are compressed by mounting intrarenal fluid hydrostatic tissue pressure, then tubular water and sodium reabsorption could be increased (Hall et al., 2002). This might explain why abdominal obesity bears a high relative risk for hypertension, but it does not account for the rapid increase in arterial pressure that occurs with weight gain (Hall et al., 2003a).

Renin-Angiotensin System (RAS)

Some components of the renin-angiotensin system are noticeably activated in obese individuals, despite its association with sodium retention and plasma volume expansion. Plasma aldosterone, angiotensinogen, angiotensin-converting enzyme activity, and plasma renin activity have all been reported to be elevated in obesity-hypertension (Hall et al., 2002; Sharma et al., 2002). A role for the RAS is further supported by studies that show angiotensin II antagonists and angiotensin-converting enzyme inhibitors blunt sodium retention, plasma volume expansion, and blood pressure elevation in obese dogs and humans (Reisin et al., 1997; Rocchini, 2000).

Sodium Retention

According to Borst and Borst-de Geus, "The blood pressure will be maintained at the exact level required for the maintenance of sodium balance (Borst and Borst-de Geus, 1963)." Extensive evidence now indicates that sodium is at least partially responsible for producing hypertension (Altun and Arici, 2006), particularly when obesity is present (Antic et al., 2003). The underlying mechanisms for this phenomenon, however, have not been fully elucidated. Sodium retention leads to an expansion of extracellular and blood fluid volumes, which, in turn leads to an increase in blood pressure. As described earlier, numerous factors that are commonly found in obesity may promote sodium retention by amplifying renal tubular sodium reabsorption. These factors include kidney compression, RAS activation, and leptin-, insulin-, or free fatty acid-induced SNS activation (Antic et al., 2003; Wofford and Hall, 2004). If obesity were to cause a chronic increase in tubular sodium reabsorption, then the compensatory mechanism, known as pressure natriuresis, would be activated to prevent infinite volume expansion. In normal situations, high salt intake leads to only very slight, if any, blood volume

expansion because of pressure natriuresis (Faber, 1996). Thus, pressure natriuresis can normally raise sodium excretion enough to offset sodium intake with only slight increases in blood pressure. It is unclear how the blood pressure elevations are transduced into changes in renal sodium excretion, but they probably lead to the inhibition of tubular sodium reabsorption because glomerular filtration rate and renal plasma flow are autoregulated to remain constant over a wide range of blood pressures (Evans et al., 2005). One possible explanation for obesity-hypertension is that pressure natriuresis is impaired so that higher blood pressures are required to increase sodium excretion sufficiently to eliminate any given level of sodium intake (Hall, 2003). Other aspects of renal function might also contribute to obesity-hypertension. For example, renal autoregulation may not be completely functional in obese subjects as suggested by reports that the glomerular filtration rate is increased in obesity (Hall et al., 2001).

Renal Injury

There is substantial evidence suggesting that obesity significantly increases the risk for renal failure, perhaps more so than hypertension (Kincaid-Smith, 2004). For example, studies have indicated that obese patients are significantly more likely to develop proteinuria and a progressive loss of renal function (Morales et al., 2003). In one study, 92% of clinically obese patients who underwent unilateral nephrectomy developed proteinuria and renal insufficiency, but only 12% of nonobese patients developed these disorders (Praga et al., 2000). The mechanisms underlying obesity-induced renal injury are unclear, but increased caloric intake (Stern et al., 2001), hypercholesterolemia (Maddox et al., 2002), hyperglycemia, insulin resistance and high blood pressure have been implicated as factors (Kincaid-Smith, 2004). Extensive renal injury may be able to

promote hypertension by interfering with the sodium-handling ability of the kidney. The ability of the kidney to excrete sodium is directly proportion to the functional renal tissue mass; when functional mass of the kidney is reduced to 10% of normal, excretion of sodium at any given arterial pressure will be only 10% of normal (Faber, 1996).

Statement of Problem

It is clear that excess weight is a major contributory factor to the development of hypertension in most patients. In fact, this well documented relationship between obesity and hypertension has become the foundation for an entire field of research alternately known as obesity-hypertension, obesity-induced hypertension, or obesity-associated hypertension. In this field, rapid advances are being made in our understanding of how obesity and hypertension are linked. Moreover, a plethora of related risk factors, mediators, and pathways have now been identified and described. Nonetheless, the relationships between these factors and the extents to which they promote obesity-hypertension are often poorly understood. Unscrambling the various cardiovascular and metabolic determinants of obesity-hypertension remains a persistent problem because both obesity and hypertension typically exhibit polygenic etiologies and diet-dependent dynamics in humans. The judicious use of appropriate animal models promises to be an important tool in this quest. Robust animal models that represent various "clustered" metabolic disturbances (e.g. insulin resistance) allow investigators to advance the frontier of knowledge regarding the underlying pathophysiological relationships between obesity and hypertension. In addition, animal models are vital tools for designing and evaluating approaches to the treatment of obesity-hypertension.

The purpose of the research described in this dissertation was to assess the obese Zucker rat as a model for obesity-hypertension. One specific aim of the research project was to determine whether obese rats were more sensitive to experimental conditions known to induce hypertension than age- and gender-matched lean controls. This was achieved by subjecting lean and obese rats to two experimental protocols that are known to induce hypertension in other rodent strains: (1) the administration of DOCA-salt to uninephrectomized rats or (2) feeding rats a moderately high fat, high salt diet. A second specific aim of this project was to test possible mechanisms through which a high fat diet might induce hypertension in obese rats. Primarily, renal NO pathways were investigated to determine if dysfunction in the renal NO system may predispose obese rats to salt-sensitive hypertension. Additionally, leptin and insulin were assessed in an effort to understand their potential prohypertensive roles in obese Zucker rats given a high fat diet.

CHAPTER II

Increased Sensitivity of The Obese Zucker Rat to Deoxycorticosterone-Salt-Induced Hypertension

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Abstract

Objective: The objective of this study was to test the hypothesis that obesity increases the sensitivity of rats to experimentally induced hypertension.

Design and Methods: To induce hypertension, unilaterally nephrectomized lean and obese Zucker rats were injected with 25 mg/kg of deoxycorticosterone acetate (DOCA) twice weekly for 5 weeks and given 1% NaCl to drink. Unilaterally nephrectomized control rats were injected with vehicle and drank tap water. Systolic blood pressure (SBP) was measured by the tail cuff method. Renal histology and urinary albumin excretion were used to assess the effects of the experimental treatment on the kidney.

Results: Obese rats exhibited a significant rise in SBP four days after the start of DOCA-salt treatment. In contrast, SBP of DOCA-treated lean rats was not significantly elevated from pretreatment measurements until day 22. Moreover, SBP was significantly higher during the plateau phase of blood pressure development in obese DOCA-salt treated rats (196 mm Hg) than in correspondingly treated lean rats (150 mm Hg). Both obesity and DOCA-salt treatment promoted glomerulosclerosis and mild tubulointerstitial damage in the kidney with DOCA-salt treatment exacerbating the effect of obesity. Urinary albumin excretion was significantly greater in obese control rats compared to lean controls and in DOCA-treated obese rats relative to vehicle-treated obese rats.

Conclusion: Results of this study indicate that Obese Zucker rats are more sensitive to mineralocorticoid-induced hypertension than lean rats. This study provides experimental evidence supporting the epidemiological findings that obesity is a risk factor for the development of hypertension.

Key words: deoxycorticosterone acetate, hypertension, kidney, Obese Zucker Rat, obesity-associated hypertension

Introduction

Obesity is a significant risk factor for diseases such as hypertension, type II diabetes, coronary heart disease and a number of other conditions that adversely affect one's health (Pi-Sunyer, 1993). Consequently, it is becoming an increasingly important health issue in developed countries where the incidence of this condition has risen dramatically over the past few decades (Kuczmarski et al., 1994; Mokdad et al., 1999; WHO, 2000).

The link between body weight and blood pressure is well known and has been clearly documented in numerous epidemiological studies (Chiang et al., 1969). Evidence from these studies indicates that overweight and obese individuals have a higher risk of developing hypertension than lean individuals of the same age and gender, with the prevalence of hypertension increasing in proportion to the degree of overweight (Kannel et al., 1967).

To fully elucidate the mechanism(s) through which obesity can predispose an individual to the development of hypertension, suitable animal models are required. One animal model that has received considerable attention in this arena is the obese Zucker rat. Blood pressures of lean and obese rats have been compared in multiple studies with varied results. Some investigators have reported that there is no difference between blood pressures of lean and obese rats (Auguet et al., 1989; Bunag and Barringer, 1988; Lavaud et al., 1996; Levin et al., 1984; O'Donnell et al., 1985; Pamidimukkala and Jandhyala, 1996; Pawloski et al., 1992; Todd and Abernethy, 1986) whereas others have concluded that blood pressure in the obese rat is elevated relative to its age- and sex-matched lean

counterpart (Alonso-Galicia et al., 1996; Carlson et al., 2000; Kasiske et al., 1985; Kurtz et al., 1989; Paradise et al., 1985; Wickler et al., 1982; Zemel et al., 1990). The fact that elevated blood pressures can be recorded for the obese Zucker rat in some laboratories but not in others when measurements were made under comparable conditions may indicate that these animals have labile blood pressure regulatory mechanisms that are sensitive to subtle differences in environment and/or experimental protocol. In fact, alterations in cardiovascular regulatory mechanisms of the obese rat that could ultimately contribute to a rise in blood pressure were described in two of the above studies reporting undetectable differences in blood pressure between lean and obese rats (Bunag and Barringer, 1988; Pamidimukkala and Jandhyala, 1996). Lability with respect to blood pressure control could make the obese rat more susceptible to the development of hypertension if this animal is exposed to an appropriate environmental stimulus.

The objective of the present study was to demonstrate experimentally that obesity is a risk factor for the development of hypertension by subjecting lean and obese Zucker rats to a stimulus known to induce hypertension and assessing the responsiveness of the two groups. Data from experiments using mineralocorticoid-induced hypertension indicate that (1) the obese Zucker rat develops hypertension more rapidly than its lean counterpart and (2) the magnitude of the hypertensive response is much greater in obese rats than in lean rats.

Methods

*Animal*s. Female obese (*fa/fa)* and lean (Fa/?) Zucker rats were purchased from Charles River Laboratories (Wilmington, MA) at 8 weeks of age and housed in the Marshall University Animal Resources Facility in plastic cages with wood chip bedding in rooms having an ambient temperature of 23 + 2^{o} C and a 12-hour light/dark cycle. All procedures using rats were approved by the Marshall University Institutional Animal Care and Use Committee.

Deoxycorticosterone acetate (DOCA)-salt hypertension. Nine-week-old lean and obese rats were unilaterally nephrectomized under ketamine HCl-xylazine (45:5 mg/kg)-induced anesthesia. During this procedure, Marshall University Animal Resources Facility guidelines were strictly adhered to for aseptic surgery on rodents. Following a recovery period of two weeks, control blood pressure measurements were obtained twice weekly over an interval of two weeks. Then DOCA (25 mg/kg), suspended in corn oil, was administered twice weekly via subcutaneous injections to one group of lean and one group of obese rats for a period of five weeks. During the treatment period, 1% NaCl was added to the drinking water of the DOCA-treated rats. Unilaterally nephrectomized control lean and obese rats were injected with vehicle (corn oil) only and were given tap water to drink. All rats had free access to Purina rat chow.

Systolic blood pressure was measured on conscious rats using a programmed electrosphygmomanometer with a pneumatic pulse transducer and tail cuff (Narco-Biosystems, Houston, TX). Practice blood pressure measurements were obtained during the week prior to the measurement of control blood pressures so that the rats could

become familiar with the technique. To alleviate any stress associated with the environment and/or protocol, the environmental conditions in the recording room and the techniques for handling the rats and measuring blood pressure were carefully controlled.

Measurement of urine albumin. After five weeks of DOCA treatment, control and DOCA-treated rats were placed in individual metabolic cages for a 24-hour urine collection. The 24-hour sample was obtained after the rats were allowed to adapt to the metabolic cage for a 24-hour period. Urine proteins were separated by one-dimensional sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis using a 5% stacking gel and a 10% separating gel. Prior to electrophoresis, urine samples were diluted with saline so that the density of the resultant band would fall within the linear portion of a curve prepared by using albumin standards. A 5 microliter aliquot of each diluted sample was mixed with 20 microliters of sample buffer containing 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.0025% bromophenol blue and heated at 95 ^{o}C for 5 minutes. Ten microliter aliquots of the mixture were then loaded onto the gel. Two lanes on each gel were loaded with 10 microliters of an albumin standard (2 mg/ml). Gels were run at 60 mA until the bromophenol blue dye front reached the bottom of the gel (approximately 1 hour) using a Bio-Rad Mini-Protean II Electrophoresis Cell. After electrophoresis the protein bands were stained with silver stain using a Silver Stain Plus kit (Bio-Rad, Richmond CA) according to the manufacturer's instructions. Gels were placed in cellophane membranes and dried overnight in a drying frame. Subsequently, protein bands were quantified using a Molecular Dynamics Personal Densitometer. To normalize data from multiple gels, the

density of a given lane was expressed as a function of the albumin standard for that gel. Results are expressed in arbitrary densitometric units times the dilution factor of the sample.

Histology. At the end of an experiment, the rats were deeply anesthetized with a ketamine HCl-xylazine mixture (45:5 mg/kg) and euthanized by exsanguination via cardiac puncture. Kidneys were removed by dissection and fixed overnight in 10% buffered formalin. Following fixation, coronal sections of the kidneys were subjected to standard processing and embedded in paraffin. The sections were cut at 2 microns and stained with hematoxylin and eosin (HandE). Additional sections were cut at 4 microns and stained with periodic acid-Schiff (PAS). The slides were reviewed without knowledge of the experimental groups. The methods of Dworkin, Feiner and Randazzo (Dworkin et al., 1987) were followed for the quantitative assessment of the glomeruli and a mesangial expansion score was obtained for each rat. Twenty-five glomeruli were examined in each section and the amount of mesangial expansion was graded according to the following scheme: "1" if of normal area, "2" if mildly increased in area, "3" if moderately increased in area and "4" if markedly increased in area. Tubular involvement was assessed semi-quantitatively with a determination of cast formation and the amount of tubular atrophy and dilatation. Both the resulting cast score and the tubular index were determined by counting the percent of tubules with casts or tubular dilatation and /or atrophy per 25 high power fields in each subject. Both determinations were scored using the following schema: "1" if 0-25% of tubules were involved, "2" if >25-50% of tubules were involved, "3" if >50-75% of tubules were involved, and "4" if >75-100% of tubules were involved.

Statistics. Data are expressed as means ± SEM. Statistical analysis was performed using Sigma Stat statistical software (Jandel Corporation, San Rafael, CA). Differences among the means of different groups were assessed by two way analysis of variance followed by a Tukey test. A Dunnett's test was used following two way analysis of variance of blood pressure data in order to compare blood pressure changes in a given group with pretreatment measurements. *P* values of 0.05 or less were interpreted as being statistically significant.

Results

At the start of the study, systolic blood pressures (SBP) for vehicle-treated unilaterally nephrectomized lean and obese rats were essentially the same (122 ± 3 and 127 ± 2 mmHg, respectively). DOCA-salt-treated obese rats exhibited a significant rise in SBP compared to their pretreatment values as early as 4 days after the start of DOCA-salt treatment (Figure 2.1A). When these blood pressure measurements were compared to the obese control group, differences in SBP reached significance at day eight. In contrast, systolic blood pressure of DOCA-salt-treated lean rats was not significantly elevated from pretreatment measurements until day 22 (Figure 2.1B). Moreover, the magnitude of blood pressure elevation during the plateau phase of blood pressure development, was significantly greater in the obese group (196 mmHg) compared to the lean group (150 mmHg). Because DOCA was administered on the basis of body weight (25 mg/kg) during the first experiment, obese rats received more DOCA than their lean counterparts. To control for this, obese rats were given only as much DOCA as the lean rats in a

subsequent experiment. This relative reduction in the administered amount of DOCA did not affect the time of onset or magnitude of the blood pressure response in the obese animals (Figure 2.2).

At the onset of DOCA-salt treatment, obese rats weighed twice as much as age- and gender-matched lean rats (427 ± 5 g versus 217 ± 2 g respectively). During the 5-week treatment period, vehicle treated lean rats gained an average of 21.5 ± 3.1 g whereas the vehicle-treated obese group gained an average of 53.3 ± 6.1 g. The gain in body weights appeared to be unaffected by DOCA-treatment in lean rats (Figure 2.3). In contrast, DOCA-treatment did affect weight gain in the obese group: after an initial gain of 12 grams during the first week of treatment, there was no further gain in weight for the remainder of the study (Figure 2.3). By week 5, approximately half of the rats in the DOCA-treated obese group were losing weight. This resulted in a mean weight for the group that was about 24 grams lower than the peak weight attained at week one.

Sections of the kidney were examined from both control and DOCA-treated lean and obese rats. Dramatic morphologic changes were noted in the glomeruli of vehicle-treated uninephrectomized obese rats (Figure 2.4b) compared to vehicle-treated lean rats (Figure 2.4a). These changes became more pronounced when obese rats were treated with DOCA (Fig.4e). While focal glomerulosclerosis was present in vehicle-treated obese rats, upon DOCA treatment, many glomeruli developed global glomerulosclerosis with almost complete replacement with eosinophillic, hyalinized material that was PAS positive. Moreover, glomerular changes in obese DOCA-treated rats were characterized by

prominent expansion of the mesangial matrix and increased mesangial cellularity. To assess the glomerular involvement, a semi-quantitative mesangial matrix expansion score was determined. In vehicle-treated uninephrectomized animals, there was a significant increase in the mesangial matrix score of kidneys from obese rats compared to lean rats (Table 2.1). In addition, DOCA-salt treatment also increased matrix scores in both lean and obese animals. Although the amount of matrix expansion in kidneys from DOCA-treated obese rats was quite dramatic relative to kidneys from DOCA-treated lean rats, analysis of the matrix scores did not reach statistical significance due to the variability both within an individual kidney and between animals.

In addition to the observed glomerular changes, tubular changes were also prominent in the DOCA-treated obese rats. These changes were characterized by a thickening of the tubular basement membranes, markedly dilated tubules with PAS-positive casts, and tubular atrophy with flattening of the tubular epithelial cells. Although some mild cast formation and tubular atrophy was noted in the kidneys of vehicle-treated obese rats, prominent tubular cast formation along with tubular dilatation and atrophy were present upon DOCA administration (Figure 2.4f). In contrast, while the lean DOCA-treated subjects showed some tubular changes, they were much less pronounced than that seen in the obese animals (Figure 2.4d). Separate scores were obtained for cast formation and for the development of tubular atrophy and dilatation. Differences between lean and obese controls and lean controls and DOCA-treated lean rats for both parameters were minor and statistically insignificant. In contrast, both the tubular index and the cast score for

kidneys from DOCA-treated obese rats were elevated significantly relative to vehicle-treated obese rats and DOCA-treated lean rats (Figure 2.5).

As a functional correlate to the histological evaluations, urine albumin content was measured for rats in all four experimental groups. Figure 2.6 is representative of the gels that were used to assess urine albumin concentration. Results of all the gels that were run are summarized in Figure 2.7A. This figure shows that the concentration of albumin in the urine of obese rats is greater than that of lean rats both in the presence and absence of DOCA-salt treatment. In addition, when total albumin excretion was determined using 24-hour urine volumes (Figure 2.7B), additional findings were highlighted: (1) DOCA-salt treatment significantly increased total albumin excretion in both lean and obese rats relative to their vehicle-treated counterparts and (2) albuminuria resulting from DOCA-salt treatment was significantly greater in obese rats than in the lean rats. Urine albumin content of the different treatment groups was consistent with their respective mesangial matrix scores (Table 2.1).

Discussion

It has been suggested that the obese Zucker rat may be a good model for the study of obesity-associated hypertension (Kurtz et al., 1989). This idea is supported by data from a number of laboratories that indicate the blood pressure of obese Zucker rats is elevated relative to lean rats (Alonso-Galicia et al., 1996; Carlson et al., 2000; Kasiske et al., 1985; Kurtz et al., 1989; Paradise et al., 1985; Wickler et al., 1982; Zemel et al., 1990). In

some of these studies, differences in blood pressure, although statistically significant, tended to be modest with little evidence of overt hypertension in animals expressing the obese phenotype. In other studies, obese rats exhibited borderline hypertension with systolic blood pressures in the range of 145-155 mmHg. This was especially the case when measurements were made in older animals (Kasiske et al., 1985; Paradise et al., 1985). In contrast, other investigators, using similar experimental conditions, have been unable to detect differences in the resting blood pressure of lean and obese Zucker rats (Auguet et al., 1989; Bunag and Barringer, 1988; Lavaud et al., 1996; Levin et al., 1984; O'Donnell et al., 1985; Pamidimukkala and Jandhyala, 1996; Pawloski et al., 1992; Todd and Abernethy, 1986). A review of these studies did not reveal any obvious factors such as the age or gender of the animals, the vendor supplying the rats or the methodology used to assess blood pressure that could readily explain why blood pressures were elevated in obese rats in some studies but not in others. Differences from study to study could have occurred because blood pressure regulation in the obese rat is more labile and, consequently, more sensitive to subtle differences in the physiological state of the animal or the environmental conditions under which it is maintained. The suggestion that obese animals may be more sensitive to physiological or environmental conditions that elevate blood pressure is consistent with the idea of obesity being a risk factor for the development of hypertension.

