THE INFLUENCE OF GENDER ON THE ASSOCIATION
BETWEEN HYPERINSULINEMIA / INSULIN RESISTANCE AND HYPERTENSION

By
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ABSTRACT

The primary objective of this study was to examine the relationship between hyperinsulinemia / insulin resistance and hypertension in female rats. A link between these two conditions has been well established in studies employing male animal models as well as in human studies, however, it has not been possible to discern what role gender plays in this relationship, if any, based on these previous reports. To investigate the effect of gender on the association between hyperinsulinemia / insulin resistance, two different hyperinsulinemic, hypertensive rat models were used; the fructose fed hypertensive rat (FHR) and a chronically insulin treated rat. In the first set of experiments using fructose fed animals, we found that fructose causes hyperinsulinemia, insulin resistance and hypertension in males, but does not affect metabolism or blood pressure at all in females. However in a separate experiment, female rats that had been ovariectomized did develop insulin resistance and hypertension, indicating that normal levels of ovarian sex hormones are involved in protecting female rats against the effects of a fructose diet. In a second set of blood pressure experiments, we employed a chronic exogenous insulin treatment to attempt to create a state of insulin resistance in females. In this study, we observed that exogenous hyperinsulinemia causes insulin resistance in both male and female rats, however, this occurs to a greater degree in males. Furthermore, hyperinsulinemia and insulin resistance were only associated with hypertension in male rats.

Several mechanisms have been proposed to play a role in the development of hypertension associated with hyperinsulinemia and insulin resistance. A secondary objective of this thesis was to identify potential mechanisms that might explain any gender differences observed. To this aim, we focused on vascular function. We examined the vascular effects of insulin and found that it tends to act more as a vasoconstrictor in female rats rather than a vasodilator, as we had previously demonstrated in males. We also demonstrated that there is a significant gender difference in the effect of U46619, a synthetic thromboxane analogue, which may be related to gender differences in the development of hypertension associated with hyperinsulinemia and insulin resistance.
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ABBREVIATIONS

Ach  Acetylcholine
ACE  Angiotensin converting enzyme
AII  Angiotensin II
ANOVA Analysis of variance
AUC  Area under the curve
BP   Blood Pressure
CEE  Conjugated equine estrogens
CSA  Cross-sectional area
COX  Cyclo-oxygenase enzyme
CVD  Cardiovascular disease
EDHF Endothelium derived hyperpolarizing factor
EDTA Ethylenediaminetetraacetic acid
ET-1 Endothelin-1
FHR  Fructose hypertensive rat
FPG  Fasting plasma glucose (16 hour)
FPI  Fasting plasma insulin (16 hour)
HDL  High density lipoprotein
ISI  Insulin sensitivity index
LDL  Low density lipoprotein
MPG  Mean plasma glucose
MPI  Mean plasma insulin
NA   Noradrenaline
NO   Nitric oxide
NOS  Nitric oxide synthase enzyme
OGTT Oral glucose tolerance test
OVX  Ovariectomy / Ovariectomized
TE   Total plasma estrogens
SEM  Standard error of the mean
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<td>SNS</td>
<td>Sympathetic nervous system</td>
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<td>VSM(C)</td>
<td>Vascular smooth muscle (cell)</td>
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INTRODUCTION

Insulin Resistance and Hyperinsulinemia in Hypertension

Hyperinsulinemia and resistance to the glucose lowering effects of insulin (insulin resistance) are often found to be associated with hypertension in both humans and several animal models [1-3]. From this observation, the "insulin hypothesis" was developed, which proposes that these metabolic impairments are directly related to the cause of hypertension in such individuals. This hypothesis was attractive because it helped to explain the apparent inability of conventional antihypertensive drugs to decrease the incidence of coronary ischemic events, since these drugs tended to worsen rather than improve insulin action [4-6]. In addition to the studies demonstrating that both obese and lean hypertensive patients exhibit insulin resistance, further evidence for this hypothesis includes the observation that insulin resistance and hyperinsulinemia are also present in normotensive offspring of hypertensive parents [7]. This can be detected as early as the second decade of life and these changes precede any rise in blood pressure. Several hypertensive rodent models also exhibit similar defects in glucose metabolism and insulin action, including the Dahl rat [8], spontaneously hypertensive rat (SHR) [9-11], Milan hypertensive rat [12], and fructose hypertensive rat (FHR) [10, 13]. As these models are etiologically distinct, the existence of these common defects lends further strength to the hypothesis that they are linked to hypertension. In addition to hypertension, studies have clearly indicated that hyperinsulinemia is an independent risk factor for coronary artery disease and that even a small degree of glucose intolerance significantly increases the risk for developing coronary artery disease [14-16].

Although several reports indicate a positive correlation among blood pressure (BP), insulin resistance, and plasma insulin concentrations, others have found that this association is weak or non-existent [17-20]. While glucose uptake during a hyperinsulinemic euglycemic clamp in lean hypertensives has been shown to be 30-40% reduced compared to normotensives [21, 22], the association between insulin resistance and blood pressure is strongest for hypertensive subjects who are obese. There also appears to be an ethnic variation in this
relationship; for example, Pima Indians and Mexican Americans often have insulin resistance but rarely have hypertension [20, 23, 24]. Despite the dispute that arises out of these conflicting reports, it appears that as many as 50% of essential hypertensive patients appear to be insulin resistant and hyperinsulinemic [25, 26]. Since there are multiple factors involved in the regulation of blood pressure, it is likely that multiple mechanisms are responsible for essential hypertension and that the same mechanisms may not be applicable to all patients. Nonetheless, the evidence is strong to suggest that hyperinsulinemia and insulin resistance may be important in the pathogenesis of hypertension in some patients.

**Mechanisms linking insulin to hypertension**

Arterial blood pressure is directly proportional to the product of cardiac output and peripheral vascular resistance. Regulation of blood pressure is accomplished via several different mechanisms including baroreflexes mediated via sympathetic nerves, humoral regulation of vascular tone, and renal mechanisms that control the volume of intravascular fluid. In hypertension, one or more of the normal homeostatic mechanisms that serve to control blood pressure are defective. It is important to note that in addition to its metabolic effects, insulin also has actions on the cardiovascular and renal system, which will be discussed in detail below. In states of hyperinsulinemia / insulin resistance, these effects of insulin may be relevant to the development and / or maintenance of hypertension.

**Vascular Effects of Insulin**

Insulin is a vasodilator and has been shown in many studies to increase blood flow [27-32]. Insulin-induced vasodilation is specific to vascular beds in skeletal muscle and occurs at physiologically relevant concentrations [29, 33, 34]. It is believed that this effect is selective for skeletal muscle because this is the major site of glucose disposal and increases in muscle blood flow will enhance insulin-stimulated glucose disposal [35, 36]. In addition to directly increasing blood flow, insulin has also been shown to reduce the pressor response to humoral
factors such as noradrenaline (NA) and angiotensin-II (AII) [37] or to reflex sympathetic activation [38]. If there is resistance to the vasodilator effects of insulin in subjects who are also resistant to insulin stimulated glucose disposal then this may contribute to the development of hypertension. It should be noted that although the majority of studies support that insulin has vasodilator effects, a few studies have been unable to demonstrate this response [39-41]. There are several possible reasons for this discrepancy, such as differences in measurement technique or variability in subject age, physical fitness, and basal vascular tone. Another explanation is that insulin evokes multiple responses that may compete against the vasodilation response to elicit an overall effect, for example activation of SNS or stimulation of antagonistic pathways in vascular tissue. These hypotheses will be discussed further below.

There is much evidence to support the hypothesis that insulin mediated vasodilation is dependent on a functional endothelium and involves the nitric oxide (NO) pathway. Infusion of an NO synthase (NOS) antagonist prevents insulin stimulated increases in blood flow in humans [28, 35, 42]. Furthermore, removal of the endothelium or infusion of a NOS antagonist in isolated arterioles converts insulin induced vasodilation to vasoconstriction [43]. Insulin may affect the NO pathway by several mechanisms. Firstly, insulin has been shown to increase endothelial-NOS (eNOS) mRNA and protein levels in aortic endothelial cells [44, 45]. Secondly, insulin may increase NOS activity by increasing the availability of tetrahydrobiopterin, a co-factor required for NOS activation [46]. In addition to its effects on NO, insulin has another effect on the endothelium to stimulate the synthesis, secretion, and gene expression of the potent vasoconstrictor endothelin-1 (ET-1) [47, 48]. A two fold increase in receptor expression has also been demonstrated in isolated vascular smooth muscle cells (VSMC) treated with insulin [47]. As well as having direct effects on ET-1, insulin appears to enhance the release of ET-1 from cultured VSMC induced by angiotensin-II and arginine vasopressin [49]. The effects of insulin to activate both the NO and ET-1 pathways appear to occur at the same time and in the same vascular tissue. Cardillo et al. have recently demonstrated that insulin infusion together with an ET-1 antagonist elicits vasodilation in the human forearm and this vasodilation is prevented if a NOS antagonist is
added to the insulin + ET-1 antagonist infusion [50]. Insulin did not alter blood flow in the absence of any antagonists, presumably because stimulation of both NO and ET-1 at the same time produces equally opposing responses, and therefore no net effect, on vascular tone. The results of this experiment help to explain some of the discrepancies related to studies of the effects of insulin on vasodilation and blood flow described above.

In addition to modulating endothelial function, insulin may alter vascular tone via direct effects on VSMC, however most of these effects have only been observed at supraphysiological concentrations of insulin. Insulin has been shown to attenuate calcium influx into VSMC, corresponding to a reduction in the degree of contraction [51]. The activity of a variety of membrane components have also been shown to be affected by insulin, including the Ca$^{2+}$ channels, Ca$^{2+}$-ATPase [52, 53], Na$^{+}$-H$^{+}$ exchanger [54], and the Na$^{+}$-K$^{+}$-ATPase [55]. While the exact mechanisms by which insulin affects vascular tone in vivo are not clear, it is possible that a variety of endothelium dependent and independent mechanisms are involved.

As mentioned above, defects in the vascular actions of insulin may be responsible for the development of hypertension associated with hyperinsulinemia and insulin resistance. Firstly, it is possible that subjects who are resistant to insulin stimulated glucose uptake also exhibit resistance to the vasodilator effects of insulin. Indeed, it has been demonstrated that the sensitivity to insulin-induced vasodilation is directly proportional to the degree of sensitivity to insulin-stimulated glucose uptake [56]. In other words, subjects with poor insulin sensitivity also exhibit blunted increases in leg blood flow compared to subjects with normal insulin sensitivity. Impairments in insulin mediated vasodilation have been demonstrated in isolated segments of vascular tissue from fructose fed rats [57], Zucker obese rats [58], and spontaneously hypertensive rats [59], animal models which all exhibit insulin resistance. Alternatively, a second possibility is that the vascular actions of insulin remain intact in insulin resistant states but the compensatory hyperinsulinemia results in excessive activation of ET-1 related vasoconstriction. Evidence in support of this theory comes from studies in both the fructose fed rat [60] and chronically insulin infused rat [61] which demonstrate that...
hypertension is associated with elevated levels of ET-1. In humans, hypertensive patients have higher insulin and ET-1 levels after a glucose load compared to control [62].

**Insulin and the Sympathetic Nervous System**

Yet another possible link between hyperinsulinemia and hypertension may be related to insulin induced stimulation of the sympathetic nervous system (SNS). Under hyperinsulinemic conditions, insulin may chronically activate the SNS, thereby leading to increased peripheral vascular tone and elevated BP. In support of this hypothesis, insulin infusion both increases plasma NA levels and sympathetic nerve activity in muscle [32, 63, 64]. However, studies in humans have shown that although physiological increases in insulin concentration can increase muscle sympathetic nerve firing rate, they result in no overall change in BP [32, 65]. The reason why insulin does not increase blood pressure, despite stimulation of the SNS, is that insulin causes vasodilation in the vasculature of skeletal muscle (as mentioned previously), which consequently leads to a redistribution of cardiac output and therefore no net effect on blood pressure [31, 42].