In the present study, we investigated obesity as a risk factor for hypertension by challenging lean and obese Zucker rats with a set of experimental conditions known to induce hypertension (DOCA-salt administration to unilaterally nephrectomized rats) in

rats and then comparing the responses of animals expressing the two different phenotypes. The onset of hypertension in the obese rat was sudden and dramatic compared to the response of the lean rat. Moreover, the severity of the hypertensive response was much greater in obese rats than in lean rats as evidenced by the fact that when the blood pressures reached a plateau during the fourth and fifth weeks of the study, the mean blood pressure in the DOCA-treated obese group exceeded that of the DOCA-treated lean group by more than 45 mmHg. These data strongly support the conclusion that obese rats are more sensitive to DOCA-salt treatment than their lean counterparts. The extreme sensitivity of obese rats to DOCA-salt treatment was further evidenced by the fact that many of the obese rats exhibited some of the more harmful effects of DOCA-salt hypertension early in the treatment period. When Gavras and associates (Gavras et al., 1975) extended DOCA-salt treatment beyond the usual 4 to 5 weeks, they observed that the rats tended to lose weight and become moribund 6 to 10 weeks after the onset of DOCA administration. Similarly, Kretzler *et al.* (1994), Wada *et al.* (1995) and Matsumura *et al.* (2000) reported deaths and evidence of stroke and neurological damage in DOCA-salt-treated rats that were severely hypertensive. In the current study, three of the DOCA-salt-treated obese rats experienced seizures and about half lost weight and became moribund before the experiment was terminated at five weeks. In contrast, only one of the DOCA-salt-treated lean rats died before the end of the experiment and none developed seizures.

An important issue with regard to rats subjected to DOCA-salt hypertension in general and the aging obese Zucker rat in particular is the structural and functional integrity of

the kidney. As the obese Zucker rat ages, it exhibits progressive renal damage. Although renal function is similar in lean and obese rats at 12 to 14 weeks of age, the obese rat is already exhibiting a small degree of proteinuria and histological evidence of mesangial matrix expansion at this time (Kasiske et al., 1985). By 17 weeks of age, approximately 3% of tubules from the kidney of the obese rat exhibit evidence of mild injury and 1% to 3% of the glomeruli exhibit early focal glomerulosclerosis (Magil, 1995). By 28 weeks of age, 5% of the glomeruli in the kidney of the obese rat exhibit focal glomerulosclerosis and there is a marked increase in urine albumin excretion. Moreover, in this age group, rats that have been unilaterally nephrectomized for a period of 24 weeks exhibit twice the incidence of focal glomerulosclerosis as two-kidney obese rats (Kasiske et al., 1989). As rats continue to age, there is an increased incidence of focal glomerulosclerosis and tubular damage. Kasiske *et al.* (1985) have reported that almost 32% of glomeruli from kidneys of 68-week-old obese rats exhibit focal glomerulosclerosis compared to just 5% of glomeruli from kidneys of age-matched lean rats. In addition, kidney sections from 51 and 61-week-old obese rats have areas with markedly dilated tubules, some of which contain PAS-positive casts (Shimamura, 1983). Casts are rare in the tubules of younger obese Zucker rats (Magil, 1995).

In the present study, rats were 9 weeks of age at the start of an experiment and 18-weeks-old at its conclusion. At 18 weeks of age, one would predict a small degree of glomerular and tubular damage in the kidneys of vehicle-treated obese rats and significant albuminuria relative to that recorded for vehicle-treated lean rats. This is consistent with our observations.

Changes in renal structure induced by chronic DOCA-salt treatment in some strains of rat are qualitatively similar to those observed in the kidney of the aging obese Zucker rat. For example, in studies using Sprague-Dawley and Munich-Wistar rats, the kidneys from DOCA-salt treated animals exhibited mesangial matrix expansion and glomerulosclerosis (Dworkin et al., 1987; Kretzler et al., 1994; Shimamura, 1990). Moreover, mild tubular damage characterized by atrophy of the tubular epithelium, tubular dilatation and the presence of hyaline casts has been observed in response to DOCA-salt treatment (Kim et al., 1994; Matsumura et al., 2000). In the present study, the renal effects of DOCA-salt treatment in the lean Zucker rat were comparable to those reported for other stains of rat: a modest increase in the mesangial matrix and mild tubular dilatation. In contrast, the effects of DOCA-salt treatment on the kidney of the obese Zucker rat were far more severe. In many glomeruli, global glomerulosclerosis was evident with the glomerulus being replaced by hyaline material. DOCA-salt effects on renal tubules were even more remarkable: many tubules in the obese rat kidney were markedly dilated, had a flattened epithelial lining and were filled with casts. Moreover, the striking structural changes in the kidney of the DOCA-salt treated obese rat were paralleled by an equally dramatic increase in the level of albuminuria. These observations suggest that DOCA-salt treatment may be accelerating the progressive renal injury that occurs in the aging obese Zucker rat.

One problem associated with research involving genetic models of obesity is that it is often difficult to determine whether the observed effect is due to obesity *per se* or to the

expression of the mutated gene. In the obese Zucker rat, obesity is due to a mutated leptin receptor (Chua et al., 1996; Takaya et al., 1996). Leptin is a peptide produced by adipose tissue that plays an important role in the long-term regulation of food intake and energy expenditure. It has been suggested that leptin may also play a role in blood pressure regulation and possibly in the development of hypertension. When administered to normotensive rats, leptin causes a modest increase in blood pressure (Carlyle et al., 2002; Dunbar et al., 1997; Fruhbeck, 1999; Shek et al., 1998), an effect that may be due to a leptin-induced increase in sympathetic activity (Dunbar et al., 1997; Hall et al., 2001). The obese Zucker rat, which has an abnormal leptin receptor, appears to be resistant to the effect of leptin on sympathetic activation (Haynes, 2000) and blood pressure elevation (Fruhbeck, 1999). It is this resistance to the effects of leptin that could make the obese Zucker rat more susceptible to DOCA-salt hypertension. When lean Zucker rats are given an IV bolus injection of leptin, there is a marked increase in urine sodium excretion (Villarreal et al., 1998). This natriuretic effect of leptin is greatly attenuated in the obese Zucker rat (Villarreal et al., 1998). If leptin is shown to play a significant regulatory role in allowing an animal to adjust to an increased sodium load, then resistance to this effect could make the obese rat more susceptible to the development of DOCA-salt hypertension. Further studies are needed to test this hypothesis and to determine whether the increased susceptibility of the obese Zucker rat to DOCA-salt hypertension is due to obesity *per se* or the consequence of a mutated leptin receptor.

In conclusion, results of this study indicate that young obese Zucker rats, with resting systolic blood pressures that are indistinguishable from age- and sex-matched lean rats,

may be more sensitive to conditions that promote the development of hypertension than their lean counterparts. Furthermore, these results support the conclusion of others that the obese Zucker rat may be a useful model for the study of obesity-associated hypertension.

Acknowledgments

This research was supported in part by the Marshall University Joan C. Edwards School of Medicine Cardiovascular Research Support Fund. We thank Yan Chen for her technical assistance, M. Aslam Chaudhry and Dr. Goran Boskovic for their technical advice and Dr. Gary Wright for his helpful discussions.

Figures and Tables

Table 2.1. Mesangial matrix scores of vehicle and DOCA-salt treated lean and obese Zucker rats.

Group	Mesangial Matrix Score
Lean 1-kidney Control	1.36±0.17
Lean 1-kidney DOCA	2.26±0.16
Obese 1-kidney Control	2.40±0.17
Obese 1-kidney DOCA	2.98±0.15

Data are expressed as the mean ± SEM for each group. The mesangial matrix score is significantly affected ($p<0.05$) by both the phenotype and DOCA-salt treatment.

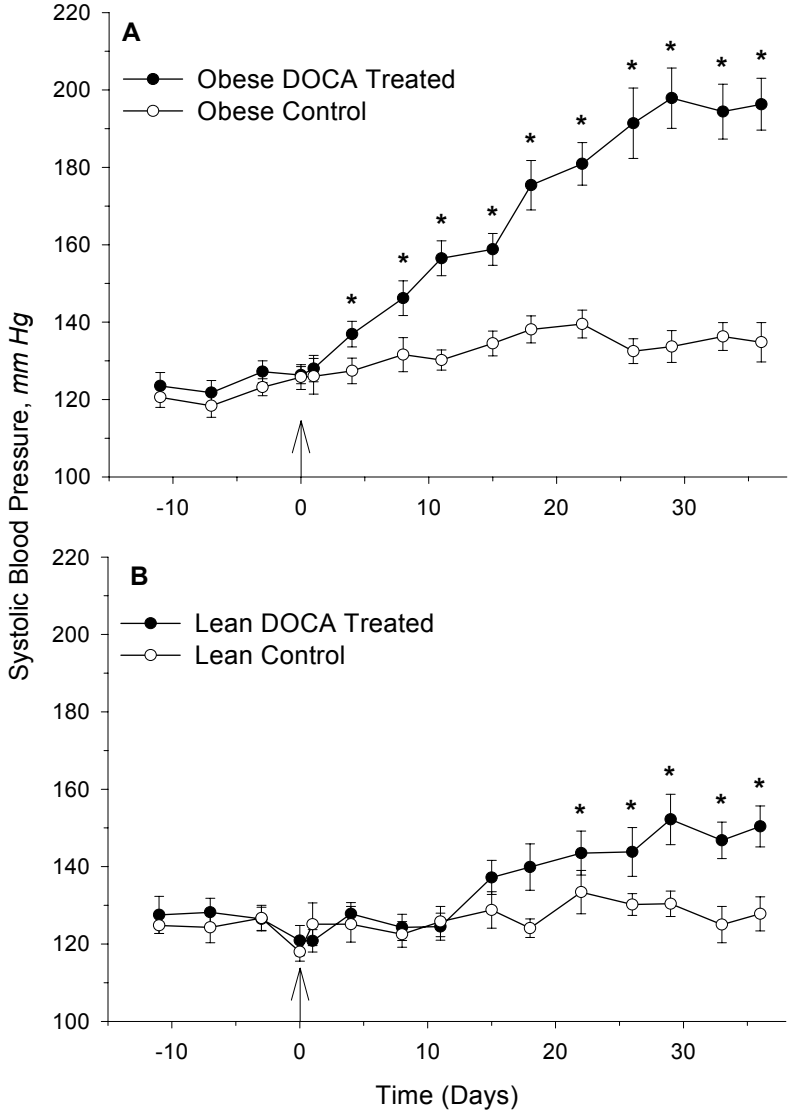

Figure 2.1. Systolic blood pressure (SBP) in obese (A) and lean (B) rats before and during DOCA-salt treatment. The arrow indicates the time when DOCA-salt treatment was initiated (Day 0). Nineteen obese rats and 13 lean rats were injected twice-weekly with DOCA (25mg/kg) in corn oil and given 1% NaCl in their drinking water. Sixteen obese rats and 13 lean rats were injected with vehicle (corn oil) and given tap water to drink. * P<0.05 compared to pretreatment SBP for that group.

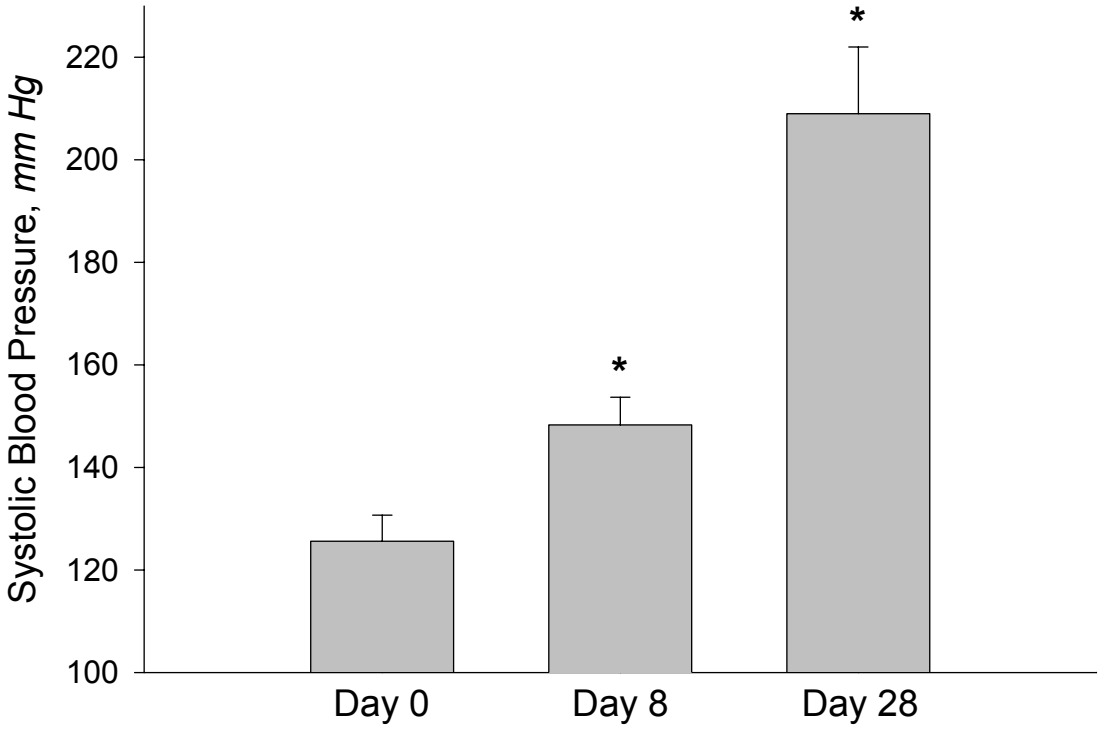

Figure 2.2. Effects of reducing the amount of DOCA administered to obese Zucker rats on SBP. In this experiment, age-matched lean and obese rats were given the same absolute amount of DOCA. The quantity of DOCA administered to the obese rats (N=7) was based upon the weight of the age-matched lean rats. The magnitude of the blood pressure response and time required to cause a significant elevation in blood pressure were essentially the same as for obese rats given DOCA at a dose of 25 mg/kg. *P<0.05 compared to both pretreatment blood pressures for the reduced-DOCA obese group and blood pressures for age-matched vehicle-treated obese rats.

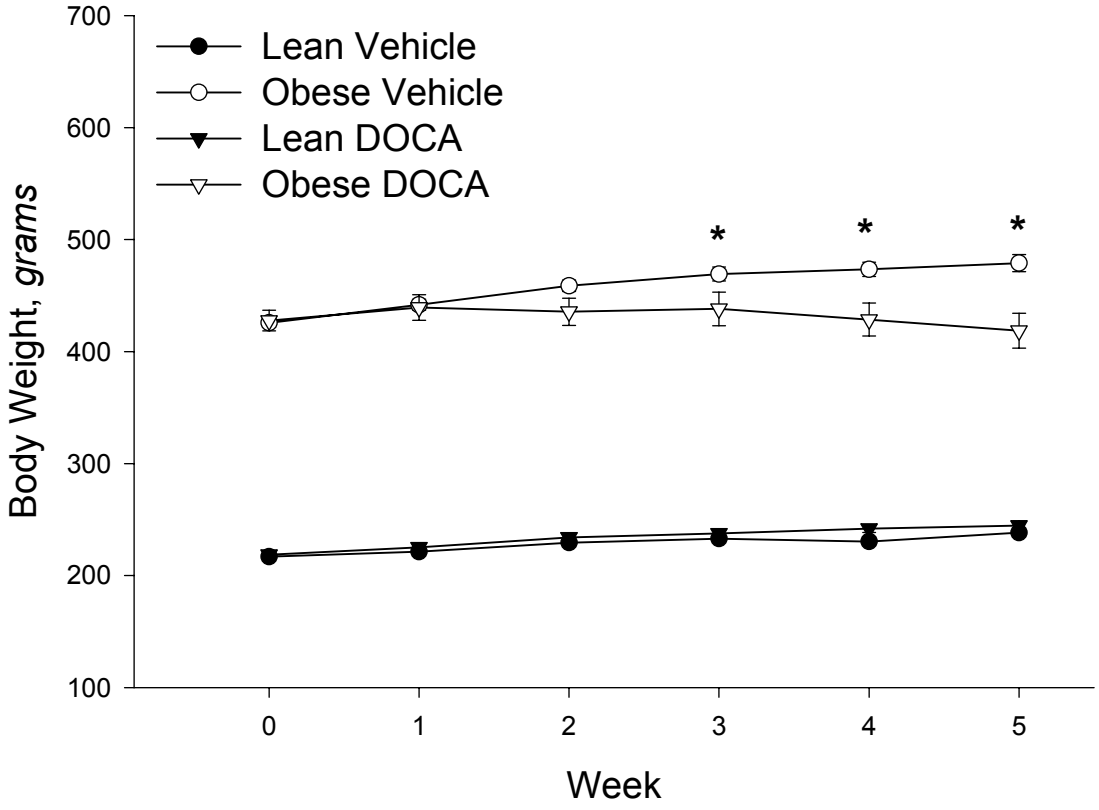

Figure 2.3. Changes in body weights of lean and obese rats in response to DOCA-salt treatment. Rats were injected twice weekly with DOCA (25 mg/kg) in corn oil and given 1% NaCl in their drinking water. Vehicle-treated rats were injected twice weekly with corn oil only and given tap water to drink. *P<0.05 compared to vehicle treated rats at the same time interval.

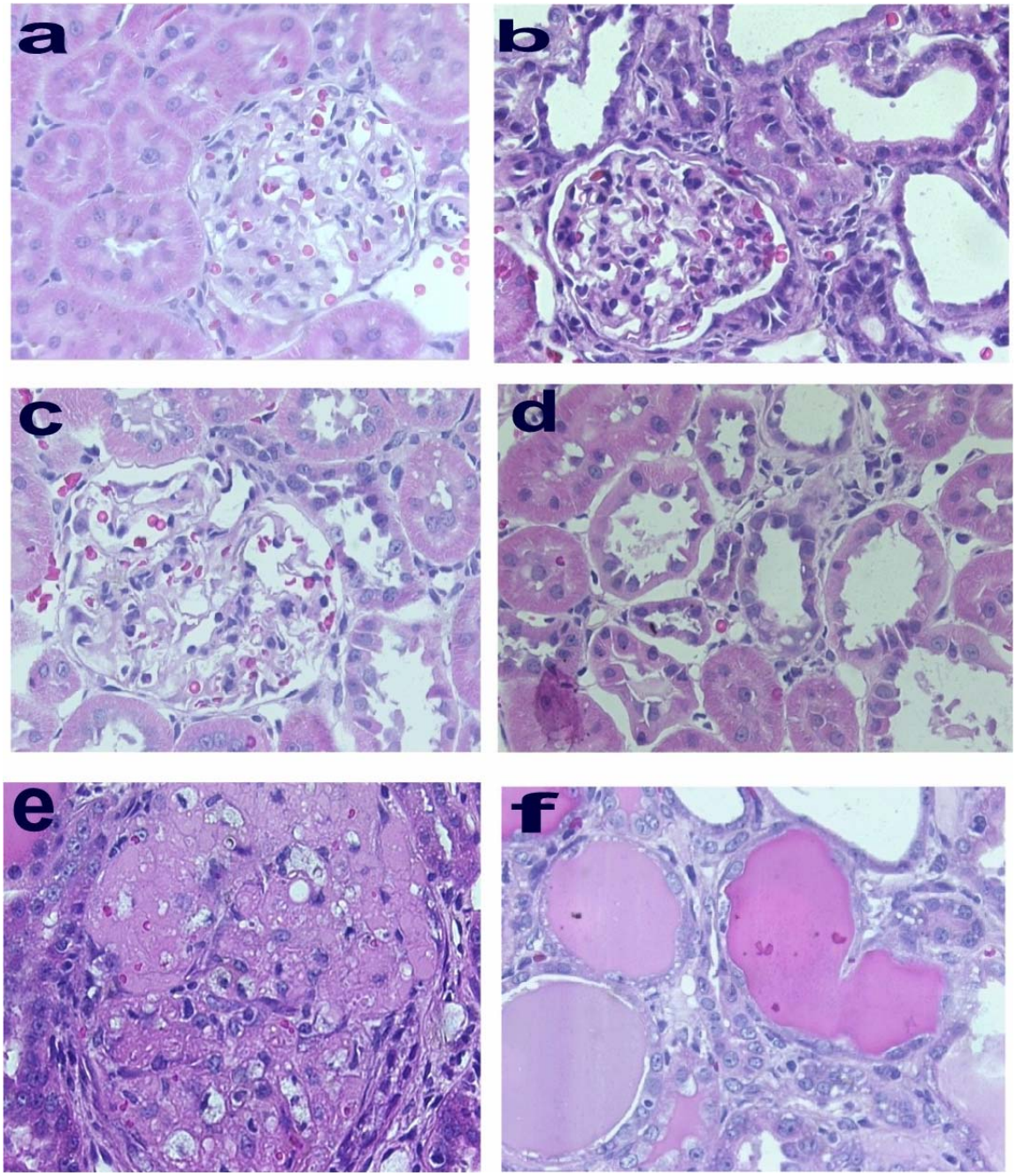

Figure 2.4. Light micrographs of hematoxylin and eosin prepared kidney sections at 600X magnification. (a) Lean control kidney showing essentially normal glomeruli and tubules. (b) Obese control kidney showing a mild increase in the mesangial matrix and some tubular dilatation. (c) Lean DOCA-treated kidney showing a glomerulus with a moderate increase in the mesangial matrix and some surrounding mild tubular dilatation. (d) Lean DOCA-treated kidney showing mild tubular dilatation. (e) Obese DOCA-treated with severe global glomerulosclerosis with the glomerulus replaced by amorphous hyaline material. (f) Obese DOCA-treated kidney showing very dilated tubules filled with casts and showing atrophy with flattening of the tubular lining epithelium.

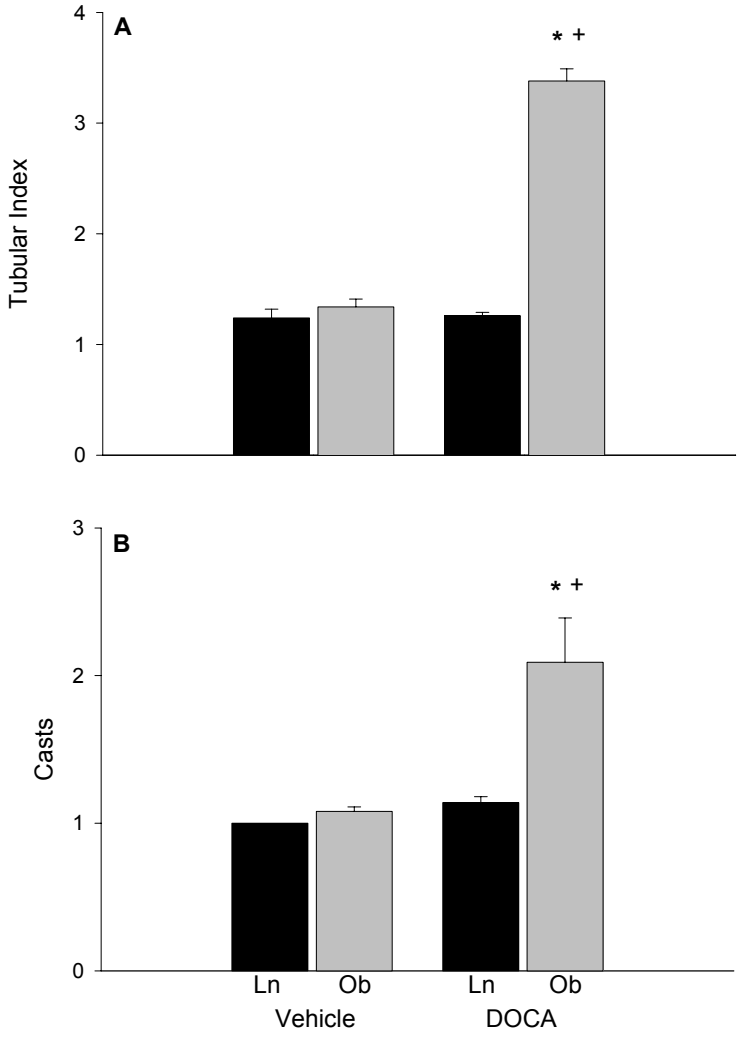

Figure 2.5. Tubular indices (A) and cast scores (B) for kidneys from DOCA- and vehicle-treated lean (Ln) and obese (Ob) Zucker rats. Each bar represents the mean and each bracket the SEM of scores from 5 to 7 different kidneys in each group. *P<0.05 compared to the vehicle-treated obese group; $^{+}$P<0.05 compared to the DOCA-treated lean group.

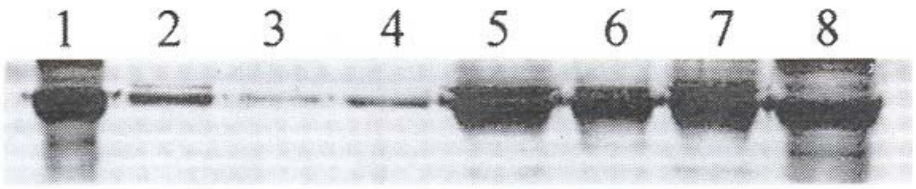

Figure 2.6. SDS-PAGE gel showing urine albumin content. Urine samples were subjected to SDS-PAGE and the gels were stained with silver stain as described in the methods section. Lanes 1 and 8 contained an albumin standard (2 mg/ml), lanes 2 and 4 contained urine from DOCA-treated lean rats, lane 3 urine from a vehicle-treated lean rat, lanes 5 and 7 urine from DOCA-treated obese rats and lane 6 urine from a vehicle-treated obese rat.

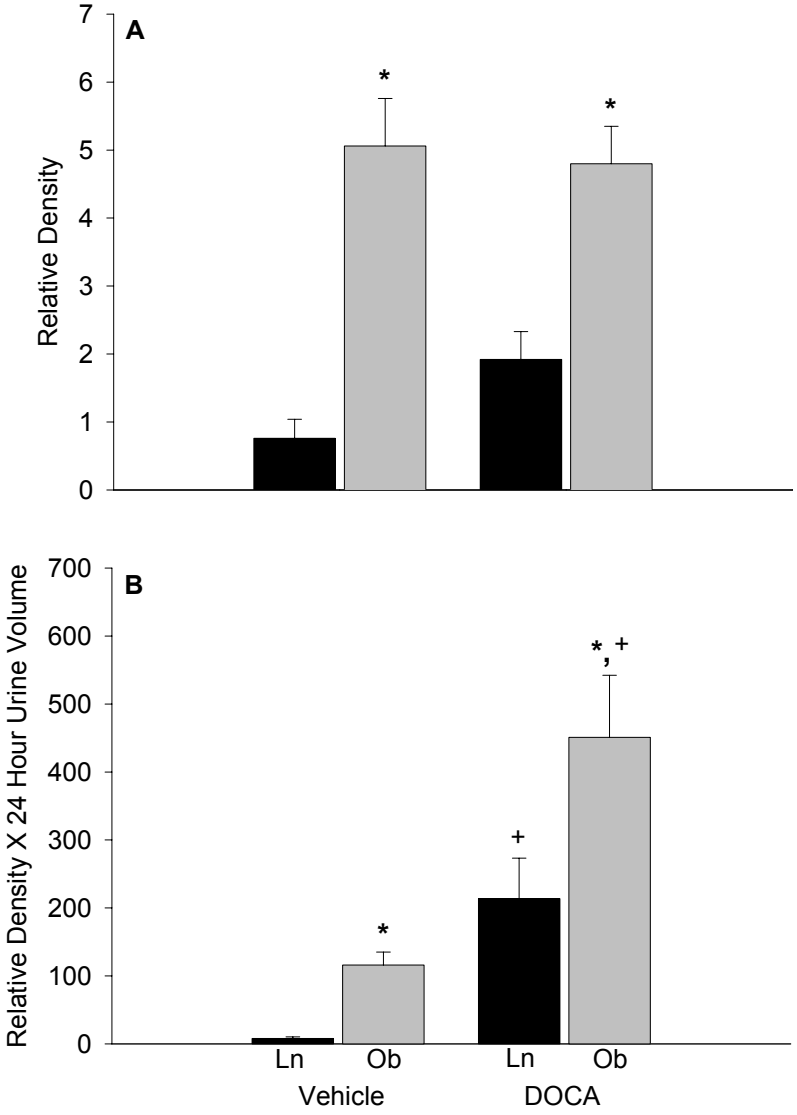

Figure 2.7. Albumin excretion in DOCA- and vehicle-treated lean (Ln) and obese (Ob) Zucker rats. Aliquots of 24-hour urine samples were diluted and subjected to SDS-electrophoresis and densitometric analysis. Urine albumin concentration, expressed as relative density in panel A, was determined by comparing the density of the albumin fraction of each urine sample with that of a known concentration of albumin standard run on the same gel. In panel B, the total amount of albumin excreted in a 24-hour period was obtained by multiplying the relative density of the albumin band times the 24-hour urine volume. Each bar represents the mean and each bracket the SEM of urine samples from 6 different rats. *P<0.05 compared to urine samples of lean rats subjected to the same treatment; $^{+}$P<0.05 compared to the vehicle-treated group of the same phenotype.

CHAPTER III

Progression of Renal Damage in the Obese Zucker Rat in Response to Deoxycorticosterone Acetate-Salt-Induced Hypertension

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Abstract

The objective of this study was to assess the progression of renal damage in obese Zucker rats in response to deoxycorticosterone acetate (DOCA)-salt-induced hypertension. Renal damage was evaluated by light microscopy and urine analysis at weekly intervals during the developmental phase of DOCA-salt hypertension and once during the plateau phase 42 days after the onset of treatment. Decreased tubular function was evident by day 8, as indicated by a significant increase in urine N-acetyl-β-D-glucosaminidase activity and glucose excretion. The tubular index, a measure of tubular damage, was significantly elevated by day 15 and continued to increase throughout the experiment. Glomerular damage, which was evident by day 8, was followed by increased urine albumin excretion by day 15. Only a few sclerotic glomeruli were apparent before the plateau phase; however, by day 42, approximately 50% of the glomeruli were sclerotic. Hyperplastic vascular changes were mild at day 8 and slowly increased in severity during the developmental phase. By day 42 the vascular changes were quite dramatic with some vessels being so hyperplastic that their lumens were almost occluded. Overall, these findings show progressive changes in renal structure and function that begin as early as day 8 and increase over time until dramatic changes are present at day 42, resulting in an end stage kidney.

Introduction

Data from multiple national surveys clearly demonstrate a dramatic rise in the incidence of obesity (a body mass index in excess of 30) in the United States over the past 25 years (Flegal et al., 1998; Flegal et al., 2002; Mokdad et al., 1999). Because obesity is a risk factor for multiple disorders such as type 2 diabetes, coronary heart disease, and hypertension (Pi-Sunyer, 2002), the progressive increase in the incidence of obesity has become a serious health concern.

Obesity is a well-established risk factor for hypertension with the incidence of hypertension for obese adults being almost three times that observed for non-obese individuals (Van Itallie, 1985). The consequences of chronic hypertension include serious end organ damage resulting in cardiac and vascular hypertrophy, stroke and/or renal damage. In the kidney, hypertension may initiate renal damage or it may promote the progression of previously established renal disease (Klahr et al., 1988). Hypertension-associated renal damage has been demonstrated in both clinical (Brazy et al., 1989; Klag et al., 1996) and experimental studies (Dworkin et al., 1984).

While much attention has been focused on the role of hypertension in progressive renal damage, less is known about the effects of obesity *per se* on renal structure and function. A recent retrospective study suggests that obesity by itself may promote the development of focal glomerulosclerosis and glomerulomegaly (Kambham et al., 2001). The glomerular changes observed in renal biopsies from this study were similar to those observed by Kasiske *et al.* (1985) and Shimamura (1983) in their work with the mature

obese Zucker rat, a genetically obese animal that has been used as an experimental model to study obesity-associated glomerulosclerosis (Gassler et al., 2001; Kamanna and Kirschenbaum, 1993; Kasiske et al., 1991; O'Donnell et al., 1985).

The obese Zucker rat may also be useful as an experimental model to assess the interaction between obesity and hypertension in the promotion of progressive renal damage. We have recently reported that obese Zucker rats exhibit an enhanced sensitivity to deoxycorticosterone-salt (DOCA-salt)-induced hypertension. In this study, systolic blood pressure rose more rapidly and the magnitude of the hypertensive response was significantly greater in obese rats treated with DOCA-salt than in correspondingly treated age- and gender-matched lean rats (Morrison et al., 2002). Moreover, marked glomerulosclerosis and tubulointerstitial damage were evident in kidneys of hypertensive obese rats at the end of the study whereas DOCA-salt treated lean rats exhibited only modest changes in renal histology.

The objective of the present study was to use biochemical and morphologic measures to assess the temporal pattern of change in the kidney of obese Zucker rats in response to a course of DOCA-salt administration. Biochemical abnormalities reflective of both tubular and glomerular damage were seen early and progressively during the developmental phase of the hypertensive response. Likewise, changes in tubular, glomerular and vascular morphology occurred early and continued to increase in extent and severity over the course of the study. The glomerular damage involved the development of mesangial hypercellularity with an endpoint of glomerulosclerosis. Progressive changes in the

tubules included tubular dilatation, atrophy of tubular epithelial cells and cast formation. The vascular changes were quite prominent with the development of hyperplastic arteriolosclerosis that increased dramatically in severity over time, culminating in the appearance of plexiform lesions and nearly complete vessel occlusion by the end of the study.

Materials and Methods

Animals. Eight-week-old female obese (fa/fa) Zucker rats were purchased from Charles River Laboratories (Wilmington, Massachusetts, USA) and housed in the Marshall University Animal Resources Facility in plastic cages with wood chip bedding in rooms having an ambient temperature of 23±2 °C and a 12 h light/dark cycle. Rats were allowed to acclimate to this facility for one week prior to being incorporated into an experimental study. All procedures using rats were approved by the Marshall University Institutional Animal Care and Use Committee.

Experimental procedures using animals. Nine-week-old rats were deeply anesthetized with a mixture of ketamine HCl and xylazine (45:5 mg/kg) and then unilaterally nephrectomized under aseptic conditions as prescribed by Marshall University Animal Resources Facility guidelines for aseptic surgery on rodents. Following surgery, rats were allowed to recover for a period of two weeks before being subjected to further experimental manipulation. After the recovery period, the rats were familiarized with the blood pressure measurement protocol for an additional week before blood pressure measurements for the experiment were begun. Throughout the study, body weights were

measured twice weekly and the systolic blood pressure (SBP) measured once weekly on conscious rats by tail cuff plethysmography using a programmed electrosphymomanometer with a pneumatic pulse transducer and tail cuff (Narco-Biosystems, Houston, Texas, USA).

Deoxycorticosterone acetate (DOCA)-salt hypertension was induced by means of biweekly subcutaneous injections of DOCA (25 mg/kg) suspended in corn oil. During the DOCA-treatment period, 1% NaCl was added to the drinking water. All rats had free access to Purina rat chow throughout the study. Five randomly selected rats were not treated with DOCA. These rats were sacrificed one day after the beginning of the DOCA-salt treatment ("Day 1") to obtain kidneys for histological analysis.

Six days prior to the start of DOCA-salt treatment and at weekly intervals during the developmental phase of the hypertensive response, 6 rats were placed in stainless steel metabolic cages to measure food and water consumption and 24-hour urine output and to collect urine for assessing renal function. The animals were given 1 day to adapt to the metabolic cages before measurements were obtained and samples collected. Urine samples were collected at 6- and 24-hours with the 6-hour urine sample being collected in a cold container. Immediately upon collection, the urine samples were centrifuged and the supernatant stored at -20^{o} C until used for analysis.

Analytical procedures. The Bradford (Bradford, 1976) technique was used to measure the total protein content of urine with the data being expressed as mg of protein excreted

per 24 hours. Urine albumin levels were measured as described previously (Morrison et al., 2002). Briefly, aliquots of a 24-hour urine sample were diluted with 0.9% NaCl and separated by one-dimensional SDS-PAGE using a 5% stacking gel and a 10% separating gel. Following electrophoresis, the gels were stained using a Silver Stain Plus kit (Bio-Rad, Richmond CA) according to the instructions of the manufacturer and then dried overnight. Protein band density was measured with a Molecular Dynamics personal densitometer and the intensity of each protein band was expressed as a function of the albumin standard run with that gel. Samples high in protein content were diluted with 0.9% NaCl before being subjected to electrophoresis so that their densities would fall within the linear portion of a densitometric curve prepared with increasing concentrations of an albumin standard. Results are expressed as arbitrary densitometric units times the dilution factor of the individual 24-hour urine sample.

Urinary excretion of the lysosomal enzyme, N-acetyl-β-D-glucosaminidase (NAG), and glucose were used as indices of renal tubular integrity. To remove inhibitors, urine samples were fractionated on a Sephadex G-50 gel filtration column before being assayed for NAG activity by means of a colorimetric assay (Roche Diagnostics, Indianapolis, IN). NAG content is expressed in terms of mU of NAG activity in a 24-hour urine sample. Urine glucose levels were measured using a Beckman Glucose Analyzer 2 (Beckman Instruments, Brea, CA) with the data being expressed as mg glucose excreted per 24 hours.

Histological methods. At weekly intervals, five randomly selected rats were deeply anesthetized with a ketamine HCL-xylazine mixture (45:5 mg/kg) and euthanized by exsanguination via cardiac puncture. Kidneys were removed and sliced into sections that were fixed overnight in 10% buffered formalin. Each kidney was entirely sectioned, and two representative sections were subjected to standard processing and embedded in paraffin. Sections were cut at 2 μm and stained with hematoxylin and eosin (HandE). Thin sections were utilized to enhance morphologic detail. Slides were reviewed without knowledge of the experimental groups. Twenty-five glomeruli were examined in each section and the amount of mesangial expansion was graded as follows: "1" if the mesangial area was normal, "2" if the area was mildly increased, "3" if it was moderately increased, and 4 if it was markedly increased. Mesangial expansion scores were calculated for each rat according to the procedure described by Dworkin et al (Dworkin et al., 1987). In addition, the percentage of sclerotic glomeruli was determined by assessing a total of 25 glomeruli in each section and counting the number of glomeruli that demonstrated sclerosis. Tubular involvement was assessed semi-quantitatively by determining a cast score and tubular index for each rat. Cast scores were obtained by counting the percentage of tubules with casts per 25 high power fields and the tubular index was determined by counting the percentage of tubules exhibiting tubular atrophy and/or dilatation per 25 high power fields. Both determinations were scored as follows: '1' if 0-25% of tubules were involved, '2' if >25-50% of tubules were involved, '3' if >50-75% were involved, and '4' if > 75-100% of tubules were involved.

Statistics. Data are expressed as means ± SEM. Statistical analysis was performed using Sigma Stat statistical software (Jandel Corporation, San Rafael, California, USA). Following normality and equal variance testing, comparisons between groups were made using one-way analysis of variance and, afterward, a Dunnett's test for making multiple comparisons against the untreated control group. When sample sizes were unequal, a Dunn's test was used instead. Three data sets failed the normality test and were subjected to Kruskal-Wallis one-way analysis of variance on Ranks followed by a Dunn's test to make multiple comparisons against the untreated control group. P values of ≤ 0.05 were interpreted as being statistically significant.

Results

In obese Zucker rats subjected to DOCA-salt treatment, systolic blood pressure increases progressively over a period of about 4 weeks (developmental phase) then tends to plateau at severely hypertensive levels (Morrison et al., 2002). In the present study, changes in the morphology of kidneys from DOCA-salt-treated obese rats were assessed at weekly intervals during the developmental phase of hypertension and at one point two weeks after systolic blood pressure reached a plateau. During the developmental phase, temporal changes in renal function were also monitored.

Twenty-four hour urine output was measured at weekly intervals and, as expected, DOCA-salt treatment caused a progressive increase in the total amount of urine excreted beginning soon after the initial injection of DOCA on day 0 (Figure 3.1). At all time

points after day 1, there was a significant increase in urine volume compared to the control measurements which were collected 6 days prior to the initial administration of DOCA. The increases in urine volume were accompanied by corresponding increases in fluid consumption (Table 1).

Randomly selected rats were sacrificed just prior to the start of DOCA-salt treatment and at weekly intervals thereafter. The remaining kidney was removed from each animal, sectioned, and examined by light microscopy using HandE staining. Thin sections from all time points were examined and significant changes were noted within the tubules. The normal architecture of the tubules was progressively altered over time and characterized by tubular disarray, dilatation of the tubular lumens, atrophy of the tubular epithelium, and loss of epithelial cell nuclei (Figure 3.2). Hyaline casts were noted within the tubules in a number of animals. Although they appeared to increase over time, the casts were focal in distribution and were not present in all animals. For a semi-quantitative assessment of tubular injury, both a tubular index and a cast score were determined for each animal. The tubular index for kidneys from DOCA-salt-treated obese rats began to rise as early as day 8 and increased progressively over time during the developmental phase of the hypertensive response: statistical significance was reached by day 15 (Figure 3.3A). Cast scores, in contrast to the tubular index, were not statistically significant until day 42 (Figure 3.3B), although there was a trend toward increased values beginning at day 22. Changes in tubular function were also evaluated during the developmental phase of the hypertensive response. NAG activity, a sensitive measure of tubular integrity (Vanderlinde, 1981), peaked in the urine of DOCA-salt-treated rats at

day 8 and remained significantly elevated compared to untreated controls throughout the collection period (Figure 3.4B). Another biochemical measure of tubular damage is glucose excretion (Figure 3.4A). Urine glucose excretion was also significantly elevated by day 8 and increased progressively throughout the collection period.