On the other hand, the SNS may be involved in this inter-relationship as the primary defect, causing both insulin resistance and hypertension. Studies have demonstrated that stimulation of β-adrenergic receptors can cause acute insulin resistance [66] and an increase in the ratio of insulin resistant fast-twitch / slow-twitch fibers in skeletal muscle [67]. Elevated SNS activity may also contribute to apparent insulin resistance via vasoconstriction, thereby reducing blood flow and glucose delivery to insulin sensitive tissues [68]. Hyperinsulinemia may occur to compensate for insulin resistance but then serves as a further stimulus for SNS activation, thereby creating a vicious circle that reinforces the insulin resistant state and elevated blood pressure. In the fructose fed rat model of hyperinsulinemia, insulin resistance, and hypertension, chemical sympathectomy prevents both the development of hyperinsulinemia and hypertension [69]. This demonstrates that a functional SNS is required for the development of both hyperinsulinemia and hypertension in this model and suggests that SNS activation may be an early defect that precedes the metabolic defects.
Effects of Insulin on the Kidney

Insulin has a direct effect on the kidney to cause sodium retention [70]. The hypothesis that insulin induced antinatriuresis is involved in the development of hypertension in hyperinsulinemic, insulin resistant subjects relies on the assumption that renal tissues maintain normal sensitivity to insulin, in contrast to peripheral tissues which are resistant to insulin stimulated glucose uptake. Indeed, in hypertensive patients who exhibit insulin resistance, insulin induced sodium retention was not impaired relative to control [71]. It follows from this hypothesis that hypertensive patients who exhibit hyperinsulinemia and insulin resistance would be salt sensitive. In humans, there is some evidence to suggest that blood pressure in both normotensive and hypertensive insulin resistant subjects is sensitive to salt [72], although there is also evidence to the contrary [73]. In contrast, experiments performed in rodent models of hypertension that are hyperinsulinemic and insulin resistant, including the fructose fed rat, showed that elevations in BP were not associated with sodium retention [74, 75].

Animal models of hyperinsulinemia, insulin resistance, and hypertension

A common animal model used to study the interaction between insulin and hypertension is the fructose hypertensive rat (FHR). This is a form of mild hypertension that also exhibits insulin resistance, hyperinsulinemia, and hypertriglyceridemia [13]. In this model, a simple chronic dietary intervention of substituting high fructose for the normal starch carbohydrate content in laboratory rodent diets has been found by several investigators to increase blood pressure within a period of 3-5 weeks [13, 76, 77]. The effects of a fructose diet have been shown to be concentration and duration dependent [76]. This cluster of symptoms, namely hypertension, hypertriglyceridemia, hyperinsulinemia, and insulin resistance, is also characteristic of other high carbohydrate fed rodent models, including sucrose [78-80] or glucose [80, 81]. These models are useful for hypertension studies because feeding with a high fructose diet does not result in any body weight gain, thus enabling one to investigate the
relationship between insulin resistance and hypertension independent of obesity. Furthermore, hypertension is acquired simply as a result of a dietary intervention and therefore provides an opportunity to study pathological mechanisms of hypertension that may not have a genetic basis.

It has been shown previously both in our laboratory and elsewhere that several drug interventions which can improve insulin sensitivity, including metformin, vanadium compounds, and thiazolidinediones, can ameliorate the hypertension observed in this animal model [82-86]. Furthermore, increasing plasma insulin levels to that seen prior to treatment reverses the effects of these drugs. These observations lend support to the hypothesis that insulin resistance / hyperinsulinemia are the primary cause of hypertension in this model.

Many of the mechanisms linking insulin to hypertension discussed above appear to be related to the pathogenesis of the blood pressure increase in FHR. We have demonstrated that FHR exhibit resistance to the vasodilator effects of insulin [57]. Several laboratories have also demonstrated that endothelium dependent relaxation of various vascular tissues is impaired in FHR [87-92], which has been attributed to defects in vasodilatory mechanisms associated with NO [90] as well as the endothelium derived hyperpolarizing factor (EDHF) [91, 92]. Alternatively, defects in endothelium derived contracting factors have also been suggested to play a role, particularly those related to ET-1. Firstly, treatment of FHR with the ET-1 receptor antagonist bosentan has been shown to prevent hypertension in this model [60, 93]. We have also shown that vascular ET-1 levels are elevated in fructose fed rats [60] and that the reactivity of mesenteric arteries to ET-1 is altered [94]. Furthermore, increased expression of both ET-1 protein and its ET<sub>A</sub> receptor subtype (which mediates vascular contraction) has been recently demonstrated in FHR [95]. The sympathetic nervous system is also believed to be involved in the pathogenesis of fructose induced hypertension as both chemical sympathectomy [69] and treatment with rilmenidine, an agent that decreases sympathetic outflow [96], have been shown to prevent fructose induced hypertension. However, an increase in sodium retention or fluid volume does not seem to be involved in FHR [75].
Other mechanisms may be involved in FHR in addition to those described above. One hypothesis involves the generation of reactive aldehydes which cause damage to proteins involved in insulin and/or calcium signalling [97, 98]. Evidence suggests that angiotensin II (All) may also play a role as plasma levels of All are increased in FHR [99, 100] and treatment with All-converting-enzyme (ACE) inhibitors or All receptor antagonists also prevent the development of hypertension [101-103]. The involvement of lipoxygenase [104] or lipid abnormalities [105] has also been proposed as drugs which specifically target these pathways prevent an increase in blood pressure.

Recent evidence suggests that there are differences between males and females in the response to feeding with a high carbohydrate diet. It has been shown that, in contrast to males, female rats do not develop hypertriglyceridemia or insulin resistance after feeding with sucrose [106]. These investigators used a euglycemic hyperinsulinemic clamp, considered the gold standard for assessing insulin sensitivity. Unfortunately, blood pressure was not measured in this study, nor were male groups included in the experiment allowing only a qualitative comparison between males and females to be made. A separate study investigating the effects of high sucrose on development of juvenile rats demonstrated that both male and female rats develop hypertension, however, the degree of blood pressure increase was greater in males than in females [107]. The male sucrose fed group was found to be insulin resistant, however, insulin sensitivity was not measured in the female groups. Therefore, it is uncertain if the elevated blood pressure in females is related to impairments in insulin sensitivity or if there were any differences between sexes in this respect. These studies leave many questions unanswered and appear to provide conflicting results, for if sucrose indeed does not produce hyperinsulinemia and insulin resistance in female rats, then according to our hypothesis, hypertension would also not develop. It seems reasonable that there may be differences between males and females in carbohydrate-induced hypertension as it is well documented that there are gender differences in the incidence and pathogenesis of cardiovascular disease [108].
Cardiovascular disease in women

While cardiovascular disease (CVD) affects both men and women, World Health Organization data shows an overall ratio of male to female incidence of $2.24 \pm 0.08$ [108]. This age-adjusted risk remains approximately 2 fold higher in males until the fourth and fifth decades of life when the risk begins to increase sharply in females and nearly equals the risk in males by the seventh decade of life. As the age of menopause coincides with the timing of increased risk for CVD in women, it has been hypothesized that estrogen provides cardiovascular protection in premenopausal women. In support of this, young women with bilateral ovariectomy show an increased risk for coronary heart disease, which is prevented by estrogen replacement therapy [109]. The concept of using estrogen replacement therapy in postmenopausal women to prevent CVD, however, remains controversial. Most of the epidemiological studies indicate that postmenopausal users of estrogen therapy have lower rates of CVD compared to non-users [110, 111]. However, results from the recent HERS trial found no benefit of hormone therapy in postmenopausal women with already established coronary artery disease [112]. This has cast doubt on the ability of hormone replacement therapy to prevent CVD, however, interpretation of the collective study data is difficult because of differences in study designs, patient populations, and hormone treatment regimens.

Hypertension is a strong risk factor for developing CVD for both men and women. The prevalence of hypertension is lower among women compared to men and increases with age in both sexes; in women, approximately 8% in premenopausal women compared to 50% postmenopause [113]. In middle age, women tend to have lower systolic and diastolic BP compared to men, but by the fifth decade of life these values begin to exceed those in males. Interestingly, women appear to tolerate high blood pressure better than men as morbidity and mortality rates for a given level of blood pressure are typically lower in women [113].
Mechanisms of cardiovascular protection related to estrogen

As a steroid hormone, estrogen acts on nuclear receptors to affect gene expression and regulation, although non-genomic (acute) effects have also been demonstrated in the vasculature (these will be discussed below). The potential protective effects of estrogen have been attributed to a variety of direct and indirect effects on the cardiovascular system. Estrogens are known to affect plasma lipid composition, glucose and insulin metabolism, hemostasis, vascular and cardiac function, as well as have anti-oxidant properties.

Effects of estrogen on carbohydrate and lipid metabolism

Confusion exists regarding the effect of estrogens on carbohydrate metabolism because of differences between estrogens used for oral contraception and endogenous estradiol. At high doses of conjugated equine estrogens (CEE) or with the potent synthetic estrogen ethinyl estradiol, adverse effects on insulin sensitivity and glucose tolerance have been reported. However, most studies using low doses of CEE or native estradiol demonstrate an improvement in insulin sensitivity [114]. Estrogen can improve insulin action in liver, muscle, and adipose tissue by increasing glycogen deposition, glucose uptake, and lipogenesis [114]. In postmenopausal women, estrogen replacement therapy has been shown to reduce fasting glucose and insulin concentrations, an indirect measure of insulin sensitivity [115]. In monkeys [116] and rats [117], ovariectomy has been shown to be associated with impaired insulin sensitivity and estrogen replacement therapy in these animals will restore it to normal.

Plasma lipids and lipoproteins are involved in the development of atherosclerosis and estrogens have beneficial effects on many of the plasma lipid parameters that are associated with the atherogenic process. Estrogens cause reductions in LDL cholesterol and elevations in HDL cholesterol, particularly the HDL₂ fraction that is protective against development of atherosclerosis [108, 118]. Furthermore, estrogens inhibit the oxidation and deposition of LDL into plaques in the arterial wall [119]. Levels of Apo-A1 are increased by estrogen,
which stimulates cholesterol efflux from cells and is another protective factor against the development of atherosclerosis [108]. Another factor which has been correlated to increased risk of atherosclerosis is Lp(a), which is also reduced by estrogen [120]. In contrast to these positive effects, estrogen can increase plasma concentrations of triglycerides in the range of 20-24% as well as VLDL secretion [121-123]. This effect is dependent on dose and type of estrogen, with the synthetic estrogens having greater potency relative to endogenous estradiol. The net effect of estrogens on lipid metabolism is believed to be favourable and accounts for approximately 20% of the cardioprotection afforded by estrogen [124]. Since effects on lipids cannot fully account for the benefits of estrogen on CVD, other mechanisms must also be present.

**Anti- and pro-thrombotic effects of estrogen**

Formation of blood clots is likely to occur at atherosclerotic lesions and has the potential to cause myocardial infarction or stroke. The effects of estrogen on the coagulation pathway are complex. Reductions in fibrinogen have been shown, which contribute to a reduction in the formation of fibrin blood clots. In addition, estrogens also increase plasminogen and reduce plasminogen activator inhibitor-1 (PAI-1), which result in net increases in plasmin, the enzyme responsible for lysis of fibrin clots [110, 125]. Low dose estrogens inhibit platelet aggregation by reducing platelet derived thromboxane (pro-aggregatory) and increase endothelium derived prostacyclin (anti-aggregatory) [108]. On the other hand, estrogen has pro-coagulant properties as well, including increasing the activation of factor VIIa and the tissue factor that converts factor X to factor Xa [110, 118]. Estrogen treatment has been linked to an increase in risk for venous thrombosis, although this may be more relevant to use of high dose synthetic estrogens [126]. Given the conflicting actions of estrogen on hemostasis, the net effect in vivo is likely to depend on individual factors such as genetic makeup and presence of other risk factors [110].
Direct vascular effects of estrogen

Acute application of estrogen causes relaxation in various vascular tissues [118, 127-131]. As this dilation can occur rapidly and in the presence of transcriptional blockade, non-genomic mechanisms appear to be involved. Multiple endothelium dependent and independent pathways have been demonstrated to mediate the vasorelaxation induced by estrogen. It is interesting to note that both males and females express estrogen receptors in cardiovascular tissues and estrogens appear to act in a similar manner in both sexes [132]. Few studies have investigated the physiological role of estrogen in the male cardiovascular system, but it is possible that differences in endogenous estrogen levels rather than inherent differences in estrogen activity are responsible for the disparity in cardiovascular risk.