Glomerular changes were also assessed using both biochemical and morphologic measures. Twenty-four-hour urine albumin excretion, an important measure of glomerular function, showed a statistically significant increase beginning at day 15 (Figure 3.5B). In addition, total urine protein was measured and exhibited changes similar to those observed for urine albumin with a significant increase being seen by day 15 (Figure 3.5A). Light microscopic review of the kidney sections revealed increased glomerular cellularity, primarily in the mesangium, beginning as early as day 8 (Figure 3.6). This progressively increased over all time points to day 29. No sclerotic glomeruli were observed in sections obtained from rats during the developmental phase of the hypertensive response. To obtain a semi-quantitative measure of the increased cellularity, a mesangial matrix score was determined. The mesangial matrix score was significantly elevated by 8 days of DOCA-salt treatment and continued to increase progressively throughout the developmental phase of the hypertensive response (Figure 3.7). Sclerotic glomeruli, which were not evident during the developmental phase, began to appear at the beginning of the plateau phase. Two weeks into the plateau phase, 50% of the glomeruli were sclerotic (Figure 3.7).

Sections from all time points were carefully examined for vascular changes. Special attention was paid to the afferent and efferent glomerular arterioles along with the peritubular vessels. No vascular changes were noted in the untreated animals. Overall, both the glomerular arterioles and peritubular vessels showed hyperplastic arteriolosclerotic changes with treatment (Figure 3.8). These changes were characterized by intimal thickening, caused by the proliferation of smooth muscle cells and the concentric layering of collagen, and a progressive narrowing of the vascular lumens. Initially, the vascular changes were mild and focal. They slowly increased in both severity and extensiveness from day 8 through day 29 (Figure 3.8). Marked findings were seen at day 42 with some vessels so hyperplastic that the lumen was almost occluded. Focal fibrinoid necrosis was present in the luminal areas of the vessels; however, the hyperplastic changes were most prominent. The hyperplastic changes were so prominent that some vessels even formed plexiform type structures (Figure 3.8f). At this time point, the kidneys were severely damaged in all areas and constituted an end stage kidney.

Discussion

In the present study, DOCA-salt hypertension was used to assess temporal changes in renal structure associated with the development of hypertension in genetically obese rats. DOCA-salt hypertension has been used by a number of laboratories as a tool to study the interactions between hypertension and other factors that lead to the progression of renal disease. Janssen and associates (Janssen et al., 2003), for example, have demonstrated

that the Goto Kakizaki rat, a model of non-obese type II diabetes, exhibits more pronounced proteinuria and tubulointerstitial damage when subjected to DOCA-salt treatment than age- and gender-matched rats of the parent strain. Investigators assessing interactions between Heymann nephritis and hypertension have also used the DOCA-salt model of hypertension and have shown that rats immunized with a renal brush border extract exhibit an increased sensitivity to the development of DOCA-salt hypertension, a greater degree of glomerulosclerosis and proteinuria (Tikkanen et al., 1980) and an enhanced production of cytokines and growth factors that are thought to promote renal damage (Tikkanen et al., 1995) than non-immunized rats that are treated with DOCA-salt. Similarly, we have shown that obesity greatly exacerbates the hypertensive response and the degree of end organ damage associated with the development of DOCA-salt hypertension (Morrison et al., 2002). Compared to lean littermates, the development of DOCA-salt hypertension is accelerated in the obese Zucker rat and the magnitude of the hypertensive response that is attained is markedly higher. Moreover, the degree of renal damage is significantly greater in obese rats than in lean rats.

Because they are inclined to develop renal injury in the absence of any external manipulation, DOCA-salt-treated obese Zucker rats appear to provide a good model for studying the interactions between two factors that independently promote the progression of renal damage: obesity and hypertension. To this end, it has been clearly demonstrated that the spontaneous development of glomerulosclerosis (Kasiske et al., 1985; Shimamura, 1983) and tubulointerstitial damage (Magil, 1995) tends to occur more frequently in Zucker rats expressing the obese phenotype than in age- and gender-

matched lean rats. In these animals, the development of glomerulosclerosis is preceded by a significant elevation in urine albumin excretion and mesangial matrix expansion (Kasiske et al., 1985).

In the present study, obese Zucker rats subjected to DOCA-salt treatment exhibited histological evidence of glomerular, tubular and vascular abnormalities early during the developmental phase of hypertension. Mesangial hypercellularity and an enlargement of the entire glomerular complex characterized the initial glomerular response. This was reflected by a statistically significant increase in the mesangial matrix score by day 8 of treatment. At this point in the experiment, the rats were exhibiting only a mild degree of hypertension with the average systolic blood pressure for the group being 143.8 $\pm$ 4.8. The extent and severity of glomerular changes increased steadily over time. By day 29, the mesangial matrix score reached its maximum and sclerotic glomeruli began to appear in kidney sections. Also by day 29, the steady rise in blood pressure reached a plateau phase characterized by systolic blood pressures in excess of 195 mmHg. It was during the plateau phase that the number of sclerotic glomeruli increased dramatically. The development of sclerosis is an end stage pathological response to injury in a glomerulus. The mechanism underlying the development of glomerulosclerosis in DOCA-salt hypertension has not been clearly defined although there is evidence that injury to the glomerulus caused by a variety of conditions leads to the excessive production of growth factors and cytokines by mesangial cells and the attraction of inflammatory cells that may promote this process (Becker et al., 2001).

Biochemical markers of the functional integrity of the glomerulus (urine albumin excretion), and tubules (urine NAG activity and glucose excretion) were also monitored during the developmental phase of the hypertensive response. Significant increases in urine NAG activity and glucose excretion were evident before there was a significant increase in urine albumin excretion, suggesting that, in the obese rat, the functional integrity of the tubules may be compromised more rapidly during the initial phase of DOCA-salt treatment than that of the glomeruli.

A significant rise in urine NAG activity and glucose excretion also preceded a statistically significant increase in the tubular index. The tubular index, a calculation used to quantify changes in tubular morphology, was significantly elevated by day 15 and continued to rise steadily throughout the remainder of the study. In contrast, the cast score, which measures the presence of casts in the tubules, was elevated significantly only after the kidney had been chronically subjected (day 42 in the process) to the severe levels of hypertension associated with the plateau phase of the hypertensive response. This observation, coupled with the findings that cast distribution was focal and that casts were not present in all kidneys during the developmental phase of the hypertensive response, suggests that cast formation may be a consequence of end stage renal disease brought on by the prolonged exposure of the kidneys to severe hypertension.

A unique feature of the present study is that we were able to observe the progression of renal damage throughout the developmental phase and well into the plateau phase of the hypertensive response. Comparisons of our results with other studies, however, are

limited to those changes associated with the plateau phase because most studies that have addressed changes in renal morphology in response to DOCA-salt administration are typically terminated only after the plateau phase has been reached. In studies that are terminated after 4 to 6 weeks of treatment, the effects of DOCA-salt administration on the glomeruli, tubules and small vessels of the kidney are qualitatively similar. The extent of the damage, however, varies from study to study with the degree of damage to the kidney and the incidence of mortality in obese Zucker rats being comparable to the more severe responses reported for DOCA-salt administration (Gavras et al., 1975; Kretzler et al., 1994; Wada et al., 1995). Variations in susceptibility to DOCA salt treatment have thus far been attributed, in part, to factors such as age (Kretzler et al., 1994; Wada et al., 1995) or strain (Hartner et al., 2003). We have demonstrated that an obese phenotype also has a profound impact upon the response of a rat to DOCA-salt administration. As shown previously (Morrison et al., 2002), the degree of glomerular and tubulointerstitial damage in the lean Zucker rat treated with DOCA-salt appears to be relatively mild. In contrast, obese littermates of the same gender exhibit profound glomerulosclerosis, tubulointerstitial damage and vascular injury when subjected to an identical treatment regimen.

The progressive changes in the glomeruli and tubules beginning at day 8 and slowly increasing at all time points during the development phase may provide a good model to study the chronic renal changes that occur with hypertension and obesity. In contrast, the dramatic vascular changes occurring most extensively at day 42 may provide a good model for studying malignant hypertension. Hyperplastic arteriolosclerosis is associated

with malignant hypertension which often occurs in patients with existing benign hypertension. Although we did not demonstrate vascular hyaline arteriolosclerosis that can be seen in patients with chronic hypertension, we did demonstrate changes in the glomeruli and tubules, as noted above. The dramatic vascular changes observed at day 42, accompanied by a marked increase in both tubular and glomerular damage, suggest that the sustained and dramatic increase in blood pressure leads to an end stage kidney.

In summary, we have used histological examination and biochemical markers to assess renal changes associated with the development of hypertension in obese rats. By doing so, we have observed that (1) there is evidence of renal injury very early in the development phase of the hypertensive response when the level of hypertension is mild, (2) the degree of renal injury increases with the rise in blood pressure during the developmental phase, (3) the functional integrity of tubules in the kidney of the obese rat may be more sensitive to injury induced by DOCA-salt hypertension than that of the glomeruli, and (4) certain indices of progressive renal damage such as glomerulosclerosis and cast formation are only evident after the plateau phase of the hypertensive response has been reached. We conclude that the obese Zucker rat may be a good model to study interactions between obesity and hypertension on the progression of renal damage.

Acknowledgements

This research was supported, in part, by the Marshall University Joan C. Edwards School of Medicine Cardiovascular Research Support Fund. The authors thank Brandi Hanshaw and Beverly Pofahl for their excellent technical assistance.

Figures and Tables

Table 3.1. Body weights, food and water consumption, and systolic blood pressure before (day -6) and during the DOCA-salt treatment period (days 1-22).

Day	-6	1	8	15	22
Body weight (grams)	380.8 ±8.6	394.5 ±7.6	410.8 ±8.2	423.2 ±9.9	416.5 ±13.7
Food Consumption (grams/24 hours)	25.5 ±1.1	12.7 ±2.1	23.0 ±1.5	25.8 ±1.6	22.4 ±0.7
Water Consumption (ml/24 hours)	47.7 ±1.0	57.8 ±6.8	110.8[a] ±5.2	150.7[a] ±12.2	171.0[a] ±2.9
Blood Pressure (mmHg)	120.4 ±2.2	132.6 ±3.6	143.8[a] ±4.8	150.5[a] ±5.6	160.3[a] ±5.7

[a]$p<0.05$ compared to day -6. Data are expressed as the mean ± SEM for each group

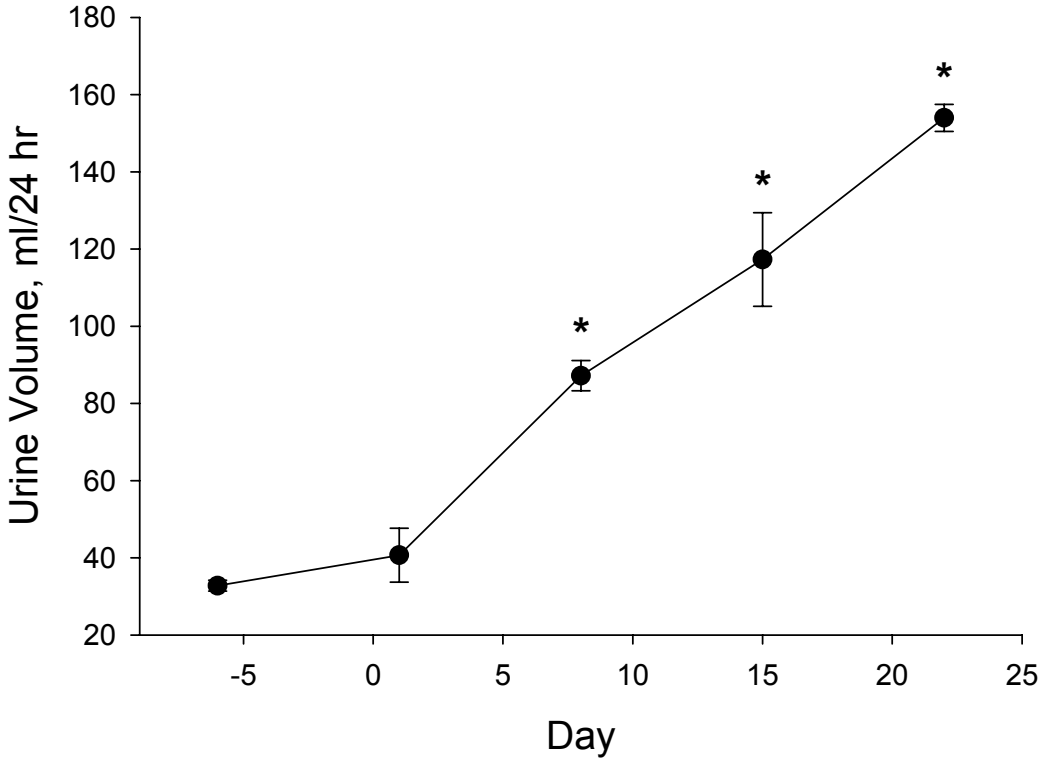

Figure 3.1. The effect of DOCA-salt treatment on urine volume. For each collection period, rats were placed in individual stainless steel metabolic cages for 48 hours. During the last 24 hours, urine output was measured. Urine samples were collected six days (day -6) prior to and 1 day after the initial DOCA injection (day 0) and at weekly intervals thereafter. Each point is the mean ± SEM for 6 rats. *P<0.05 compared to urine formation on day -6.

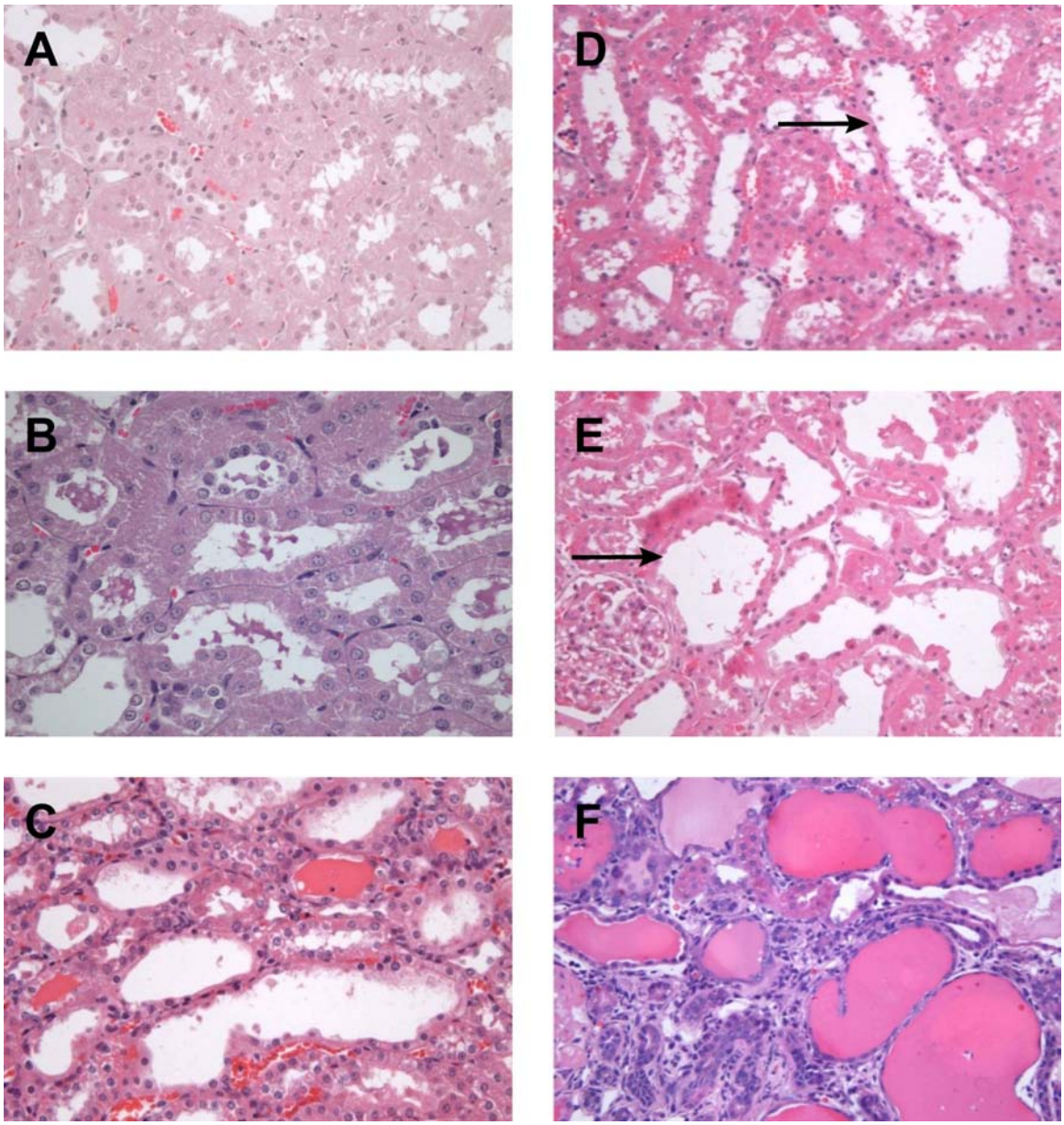

Figure 3.2. Histological changes occurring in the kidney tubules examined at the following time points: A-control, B-day 8, C-day 15, D-day 22, E-day 29, and F-day 42. All pictures were taken at 600x except panel A which was taken at 400x. The lower power was utilized for the control kidneys (panel A) to allow assessment of a larger field and confirm the lack of histological changes.

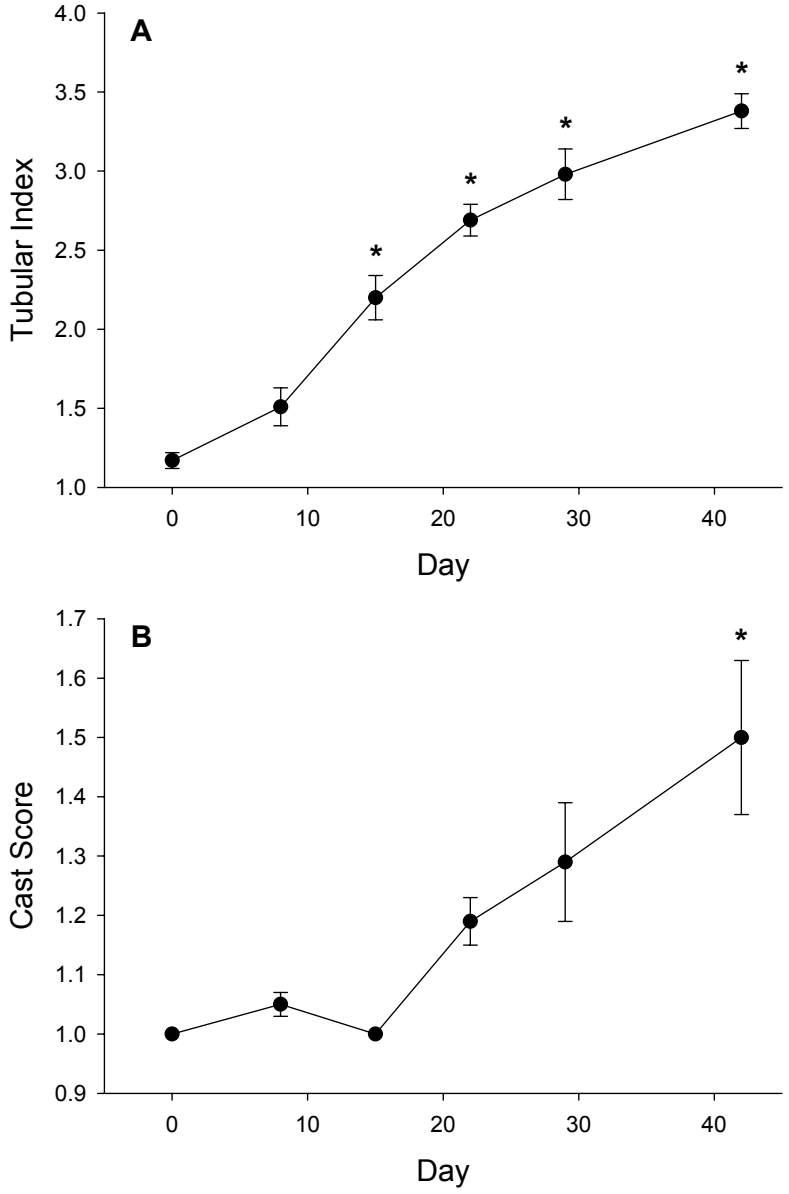

Figure 3.3. The effect of DOCA-salt treatment on renal tubules. Tubular indices and cast scores were determined on HandE sections of kidneys as described in the methods. Each point represents the mean ± SEM of the tubular index (A) or cast scores (B) from 5 rats subjected to DOCA-salt treatment for the number of days indicated on the X-axis. *P<0.05 compared to the untreated uninephrectomized controls (Day 0).

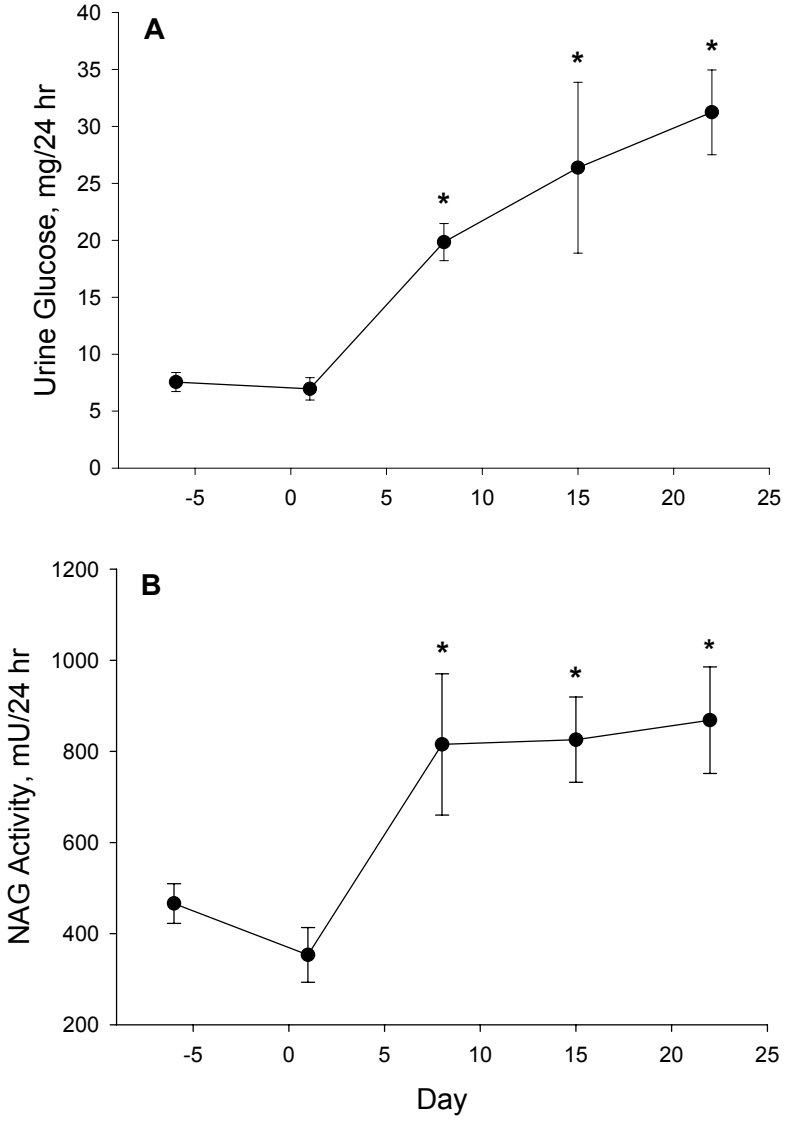

Figure 3.4. The effect of DOCA-salt treatment on the excretion of glucose and N-acetyl-glucosaminidase (NAG). Urine glucose levels (A) and NAG activity (B) were determined in 24-hour urine samples collected six days (day -6) prior to the initial DOCA injection (day 0), one day after the first DOCA injection and at weekly intervals thereafter. Urine NAG content is expressed in terms of mU of NAG activity excreted in 24-hours. Each point is the mean ± SEM for 6 rats. *P<0.05 compared to glucose content or NAG activity in urine collected from rats on day -6.

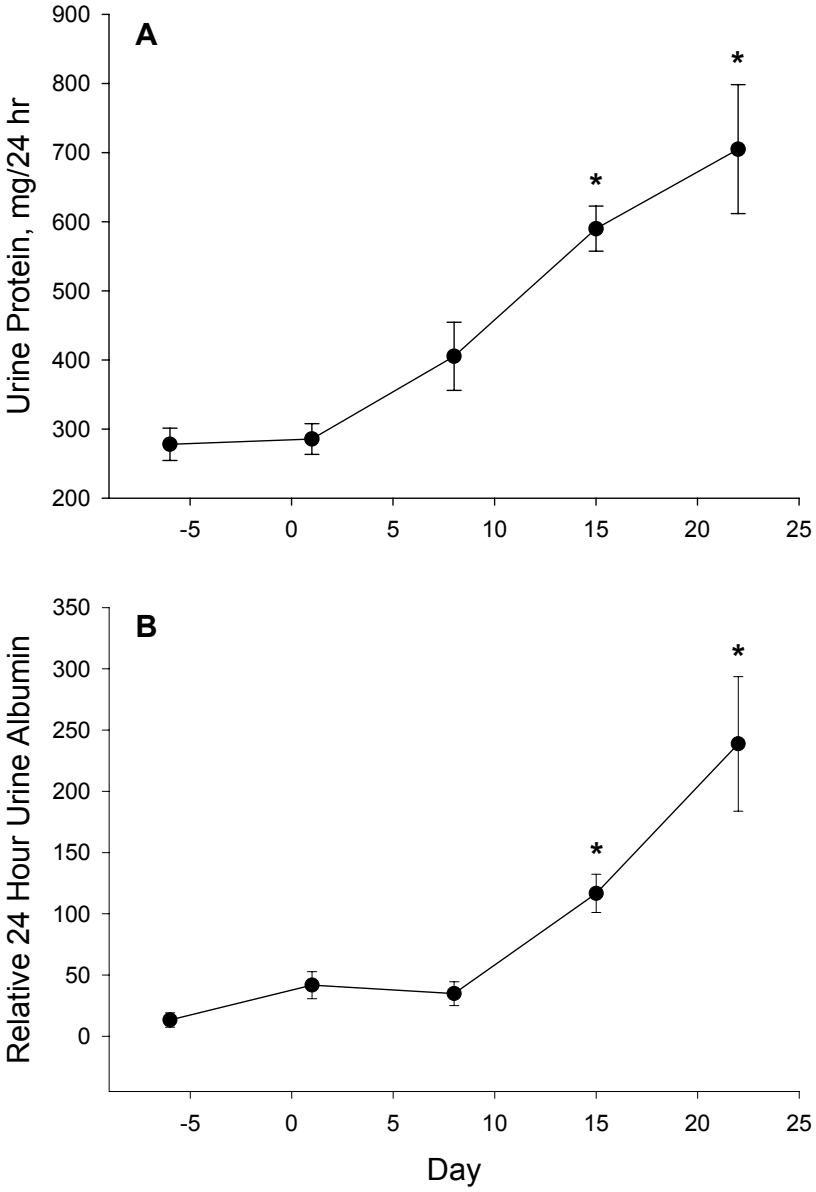

Figure 3.5. The effect of DOCA-salt treatment on protein and albumin excretion. Total protein (A) and albumin (B) levels were measured in 24-hour urine samples obtained six days (day -6) prior to the initial DOCA injection (day 0), one day after the first DOCA injection and at weekly intervals thereafter. Total protein is expressed as mg of protein excreted per 24 hours. Urine albumin content is expressed as arbitrary densitometric units per unit volume times the appropriate dilution factor for each 24-hour urine sample. Each point is the mean ± SEM for 6 rats. *P<0.05 compared to total protein or albumin content of urine collected from rats on day -6.

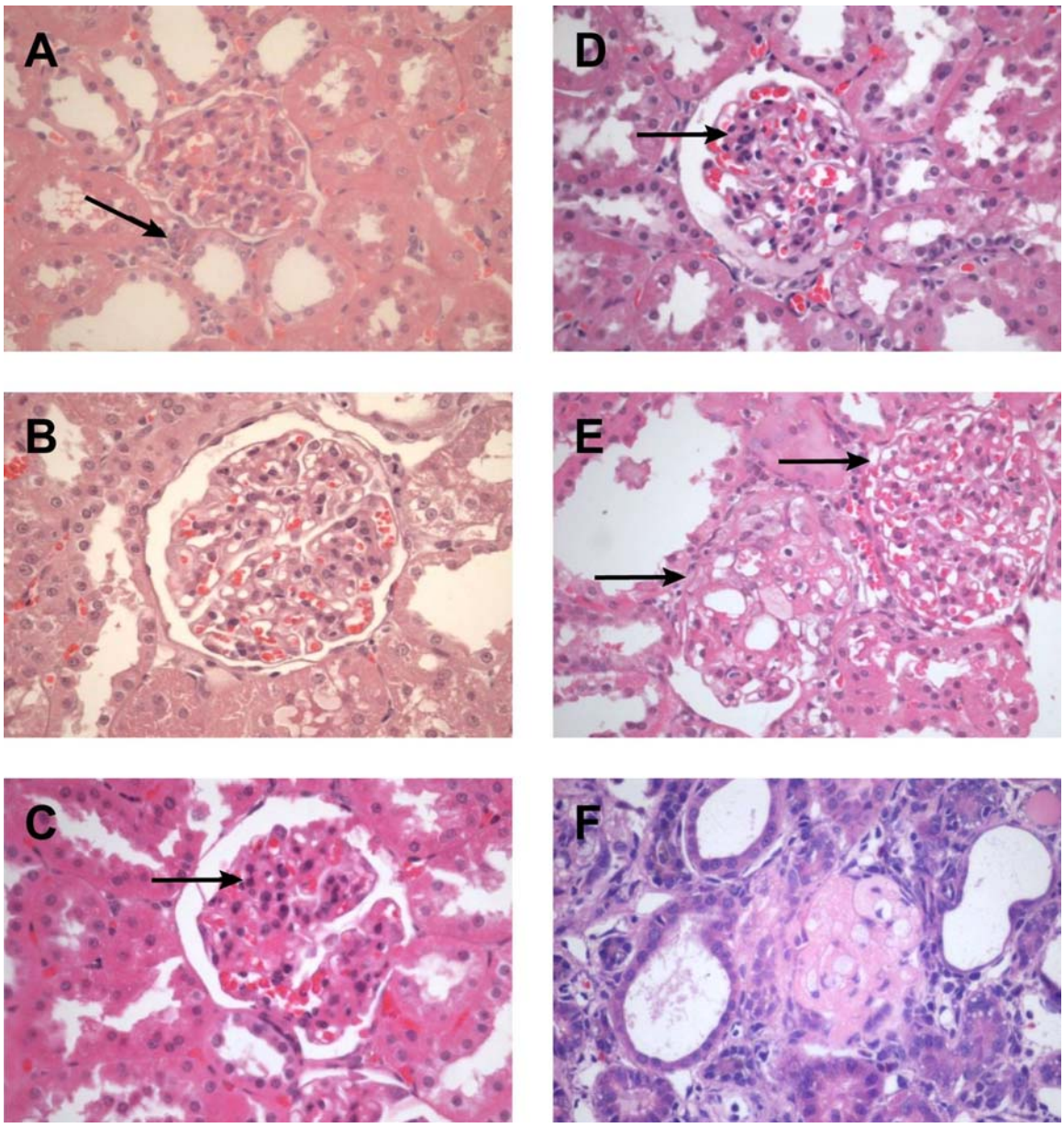

Figure 3.6. Histological changes occurring in the glomeruli examined at the following time points: A-control, B-day 8, C-day 15, D-day 22, E-day 29, and F-day 42. All pictures were taken at 600x, except panel E was taken at 400x. Note the arrow in panel a. This is highlighting a small vessel in the control animal. Panel E was taken at a lower power to allow visualization of two glomeruli, illustrating the mesangial proliferation present.

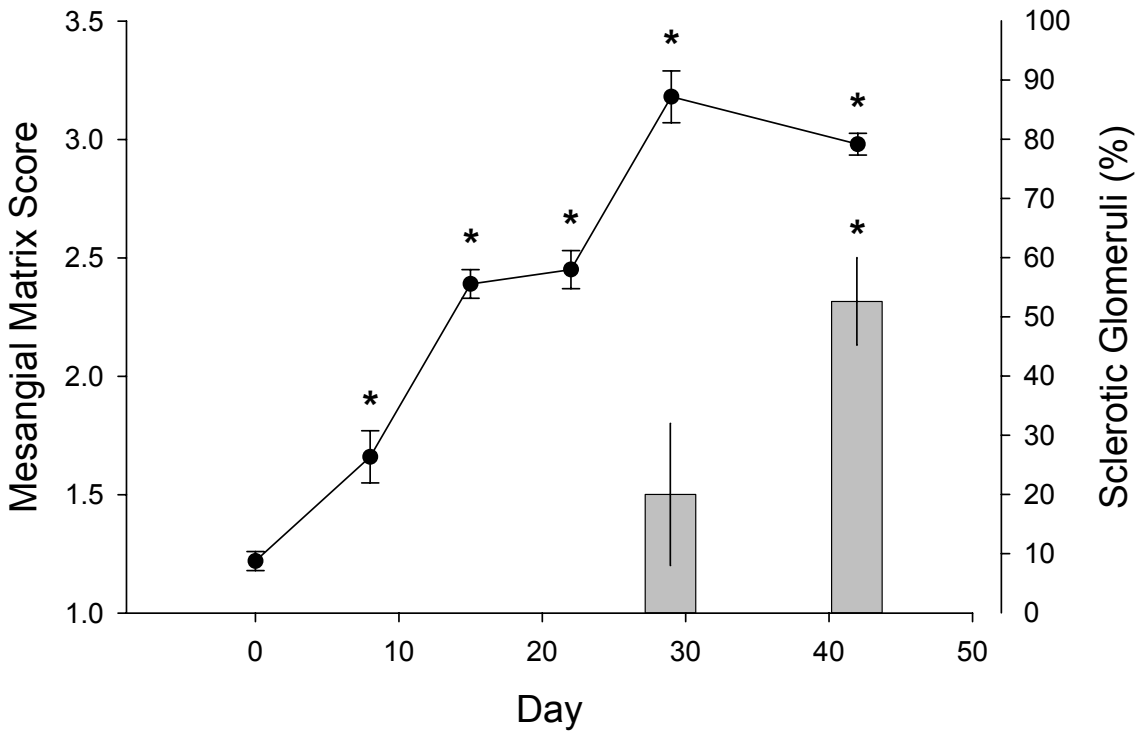

Figure 3.7. The effect of DOCA-salt treatment on mesangial expansion and the formation of sclerotic glomeruli. Mesangial matrix scores were determined on HandE sections of kidneys as described in the methods. Each point represents the mean ± SEM of matrix scores from 5 rats subjected to DOCA-salt treatment for the number of days indicated on the X-axis. The vertical bars represent the percentage of sclerotic glomeruli at the indicated days. Sclerotic glomeruli were not evident before day 28. *P<0.05 compared to the untreated uninephrectomized controls (Day 0).

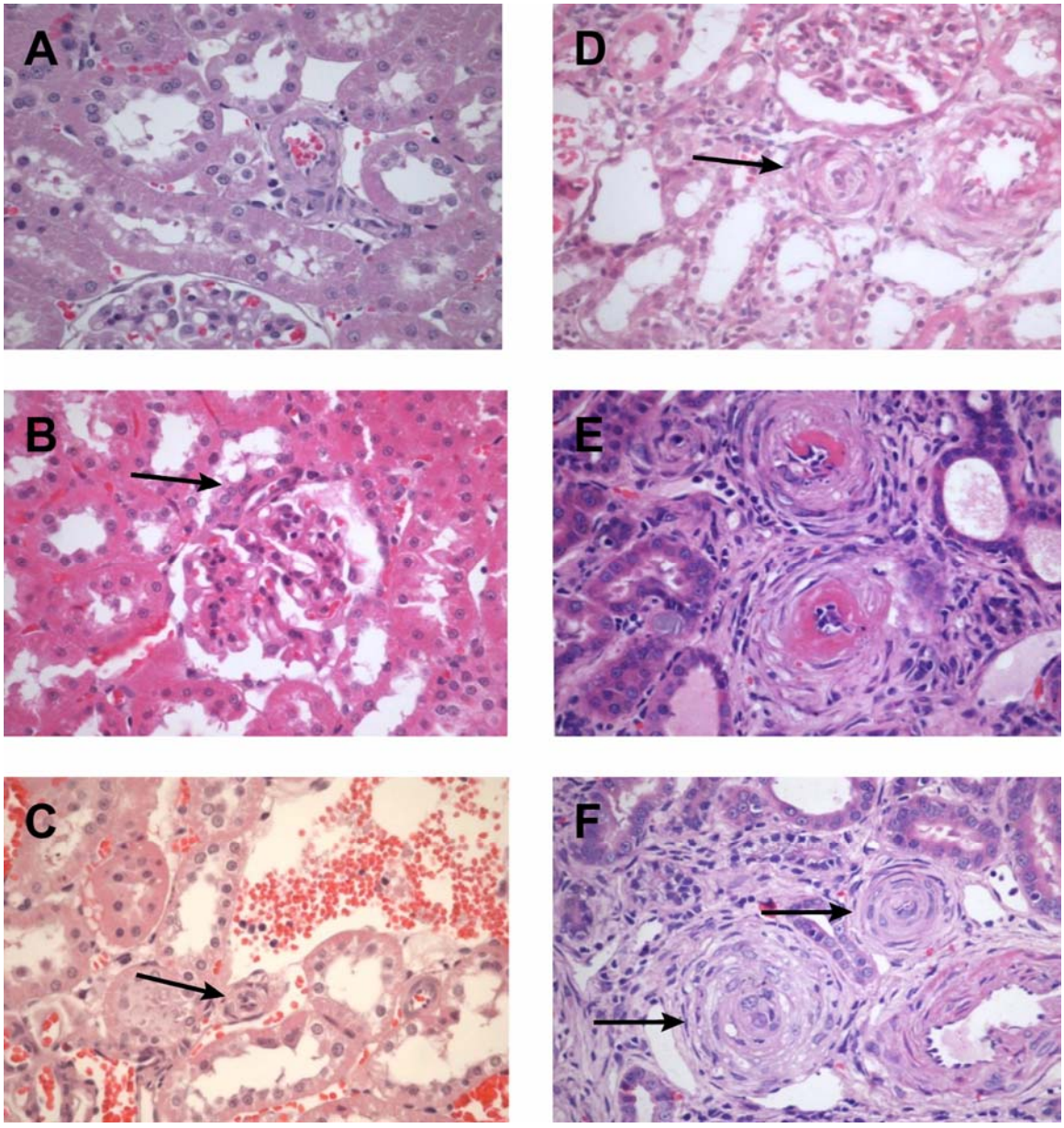

Figure 3.8. Histological changes occurring in the vessels examined at the following time points: A-day 8, B-day 15, C-day 22, D-day29, E-day 42, and F-day 42. All pictures were taken at 600x. Part F demonstrates the plexiform structures present.

CHAPTER IV

A Moderately High Fat, Salt-Supplemented Diet May Promote Hypertension in Obese Zucker Rats by Inhibiting Renal Nitric Oxide Synthase Activity

A Manuscript to be Submitted for Publication

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Abstract

Objective(s): The objective of the present study was to evaluate the obese Zucker rat as model of obesity-hypertension. A high fat, salt supplemented diet was used to determine whether the obese Zucker rat was more susceptible to the development of hypertension.

Design: Eight-week-old female lean and obese Zucker rats were fed a diet high in fat content or standard rat chow (control diet) for 10 weeks. From the 4th week until the 10th week, 1% NaCl was added to their drinking water. Animals were sacrificed at the end of the 10th week for analysis of blood leptin and insulin levels.

Methods: Blood pressure was measured at weekly intervals. Urinary excretion of nitric oxide (NO) metabolites was determined by colorimetric assay at week 4 and 10. At the end of the experiment, plasma insulin and leptin concentrations were determined by radioimmunoassay and renal nitric oxide synthase (NOS) activity was assessed in kidney homogenates.

Results: The blood pressure of obese, but not lean, Zucker rats on a high fat diet was increased by 2 weeks after onset of salt supplementation. The excretion of NO metabolites, a measure of systemic NO production, was inhibited by the high fat diet, especially in obese rats. The high fat diet suppressed renal NOS activity in obese Zucker rats on the high fat diet. The high fat diet increased plasma leptin in lean, but not obese, Zucker rats. Conversely, the diet increased plasma insulin in obese, but not lean zucker rats.

Conclusions: Data from these experiments indicate that obese Zucker rats are more sensitive than age- and gender-matched lean counterparts to the development of diet-induced hypertension in response to a moderately high fat, salt-supplemented diet. Furthermore, this obesity-associated susceptibility to hypertension is linked to deficiencies in NO production and availability caused, in part, by dietary NO synthase inhibition.