The most well studied pathway of estrogen induced vasodilation is the NO system [133]. Estrogen causes the rapid release of NO from endothelial cells in vitro [128]. Basal and stimulated release of NO is greater in female normotensive rats compared to males [134], and this may be an important factor in the gender differences in CVD incidence. In addition to the rapid effect of estrogen on NO activity, prolonged exposure can also increase the expression of NOS enzyme [135], but whether this action plays a predominant role in the physiological effects of estrogen is unknown. Estrogen also stimulates the secretion of another vasodilator, prostacyclin, from endothelial cells [136]. Furthermore, estrogen selectively enhances the effects of ET-1 to stimulate prostacyclin but not thromboxane production [137]. Another direct effect of estrogen on vascular tissue involves inhibition of various contracting factors, including ET-1 [138, 139] and thromboxane A2 [140]. Reduction in the expression of ACE in endothelial cells has also been demonstrated [141], which would lead to reductions in the synthesis of AII, yet another vasoconstrictor hormone.

Endothelium independent mechanisms are also involved in the vasodilation mediated by estrogen. Inhibition of Ca\(^{2+}\) entry via voltage gated calcium channels and increases in efflux of calcium in VSMC reduces intracellular Ca\(^{2+}\) and therefore reduces vascular smooth muscle contraction [129, 142]. This effect does not require protein synthesis and may involve a direct
interaction between estrogen and calcium channels. It has recently been demonstrated that estrogen can bind directly to and activate $K^+$ channels, which causes hyperpolarization of the VSMC [143]. This would also lead to a reduction in vascular contraction. Both of these mechanisms have been shown to contribute to the acute vasodilatory effects of estrogen in coronary arteries [144].

**Rationale and experimental objectives**

The main objective of this study was to examine the relationship between hyperinsulinemia / insulin resistance and hypertension in female rats. A secondary objective of this thesis was to identify potential mechanisms that might explain any sex differences observed. The link between these two conditions has been well established in studies employing male animal models as well as in human studies [145-147], however, it has not been possible to discern what role sex plays in this relationship, if any, based on these previous reports. The limited studies performed with female sucrose fed rats are inconsistent and incomplete in order to address this question [106, 107]. Given the differences in incidence and pathogenesis of CVD in men and women, we hypothesized that gender may affect the relationship between hyperinsulinemia / insulin resistance and hypertension. In order to investigate this hypothesis, experiments were designed with the following specific aims:

1. Clarify the effect of a high carbohydrate (fructose) diet in both male and female rats on development of hyperinsulinemia, insulin resistance, and hypertension.
2. Examine the role of the sex hormones in the response to a fructose diet in females.
3. Determine if there are differences in the vascular actions of insulin between fructose fed male and female rats.
4. Evaluate the effects of exogenous insulin treatment on insulin sensitivity and blood pressure in male and female rats.
5. Determine if there are differences in vascular response to the vasoconstrictor U46619, a thromboxane analogue, between males and females.
MATERIALS AND METHODS

General Methodology

1. Systolic Blood Pressure Measurement

Blood pressure was measured in conscious rats using the tail cuff method without external preheating as previously described [82, 148]. This method is an indirect and non-invasive method suitable for studies requiring chronic measurement of blood pressure. Rats were placed in Plexiglas holders and their tails inserted into an inflatable cuff containing a photoelectric sensor, connected to a multi-sensor manual scanner (Model 65-120) and blood pressure amplifier with an analog / digital recorder (Model 179) from IITC Inc. (Woodland Hills, Ca., USA). Cuffs were inflated to a pressure of 200 mm Hg and the reappearance of pulsations upon gradual deflation of the cuff was detected by the photoelectric sensor and recorded as the systolic blood pressure. The tail cuff method has been validated in our laboratory [82] and shown by others to record values similar to direct intra-arterial measurements [148-150]. For all blood pressure studies, rats were preconditioned to the procedures before baseline measurements were recorded. A minimum of 5 readings were taken for each rat at each time point in the studies.

2. Assessment of Insulin Sensitivity / Resistance

In all blood pressure studies, an oral glucose tolerance test (OGTT) was performed to assess insulin sensitivity / resistance. After an overnight fast (16 h), rats were administered a 40% glucose solution via oral gavage (1 g / kg body weight). Blood samples were obtained from the tail vein at times 0, 10, 20, 30, and 60 minutes. A 90 minute time point was included in the chronic insulin treatment study. An index of insulin sensitivity (ISI) was calculated in some studies as follows:
The value of $k$ was set to 100 in order to obtain values between 1 and 15. Fasting plasma glucose (FPG) and insulin (FPI) values were the time 0 (16 h fasted) values, and mean plasma glucose (MPG) and insulin (MPI) values were calculated as the mean value of all time points measured in the test (i.e. 0 to 60 or 90 minutes inclusive). This equation serves as a composite index of both hepatic and peripheral tissue sensitivity to insulin; higher values indicate high sensitivity to insulin. Results derived from this equation have been shown to correlate highly to those obtained from the euglycemic insulin clamp test [151], which is considered the "gold standard" method for measuring insulin sensitivity. Area under the curve values for glucose (AUC$_g$) and insulin (AUC$_i$) were also calculated from OGTT data according to the trapezoidal rule.

3. Isolated Blood Vessel Preparation

Vascular smooth muscle (VSM) function was assessed in some of the same animals used in the blood pressure studies. For these experiments, similar procedures were used to prepare the tissue, but with different drug protocols that will be described separately. Rats were anaesthetized with intraperitoneal injection of pentobarbital. When loss of foot and blink reflexes was observed the abdomen was opened and the thoracic aorta excised. The tissue was placed in cold oxygenated modified Krebs-Ringer solution of the following composition (mM); NaCl (118), KCl (4.7), CaCl$_2$ (2.5), KH$_2$PO$_4$ (1.2), MgSO$_4$ (1.2), NaHCO$_3$ (25), glucose (11.1), and ethylenediaminetetraacetic acid (EDTA 0.026), maintained at 37°C and gassed with 95% O$_2$ / 5% CO$_2$. Tissues were cleaned of any excess connective tissue and cut into 5 mm rings. The endothelium of some rings was intentionally removed by gently rubbing the lumen with a stainless steel rod. Loss of the vasorelaxation response to acetylcholine (Ach) was taken to indicate successful removal of the endothelium. Each ring was placed under a resting tension of 2 g on stainless steel hooks and equilibrated for 60 minutes with periodic washing and readjustment of tension. Changes in tension were detected with a force...
transducer and recorded on a Grass polygraph machine (model 79D). After completion of the experimental protocol, the length and weight of each ring was measured after lightly blotting. The cross-sectional area (CSA) of each tissue was calculated as follows:

\[
\text{CSA (mm}^2\text{)} = \frac{\text{weight (mg)}}{\text{length (mm)} \times \text{density (mg/mm}^3\text{)}}
\]

The density was assumed to be 1.05 mg/mm³. Tension responses were expressed either as percent of control contraction to noradrenaline (%) or as tension normalized to CSA (g/mm²). Values for pD₂ (-log M) and curve maximum for each agonist were calculated by nonlinear regression analysis of the concentration response curves.

4. Biochemical Analyses

Blood samples for biochemical analyses were obtained from the tail vein, except at termination when samples were obtained by cardiac puncture. Samples were centrifuged in a desktop microcentrifuge (14000 rpm for tail vein samples and 4500 rpm for cardiac samples, 4°C, Beckman Allegra 21R) to separate plasma. Plasma was aliquoted and stored at -70°C until assay. Plasma glucose was measured with an automatic Beckman Glucose Analyzer II. Enzymatic colorimetric kits were used to measure plasma triglycerides (Boehringer Mannheim, Canada, and Sigma, USA for chronic insulin treatment study only). Plasma insulin and total estrogens were measured with radioimmunoassay kits from Linco Diagnostics Inc., USA and ICN Biochemicals, USA respectively.
Experimental Protocols

1. Effect of a high fructose diet in male and female rats

To directly examine gender differences in the response to high carbohydrate feeding, male and female rats were fed with a 60% fructose diet for 9 weeks as described previously [82]. Specifically, the variables of interest were blood pressure and markers of insulin resistance. Four experimental groups of Wistar rats were used in this study; male (M, n=4), male fructose treated (MT, n=4), female (F, n=8), and female fructose treated (FT, n=8). Animals were obtained at 5 weeks of age and allowed one week for acclimatisation prior to beginning the study. At age 6 weeks, treatment groups were started on a diet of 60% fructose for 9 weeks, while control groups were maintained on normal laboratory rat diet. The electrolyte, protein, and fat contents of the fructose diet were comparable to the normal diet. Systolic blood pressure was measured prior to treatment and weekly throughout the study period as described above. Animals were monitored for weight gain and food and fluid intake on the same day each week. Blood samples for determination of 5 hour fasted plasma insulin, glucose, and triglycerides were obtained at study weeks 0, 2, 5, and 7. An oral glucose tolerance test after an overnight fast was performed at study weeks 4 and 8.

2. Effect of ovariectomy on the response to a fructose diet in female rats

To determine if sex hormones are responsible for protecting female rats from development of hypertension and hyperinsulinemia / insulin resistance, we evaluated the effects of a fructose diet in ovariectomized rats. Four groups of 15 week old female Wistar rats were used in this experiment (n=8 per group); control (C), fructose (F), ovariectomized (OVX), and OVX + fructose (FOVX). Ovariectomy was accomplished with bilateral dorsal incisions to expose and remove the ovaries. Surgery was performed under anaesthesia initiated in a halothane chamber, then maintained with 95% N₂O / 5% O₂ for the duration of the procedure. Control animals were subjected to the same procedures without excision of the ovaries. Wounds were
closed with stainless steel suture clips and animals were allowed two weeks to recover before taking any measurements. During this recovery period, some rats were treated with topical Polysporin ointment to prevent infection. Treatment groups received a 60% fructose pellet diet that was begun on the same day as ovariectomy while control and OVX received normal laboratory rodent diet. Body weight gain and 24 hour food and fluid intake were monitored on a weekly basis. Systolic blood pressure was measured weekly beginning at week 2. Prior to surgery, animals were trained for the procedure and baseline measurements were taken. Blood samples were collected after a 5 hour fast at study weeks 0, 2, 4, and 6. At week 7, an OGTT was performed. At termination, ovariectomy was confirmed by visual inspection and blood was collected into a heparinized tube via cardiac puncture for measurement of plasma total estrogens (TE).

3. Vascular responses to insulin in fructose fed female rats

We have previously demonstrated that male FHR exhibit resistance to the vasodilatory actions of insulin [57], which may help explain the link between insulin resistance and hypertension. To determine if the same abnormalities were present in female fructose fed rats, we evaluated the vascular responses to insulin in two different sets of female rats. Rats in set #1 consisted of 15 week old female Wistar rats from protocol 2 above and were fed with fructose (n=8) or control diet (n=8) for a period of 11-12 weeks prior to termination. Rats in set #2 consisted of 6 week old female Wistar rats also fed with fructose (n=8) or control diet (n=6) for 11-12 weeks. Systolic blood pressure and a 5 hour fasted blood sample were obtained at baseline and at week 11 from set #2 for comparison to values obtained for female rats in protocols 1 and 2 described above. Measurements for systolic blood pressure were not taken throughout the treatment period, however, rats were periodically placed in Plexiglas holders to maintain training for the week 11 measurement.