Keywords: hypertension, nitric oxide, obese Zucker rat, diet-induced, high fat

Introduction

The prevalence of obesity has recently skyrocketed in the United States and other industrialized countries. According to the National Health and Nutrition Examination Studies (NHANES), the prevalence of obesity, defined as a body mass index equal to or greater than 30 kg/m^2, more than doubled among adults in the United States from 1980 to 2000 (Flegal et al., 2002). The rise in the prevalence of obesity is a chief factor in the increasing incidence of hypertension (Care, 2005) and other obesity-related disorders such as coronary heart disease (Pi-Sunyer, 2002). According to risk estimates from the Framingham Heart Study, obesity is linked to about 75% of male and about 65% of female cases of hypertension (Davy and Hall, 2004; Garrison et al., 1987). Thus, obesity-hypertension appears to be the most common form of essential hypertension.

Meaningful advances in the field of obesity-hypertension depend upon the development of an assortment of animal models that have characteristics in common with those observed in obesity-hypertension in humans. Thus far, several very good models of obesity-hypertension have been developed using rats (Ernsberger et al., 1999b), dogs (Rocchini et al., 1987), and rabbits (Carroll et al., 1996). Still, the field may benefit from additional animal models because of the complex polygenic nature of obesity-hypertension. This view is further reinforced by the observation that factors commonly clustering with obesity (e.g. insulin resistance) that may play a role in promoting hypertension are not uniformly present in obese hypertensives. At this juncture, it appears that the most practical approach to the study of obesity-hypertension would be to use a wide range of species/models.

The obese Zucker rat (OZR), which is used extensively as a model of metabolic syndrome X (Takaya et al., 1996), is also considered by some to be a viable model for obesity-associated hypertension (Kurtz et al., 1989). Controversy exists, however, as to whether the OZR is actually hypertensive. Some investigators have reported that the OZR is not hypertensive relative to age- and gender- matched lean Zucker rats (Bunag and Barringer, 1988; Morgan et al., 1995; Pamidimukkala and Jandhyala, 1996; Pawloski et al., 1992; Reddy and Kotchen, 1992), whereas others have indicated that the OZR is hypertensive (Alonso-Galicia et al., 1996; Ambrozy et al., 1991; Carlson et al., 2000; Kurtz et al., 1989; Maher et al., 1995; Turner et al., 1995). In those studies where hypertension was present, it was generally mild. The argument that the obese Zucker rat is at a greater risk of developing hypertension has been bolstered by experiments from our laboratory showing that obese rats are far more sensitive to deoxycorticosterone-salt-induced hypertension than lean rats of the same age and gender (Morrison et al., 2002).

Models of obesity that are based on dietary manipulations, such as increased fat consumption, have been suggested to be particularly applicable to understanding human obesity (Lauterio et al., 1994). In addition to promoting obesity, high dietary fat consumption *per se* may be independently associated with hypertension. For example, in the Dietary Approaches to Stop Hypertension (DASH) clinical trials, patients' blood pressures were lowered in the absence of weight loss by reducing the dietary content of saturated fat (Delichatsios and Welty, 2005). Furthermore, high fat diets promote insulin resistance, a condition implicated in the pathogenesis of hypertension, independently of

obesity in animal models (Vessby, 2003; Woods et al., 2004). Still, it is not clear whether high fat diets interact with obesity to promote hypertension. In part, this lack of understanding is due to a very close and confounding relationship between high fat diets and weight gain.

Another dietary factor which has received considerable scrutiny regarding its role in hypertension is sodium chloride. Increased dietary salt consumption induces hypertension in salt-sensitive, but not salt-resistant individuals (Facchini et al., 1999). Since salt-sensitivity occurs at a much higher rate in obese individuals, obesity-hypertension is thought by some to be a salt-sensitive form of hypertension (Diaz, 2002; Rocchini, 2000). There are many hypotheses that have been proposed to explain the link between salt-sensitivity and hypertension. One hypothesis, based on studies using both human subjects and laboratory animals, suggests that salt-sensitivity of blood pressure can be explained, at least in part, by an inability of the salt-sensitive subject to sustain or increase NO production in response to high salt intake (Bragulat and de la Sierra, 2002; Cubeddu et al., 2000; Kopkan and Majid, 2005; Manning et al., 2001). NO plays an important role in the long term regulation of blood pressure through its effects on the kidney: it mediates the tubuloglomerular feedback response to luminal sodium, renal blood flow, and pressure natriuresis (Bachmann and Mundel, 1994; Cowley et al., 2003; Raij and Baylis, 1995).

The objective of the present study was to further evaluate the obese Zucker rat as a model of obesity-hypertension by exposing it to dietary factors that may promote hypertension.

Lean and obese Zucker rats were fed a high fat diet that was later supplemented with salt in the drinking water, in order to investigate whether obese rats were more inclined to develop hypertension on this diet than lean rats. Our experiments indicate that (1) obese Zucker rats develop marked hypertension in response to a high fat, salt-supplemented diet, whereas lean Zucker rats do not (2) NO production and availability may be reduced by a high fat diet, and (3) the resulting NO deficiency may be due, in part, to decreased NO synthase activity.

Methods

Animals. Seven-week-old female lean (Fa/?) and obese (fa/fa) Zucker rats were purchased from Charles River Laboratories (Wilmington, MA, USA) and allowed to acclimate to the Marshall University Animal Resources Facility for one week prior to the start of an experiment. The rats were housed in plastic cages with corn chip bedding in a room having a 12 h light/dark cycle and maintained at an ambient temperature of 23±2 °C. All procedures using animals were reviewed and approved by the Marshall University Institutional Animal Care and Use Committee.

Experimental protocol. Systolic blood pressure (SBP) was measured once weekly using tail cuff plethysmography. Recordings were taken with a programmed electrosphygmomanometer coupled to a pneumatic pulse transducer and tail cuff (Narco-Biosystems, Houston, Texas, USA).

Food consumption, water consumption, and 24-hour urine output were measured weekly by placing rats in stainless steel metabolic cages. The rats were given 24 hours to adapt to the cages before measurements were taken or urine was collected. To help ensure a consistent environment, the metabolic cages were placed in the same room in which the rats were normally housed. After the 24-hour urine collection, the urine was centrifuged to remove any particulate matter. Aliquots of the supernatant were then stored at -20^{o}C until used for analysis.

After basal measurements were obtained (week 0), the rats were divided into four groups. One group of lean rats and one group of obese rats were maintained on a standard balanced rodent diet (Rodent Diet 5001) for the duration of the experiment and one group of lean rats and one group of obese rats were fed a high fat diet (formula D12266B: Research Diets, New Brunswick, New Jersey, USA) during the same 10 week interval. The high fat diet (Table 4.1) was the purified ingredient-version of the condensed milk diet that is used to separate lean from obesity-prone outbred rats (Lauterio et al., 1994). After 4 weeks, 1% NaCl was added to the drinking water of all four groups until the end of the experiment. At the end of the experiment, the rats were euthanized by exsanguination via cardiac puncture after being deeply anesthetized with an intraperitoneal injection of ketamine HCl:xylazine (45:5 mg/kg). Blood was drawn into heparinized syringes and the plasma was recovered by centrifugation and stored at -20^{o}C until used for analysis. After the rats were exsanguinated, their kidneys were removed and homogenized in ice-cold EDTA-free homogenization buffer (50 mM phosphate buffer, pH 7.5, containing 250 mM sucrose, 10 μg/ml aprotinin, 1 μg/ml leupeptin, and

87 μg/ml phenylmethylsulfonylfluoride) using Duall glass homogenizers. The homogenate was then centrifuged to remove cellular debris and the resultant supernatant was stored at -70^{o}C until used for analysis.

Analytical techniques. Plasma insulin and leptin levels were determined using [^{125}I] rat insulin and leptin radioimmunoassay kits according to the manufacturer's instructions (Linco, St. Charles, MO, USA). Nitric oxide production was estimated by measuring the concentration of NO_3 + NO_2 in the urine with a nitric oxide quantitation kit (Active Motif, Carlsbad, CA, USA). Total nitric oxide synthase (NOS) activity in whole kidney homogenates was determined in duplicate using an assay kit (Cayman Chemical, Ann Arbor, MI, USA) that measures the conversion of L-[^{3}H] arginine to [^{3}H] citrulline. Reaction conditions were verified using 1 mM N^{G}-nitro-L-arginine as a negative control. All samples were measured in duplicate.

Statistics. Data is expressed as means ± SE. Statistical differences among means were determined by two-way analysis of variance (2WANOVA) followed by a Tukey test. In the case of leptin, multiple t-tests were subsitituted for 2WANOVA because the obese and lean data sets were too unbalanced for ANOVA comparison. Differences were determined to be statistically significant at $p \leq 0.05$. Statistical analysis was performed using SigmaStat software (Jandel Scientific Software, San Rafael, CA, USA).

Results

Consumption of a high fat diet induced a greater weight gain than a standard balanced rodent diet in both lean and obese rats, with the weight gain in obese rats being considerably larger than that of lean rats (Fig. 4.1A). Despite substantial weight gain, obese rats consuming a high fat diet had a reduced cumulative energy intake (CEI) relative to obese rats on the standard control diet (Fig. 4.1B). We also observed that the high fat diet did not affect the cumulative energy intake of lean animals. In addition, obese rats on either diet had a greater energy efficiency ratio than lean rats fed the same diet (the energy efficiency ratio measures how efficiently energy is converted to body weight). This difference in energy efficiency ratios between lean and obese rats was particularly noticeable when comparing rats on the high fat diet (Fig 4.1C).

Regardless of diet, plasma leptin and insulin levels were markedly elevated in obese rats relative to lean rats by the time blood samples were obtained at the end of the study (Fig. 4.2). The high fat diet, however, had no effect on leptin levels in obese rats which is in contrast to the significant rise in plasma leptin levels observed in lean rats on the high fat diet (Fig. 4.2A). Conversely, plasma insulin levels were unaffected by the high fat diet in lean rats, whereas they were significantly elevated in obese animals on the same diet (Fig. 4.2B).

A principal objective of this study was to determine whether obesity increases the risk of developing salt-sensitive-hypertension in animals consuming a high fat diet. Both lean and obese rats were fed either a high fat diet or a diet of standard rat chow for the

duration of the study. Four weeks into the study, 1% NaCl was added to the drinking water of all animals. Within 2 weeks of adding NaCl to the drinking water, the obese rats on the high fat diet developed moderate hypertension (Fig. 4.3A). The peak systolic blood pressure for obese rats on the high fat, salt-supplemented diet was 158 mm Hg. From the 6^{th} week until the end of the study, the blood pressures of obese rats on the high fat, salt-supplemented diet were significantly higher than those of obese rats on the salt-supplemented control diet (Fig. 4.3A). By contrast, the blood pressures of lean rats were hardly affected by dietary manipulation. Average blood pressures of lean rats on a high fat, salt-supplemented diet never rose above 136 mm Hg, and only at one point was the elevation in blood pressure statistically significant (Fig. 4.3B).

To test the hypothesis that a diminished capacity to produce nitric oxide (NO) is involved in the development of hypertension in obese rats on the high fat, salt-supplemented diet, urinary excretion of stable NO metabolites (NO_2 and NO_3) was measured in all the treatment groups before and after salt supplementation. As shown in Figure 4.4, obese Zucker rats on the control diet excreted significantly more stable NO metabolites (NO_x) than their lean counterparts on the same diet. Also, there was an increase in NO_x excretion in both lean and obese rats when the control diet was supplemented with NaCl (Figure 4.4). This increase in NO_x excretion was particularly striking in obese rats on the control diet.

Results of this study also show that the high fat diet suppresses NO_x excretion (Figure 4.5A). Again, the effect in obese rats is quite dramatic because of the pronounced rise in

urinary NO_x excretion associated with an increased NaCl load. To test the hypothesis that the decrease in NO_x excretion by rats on the high fat diet is due, at least in part, to a decrease in NO production, renal NO synthase activity was measured in whole kidney homogenates obtained from week 10 animals. There was a significant suppression of NO synthase activity in renal homogenates from obese rats on the high fat, salt-supplemented diet relative to the obese control group (Fig. 4.5B). Renal NO synthase activity levels in lean control, lean high fat, and obese control animals were similar.

Discussion

The objective of the present study was to evaluate the obese Zucker rat as a model of obesity-associated hypertension using a high fat, salt-supplemented diet to induce hypertension. Obese rats that were fed the high fat diet developed hypertension when subjected to a salt load whereas lean rats did not. Moreover, blood pressures did not go up in response to a salt load in either the lean or obese rats that were fed a diet of standard rat chow. These results suggest that obesity increases the risk of developing hypertension when the obese subject is exposed to a hypertensive challenge. This observation is consistent with previous work in which we demonstrated that the obese Zucker rat was more sensitive to DOCA-salt-induced hypertension than age- and gender-matched lean rats (Morrison et al., 2002).

Blood pressures in the obese rats that were fed the high fat, salt supplemented diet reached a plateau within a few weeks of being subjected to a salt load with the peak blood pressure reaching 158 ± 4 mmHg. There was no overt evidence of renal damage at

the end of the study and the practically all animals survived. Thus, this model of obesity-hypertension could prove to be a useful model for studying long-term effects of moderate hypertension. This model may also be particularly useful for studying the interaction of obesity and hypertension on renal function since both hypertension (Morrison et al., 2005; Morrison et al., 2002) and obesity (Kasiske et al., 1985; Magil, 1995; Shimamura, 1983) affect renal function in the Zucker rat.

The moderate hypertension induced by a high fat, salt-supplemented diet stands in sharp contrast to the severity of the response of the obese Zucker rat to DOCA-salt-induced hypertension. In the DOCA-salt model, a significant elevation in blood pressure was evident within 4 days of the onset of treatment. The blood pressure peaked at levels in excess of 195 mmHg. The mortality rate was high and histological analysis revealed an end-stage kidney (Morrison et al., 2005). Because of the high mortality rate, the DOCA-salt model is only useful for short-term studies.

There is substantial evidence that the sensitivity of blood pressure to sodium intake may be modulated by a variety of nutritional factors (Kotchen and Kotchen, 1997). In particular, high fat diets have been shown to promote sodium and water retention (Strazzullo et al., 2001). In the present study, the obese Zucker rat appears to exhibit diet-induced salt-sensitivity of blood pressure in response to a high fat diet. The blood pressure did not rise appreciably in obese rats on the high fat diet during the 4 week interval prior to being subjected to a salt load. This is consistent with the observations of Alavi *et al.* (1995) and Maher *et al.* (1995). When subjected to a salt load, blood pressure

rose in the obese Zucker rat on a high fat diet, but hardly changed in obese rats on a diet of standard rat chow.

One mechanism through which high fat diets may promote salt-sensitivity involves the disruption of renal NO function. NO plays an important role in the long term regulation of blood volume and pressure through its effects on the kidney. NO mediates the tubuloglomerular feedback response to luminal sodium, renal blood flow, and pressure natriuresis (Bachmann and Mundel, 1994; Cowley et al., 2003; Raij and Baylis, 1995). Furthermore, NO has well known vasodilatory properties in the vasculature (Cockcroft, 2005). Studies in laboratory animals and humans have suggested that salt-sensitivity of blood pressure can be at least partly explained by an inability to sustain or increase NO production in response to high salt intake (Cubeddu et al., 2000; Kopkan and Majid, 2005; Manning et al., 2001). Thus, increased production of NO appears to be a vital physiological response for homeostatic management of blood pressure and volume under conditions of increased salt consumption. Accordingly, NO metabolite excretion was markedly increased after salt supplementation, reflecting increased systemic nitric oxide production in response to sodium loading. Interestingly, obese Zucker rats on the control diet exhibited higher systemic NO production than lean counterparts both before and after salt supplementation, suggesting that this mechanism may be playing a greater regulatory role in the obese rat than in the lean rat. Ingestion of the high fat diet markedly reduced this effect, suggesting that it has a negative impact on systemic NO production. This potential inhibitory effect of a high fat diet on NO production is also evidenced to a lesser

extent in the lean Zucker rats, where NO_x excretion was reduced slightly, but significantly.

The phenotypic differences in physiologic responses to salt loading were further investigated by directly measuring NO production in the kidney, where NO may play a critical role in the chronic regulation of sodium retention and blood pressure (Cowley et al., 2003). Renal NOS activity was reduced by the high fat diet in obese rats, but lean rats were unaffected by the dietary modification, further demonstrating the increased susceptibility of obese rats to diet-induced dysregulation of nitric oxide production. These data suggest the effects of the high fat diet on nitric oxide production may promote salt-sensitivity of blood pressure in obese Zucker rats.

The high fat diet significantly reduced urinary NO metabolite excretion in lean animals by the end of the study, but it had no effect on renal NO production at this point. This underscores the complexity of NO metabolism and the importance of other mechanisms that ultimately may play a role in determining NO availability, such as the inactivation of NO by oxidative stress. Recent studies have indicated that oxidative stress may rapidly inactivate NO, reducing functional NO availability (Dobrian et al., 2003; Roberts et al., 2003).

The underlying cause of obesity in obese Zucker rats is a missense point mutation in the leptin receptor (Chua et al., 1996) that leads to severe leptin resistance and hyperleptinemia. In our study, the high fat diet did not further exaggerate this

genetically-produced hyperleptinemia in OZRs, despite significant diet-induced weight gain, suggesting that plasma leptin may have already achieved peak levels in response to the aforementioned receptor defect. On the other hand, leptin levels were increased more than five-fold after high fat feeding in lean Zucker rats, which have a normally functioning leptin receptor. These phenotypic differences in leptin physiology may be responsible, in part, for the divergence of blood pressure responses between lean and obese rats on the experimental diet. Villarreal *et al.* (1998) have demonstrated a significant natriuretic effect of exogenous leptin that is attenuated in obese Zucker rats, suggesting that the genetically determined inability to respond to leptin in the obese Zucker rat may lead to impaired natriuretic capacity. This impairment may promote salt-sensitivity of blood pressure in obese Zucker rats. In contrast, lean rats with their functioning leptin receptor may be able to maintain salt resistance. In addition to this potential role in maintaining resistance to salt, a role for leptin-induced release of NO in maintaining normal blood pressure homeostasis has also been suggested (Fruhbeck, 1999).

In conclusion, our data indicates that high dietary fat intake dramatically compounds salt-sensitivity of blood pressure in genetically obese Zucker rats. This sensitivity is closely correlated to derangements in the compensatory production of NO, which is inhibited by high fat feeding in obese Zucker rats. In addition, leptin is implicated as a potential mediator in the dysregulation of blood pressure in obese Zucker rats on a moderately high fat, salt-supplemented diet. Taken together, the data suggests that multiple factors are probably responsible for the salt-sensitivity of blood pressure in Zucker rats.

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Figures and Tables

Table 4.1. Energy composition of the control and high fat diets. Macronutrient components are given in kcal percentage.

Diet	Control	High Fat
Protein	23	17
Carbohydrate	65	52
Fat	12	32
Total kcal/g	4.00	4.41

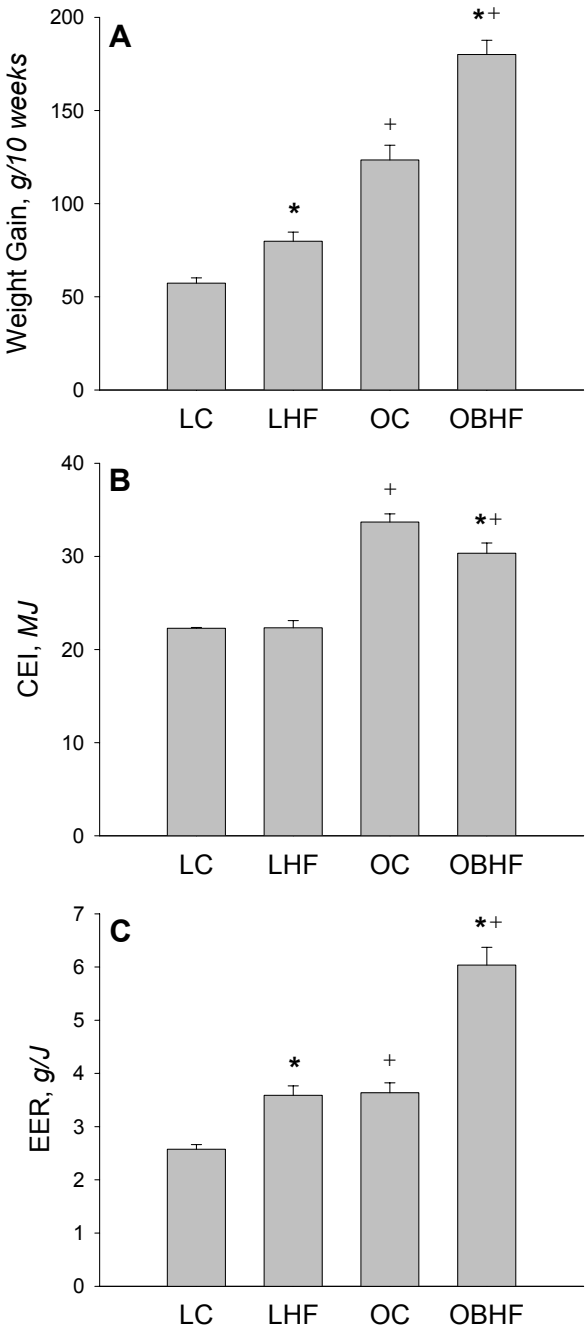

Figure 4.1. Weight gain (A), cumulative energy intake (B) and energy efficiency ratio (C) for lean and obese Zucker rats that were fed a high fat diet or standard balanced rodent (control) diet ad libitum. LC = lean rats, control diet; LHF = lean rats high fat diet; OC = obese rats, control diet; and OHF = obese rats, high fat diet. The cumulative energy intake (CEI) and energy efficiency ratio (EER) were calculated as described in the methods. The number of rats per group: LC =17, LHF = 17, OC = 16 and OHF = 14. *p<0.05 for a diet comparison within a given phenotype; +p<0.05 for a phenotype comparison within a given dietary treatment.