At termination, aortae were excised and prepared as described above. For experiments with set #2, indomethacin (10^{-5} M) was included in the Krebs-Ringer buffer to inhibit cyclo-
oxygenase and thus prevent prostacyclin dependent mechanisms of vasodilation. Therefore, insulin mediated inhibition of contraction (i.e. vasodilation) could be attributed to primarily NO dependent mechanisms. All variables and procedures used with set #2 were the same as those described in Verma et al. [57] which used male FHR of similar age and duration of fructose treatment. Drug treatment protocols for set #1 and set #2 began with two separate additions of 40 mM KCl to stretch the tissues, followed by $10^{-6}$ M NA and $10^{-5}$M Ach to assess endothelial viability. After washing the tissues and tension returned to baseline, a concentration response curve to NA was constructed ($10^{-9}$ – $10^{-5}$M). Tissues were then incubated with 100 mU/ml insulin (pork/beef diluted in 0.1% albumin) for 2h prior to constructing a second NA concentration response curve. In each experiment, one ring was used as a time control and was not exposed to insulin.

4. Effect of chronic insulin treatment in male and female rats

Since fructose fed female rats did not develop hyperinsulinemia, insulin resistance or hypertension, we employed a chronic exogenous insulin treatment to attempt to create a state of insulin resistance. The effects of chronic hyperinsulinemia on blood pressure and insulin sensitivity were evaluated in groups of male and female rats. Four experimental groups of 6 week old Wistar rats were used: male (M, n=6), male treated (MT, n=10), female (F, n=6), and female treated (FT, n=10). Treatment with bovine insulin via sustained release implants began at age 7 weeks (study week 1) (Linplant, produced by Linshin Canada). These implants are designed to continuously release a dose of 2 U/d lasting for a period of up to 60 days. Similar insulin treatment regimens have previously been shown to elevate blood pressure and cause insulin resistance in male rats [10, 61, 152, 153]. Control animals were implanted with placebo containing the vehicle, palmitic acid, only. All implants were dipped in 2% iodine for sterilisation purposes prior to subcutaneous insertion. Animals were briefly anaesthetised in a halothane chamber for the procedure. The drinking water of insulin treated animals was supplemented with 10% glucose for the first 4 days of treatment to avoid hypoglycemia. Control animals also received 10% glucose to control for possible effects on blood pressure.
Body weight, food and fluid intake were monitored daily during the first 4 days and weekly thereafter. Prior to treatment, baseline systolic blood pressure and 16 hour fasted blood samples were obtained. Systolic blood pressure was measured again at week 4 and an OGTT was performed at week 5. Three male treated rats and one female treated rat were removed from the study because fasted plasma insulin levels were < 1ng/ml, suggesting failure of the implant. Another male rat in the treated group was excluded from the study due to an abnormal muscle condition that resulted in poor hindlimb muscle development and weakness, thus impacting on the rat’s ability to walk and feed normally. Final n numbers included in the statistical analysis were; M n=6, MT n=6, F n=6, FT n=9.

5. Vascular responses to insulin and U46619 in Male and Female Hyperinsulinemic Rats

There is evidence to suggest that thromboxane may play a role in the pathogenesis of hypertension related to hyperinsulinemia / insulin resistance [154]. To evaluate the effect of chronic insulin treatment on vascular responses to thromboxane and compare these responses between sexes, the same rats from study 4 were used in this experiment. At termination, aortae were excised and prepared as described above. After equilibration, tissues were challenged with 40 mM KCl to stretch the tissue, followed twice with $10^{-6}$ M NA and $10^{-5}$ M Ach to assess endothelial function and ensure that responses to NA were consistent and stable. After washing, one ring of each pair of endothelium intact or denuded rings was incubated with 100 mU/ml insulin (pork / beef, diluted in buffer containing 0.1% albumin) for 2 hours. Rings were then contracted with increasing doses of NA ($10^{-9}$ to $10^{-5}$ M), washed 3 times, then contracted with increasing doses of U46619, a stable thromboxane A$_2$ (TXA$_2$) mimetic ($10^{-12}$ to $10^{-7}$ M). Insulin was added to all washes and included in the bath medium during construction of both concentration – response curves.
Reagents

Fructose diet was obtained from Teklad Laboratory Diets. Noradrenaline, U46619, insulin, albumin, and EDTA were obtained from Sigma. Buffer chemicals were obtained from BDH except for MgSO\(_4\) and KH\(_2\)PO\(_4\), which were purchased from Fischer.

Statistical Analyses

All data are presented as the mean ± standard error of the mean (SEM). For studies with multiple time points, variables were analysed by the general linear model ANOVA, including plasma parameters, body weight, food and fluid intake, and systolic blood pressure. The GLM ANOVA model was also used to analyse each data point of the OGTT and concentration-response curves. Insulin sensitivity indices (ISI), AUC, pD\(_2\), and curve maximum values were examined with a one-way ANOVA. Unpaired t-tests were used to compare fructose and control groups in protocol 3 (set #2) as well as the effect of treatment within sex in the chronic insulin treatment study (protocol 4). Mean values were considered significant at p < 0.05. When a mean difference was detected, a Newman-Keuls multiple comparison test was applied. All statistical analyses were performed using the Number Cruncher Statistical System (NCSS) software package.


RESULTS

1. Comparison of the Effects of a Fructose Diet in Male and Female Rats

Males (M and MT) had significantly higher body weights than females (F and FT) throughout the study, but fructose treatment did not affect body weight (figure 1). The pattern of 24 hour food intake was similar for all groups throughout the study, indicating that males and females had similar caloric intake based on body weight, regardless of diet. Fluid consumption tended to be lower in fructose treated groups, but this was only significant in males during the first 3 weeks of the study period.

By week 7, fasted plasma insulin values were significantly higher in males treated with fructose compared to females while plasma glucose levels were similar (table 1). Elevated plasma insulin in the presence of normal glucose levels suggests impaired insulin activity. Plasma insulin levels were not elevated significantly in fructose fed males compared to their control group, however, it should be noted that this experiment was not powered to adequately demonstrate statistical differences between male and male treated groups. Plasma triglyceride values were significantly higher in fructose treated males compared to both control and their female counterparts by the end of the study. It is noteworthy that fructose fed female rats did not exhibit a change in any of the plasma parameters measured in this study. An age related increase in systolic blood pressure was observed for all groups (figure 2). In the male fructose treatment group, blood pressure began to increase significantly compared to control by 3 weeks of treatment and continued to increase throughout the study (115 ± 5 vs. 150 ± 5 mm Hg at week 9). The blood pressure of female rats was not significantly different between fructose fed and control at any time point (126 ± 5 and 125 ± 3 mm Hg at week 9 for fructose fed and control respectively).

Results of the OGTT performed at weeks 4 and 8 of fructose feeding are shown in figures 3 and 4. At week 4, fructose fed males secreted more insulin compared to male control and both female groups. Plasma glucose tended to peak higher in males than females, but this was
not different. Furthermore, there were no differences between male control and fructose fed groups. Based on AUC data (figure 5), fructose fed males were insulin resistant as they had significantly higher insulin but similar glucose levels. At week 8, differences between fructose fed males and females were still evident however, the difference between male control and treated groups did not reach significance. We, and other laboratories have previously demonstrated that male fructose fed rats are insulin resistant as assessed by the euglycemic hyperinsulinemic clamp method [82, 85, 155, 156]. No differences between female control and fructose fed groups were observed at either week 4 or 8.

2. Effect of Ovariectomy on the Response to Fructose Diet in Female Rats

Ovariectomy of female rats was successful as indicated by visual inspection and by a significant decrease in plasma total estrogen levels (at termination in ovariectomized groups (table 2). Body weights of ovariectomized rats (OVX and FOVX) tended to be higher throughout the study period, but this was not significant until termination (figure 6, table 2). The pattern of food and fluid intake was similar among all groups. Fructose fed groups (F and FOVX) tended to eat less compared to control (C and OVX), which was significant only at weeks 3 and 5. Only the FOVX group experienced a significant increase in blood pressure from baseline measurements (figure 7). The systolic blood pressures at study week 7 of the control, OVX, fructose, and fructose + OVX groups were 118 ± 3, 120 ± 5, 116 ± 3, and 135 ± 6 mm Hg, respectively.

All groups were normoglycemic, but fructose + OVX were hyperinsulinemic compared to OVX alone (table 3). Plasma triglycerides were elevated in the fructose fed only group and the fructose + OVX group at weeks 4 and 6. The results from the week 7 OGTT are depicted in figures 8 and 9. While the fructose groups tended to have elevated plasma insulin values, denoted by the AUC_t, this was not significant. Plasma glucose values were nearly identical. A comparison of the ISI values indicates that the fructose + OVX group was insulin resistant compared to OVX rats (figure 10).
3. Vascular Effects of Insulin in Fructose Fed Female Rats

Experiments with female rats from set #1 were designed to investigate the effect of insulin on the vascular responses to NA. Incubation with insulin increased the response to NA in endothelium intact VSM of both control (C) and fructose fed female rats (F) (figure 11). The response was similar regardless of diet group. Insulin had no effect on sensitivity to NA, as the pD₂ values are similar for all groups, but AUC is significantly higher in the insulin treated rings compared to untreated (table 4). Maximum values obtained by linear regression were not different. Insulin treatment did not affect the response to NA in endothelium denuded VSM of either diet group, suggesting that the vasoconstrictor effects of insulin observed in this experiment were mediated via the endothelium.

Female rats in set #2 did not develop hypertension after 11 weeks of fructose feeding, which agrees with results from the experiment in protocol 1 in which female rats were fed with fructose for 9 weeks (table 5). After this duration of feeding, plasma triglycerides were elevated in the fructose fed rats compared to control, whereas in the 9 week protocol they were not different. Plasma glucose levels were not different but insulin levels were significantly elevated after 11 weeks, however, this difference (1.3 ± 0.2 ng/ml vs 1.7 ± 0.1 ng/ml) is very small and considered clinically insignificant. In our laboratory, male rats fed with fructose typically develop plasma insulin levels >3 ng/ml [10, 57, 83, 157].

The experiments in set #2 were designed to investigate the effect of insulin on nitric oxide mediated responses in VSM. Incubation with insulin under these experimental conditions did not significantly alter VSM responses to NA in any way (figure 12, table 6). In endothelium denuded tissues, insulin tended to reduce the response to NA in both control (C) and fructose fed groups (F), but this was not significant (figure 13). Previous experiments in our laboratory using the same conditions have demonstrated that male rats exhibit an endothelium-dependent vasodilatory response to insulin and that this response is abolished if rats are fed with fructose (figure 14).
4. Effect of Chronic Insulin Treatment in Male and Female Rats

Male rats (M and MT) had higher body weights than females (F and FT) at all time points in the study (figure 15). Insulin treatment did not affect body weight in females, but caused small but significant weight gain in males by 2 weeks of treatment. During the initial 4 days of insulin treatment and during the time in which drinking water was supplemented with 10% glucose, fluid intake increased and food intake in all groups dropped slightly. Females drank significantly more than males during this period. After removal of glucose from the drinking water, food and fluid intake stabilised near baseline levels. Females treated with insulin ate significantly more than F or MT at all time points during the study, however, amounts varied little from the baseline for this group.

Fasting values of glucose, insulin, and TG are shown in table 7. Animals treated with insulin had significantly higher plasma values for insulin compared to their respective control groups. It is of note that post-treatment insulin values were very similar for both the male and female treated groups. Plasma glucose values were significantly lower in treated rats compared to control, and treated females were lower than treated males. A small but significant increase in plasma triglycerides from baseline was observed in both the male and female control groups; this may be related to the placebo vehicle, palmitate. Females treated with insulin had lower plasma triglycerides compared to treated males.

Systolic blood pressure, measured after 4 weeks of treatment, was significantly higher in hyperinsulinemic male rats only (figure 16). Mean values for the F, FT, M, and MT groups were as follows, respectively; 120 ± 3, 120 ± 3, 123 ± 2, 137 ± 6 mm Hg.