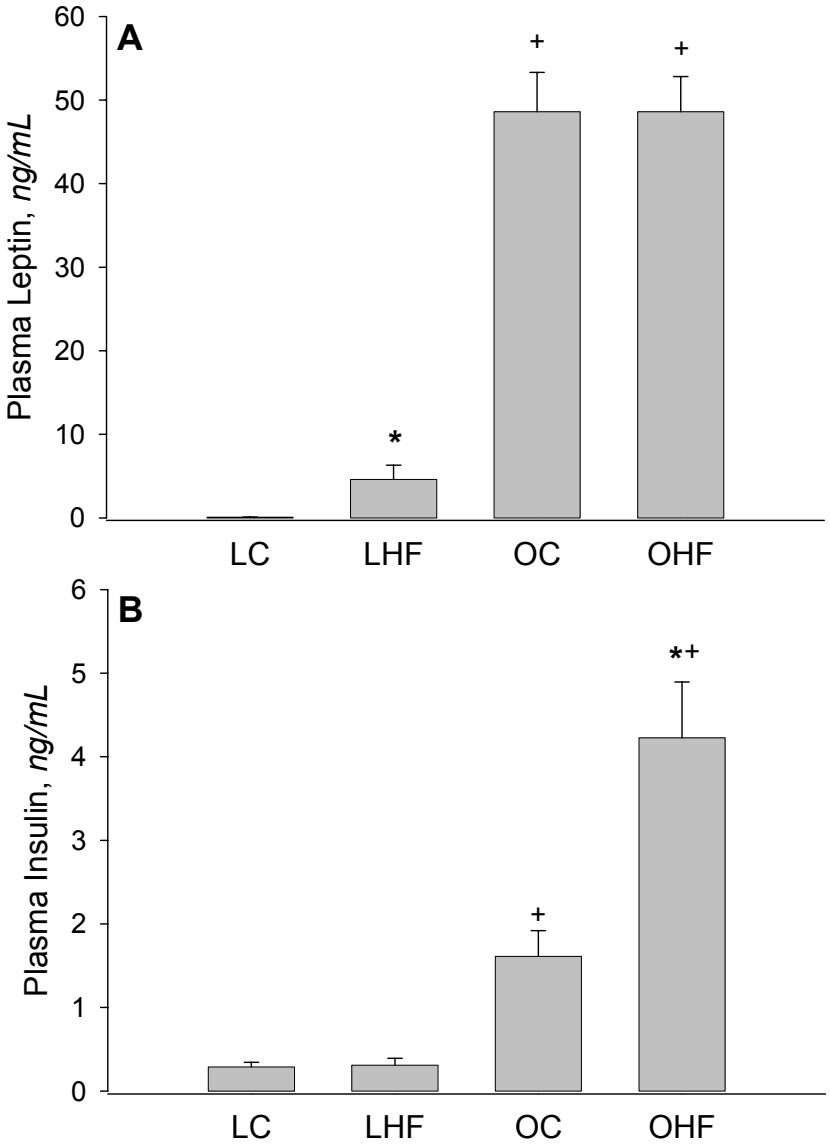

Figure 4.2. Plasma leptin (A) and insulin (B) concentrations in lean control (LC), lean high fat (LHF), obese control (OC) and obese high fat (OHF) groups after 10 weeks of dietary treatment. The number of rats per group: 7 for LHF and n=8 for all other groups. *p<0.05 for a diet comparison within a given phenotype; +p<0.05 for a phenotype comparison within a given dietary treatment.

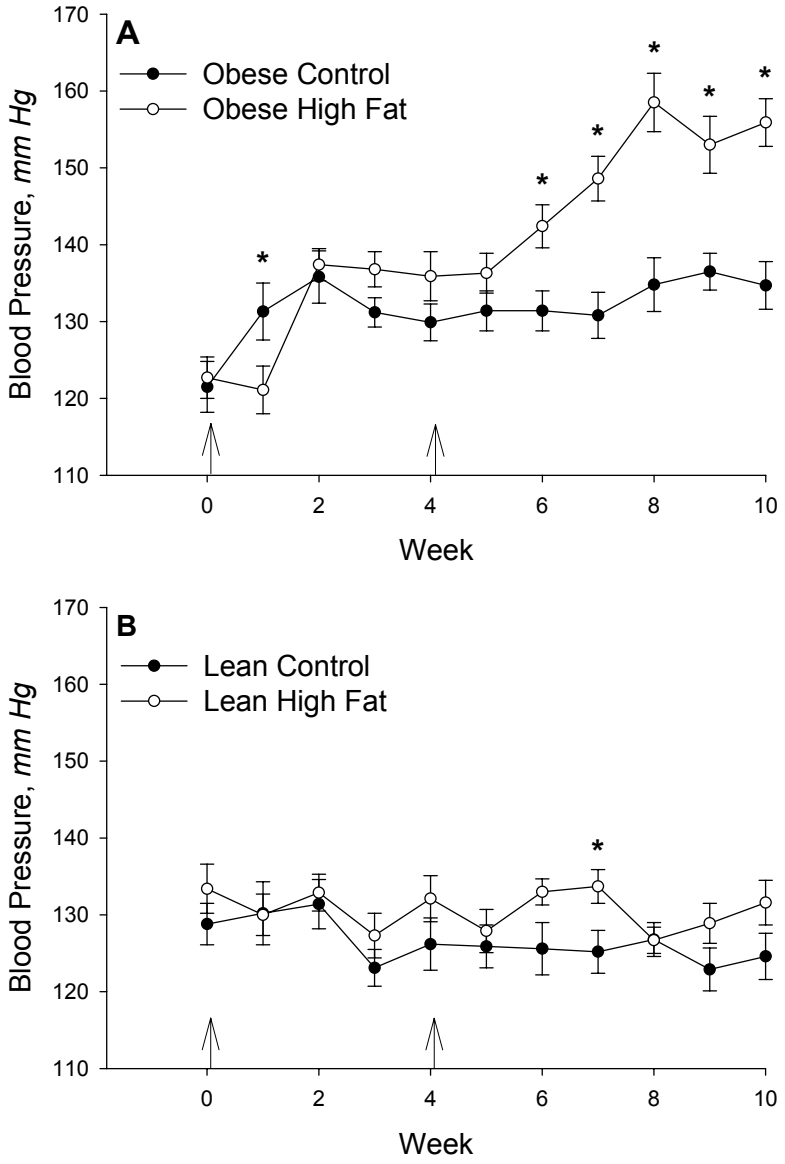

Figure 4.3. Blood pressures of obese (A) and lean (B) Zucker rats on either a high fat or control diet. All rats were being fed a control diet when blood pressures were obtained at week zero. Immediately after week zero measurements were recorded, half of the lean and obese rats were placed on a high fat diet (indicated by first arrow). After blood pressure measurements were recorded at week 4, the drinking water of all groups was supplemented by 1% saline (indicated by second arrow). The number of rats per group: 16 for obese groups and 18 for lean groups. *$p<0.05$ for comparison of dietary treatment within the same phenotype at the same week.

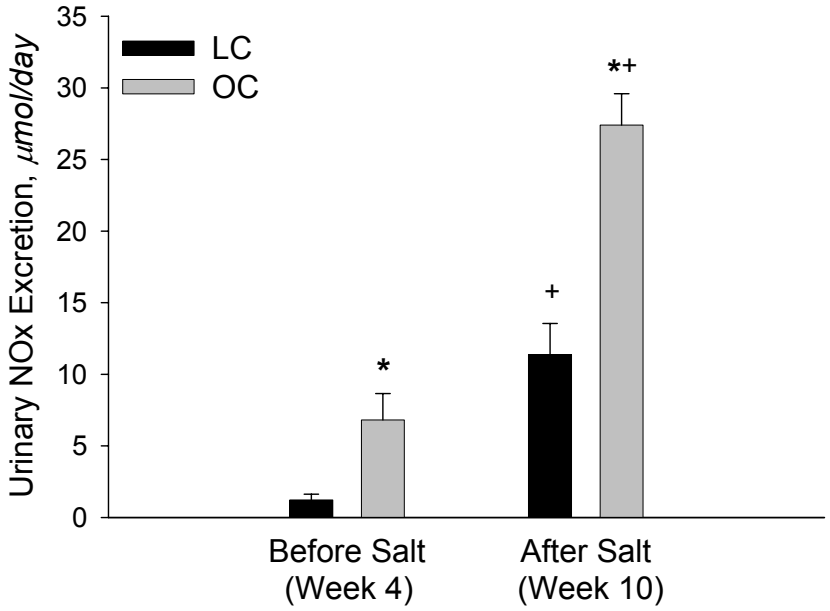

Figure 4.4. NO_x (NO_2 plus NO_3) excretion by lean control (LC) and obese control (OC) groups on weeks 4 and 10. The number of rats per group: 9 for lean groups and 8 for obese groups. *p<0.001 for diet comparisons within a given phenotype at the same week; +p<0.05 for a phenotype comparison within a given dietary treatment at the same week.

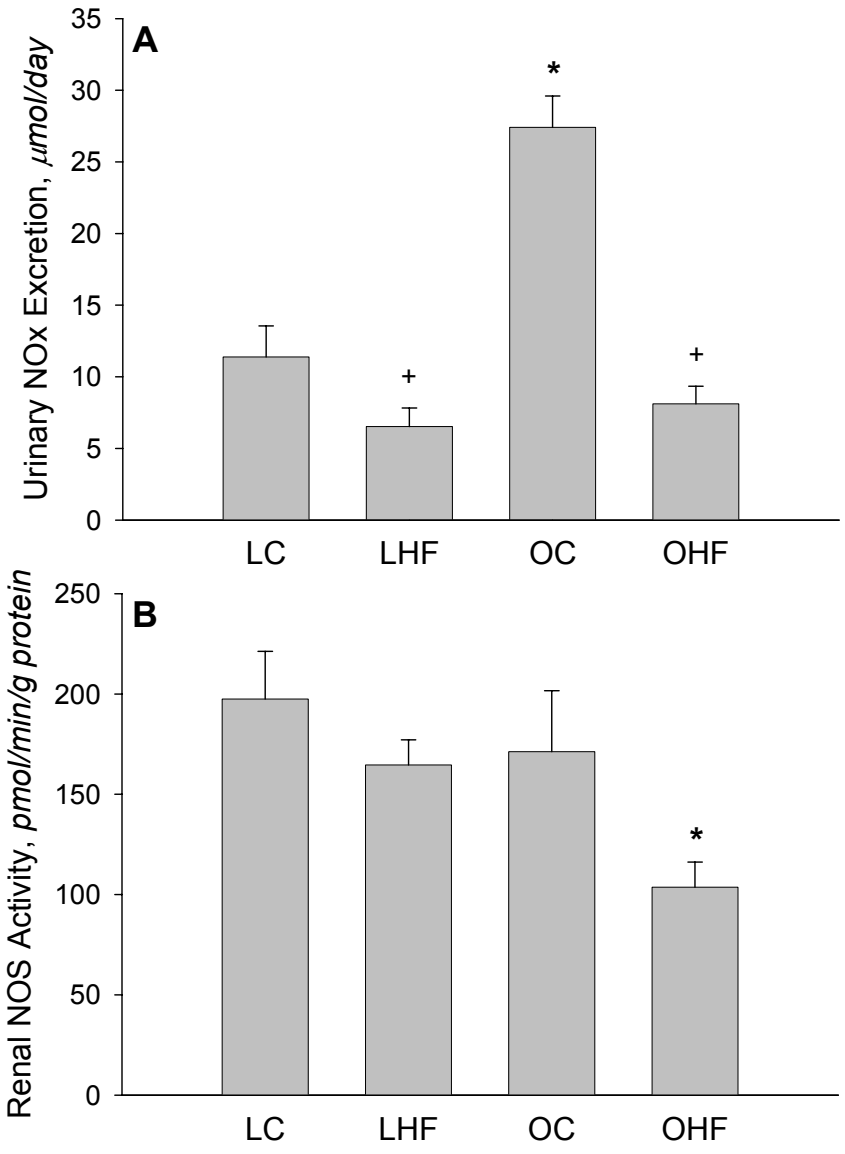

Figure 4.5. NO_x excretion and renal nitric oxide synthase (NOS) activity in lean control (LC), lean high fat (LHF), obese control (OC), and obese high fat (OHF) groups after 10 weeks of treatment. Number of rats per group: 7 for LHF and n=8 for all other groups. *p<0.05 for a diet comparison within a given phenotype; +p<0.05 for a phenotype comparison within a given dietary treatment.

CHAPTER V

Conclusions and Future Directions

Conclusions

We have characterized two models of obesity-hypertension using the obese Zucker rat. Both models have the potential to yield new insights into the nature of obesity-hypertension and may be useful in developing a better understanding of this complex disease. In one model, hypertension was induced by the administration of deoxycorticosterone acetate (DOCA) and salt excess. DOCA induces salt retention and a volume-expansion-dependent form of hypertension. The hypertensive response was clearly exacerbated in obese rats relative to age- and gender-matched lean controls that were subjected to the same treatment regimen. The DOCA-salt treated obese Zucker rat (DST-OZR) rapidly advanced to a state of severe hypertension and exhibited signs of end stage kidney disease by the end of the study (Morrison et al., 2005). By six weeks, the mortality rate in DST-OZRs climbed dramatically, making this model unsuitable for longer-term investigations. The second model of obesity-hypertension involved feeding rats a high fat diet and then subjecting them to a salt load. In this model, a more moderate form of hypertension with a lower mortality rate (Morrison et al., 2002) and less noticeable renal damage (personal communication, Dr. Betts Carpenter) developed in the obese Zucker rat. In contrast, the response of lean rats to this treatment was marginal and statistically insignificant. While few DST-OZRs survived more than 6 weeks beyond the onset of treatment, HF-OZRs had barely developed hypertension by that point; moreover, practically all HF-OZRs survived the 10-week long experimental treatment

(n=32, data not shown). An impaired ability to respond to a salt load appears to be a common characteristic of both of these models of obesity-hypertension. This may be a useful feature of these models since dietary salt consumption has been linked to human hypertension in general (MacGregor, 1983; MacGregor et al., 1989) and obesity-associated hypertension (Hsueh and Buchanan, 1994) in particular.

One aim of this research was to investigate a probable mechanism that could promote the development of hypertension in the HF-OZR model. In this regard, our work indicates that a dysfunction in the nitric oxide pathway may play a role in the pathophysiology of obesity-hypertension in the HF-OZR. Results presented as part of this dissertation clearly indicate that NO production is markedly suppressed by the high fat diet and that this effect is due, in part, to the inhibition of NO synthase activity. In light of the dramatic increase in NO production by obese rats that remain normotensive when subjected to a salt load while on a standard control diet, the inhibition of NO synthase by a high fat diet may indeed be a critical component of the mechanism underlying the development of hypertension in this model.

Future Directions

Future investigations will attempt to further elucidate the roles of genetic and environmental factors in the HF-OZR. Gene microarray analysis of cortical kidney homogenates from HF-OZR rats has already been performed, providing semiquantitative comparisons of the expression levels of over 9000 genes. Eventually, this data will be probed using a sophisticated technique known as gene set enrichment analysis (GSEA),

which allows the detection of cumulative changes in gene expression throughout multiple *a priori* defined biological pathways (Curtis et al., 2005). This analysis could provide a more comprehensive view of the mechanisms that may promote hypertension in response to a high fat diet and genetic obesity. Moreover, experiments can be designed to investigate whether the pathways that are implicated by the GSEA analysis may be involved in the pathogenesis of obesity-hypertension.

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APPENDIX A

Copywright Permissions for Chapter II

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February 7, 2006

Ryan Morrison
946 Madison Avenue Apt. 13
Huntington, WV 25704

VIA EMAIL TO: r_morrison27@hotmail.com sent February 7, 2006

FEE: NONE

RE: Morrison RG, Carpenter AB, Moore SK, Mangiarua EI, Valentovic MA, Walker EM Jr, Wehner PS, Rhoten WB, Touchon RC, McCumbee WD. "Increased sensitivity of the obese Zucker rat to" Journal of Hypertension 2002 Nov;20(11):2247-55

USE: Dissertation

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Subject :	Re: copywright release for dissertation (ACLS Feedback Form)

Dear Mr Morrison: Thank you for your message. On behalf of the Association of Clinical Scientists I am pleased to provide this release so that you may legally reproduce your work printed in the Annals of Clinical and Laboratory Science in the body of your academic dissertation. Sincerely, Bill Sunderman, Editor of the Annals and Secretary-Treasurer of the Association.

APPENDIX B

Cortical Kidney Microarray in HF-OZR

Methods: At the end of the high fat dietary study, whole kidneys were excised and flash-frozen in liquid nitrogen. Frozen kidneys were stored at -70 ºC until thawed on RNA*later*®-ICE according to the manufacturer's instructions (Ambion, USA). Cortical kidneys were excised and total RNA was extracted using RNEasy MIDI kit (Qiagen, USA). RNA integrity was assessed using the Agilent Bioanalyzer® (Agilent Technologies, Palo Alto, CA). 40 μg total RNA for 4 biological repeats per experimental group were used in a balanced block experimental design with a MWG rat 10K array (MWG, UK). Superscript Direct cDNA Labeling for Microarray (Invitrogen, USA) was used for labeling. mRNA samples were fluorescently labeled by incorporating Cy3-dCTP or Cy5-dCTP (Perkin-Elmer, USA) during reverse transcription. Hybridization was done on Hybe Station (Genomic Solutions, USA). Microarrays were scanned using ScanArray Express and quantified with ScanArray Express Software (Perkin-Elmer, USA), and then analyzed with Genespring Software (Agilent, USA).