Results from the OGTT performed at week 5 are illustrated in figures 17 and 18. Control male and female rats had similar plasma glucose profiles, however, plasma insulin was significantly higher in males than females throughout the glucose challenge. This indicates that female rats required less insulin to handle the same glucose load, and therefore implies that females have enhanced insulin sensitivity compared to males. Chronic insulin treatment in both male and female rats resulted in a reduction in the OGTT glucose profile, although
this was reduced more so in females than males despite similar insulin responses. This observation again supports that females in the treatment cohort were more insulin sensitive relative to their male counterparts.

A comparison of ISI values (figure 19) derived from the OGTT data shows that female rats were highly sensitive to insulin compared to males and that chronic insulin treatment reduced insulin sensitivity in both males and females (F 68 ± 20, FT 13 ± 2, M 15 ± 1, MT 8 ± 1). A comparison of the MT and FT groups indicates that insulin sensitivity was impaired to a greater degree in hyperinsulinemic males than females. The slopes of the regression lines for systolic blood pressure and ISI were $r = -0.351$ (male) and $r = -0.003$ (females) (figure 20). The correlations were not significant for either group.

5. Vascular Responses to Insulin and U46619 in Male and Female Hyperinsulinemic Rats

Concentration response curves to NA in the presence and absence of 100 mU/ml insulin are depicted in figures 21 and 22. There were no differences in response to NA between groups in all endothelium intact tissues. In endothelium denuded rings, however, chronically hyperinsulinemic male rats (MT) developed greater tension in the presence of insulin compared to control males (M) (table 8). Chronic hyperinsulinemia did not alter the response to NA in any of the female groups. When comparing the effect of chronic hyperinsulinemia or acute insulin incubation on the response to U46619, no differences were observed within sexes (figures 23 and 24). A striking gender difference was noted, however, with respect to sensitivity to U46619. The potency of this agonist was approximately two-fold greater in males compared to females (table 9) in both endothelium intact and denuded tissues and regardless of treatment group.
FIGURE 1. Body weight (a), fluid intake (b), and food intake (c) of male and female rats fed with fructose. Measurements were taken on the same day each week. Values are means ± SEM, n=4 (M and MT), n=8 (F and FT). *p<0.05 vs control diet, #p<0.05 vs opposite sex.
## TABLE 1

Plasma Parameters in Male and Female Rats Fed with Fructose

<table>
<thead>
<tr>
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<th>Glucose (mM)</th>
<th>Insulin (ng/ml)</th>
<th>Triglycerides (mM)</th>
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<tbody>
<tr>
<td></td>
<td>M</td>
<td>MT</td>
<td>F</td>
</tr>
<tr>
<td>Basal</td>
<td>7.7±0.3</td>
<td>7.7±0.2</td>
<td>6.8±0.1</td>
</tr>
<tr>
<td>Week 2</td>
<td>8.2±0.2</td>
<td>8.6±0.4</td>
<td>7.2±0.1</td>
</tr>
<tr>
<td>Week 5</td>
<td>8.3±0.2</td>
<td>8.1±0.3</td>
<td>7.7±0.1</td>
</tr>
<tr>
<td>Week 7</td>
<td>7.1±0.3</td>
<td>7.5±0.2</td>
<td>7.2±0.2</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>MT</td>
<td>F</td>
</tr>
<tr>
<td>Basal</td>
<td>0.75±0.23</td>
<td>1.01±0.18</td>
<td>1.08±0.31</td>
</tr>
<tr>
<td>Week 2</td>
<td>1.70±0.33</td>
<td>2.21±0.31</td>
<td>1.32±0.11</td>
</tr>
<tr>
<td>Week 5</td>
<td>2.21±0.44</td>
<td>3.27±0.48*</td>
<td>1.40±0.20</td>
</tr>
<tr>
<td>Week 7</td>
<td>1.75±0.42</td>
<td>2.70±0.44*#</td>
<td>1.11±0.12</td>
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<tr>
<td></td>
<td>M</td>
<td>MT</td>
<td>F</td>
</tr>
<tr>
<td>Basal</td>
<td>0.35±0.05</td>
<td>0.51±0.07</td>
<td>0.83±0.1</td>
</tr>
<tr>
<td>Week 2</td>
<td>0.99±0.20</td>
<td>3.03±0.33*#</td>
<td>1.05±0.18</td>
</tr>
<tr>
<td>Week 5</td>
<td>3.05±0.93*</td>
<td>2.22±0.82</td>
<td>1.00±0.16</td>
</tr>
<tr>
<td>Week 7</td>
<td>1.19±0.34</td>
<td>6.33±0.40*#</td>
<td>0.68±0.10</td>
</tr>
</tbody>
</table>

Animals were fasted for 5 hours prior to obtaining a blood sample from the tail vein. Values are means ± SEM, n = 4 (M and MT), n = 7 (F), and n = 8 (FT). *p<0.05 vs same sex control, #p<0.05 vs opposite sex counterpart.
FIGURE 2. Systolic blood pressure of male and female rats fed with fructose. Blood pressure was measured via the tail cuff at baseline and once weekly for 9 weeks. Values are means ± SEM, *p<0.05 vs control diet, #p<0.05 vs opposite sex.
**FIGURE 3.** Plasma insulin (a) and glucose (b) during an oral glucose tolerance test after 4 weeks of fructose diet. Glucose (1g/kg) was administered via oral gavage and blood samples obtained from the tail vein at the times indicated. Values are means ± SEM, #p<0.05 vs opposite sex.
FIGURE 4. Plasma insulin (a) and glucose (b) during an oral glucose tolerance test after 8 weeks of fructose diet. Glucose (1g/kg) was administered via oral gavage and blood samples obtained from the tail vein at the times indicated. Values are means ± SEM, #p<0.05 vs opposite sex.
**FIGURE 5.** Area under the curve for insulin (a) and glucose (b) based on oral glucose tolerance test data at weeks 4 and 8. AUC was calculated using the trapezoidal rule. *p<0.05 vs control diet, #p<0.05 vs opposite sex.
**TABLE 2**

Termination Measurements in the Ovariectomy Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>OVX</th>
<th>F</th>
<th>FOVX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>370 ± 9</td>
<td>424 ± 15*</td>
<td>344 ± 21</td>
<td>439 ± 17*</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.18 ± 0.03</td>
<td>1.13 ± 0.02</td>
<td>1.13 ± 0.08</td>
<td>1.08 ± 0.05</td>
</tr>
<tr>
<td>TE (pg/mL)</td>
<td>47 ± 4</td>
<td>31 ± 2*</td>
<td>54 ± 3</td>
<td>28 ± 2*</td>
</tr>
</tbody>
</table>

Measured plasma total estrogens (TE) include both estradiol and estrone.
Values are means of 7-8 measurements ± SEM, *p < 0.05 vs ovary intact (C or F).
**FIGURE 6.** Body weight (a), food (b), and fluid (c) intake characteristics. Measurements were taken on the same day each week. Values are means ± SEM, n=8 per group. * p<0.05 vs untreated control (C or OVX), # p<0.05 vs ovary intact (C or F).
FIGURE 7. Change in systolic blood pressure from baseline. Ovariectomy and fructose treatment began concurrently at study week 1. Baseline systolic blood pressures were 117 ± 1, 119 ± 2, 116 ± 2, 114 ± 3 mm Hg for C, F, OVX, and FOVX respectively. Values are means ± SEM, n=8 per group, * p<0.05 vs F.
## TABLE 3

Plasma Parameters in the Ovariectomy Study

<table>
<thead>
<tr>
<th></th>
<th>Glucose (mM)</th>
<th></th>
<th>Insulin (ng/ml)</th>
<th></th>
<th>Triglycerides (mM)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>OVX</td>
<td>F</td>
<td>FOVX</td>
<td>C</td>
<td>OVX</td>
</tr>
<tr>
<td>Basal</td>
<td>6.9 ± 0.2</td>
<td>6.6 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>6.7 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>6.9 ± 0.1</td>
<td>6.6 ± 0.1</td>
<td>6.7 ± 0.2</td>
<td>7.0 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>7.2 ± 0.3</td>
<td>6.6 ± 0.1</td>
<td>7.2 ± 0.2</td>
<td>6.7 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 6</td>
<td>7.2 ± 0.1</td>
<td>7.1 ± 0.2</td>
<td>7.2 ± 0.2</td>
<td>7.0 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.5 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>1.7 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>2.5 ± 0.5*#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>1.5 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>2.5 ± 0.2 #</td>
<td>2.3 ± 0.3 #</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 6</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>2.5 ± 0.3</td>
<td>2.7 ± 0.2 #</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.9 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>1.4 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>1.4 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>3.8 ± 0.7 #</td>
<td>1.3 ± 0.2 §</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>2.3 ± 0.3</td>
<td>0.7 ± 0.1</td>
<td>3.0 ± 0.4 #</td>
<td>1.3 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 6</td>
<td>2.3 ± 0.4</td>
<td>1.2 ± 0.2</td>
<td>5.6 ± 1.2 #*</td>
<td>2.6 ± 0.5 §</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Animals were fasted for 5 hours prior to obtaining a blood sample from the tail vein. Values are means ± SEM, n=8 for each group.

* p<0.05 vs C, # p<0.05 vs OVX, § p<0.05 vs F.
**FIGURE 8** Plasma glucose during an oral glucose tolerance test. Glucose (1g/kg) was administered via oral gavage and blood samples obtained from the tail vein at the times indicated. Area under the curve values are illustrated in the inset. Values are means ± SEM. No significant differences were noted.
**Figure 9.** Plasma insulin during an oral glucose tolerance test. Glucose (1g/kg) was administered via oral gavage and blood samples obtained from the tail vein at the times indicated. Area under the curve values are illustrated in the inset. Values are means ± SEM. No significant differences were noted.
**FIGURE 10.** Insulin sensitivity indices obtained from oral glucose tolerance testing. Refer to materials and methods for calculation method and see reference 10. Values are means ± SEM, n=8 per group, * p< 0.05 vs OVX.
FIGURE 11. Cumulative concentration – response curve to noradrenaline in (a) control and (b) fructose fed female rats (set #1). Rings of endothelium intact aorta were incubated with or without insulin (100 mU/ml, 2h). Values are means ± SEM, n=7 or 8 rings per group, *p<0.05 vs without insulin.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>pD₂ (-logM)</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endothelium +</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.01 ± 0.09</td>
<td>214 ± 18</td>
</tr>
<tr>
<td>C + insulin</td>
<td>6.04 ± 0.13</td>
<td>260 ± 13*</td>
</tr>
<tr>
<td>Fructose</td>
<td>5.80 ± 0.12</td>
<td>181 ± 11</td>
</tr>
<tr>
<td>F + insulin</td>
<td>6.03 ± 0.15</td>
<td>234 ± 16*</td>
</tr>
<tr>
<td><strong>Endothelium -</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.58 ± 0.08</td>
<td>266 ± 15</td>
</tr>
<tr>
<td>C + insulin</td>
<td>6.42 ± 0.04</td>
<td>273 ± 13</td>
</tr>
<tr>
<td>Fructose</td>
<td>6.41 ± 0.17</td>
<td>268 ± 24</td>
</tr>
<tr>
<td>F + insulin</td>
<td>6.37 ± 0.12</td>
<td>270 ± 18</td>
</tr>
</tbody>
</table>

Female rats were 15 weeks of age and fructose fed for 11 weeks prior to termination. Rings of aorta were treated with or without 100 mU/ml insulin for 2 hours. Values are mean ± SEM of 8 values, * p < 0.05 vs untreated.
TABLE 5

Systolic Blood Pressure and Plasma Parameters at Termination of Female Rats in Set#2

<table>
<thead>
<tr>
<th>GROUP</th>
<th>BP (mm Hg)</th>
<th>Glucose (mM)</th>
<th>TG (mM)</th>
<th>Insulin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>113 ± 3</td>
<td>7.7 ± 0.3</td>
<td>1.6 ± 0.1</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Fructose</td>
<td>115 ± 4</td>
<td>8.0 ± 0.3</td>
<td>2.9 ± 0.3*</td>
<td>1.7 ± 0.1*</td>
</tr>
</tbody>
</table>