Obese High Fat vs. Obese Control Diet

GENE ID	FOLD INCREASE	DESCRIPTION
D26111_1	2.06	chloride channel putative; clc-k2s; clc-k2l
RATTUS01236	2.16	expression: liver kidney heart brain strains: shrsp wistar_kyoto trembl||q9jmf4 ; complete cds; clone:2-31
RATTUS01691	2.50	expression: liver brain kidney strains: shrsp sprague_dawley wistar_kyoto gbp|ak018016|ak018016_1 riken full-length enriched library; clone:5830455m19 - mus musculus
RATTUS01645	2.51	expression: kidney strains: shrsp wistar_kyoto trembl||q9qxp0 rh type c glycoprotein
NM_033349_1	2.71	expression: liver kidney strains: sprague_dawley wistar_kyoto gbp|u97667|u97667_1 rsp29 - rattus norvegicus; hydroxyacyl glutathione hydrolase hagh
NM_030833_1	2.73	interferon-inducible protein 16 loc80875
RATTUS00354	3.06	expression: liver brain heart kidney strains: shrsp sprague_dawley wistar_kyoto trembl||q9nwu5 cdna flj20594 fis; clone kat08731
X62660_1	3.17	glutathione transferase
NM_052802_1	3.34	kidney androgen-regulated protein kap
RATTUS01543	3.60	expression: liver heart strains: sprague_dawley wistar_kyoto gbp|ak012051|ak012051_1 riken full-length enriched library; clone:2610319b19 - mus musculus
RATTUS02152	3.78	expression: brain kidney strains: shrsp gbp|ak027689|ak027689_1 cdna flj14783 fis; clone nt2rp4000541 unnamed protein product - homo sapiens
RATTUS00919	4.73	expression: liver brain strains: shrsp sprague_dawley wistar_kyoto trembl||o88783 coagulation factor v
RATTUS01707	4.76	expression: kidney strains: wistar_kyoto gbp|ak003647|ak003647_1 riken full-length enriched library; clone:1110012o14 - mus musculus

GENE ID	FOLD DECREASE	DESCRIPTION
NM_020976_1	2.05	kidney-specific membrane protein nx-17
U00926_1	2.08	delta subunit of f1f0 atpase

M36410_1	2.13	sepiapterin reductase ec 1.1.1.153
NM_017081_1	2.16	hydroxysteroid dehydrogenase; 11 beta type 2 hsd11b2; 11-beta-hydroxylsteroid dehydrogenase
AF029886_1	2.17	microphthalmia associated transcription factor microphthalmia
NM_017314_1	2.25	polyubiquitin ubc; ubiquitin c
NM_017314_1	2.29	polyubiquitin ubc; ubiquitin c
NM_022399_1	2.34	d-beta-hydroxybutyrate dehydogenase; calreticulin; calr; precursor aa -17 to 399
NM_031090_1	2.44	ras-related protein rab1; ras
NM_053576_1	2.44	acidic calcium-independent phospholipase a2 aipla2; thiol-specific antioxidant protein prdx5; tsa
M96377_1	2.58	neurexin ii-beta-a
S61973_1	2.64	nmda receptor glutamate-binding subunit
RATTUS01564	2.64	expression: liver brain kidney strains: shrsp sprague_dawley wistar_kyoto gbp|ak009057|ak009057_1 riken full-length enriched library; clone:2310001a20 - mus musculus
RATTUS01409	2.67	expression: liver brain kidney strains: shrsp sprague_dawley wistar_kyoto gbp|af195142|af195142_1 selenoprotein r - mus musculus
NM_017245_1	2.76	eukaryotic translation elongation factor 2 eef2; ef-2; aa 1-858
RATTUS02428	2.87	expression: brain strains: shrsp sprague_dawley wistar_kyoto mwg own new gene sequence
NM_031012_1	3.02	kidney zn-peptidase precursor; aminopeptidase m apm; anpep
NM_017147_1	3.21	cofilin 1; non-muscle cfl1
NM_031354_1	3.24	mitochondrial voltage dependent anion channel vdac2; voltage-dependent 2
S78284_1	3.27	bcl-x short form; bcl-xshort; bcl-x-long; bcl-xalpha
NM_012783_1	3.31	basigin ox47 antigen or ce-9 emmprin in human neurothelin; ht7 or 5a11 in avian bsg; mrc; transmembrane glycoprotein
RATTUS00028	3.40	expression: heart brain strains: shrsp sprague_dawley wistar_kyoto gbp|ab055737|ab055737_1 nd1-l kelch family protein nd1-l - mus musculus
NM_053840_1	3.56	put. ggt light subunit 380-568 protein sequence is in conflict with the conceptual translation; gamma-glutamyl transpeptidase ec

		2.3.2.2; m33821; precursor aa 4-568; gamma-glutamyltranspeptidase 1-568
NM_013013_1	3.68	prosaposin sulfated glycoprotein; sphingolipid hydrolase activator psap; glycoprotein precursor; glycoprotein-1 glycoprotein-1; sgp-1; prosapo>; prosaposin; sgp-1
NM_023950_1	4.19	rab7; member ras oncogene family rab7; gtp-binding protein; ras-related p23
NM_017173_1	4.26	serine proteinase inhibitor; clade h heat shock protein 47; member 1 serpinh1; collagen-binding gp46
NM_020076_1	4.28	3-hydroxyanthranilate 3;4-dioxygenase haao
NM_031605_1	4.44	cytochrome p450; 4a10 cyp4a10; p450
NM_022866_1	4.68	sodium-dependent high-affinity dicarboxylate transporter 3 nadc3; sdct2
NM_024149_1	4.96	adp-ribosylation factor 5 arf5
S69315_1	6.51	endoplasmin grp94
RATTUS00063	11.31	expression: liver brain heart kidney strains: shrsp sprague_dawley wistar_kyoto gbp|x02344|x02344_1 beta 2 beta-tubulin - homo sapiens; trembl||q9jjy6 fragment

Obese High Fat vs. Lean Control Diet

GENE ID	FOLD INCREASE	DESCRIPTION
D16478_1	2.05	mitochondrial long-chain enoyl-coa hydratase/3-hydroxycyl-coa dehydrogenase alpha-subunit rtp-alpha
NM_052802_1	4.16	kidney androgen-regulated protein kap

GENE ID	FOLD DECREASE	DESCRIPTION
AJ238391_1	2.06	sulfotransferase k1 sultk1; k2 sultk2
NM_053864_1	2.10	transitional endoplasmic reticulum atpase; valosin-containing protein vcp
RATTUS03589	2.12	expression: brain strains: shrsp sprague_dawley trembl||q9nsn1 hypothetical 110.4 kda protein fragment
RATTUS00172	2.14	expression: kidney heart brain strains: shrsp sprague_dawley wistar_kyoto gbp|m74773|m74773_1 spnb-2 brain beta spectrin - mus musculus
NM_012504_1	2.20	atpase; na+k+ transporting; alpha 1 polypeptide atp1a1; na+;k+-atpase alpha-subunit precursor; na+ and k+ catalytic subunit; na;k-atpase alpha-1; atpase aa -4 to -1
NM_053837_1	2.21	rat assembly protein ap50 associated with clathrin-coated vesicles; adaptor-related complex 2; mu 1 subunit ap2m1
NM_053576_1	2.28	acidic calcium-independent phospholipase a2 aipla2; thiol-specific antioxidant protein prdx5; tsa
RATTUS02848	2.31	expression: kidney heart strains: shrsp sprague_dawley wistar_kyoto gbp|ak010581|ak010581_1 riken full-length enriched library; clone:2410024g15 - mus musculus; gbp|u51014|u51014_1 pep4 prolidase
NM_017233_1	2.32	4-hydroxyphenylpyruvic acid dioxygenase hpd; 4-hydroxyphenylpyruvate hppd; f alloantigen
NM_053752_1	2.41	succinate-coa ligase; gdp-forming; alpha subunit suclg1; succinyl-coa synthetase ec 6.2.1.4
NM_022399_1	2.60	d-beta-hydroxybutyrate dehydogenase; calreticulin; calr; precursor aa -17 to 399
AF029886_1	2.78	microphthalmia associated transcription factor microphthalmia

NM_019134_1	2.86	solute carrier family 12; member 1 bumetanide-sensitive sodium-[potassium]-chloride cotransporter slc12a1; sodium- potassium – chloride
U30290_1	3.05	galanin receptor galr1
NM_012615_1	3.09	ornithine decarboxylase ec 4.1.1.17; odc; ornitine odc1
NM_021594_1	3.32	erm-binding phosphoprotein loc59114
S61973_1	3.33	nmda receptor glutamate-binding subunit
NM_013085_1	3.46	urinary plasminogen activator; urokinase plau; urikinase-type activator; upa
NM_021266_1	3.55	drosophila polarity gene frizzled homologue fzd1
RATTUS00794	3.66	expression: liver brain kidney strains: shrsp sprague_dawley wistar_kyoto gbp|ak007469|ak007469_1 riken full-length enriched library; clone:1810013b01 - mus musculus
RATTUS00028	3.67	expression: heart brain strains: shrsp sprague_dawley wistar_kyoto gbp|ab055737|ab055737_1 nd1-l kelch family protein nd1-l - mus musculus
NM_021740_1	3.86	alpha-prothymosin myc-regulated gene; prothymosin precursor; prothymosin-alpha; alpha ptma
RATTUS03021	4.56	expression: liver brain kidney strains: shrsp sprague_dawley wistar_kyoto gbp|bc003903|bc003903_1 glutathione s-transferase theta 1 - mus musculus
M24359_2	5.33	m2 pyruvate kinase pk
NM_012886_1	5.70	tissue inhibitor of metalloproteinase 3 timp-3; timp3
Z11994_1	6.36	45kda protein
NM_013214_1	6.72	cytosolic peroxisome proliferator-induced acyl-coa thioesterase; hydrolase rbach; rlach1; acyl coenzyme a thioester

Obese Control vs. Lean Control Diet

GENE ID	FOLD INCREASE	DESCRIPTION
NM_012939_1	2.12	cathepsin h pre-pro-peptide; rch11; ctsh
AJ309926_1	2.19	ion channel asic 1b; asic-beta
NM_020976_1	2.24	kidney-specific membrane protein nx-17
S76779_1	2.25	apolipoprotein e rapoe; apoe
NM_031012_1	2.30	kidney zn-peptidase precursor; aminopeptidase m apm; anpep
M35052_1	2.55	f-0-atpase subunit b precursor
RATTUS00729	2.56	expression: liver brain kidney strains: shrsp sprague_dawley wistar_kyoto gbp|ak004817|ak004817_1 riken full-length enriched library; clone:1200017c17 - mus musculus
RATTUS01564	2.63	expression: liver brain kidney strains: shrsp sprague_dawley wistar_kyoto gbp|ak009057|ak009057_1 riken full-length enriched library; clone:2310001a20 - mus musculus
M36410_1	2.72	sepiapterin reductase ec 1.1.1.153
RATTUS01409	2.80	expression: liver brain kidney strains: shrsp sprague_dawley wistar_kyoto gbp|af195142|af195142_1 selenoprotein r - mus musculus
NM_022266_1	2.80	connective tissue growth factor ctgf
RATTUS00443	2.90	expression: heart brain kidney strains: shrsp sprague_dawley wistar_kyoto gbp|aj310346|aj310346_1 paf67 67 kda polymerase-associated factor paf67 - mus musculus
NM_031031_1	3.33	l-arginine: glycine amidinotransferase gatm; l-arginine:glycine
NM_021757_1	3.45	pleiotropic regulator 1 plrg1
RATTUS00249	3.51	expression: liver heart kidney brain strains: shrsp sprague_dawley wistar_kyoto trembl||caa02861 sequence 56 from patent wo9520654
NM_022947_1	3.52	suppressor of k+ transport defect 3 skd3
NM_017306_1	4.40	dodecenoyl-coenzyme a delta isomerase 3;2 trans-enoyl-coenyme a isomerase dci; delta-3;delta-2-enoyl-coa; 3-2trans-enoyl-coa
NM_053314_1	4.54	potassium inwardly-rectifying channel; subfamily j; member 16 kcnj16; kir5.1; inward rectifier potassuim channel 9

NM_031605_1	5.63	cytochrome p450; 4a10 cyp4a10; p450
NM_013098_1	17.43	glucose-6-phosphatase catalytic subunit g6pase; g6pc

GENE ID	FOLD DECREASE	DESCRIPTION
RATTUS00548	2.47	expression: heart brain kidney strains: shrsp sprague_dawley wistar_kyoto gbp|ak010275|ak010275_1 riken full-length enriched library; clone:2400003o04 - mus musculus
NM_053319_1	2.65	protein inhibitor of neuronal nitric oxide synthase pin; dynein; cytoplasmic; light peptide
X61296_2	2.66	orf2 consensus sequence encoding endonuclease and reverse transcriptase minus rnaseh; unknown protein; orf 2; open reading frame
RATTUS02304	2.87	expression: liver kidney strains: shrsp sprague_dawley wistar_kyoto gbp|ak002802|ak002802_1 riken full-length enriched library; clone:0610038k03 - mus musculus
U67995_1	4.54	stearyl-coa desaturase 2
NM_013214_1	17.53	cytosolic peroxisome proliferator-induced acyl-coa thioesterase; hydrolase rbach; rlach1; acyl coenzyme a thioester

Lean High Fat vs. Lean Control Diet

GENE ID	FOLD INCREASE	DESCRIPTION
NM_012793_1	2.07	guanidinoacetate methyltransferase precursor; gamt
NM_031012_1	2.18	kidney zn-peptidase precursor; aminopeptidase m apm; anpep
M35052_1	2.29	f-0-atpase subunit b precursor
NM_021765_1	2.57	beta prime cop copb
NM_012771_1	2.64	lysozyme lyz
RATTUS03013	2.78	expression: kidney strains: wistar_kyoto trembl||q9jmg1 ; complete cds; clone:1-9
RATTUS00582	2.80	expression: kidney heart strains: shrsp sprague_dawley wistar_kyoto gbp|ak007386|ak007386_1 riken full-length enriched library; clone:1810008o21 - mus musculus
NM_053021_1	3.50	testostrone-repressed prostate message 2 clu; clusterin; putative open reading frame; sulfated glycoprotein precursor; trpm-2
NM_012992_1	4.57	nucleoplasmin-related protein nuclear protein b23 npm1; nucleolar; b23.1
RATTUS01085	5.88	expression: liver brain heart strains: shrsp sprague_dawley wistar_kyoto gbp|ak014338|ak014338_1 riken full-length enriched library; clone:3230402m22 - mus musculus
NM_017306_1	7.16	dodecenoyl-coenzyme a delta isomerase 3;2 trans-enoyl-coenyme a isomerase dci; delta-3;delta-2-enoyl-coa; 3-2trans-enoyl-coa
RATTUS01484	13.43	expression: liver strains: shrsp wistar_kyoto gbp|m17440|m17440_1 c4a complement component c4a - mus musculus

GENE ID	FOLD DECREASE	DESCRIPTION
NM_012615_1	1.98	ornithine decarboxylase ec 4.1.1.17; odc; ornitine odc1
NM_012615_1	2.05	ornithine decarboxylase ec 4.1.1.17; odc; ornitine odc1
NM_012615_1	2.08	ornithine decarboxylase ec 4.1.1.17; odc; ornitine odc1
NM_053792_1	2.08	selective lim binding factor; rat homolog slb; factor
NM_031535_1	2.09	b cell lymphoma 2 like bcl2l; bcl-xbeta

NM_053598_1	2.10	diphosphoinositol polyphosphate phosphohydolase type ii nudt4
D30804_1	2.13	proteasome subunit rc6-1
NM_012615_1	2.16	ornithine decarboxylase ec 4.1.1.17; odc; ornitine odc1
NM_013059_1	2.18	tissue-nonspecific alp alkaline phosphatase alpl; precursor polypeptide aa -17 to 507
NM_031774_1	2.29	rab acceptor 1 prenylated rabac1; pra1
X67788_1	2.35	ezrin; p81
NM_012497_1	2.54	aldolase c; fructose-biphosphate aldoc; c
NM_032617_1	2.62	rab11b; member ras oncogene family rab11b; gtp-binding protein; gtp binding rab11-p24-b
NM_012615_1	2.79	ornithine decarboxylase ec 4.1.1.17; odc; ornitine odc1
NM_031523_1	2.80	nerve growth factor; gamma polypeptide ngfg; kallikrein precursor; ps; s3; kal; 1; renal/pancreas/salivary klk1
NM_017248_1	3.00	heterogeneous nuclear ribonucleoprotein a1 hnrpa1; helix destabilizing protein
RATTUS03589	3.02	expression: brain strains: shrsp sprague_dawley trembl||q9nsn1 hypothetical 110.4 kda protein fragment
RATTUS01421	3.07	expression: heart brain kidney strains: shrsp wistar_kyoto trembl||q9jj97 brain cdna; clone mncb-0091
AF273025_1	3.22	amino acid system n transporter
M24930_1	3.27	mhc a-beta rt1.b-b-beta cell surface glycoprotein; class ii antigen b beta-chain rt1.bbeta; integral membrane protein rt1.b; a<beta>; rt1.b-1
NM_031523_1	3.32	nerve growth factor; gamma polypeptide ngfg; kallikrein precursor; ps; s3; kal; 1; renal/pancreas/salivary klk1
RATTUS00028	3.44	expression: heart brain strains: shrsp sprague_dawley wistar_kyoto gbp|ab055737|ab055737_1 nd1-l kelch family protein nd1-l - mus musculus
NM_012615_1	3.49	ornithine decarboxylase ec 4.1.1.17; odc; ornitine odc1
S62903_1	3.60	genetic hypertension component sa
RATTUS01155	3.64	expression: brain strains: shrsp sprague_dawley wistar_kyoto trembl||o08551 serine/arginine-rich protein specific kinase 2 ww domain binding protein 6 fragment
NM_012615_1	3.65	ornithine decarboxylase ec 4.1.1.17; odc; ornitine odc1

NM_031763_1	3.98	platelet-activating factor acetylhydrolase beta subunit paf-ah beta pafah1b1
NM_023102_1	5.35	casein kinase 1 gamma 2 isoform csnk1g2
U67995_1	7.17	stearyl-coa desaturase 2

Curriculum Vitae

Ryan Morrison

Department of Pharmacology, Physiology, and Toxicology

Marshall University, Joan C. Edwards School of Medicine

1542 Spring Valley Drive

Huntington, WV 25704

EDUCATION

Marshall University:

- Ph.D. in Biomedical Sciences (January 2000 - May 2006)
- M.B.A. (January 2003 – August 2005)
- B.S. Biology (August 1995 – December 2000)

Graduate GPA: 3.62 *Total Undergraduate/Graduate Hours: 182/204*

PUBLICATIONS IN PEER-REVIEWED JOURNALS

- **Morrison RG**, Carpenter AB, Adams VL, Mangiarua EI, Wehner PS, McCumbee WD. Progression of renal damage in the obese Zucker rat in response to deoxycorticosterone acetate-salt-induced hypertension. Ann Clin Lab Sci. 2005 Winter;35(1):54-65.

- Wright GL, **Morrison R**, Fultz ME, Wright G, McCumbee W, Wehner P, Studeny M. Effect of fasting on vascular contractility in lean and obese Zucker rats. Clin Nutr. 2003 Aug;22(4):359-63.

- **Morrison RG**, Carpenter AB, Moore SK, Mangiarua EI, Valentovic MA, Walker EM Jr, Wehner PS, Rhoten WB, Touchon RC, McCumbee WD. Increased sensitivity of the obese Zucker rat to deoxycorticosterone-salt- induced hypertension. J Hypertens. 2002 Nov;20(11):2247-55.
    - *Selected for Editorial Commentary:*

      Kenyon CJ. Mineralocorticoid-induced hypertension in obese Zucker rats. J Hypertens. 2002 Nov;20(11):2151-2. Review. No abstract available.

PRESENTATIONS AT NATIONAL SCIENCE MEETINGS

- Annual Meeting of the American Society of Hypertension

  San Francisco, California, May 2001

  "Increased Sensitivity of the Obese Zucker Rat to DOCA-Salt-Induced Hypertension"

  R.G. Morrison, S.K. Moore, E.I. Mangiarua, G.L. Wright, and W.D. McCumbee

- Annual Meeting of the Federation of American Societies for Experimental Biology

  San Diego, California, May 2005

  "Nitric Oxide and Diet-Induced Hypertension in the Obese Zucker Rat"

  Ryan G. Morrison, Antoinette L. Moran, Chelsea E. Walton, Mohamed H. Sadek, Paulette S. Wehner, Elsa I. Mangiarua, and William D. McCumbee

PRESENTATIONS AT LOCAL SCIENCE MEETINGS

- Marshall University Research Day

  Huntington, West Virginia, 2003

  "Effects of Zocor on Blood Pressure and Kidney Function in Obese Rats With Mineralocorticoid-Induced Hypertension"

  R.G. Morrison, I. Arif, E. Dager, A.B. Carpenter, E.I. Mangiarua, W.D. McCumbee

- MU Research Day, 2001

  Huntington, West Virginia, 2001

  "Increased Sensitivity of the Obese Zucker Rat to DOCA-Salt-Induced Hypertension"

  R.G. Morrison, S.K. Moore, E.I. Mangiarua, G.L. Wright, and W.D. McCumbee

- Listed as coauthor and helped present posters for 3 different BRIN student projects at WV-BRIN meetings, 2003-2004

- 2 Undergraduate Sigma Xi poster presentations, 1999

ADDITIONAL RESEARCH EXPERIENCE

- Undergraduate Research in Chemistry, 1998

  developed a procedure for detecting lead in water utilizing scanning electron microscopy of electrodeposited samples on a microscale surface

PRESENTATIONS AT REGIONAL BUSINESS MEETINGS

- Proceedings of the Southeast Case Research Association Annual Meeting

  Myrtle Beach, South Carolina, February 2006

  "Jacob Supply Company"

  with Uday Tate, Ashley Collins, Kim Congrove, and Blaker Bolling

INVITED LECTURES

- Continuing Medical Education Lecture for Cardiology Group

  Saint Mary's Hospital, Huntington, WV, 2004

  "Obesity and Renal Disease"

- Continuing Medical Education Lecture for Cardiology Group

  Saint Mary's Hospital, Huntington, WV, 2005

  "Molecular Mechanisms of Cardiac Hypertrophy"

PUBLISHED ILLUSTRATIONS

- Designed and Produced Scientific Figures

  Carpenter, AB. "Immunoassays for Diagnosis of Infectious Diseases" in Manual of Clinical Microbiology. 9th Ed. In Press 2006. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (Eds.)

AWARDS

- WV PROMISE scholarship, August 1995
- COS Undergraduate Research Scholarship, January 1999
- Special Travel Award from Dean of Graduate Studies, May 2005
- Special Travel Award from Provost, May 2005

TECHNICAL PROFICIENCIES

- Biomedical Science:

  rodent handling (anesthesia and surgery), enzymatic/colorimetric assays, ELISAs, RIAs, RNA extraction and analysis, protein extraction and analysis

- Computer:

  highly developed computer-literacy , skilled in all Microsoft Office products (65 wpm and numpad proficient), Sigmastat/Sigmaplot Statistical Software, Adobe Photoshop and Illustrator, Endnote Citation Management Software

MANAGEMENT EXPERIENCE

- Managed research lab, 2003-2006

  trained new graduate students, technicians, and BRIN undergraduate students, organized and maintained inventory

CLASSROOM-BASED PRESENTATIONS

- Japanese Healthcare System
- The Electronic Medical Record
- The Metabolic Syndrome