Female rats were 6 weeks of age and fed with fructose for 11 weeks prior to termination. n= 6 (control), n=8 (fructose), values are means ± SEM, *p<0.05 vs control.
FIGURE 12. Cumulative concentration – response curves to noradrenaline in (a) control and (b) fructose fed female rats (set #2) - endothelium intact. Rings of aorta were incubated with indomethacin (10^{-5} M) and with or without insulin (100 mU/ml, 2h). Values are means ± SEM, n=6 (control), n=8 (fructose). No significant differences were detected.
FIGURE 13. Cumulative concentration – response curves to noradrenaline in (a) control and (b) fructose fed female rats (set #2) - endothelium denuded. Rings of aorta were incubated with indomethacin (10^{-5} M) and with or without insulin (100 mU/ml, 2h). Values are means ± SEM, n=6 (control), n=8 (fructose). No significant differences were detected.
FIGURE 14. Cumulative concentration – response curve to noradrenaline in endothelium intact aorta of (a) control and (b) fructose fed male rats. Experimental conditions are similar to conditions used for female rats in set #2. Compare to figure 12. (Reproduced from Verma, 1997 with permission).
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + Insulin</th>
<th>Fructose</th>
<th>Fructose + Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endothelium +</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pD$_2$ (-log M)</td>
<td>7.20 ± 0.09</td>
<td>6.96 ± 0.12</td>
<td>7.38 ± 0.15</td>
<td>7.10 ± 0.21</td>
</tr>
<tr>
<td>Max (%)</td>
<td>82 ± 6</td>
<td>104 ± 15</td>
<td>109 ± 12</td>
<td>96 ± 5</td>
</tr>
<tr>
<td>AUC</td>
<td>184 ± 15</td>
<td>104 ± 15</td>
<td>264 ± 35</td>
<td>208 ± 27</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><strong>Endothelium -</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pD$_2$ (-log M)</td>
<td>8.17 ± 0.20</td>
<td>7.75 ± 0.13</td>
<td>7.95 ± 0.08</td>
<td>7.64 ± 0.15</td>
</tr>
<tr>
<td>Max (%)</td>
<td>110 ± 18</td>
<td>91 ± 11</td>
<td>124 ± 13</td>
<td>109 ± 5</td>
</tr>
<tr>
<td>AUC</td>
<td>316 ± 43</td>
<td>250 ± 25</td>
<td>356 ± 49</td>
<td>274 ± 19</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

Fructose treatment began at age 6 weeks and continued for 11 weeks. Aortae were incubated with 100 mU/ml insulin for 2h. Values are means ± SEM, no significant differences were noted.
FIGURE 15. Body weight (a), food intake (b), and fluid intake (c) of male and female rats chronically treated with insulin. Rats were treated with insulin (2U / d) or vehicle via sustained release implants. Measurements were taken on days 1-4 and on the same day of each week thereafter. Values are means ± SEM, *p<0.05 vs untreated, #p<0.05 vs opposite sex. In panel (a), males weighed significantly greater than females at all time points in the study.
<table>
<thead>
<tr>
<th>GROUP</th>
<th>Insulin (ng/mL)</th>
<th>Glucose (mM)</th>
<th>Triglycerides (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>0.26 ± 0.02</td>
<td>5.9 ± 0.2</td>
<td>1.1 ± 0</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>0.21 ± 0.09</td>
<td>6.2 ± 0.3</td>
<td>1.8 ± 0*</td>
</tr>
<tr>
<td><strong>Female Treated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>0.48 ± 0.16+</td>
<td>6.3 ± 0.1</td>
<td>1.2 ± 0</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>1.95 ± 0.11*#</td>
<td>3.4 ± 0.4*#+</td>
<td>1.3 ± 0.1*#+</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>0.25 ± 0.02</td>
<td>5.5 ± 0.2</td>
<td>1.2 ± 0*</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>0.60 ± 0.05</td>
<td>6.8 ± 0.2</td>
<td>1.7 ± 0.3*</td>
</tr>
<tr>
<td><strong>Male Treated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>1.08 ± 0.20*#</td>
<td>6.4 ± 0.2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>2.05 ± 0.28*#</td>
<td>4.8 ± 0.5*##+</td>
<td>1.9 ± 0.1*</td>
</tr>
</tbody>
</table>

Rats were treated with 2U / day bovine insulin via sustained release implant for 4 weeks. Blood samples were obtained from the tail vein after a 16 h fast. 

*p<0.05 vs basal, #p<0.05 vs control group, +p<0.05 vs opposite sex, p<0.05.
FIGURE 16. Systolic blood pressure of male and female rats chronically treated with insulin. Rats were treated with insulin (2U / d) via sustained release implant for a period of 4 weeks. Systolic blood pressure was measured via the tail cuff method each week. Values are means ± SEM, *p<0.05 vs all other groups.
**Figure 17.** Plasma glucose during an oral glucose tolerance test in male and female rats chronically treated with insulin. Rats were treated with insulin (2 U/d) via sustained release implant. Rats were challenged with glucose (1 g/kg) via oral gavage and blood samples obtained from the tail vein at the times indicated. Area under the curve (AUC) is illustrated in the inset. Values are means ± SEM, *p<0.05 vs untreated, #p<0.05 vs opposite sex.
**FIGURE 18** Plasma insulin during an oral glucose tolerance test in male and female rats chronically treated with insulin. Rats were treated with insulin (2 U/d) via sustained release implant. Rats were challenged with glucose (1 g/kg) via oral gavage and blood samples obtained from the tail vein at the times indicated. Area under the curve (AUC) is illustrated in the inset. Values are means ± SEM, \*p<0.05 vs treated, #p<0.05 vs opposite sex.
FIGURE 19. Insulin sensitivity indices obtained from the oral glucose tolerance test. Indices were calculated as described in the materials and methods. *p<0.05 vs untreated, #p<0.05 vs opposite sex.
Figure 20. Correlation of systolic blood pressure to insulin sensitivity in (a) male and (b) female rats. No significant correlation was detected for either sex.
FIGURE 21. Cumulative concentration – response curves to noradrenaline in endothelium intact aorta of (a) male and (b) female rats chronically treated with insulin. Chronic insulin treatment was 2 U/d via sustained release implant. Rings of aorta were incubated with or without insulin (100 mU/ml, 2h). Values are means ± SEM. No significant differences were detected.
FIGURE 22. Cumulative concentration – response curves to noradrenaline in endothelium denuded aorta of (a) male and (b) female rats chronically treated with insulin. Chronic insulin treatment was 2 U/d via sustained release implants. Rings of aorta were incubated with or without insulin (100 mU/ml, 2h). Values are means ± SEM. *p<0.05 vs M + insulin.
TABLE 8

Concentration – Response Data to Noradrenaline in Male and Female Rats
Chronically Treated with Insulin

<table>
<thead>
<tr>
<th>Group</th>
<th>Control pD₂</th>
<th>Max (g/mm²)</th>
<th>+ 100 mU/ml insulin pD₂</th>
<th>Max (g/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endothelium +</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>6.69 ± 0.19</td>
<td>2.73 ± 0.29</td>
<td>6.91 ± 0.24</td>
<td>2.69 ± 0.24</td>
</tr>
<tr>
<td>MT</td>
<td>6.77 ± 0.18</td>
<td>3.38 ± 0.21</td>
<td>6.74 ± 0.16</td>
<td>3.31 ± 0.31*</td>
</tr>
<tr>
<td>F</td>
<td>6.71 ± 0.16</td>
<td>2.34 ± 0.28</td>
<td>6.66 ± 0.14</td>
<td>2.81 ± 0.35</td>
</tr>
<tr>
<td>FT</td>
<td>6.76 ± 0.06</td>
<td>2.50 ± 0.26</td>
<td>6.44 ± 0.14</td>
<td>2.07 ± 0.37</td>
</tr>
<tr>
<td><strong>Endothelium -</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>7.36 ± 0.23</td>
<td>2.69 ± 0.31</td>
<td>7.17 ± 0.21</td>
<td>2.26 ± 0.2</td>
</tr>
<tr>
<td>MT</td>
<td>7.32 ± 0.12</td>
<td>2.98 ± 0.29</td>
<td>7.71 ± 0.09</td>
<td>3.37 ± 0.24</td>
</tr>
<tr>
<td>F</td>
<td>6.80 ± 0.16</td>
<td>2.42 ± 0.22</td>
<td>6.54 ± 0.18</td>
<td>2.45 ± 0.35</td>
</tr>
<tr>
<td>FT</td>
<td>6.64 ± 0.12</td>
<td>2.76 ± 0.29</td>
<td>6.37 ± 0.15</td>
<td>1.85 ± 0.14</td>
</tr>
</tbody>
</table>

Treatment was with 2U / day bovine insulin via sustained release implant.
Rings of aorta were incubated with or without insulin (100 mU/ml for 2 hours).
Values are means ± SEM, pD₂ values are calculated as -log M, *p<0.05 vs M + insulin
FIGURE 23. Cumulative concentration – response curves to U46619 in endothelium intact aorta of (a) control and (b) chronically insulin treated male and female rats. Chronic insulin treatment was 2 U/d via sustained release implants. Rings of aorta were incubated with or without insulin (100 mU/ml, 2h). Values are means ± SEM.
FIGURE 24. Cumulative concentration – response curves to U46619 in endothelium denuded aorta of (a) control and (b) chronically insulin treated male and female rats. Chronic insulin treatment was 2 U/d via sustained release implants. Rings of aorta were incubated with or without insulin (100 mU/ml, 2h). Values are means ± SEM.
**TABLE 9**

Concentration - Response Data to U46619 in Male and Female Rats Chronically Treated with Insulin

<table>
<thead>
<tr>
<th>Group</th>
<th>Control pD₂</th>
<th>Control Max (g/mm²)</th>
<th>+ 100 mU/ml insulin pD₂</th>
<th>+ 100 mU/ml insulin Max (g/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endothelium +</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>M</td>
<td>10.14 ± 0.44*</td>
<td>2.66 ± 0.23</td>
<td>10.62 ± 0.90*</td>
<td>2.77 ± 0.28</td>
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<tr>
<td>MT</td>
<td>9.39 ± 0.68*</td>
<td>3.82 ± 0.38</td>
<td>9.61 ± 0.59*</td>
<td>3.52 ± 0.51</td>
</tr>
<tr>
<td>F</td>
<td>8.02 ± 0.34</td>
<td>3.06 ± 0.36</td>
<td>8.59 ± 0.49</td>
<td>3.62 ± 0.23</td>
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<tr>
<td>FT</td>
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<td>3.66 ± 0.32</td>
<td>7.69 ± 0.18</td>
<td>3.59 ± 0.36</td>
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<tr>
<td><strong>Endothelium -</strong></td>
<td></td>
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</tr>
<tr>
<td>M</td>
<td>10.56 ± 0.49*</td>
<td>3.00 ± 0.28</td>
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<td>2.92 ± 0.25</td>
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<tr>
<td>MT</td>
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<tr>
<td>F</td>
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<td>2.99 ± 0.50</td>
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<tr>
<td>FT</td>
<td>8.37 ± 0.45</td>
<td>3.56 ± 0.29</td>
<td>8.41 ± 0.32</td>
<td>2.92 ± 0.2</td>
</tr>
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Rats were treated with 2U / day bovine insulin via sustained release implant.
Rings of aorta were incubated with or without insulin (100 mU/ml for 2h).
Values are means ± SEM, pD₂ values are calculated as –log M, *p<0.05 vs opposite sex.
DISCUSSION

The primary objective of this study was to examine the relationship between hyperinsulinemia / insulin resistance and hypertension in female rats. A link between these two conditions has been well established in studies employing male animal models as well as in human studies [145-147], however, it has not been possible to discern what role gender plays in this relationship, if any, based on these previous reports. To investigate the effect of gender on the association between hyperinsulinemia / insulin resistance, two different hyperinsulinemic, hypertensive rat models were used; the fructose fed hypertensive rat (FHR) and a chronically insulin treated rat. Specific questions addressed by experiments using these models were (a) does fructose produce hyperinsulinemia, insulin resistance, and hypertension in female rats as it does in males? and (b) does exogenous insulin treatment cause insulin resistance and hypertension in female rats as in males?

Several mechanisms have been proposed to play a role in the development of hypertension associated with hyperinsulinemia and insulin resistance. A secondary objective of this thesis was to identify potential mechanisms that might explain any sex differences observed. To this end, the role of the female sex hormones in the response to a fructose diet was examined using ovariectomized and control rats. In addition, the vascular actions of insulin and thromboxane, a potential mediator of hypertension related to hyperinsulinemia were also studied.

Metabolic and Cardiovascular Protection

Experiments in Fructose Fed Rats

The experiments described in this thesis demonstrate that female rats are protected against the metabolic defects and hypertension typically produced by fructose feeding in male rats. In
other animal models of hypertension, for example the spontaneously hypertensive rat [158-160] and deoxycorticosterone – salt hypertensive rat [161], similar disparities have been observed where the male sex commonly develops a more severe degree of hypertension compared to females. A previous report in sucrose fed rats, another high carbohydrate fed model of hypertension that is similar to the fructose fed, also demonstrated higher blood pressure development in males compared to females [107].

It is noteworthy that there were no significant changes in any parameter investigated in the female rats used in protocol 1, including fasting triglycerides (TG), insulin, and glucose as well as blood pressure and glucose tolerance. Hyperinsulinemia and insulin resistance are believed to be the primary defects which cause hypertension in the fructose fed male rat model [82, 84-86, 162]. Based on this hypothesis, it follows that hypertension could not have developed in the fructose fed female rats because the necessary metabolic abnormalities were absent. In the studies by Hulman et al. [107], the blood pressure of juvenile female rats fed with sucrose was reported to be elevated, although the blood pressure increase was not as severe as in males. While these authors showed that the male rats used in their study demonstrated insulin resistance as detected by the euglycemic clamp method, they did not measure any metabolic parameters in the female group. Another group has reported that sucrose fed female rats do not develop changes in insulin or triglyceride levels and are not insulin resistant as measured by the euglycemic hyperinsulinemic clamp [106]. The data presented here are in closer agreement with the latter study, which unfortunately did not measure the blood pressure changes in response to sucrose.

Of the experiments described in this thesis, groups of female rats were also fed with fructose in protocols 2 and 3. In both these experiments the fructose diet failed to cause any increase in blood pressure, thus confirming the observations made in the first protocol. However, some changes in metabolic parameters were noted in these groups of rats. Significant increases in plasma TG concentrations were observed in protocols 2 and 3, but this discrepancy may be explained by differences in duration of fructose feeding and age at which the diet was initiated. In protocol 2, the female rats were 15 weeks of age compared to 6
weeks at the start of the experiment. Rats were 6 weeks of age to begin with in both protocols 1 and 3, however the fructose treatment period lasted for 11 weeks in protocol 3 compared to 7 weeks in protocol 1 at the time of fasting plasma TG measurements. A closer look at the plasma TG concentrations from female rats in protocol 1 does suggest a trend towards increasing values, though not significant. It is possible that had this group of rats been followed to an older age for a longer duration of feeding that a significant increase would also have eventually been observed. Taken together, these results suggest that female rats may be susceptible to an increase in plasma TG concentrations in response to a fructose diet in an age- and duration-dependent manner. Age is known to have an effect on metabolism as reductions in insulin sensitivity have been reported in older subjects [163-165]. Plasma insulin values were not significantly different between control and fructose fed females in protocols 1 and 2, but there was a small increase in the fructose fed group in protocol 3. Again, this may be explained by the longer study period length used in this protocol. It is important to note that the difference observed in this group of rats was very small and is considered clinically insignificant (1.3 ± 0.2 vs 1.7 ± 0.1). An OGTT was not performed in this group of rats, but it is believed that such a small increase in fasting insulin values would not result in a decrease in insulin sensitivity.

The mechanisms by which fructose alters metabolism and insulin action in males are not fully understood, but many systems have been reported to be affected. Changes in hepatic enzyme activities involved in fatty acid synthesis [166] and carbohydrate metabolism [167] have been demonstrated. Insulin resistance in male FHR has been attributed to the inability of insulin to suppress hepatic glucose production in fructose fed male rats [168]. Furthermore, alterations in muscle fiber composition [156] and defects in insulin signalling pathways in muscle [169] have been reported and thereby suggest that insulin resistance is also expressed in muscle tissue. The experiments described in this thesis clearly demonstrate that fructose affects metabolism differently in male and female rats. The only relevant effect of fructose observed in female rats was an increase in plasma triglycerides, which developed only after an extended period of feeding or at older age. This still represents a sex difference as the increase in plasma TG response usually develops in young male rats within 2-3 weeks of
starting the fructose diet. Two possibilities exist for these sex differences; there may be mechanisms present only in male rats necessary to facilitate the effects of fructose on metabolism or alternatively, female rats may possess counter mechanisms that protect against the adverse effects of fructose. These mechanisms, if present, may be linked to sex hormones.

In general, physiological concentrations of estrogen have beneficial effects on lipoproteins, insulin and glucose metabolism [114, 120]. Interestingly, the only adverse effect of estrogen on lipid metabolism is to increase hepatic triglyceride synthesis and circulating plasma triglyceride levels [121-123]. This may explain why the only metabolic change observed in fructose fed females was an increase in plasma triglyceride levels, as the effects of fructose and estrogen would be synergistic in this respect only.

To determine if the female sex hormones were involved in preventing the effects of fructose on metabolism and blood pressure, we examined the response to fructose in ovariectomized female rats. This experiment demonstrated that females, if in the absence of normal levels of ovarian sex hormones, will develop hypertension and the metabolic defects seen in male rats after feeding with fructose. Similar to these results, ovariectomy has been shown to further augment blood pressure in other rat models with established hypertension, such as DOCA [161], Dahl salt sensitive [170], and sucrose fed [171]. Even though it is well established that estrogen has many cardiovascular benefits [172, 173], a decrease in sex hormones alone in OVX animals did not cause a significant change in blood pressure. In the current experiment, ovariectomy itself also did not affect any of the metabolic parameters measured, although insulin sensitivity appeared to slightly increase, but not significantly. Comparing the estrogen deficient rat groups (i.e. ovariectomized), insulin sensitivity was significantly reduced upon feeding with fructose. In contrast, in rats with intact ovaries, fructose did not significantly reduce insulin sensitivity. This data indicates that the combination of fructose diet and estrogen / sex hormone deficiency are required to elevate blood pressure in female rats.

Menopause is an example of an estrogen deficient state in humans that is generally accepted as a risk factor for adverse cardiovascular outcomes in women. Prior to menopause, age matched men have a higher risk of developing cardiovascular disease compared to women [108]. Other risk factors such as activity level, diet, and smoking status are also recognized.
for both sexes and the risk for developing cardiovascular disease is heightened when two or more risk factors are combined [108, 174]. To draw parallels between humans and this study, it can be said that fructose diet and ovariectomy are independent risk factors for cardiovascular disease. In the presence of a single risk factor (fructose diet), female rats have a low risk of developing hypertension while males are highly susceptible; but when this risk factor is combined with estrogen / sex hormone deficiency in females, the risk of hypertension increases greatly.

For this experiment, we performed ovariectomy to create an estrogen deficient state as it is the hormone we believe to be more important in protecting against the effects of fructose, although clearly other hormone systems were also removed or altered by ovariectomy. As indicated by the total plasma estrogen concentrations, the ovariectomized rats in this experiment did have significantly lower levels of the hormone, however it was not completely absent. It is not known how much of the hormone present was as estradiol compared to the less active estrone, nor if the ratio between the two differed in intact vs OVX groups since the assay method used did not distinguish between these two types of estrogens. Nonetheless, the data indicate that estrogens were still being secreted from another source in our ovariectomized model, likely the adrenal cortex. As well, it is also important to note that the ovaries are a source of other hormones in addition to estrogen, such as progesterone and androgens, that could impact upon multiple other hormone systems which also affect metabolism and the cardiovascular system. It cannot be determined from this experiment how these other hormone systems were affected by ovariectomy. Therefore, it is difficult to draw definitive conclusions based on this experiment alone on the identity or specific role of the female sex hormones in protecting against fructose induced alterations in metabolism and hypertension. Further experiments with ovariectomized rats using estrogen treatment protocols would be required to shed light on these issues. This experiment does, however, help to rule out other genetic or physiological mechanisms not related to sex hormones.

Several pieces of evidence do suggest that female sex hormones, estrogen in particular, have beneficial effects on insulin sensitivity in both humans and animals. Estrogen can improve
insulin action in liver, muscle, and adipose tissue by increasing glycogen deposition, glucose uptake, and lipogenesis [114]. In postmenopausal women, estrogen replacement therapy has been shown to reduce fasting glucose and insulin concentrations, an indirect measure of insulin sensitivity [115]. In monkeys [116] and rats [117], ovariectomy has been shown to be associated with impaired insulin sensitivity and estrogen replacement therapy in these animals will restore it to normal. In this experiment, ovariectomy itself was not associated with a reduction in insulin sensitivity, but the difference between this and other studies may be explained by differences in age at time of ovariectomy, duration of ovariectomy at time of study, species or strain, and/or method of assessing insulin sensitivity.

**Experiments in Chronically Insulin Treated Male and Female Rats**

Since fructose feeding did not induce hyperinsulinemia or insulin resistance in female rats, it remained unclear whether these conditions are causative factors for hypertension in females as in males. Therefore, we used a chronic exogenous insulin treatment to create hyperinsulinemia and compared the response in both male and female rats. The results of this experiment demonstrated that chronic insulin treatment can impair insulin sensitivity in both male and female rats. However, only male hyperinsulinemic rats experienced a rise in blood pressure. The increase in blood pressure was mild but significant and is of similar severity to that observed in our hyperinsulinemic, insulin resistant male fructose fed model. We believe that the mechanisms of hypertension in these two male rat models are similar and directly related to hyperinsulinemia and insulin resistance.

Strikingly, the female control rats in this experiment had an insulin sensitivity index 4.5 fold greater than male controls. To our knowledge, this is the first report demonstrating a gender difference in insulin sensitivity in rats, but this does agree with previous observations made in humans. An experiment comparing age matched male and female subjects demonstrated that females were inherently more sensitive to insulin as assessed by the euglycemic insulin clamp [175]. The enhancement in insulin sensitivity in females was accounted for by a 50% greater rate of insulin stimulated glucose uptake in femoral muscle tissue. Using chronic exogenous insulin treatment, we were able to impair insulin sensitivity in female rats,
although compared to chronically hyperinsulinemic males, the data clearly support that hyperinsulinemic females remained significantly more sensitive to insulin. Firstly, despite similar plasma insulin levels, greater glucose lowering effects were seen in treated females (FT) than males (MT) in both the fasted state as well as during a glucose load. Secondly, ISI values were higher for the treated female group. A study which employed a chronic insulin treatment regimen with an osmotic pump in female rats demonstrated that hyperinsulinemia actually increased muscle glycogen synthesis with no change in insulin stimulated glucose uptake compared to control [176]. Blood pressure was not assessed in this study. An important difference between this experiment and ours was that the rats were also adrenalectomized and treated with propranolol and corticosterone replacement to prevent elevations in adrenergic activity. This may explain why we were able to show an impairment in insulin sensitivity and they were not.

Several possibilities may explain the lack of any significant rise in blood pressure of hyperinsulinemic female rats. Firstly, there may be a critical degree to which insulin sensitivity must be impaired before adverse effects on blood pressure can be observed and if so, the conditions used in this experiment may not have sufficiently impaired insulin sensitivity in female rats. Hyperinsulinemic females had greater sensitivity than hyperinsulinemic males, and in fact, were comparable to the male control group, which also did not show any elevation in blood pressure. Another explanation may be that hyperinsulinemia and insulin resistance are not associated with high blood pressure in females. The mechanisms which link hyperinsulinemia / insulin resistance to hypertension may be absent in females or there may be compensatory mechanisms that counteract the adverse effects of hyperinsulinemia / insulin resistance on blood pressure.

**Vascular Effects of Insulin**

In addition to its effects on metabolism, insulin is known to have cardiovascular effects at physiologically relevant concentrations [31]. As discussed in the introduction, insulin is
typically considered a vasodilator and defects in the vascular actions of insulin are believed to be involved in the mechanisms linking insulin resistance to hypertension. Therefore, sex differences in the relationship between hypertension and hyperinsulinemia / insulin resistance may be related to differences in the vascular actions of insulin.

**Effect of insulin on VSM in female rats**

Incubation of aortic rings from control and fructose fed female rats (protocol 3, set #1) with insulin caused an increase in the response to NA. This effect was observed only in endothelium-intact tissues and likely reflects the stimulation of endothelium derived contracting factors by insulin, such as endothelin-1 [47, 50, 62, 177] and / or thromboxane [178]. As expected, the response was the same in both diet groups since neither group experienced a change in blood pressure. The vasoconstrictor response observed *in vitro* here might be expected to correlate to increases in blood pressure in female rats *in vivo* under hyperinsulinemic conditions. This reasoning would seem to support that the link between hyperinsulinemia / insulin resistance and hypertension is present in females as well as males, although via different mechanisms. Of course, an insulin-induced pressor effect, reflected by a change in blood pressure, was not observed in this group of female rats since fructose did not cause hyperinsulinemia. However, contrary to this hypothesis blood pressure was not affected by hyperinsulinemia in exogenously insulin treated female rats. Thus, either the effects of insulin observed in this tissue bath experiment (set #1) are not relevant to *in vivo* blood pressure control and hemodynamics in female rats or counter regulatory mechanisms exist to maintain normal blood pressure homeostasis even under hyperinsulinemic conditions. Based on this experiment alone, we are unable to draw conclusions about the influence of sex on the vascular actions of insulin. Therefore, we designed a second experiment to more clearly address this issue.

We have previously demonstrated that insulin reduces the maximum contraction induced by NA in endothelium-intact aortae of male control rats and that this response is abolished by fructose feeding [57]. This implies that insulin, at the concentrations used, acts as an
endothelium-dependent vasodilator in the aorta and that fructose fed male rats exhibit vascular insulin resistance, which may be a contributing factor to the hypertension in this model. In order to compare the vascular effects of insulin and the impact of fructose diet on these effects between the sexes, we carefully repeated the experiment using vascular tissue obtained from female control and fructose fed rats (protocol 3, set #2). The conditions used in this experiment utilized pharmacological inhibition of prostacyclin synthesis in order to highlight the effects of insulin on nitric-oxide (NO) mediated vasodilation. Based on the results showing that insulin by itself increases the response to NA in female aortae, we expected that prostacyclin inhibition would either not alter or potentiate the enhanced response to NA by insulin. However, insulin did not significantly alter the response to NA in this experiment, irrespective of diet group or status of the endothelium. Since this differs from our previous results in males and we used the same protocol in both experiments, we can clearly conclude from these results that there is a sex difference in the vascular actions of insulin in our rat model. As this difference in response was observed in tissues with an intact endothelium, it is likely related to sex differences in endothelial function. Indeed, sex differences in the insulin stimulated release of endothelial factors have been reported in human subjects [179]. If the hypothesis that hyperinsulinemia / insulin resistance is related to hypertension only in males is true, then it may be due to gender differences in the role and function of insulin as a cardiovascular hormone.

As mentioned above, the response to insulin in female aortae was not expected to differ between the conditions used for these experiments, but in fact insulin produced a vasoconstrictor response in endothelium intact tissue only in set #1. There are two possible explanations for this discrepancy. Firstly, the rationale for inhibiting prostacyclin in order to investigate the effects of insulin on NO arose from reports that insulin induces the expression and activity of NO synthase enzymes (NOS) [44, 45, 50]. It was hypothesized that insulin resistance may lead to defects in NO signalling in the vasculature. It has become well established that females have a greater capacity than males to generate NO [134] and this is related to the ability of estradiol to increase NOS expression and activity [128, 135, 180, 181], effects which are qualitatively similar to insulin. In females, insulin may not stimulate
the expected NO-mediated vasodilator response because activity of the enzyme is already so high that it cannot be further increased by insulin.

A second explanation for the apparent discrepancy is that the pharmacological method for preventing prostacyclin mediated vasodilation used in set #2 is actually a non-specific inhibition of all cyclo-oxygenase (COX) derived products, which also includes the vasoconstrictor thromboxane. By inhibiting thromboxane synthesis, it is possible that the mechanism by which insulin produces vasoconstriction in female aorta was blocked as a consequence. Experiments in male porcine coronary artery have demonstrated that insulin can enhance the vasoconstrictor effects of the synthetic thromboxane agonist U46619. This has recently led us to hypothesize that insulin may be a stimulus for thromboxane production or action in addition to the known effects on endothelin-1 and nitric oxide. Conditions of hyperinsulinemia / insulin resistance may affect the balance between these factors to favour elevations in thromboxane production and lead to the development of hypertension. Indeed, inhibition of thromboxane synthesis prevents the development of hypertension in both male rats chronically infused with insulin [182] and hyperinsulinemic, insulin resistant, fructose fed rats (D. Galipeau et al, unpublished observations).

**Role of thromboxane in VSM function**

In addition to the sex differences observed for the vascular actions of insulin, we also observed a striking sex difference in the vascular actions of thromboxane. Male aortae were 2 fold more sensitive to the thromboxane analogue U46619 compared to females. This difference in potency was not affected by removal of the endothelium, which indicates that this relates to the function of vascular smooth muscle itself, rather than modulation by the endothelium. Neither chronic insulin treatment, nor acute incubation with insulin affected the response to U46619 in either gender. This finding is in contrast to the observation that insulin increases contraction to U46619 in male porcine coronary artery [178]. The difference may be species or tissue related. In interpreting our data, it should be noted that the aorta is considered a conduit blood vessel and does not play a large role in the control of blood
pressure. Therefore, it remains possible that other resistance arteries from this model may still exhibit enhanced responsiveness to thromboxane agonists. Regardless of regional variations on the effects of insulin on the actions of thromboxane, the gender difference in sensitivity to thromboxane seems to apply to various blood vessel types and species. The same gender difference in the actions of U46619 have been shown in rat aorta and canine coronary and renal arteries [183, 184]. Furthermore, it has also been shown that infusion of U46619 in rats can cause a pressor response in male rats, but not in female rats [185]. Since thromboxane is involved in the pathogenesis of hypertension associated with hyperinsulinemia [182] (D. Galipeau et al, unpublished), such a profound gender difference in the vascular actions of this hormone provides an appealing explanation for why female rats do not develop hypertension after chronic hyperinsulinemia. It is expected that females would be less susceptible to developing hypertension secondary to hyperinsulinemia because they have significantly reduced sensitivity to this factor.

**Limitations and Future Research Directions**

For the experiments described in this thesis using female rats, we did not feel that it was important to identify the stage of estrus at the time various procedures were performed. Firstly, reports of the effects of estrus / menstrual cycle are conflicting and show small differences at best. Studies of the effects of menstrual cycle on insulin sensitivity in humans have demonstrated either no change [186, 187] or small increases in insulin sensitivity during the follicular phase when estrogen levels are highest [188, 189]. The one study available in rats shows that glucose tolerance and plasma insulin levels are slightly higher at estrus, when estradiol levels are also highest [190]. Secondly, the development of hypertension secondary to hyperinsulinemia / insulin resistance requires chronic exposure in the order of weeks and any effects related to the 4 day estrus cycle are likely to be related to the acute exposure of hormones and short-lived. As we were interested in the net influence of gender on the variables we measured, knowledge only of the overall hormonal status was necessary and
fluctuations due to the estrus cycle, if any, were not important to the overall goals of the experiments.

Conclusions regarding the role of female sex hormones in protecting against fructose induced changes to metabolism and blood pressure are difficult based on the single experiment described here. This data would be strengthened greatly by further experiments using ovariectomized rats and sex hormone treatment protocols to elicit the identity and mechanisms by which females are prevented from developing hyperinsulinemia or hypertension in response to fructose. Parallel experiments in gonadectomized male rats may also provide interesting results.

All the tissue bath experiments described here were performed on aorta. This vessel was chosen because it is simple to dissect, large amounts of tissue can obtained from a single animal, and for consistency and comparison with previous experiments from our laboratory. The aorta is typically considered a conduit blood vessel only, so extrapolating results to *in vivo* mechanisms of blood pressure control may not be justified. However, effects observed in the aorta provide useful guidance for studies of more relevant tissues. Furthermore, the insights gained into the differences in VSM function based on sex are not diminished on this account.

**Gender and Hypertension**

The purpose of these experiments was to determine if hyperinsulinemia and insulin resistance are causally related to hypertension in female rats. We showed that feeding with a high carbohydrate diet, such as fructose, does not produce the same effects as in males, namely hyperinsulinemia, insulin resistance, or hypertension. Using a second method to study the relationship between these conditions, chronic exogenous insulin treatment, we showed again that female rats will not develop hypertension under conditions which increase blood pressure in males. Females were highly sensitive to insulin compared to males. It is possible
that females did not develop hypertension because they have inherently greater insulin sensitivity than males and require even more severe impairments than observed in these experiments to become "adequately" insulin resistant. In addition to differences in insulin sensitivity, we also showed that insulin has different vascular effects in males and females and that VSM function itself also differs.

It is concluded, therefore, from these results that the link between hyperinsulinemia, insulin resistance, and hypertension is gender dependent and applies most clearly to males (figure 25). This is not to say, however, that hypertension does not affect females. Rather, it suggests that the pathological mechanisms of hypertension may differ between males and females. There is very little evidence on the effect of sex on the relationship between hyperinsulinemia, insulin resistance, and hypertension in humans. The results of these experiments, at the very least, indicate that this question warrants further study in a human population. The consequences of chronic high blood pressure can be severe but maintaining adequate control of blood pressure is often difficult for many patients. If the results of these studies in rats can be extrapolated to humans, this may lead to new gender based treatment strategies that improve success at blood pressure control.
**Figure 25.** Mechanisms linking insulin resistance / hyperinsulinemia to hypertension. Pathways illustrated in boxes may be altered in females (see text for details).
CONCLUSIONS

1. Females are protected against the metabolic changes (hyperinsulinemia and hypertriglyceridemia) and development of hypertension that are observed in males upon fructose feeding.

2. The female sex hormones appear to be involved in protecting against high carbohydrate induced hyperinsulinemia / insulin resistance and hypertension. Loss of the influence of these hormones, as with ovariectomy, permits female rats to become hyperinsulinemic, insulin resistant, and hypertensive upon feeding with fructose.

3. Chronic hyperinsulinemia impairs insulin sensitivity in both male and female rats, however, this occurs to a greater degree in males. The hyperinsulinemic, insulin resistant state is associated with hypertension in male rats but not females, indicating that the link between these two conditions may be gender dependent.

4. Females are inherently more sensitive to insulin compared to males. Females may not have developed hypertension secondary to hyperinsulinemia / insulin resistance because the experimental conditions used in these studies (i.e. fructose diet and chronic insulin treatment) did not adequately impair insulin sensitivity to below a critical threshold level required for hypertension to develop.

5. Alternatively, the link between hyperinsulinemia / insulin resistance and hypertension may not be present in females because of differences in vascular smooth muscle function. In male FHR, insulin acts as a vasodilator, whereas in females, this effect is absent or less pronounced. Furthermore, vascular tissue from female rats is significantly less sensitive to thromboxane, a possible mediator of hyperinsulinemia induced hypertension, compared to males.
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