CENTRAL LEPTIN, BUT NOT CENTRAL INSULIN, ATTENUATES THE DECREASE OF ADIPONECTIN CONCENTRATIONS AND INCREASES INSULIN SENSITIVITY IN STREPTOZOTOCIN (STZ)-INDUCED DIABETICS RATS

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# VITA

Jinpin Wang, son of Junke Wang and Xiujing Hou, was born June 6, 1974, in Chengdu, Sichuan, the People's Republic of China. He graduated from Tongji Medical University, Wuhan, the People's Republic of China with a Bachelor of Clinic Medicine in 1997. After that he worked as a resident in No.1 Municipal Hospital of Chengdu. In the fall of 2001, he was accepted as a doctoral student in Department of Nutrition and Food Science at Auburn University.

DISSERTATION ABSTRACT

CENTRAL LEPTIN, BUT NOT CENTRAL INSULIN, ATTENUATES THE DECREASE OF ADIPONECTIN CONCENTRATIONS AND INCREASES INSULIN SENSITIVITY IN STREPTOZOTOCIN (STZ)-INDUCED DIABETIC RATS

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Central leptin increases peripheral insulin sensitivity through unknown mechanisms. Central insulin signaling may also contribute to peripheral insulin sensitivity. To clarify the relationships among central leptin, central insulin, peripheral insulin sensitivity, and adiponectin, we examined the effects of intracerebroventricular leptin and insulin on peripheral insulin sensitivity and adiponectin concentrations in streptozotocin (STZ)-induced diabetic rats. Rats were cannulated in the lateral ventricle. Intravenous STZ was injected to induce diabetes. After establishment of hyperglycemia in STZ-treated rats, insulin (10 mU/day), leptin (10 μg/day), or vehicle was administered daily for 10 days. After one week of central administration, in vivo insulin sensitivity was measured by injecting IV insulin (0.025 U/kg body weight) and measuring blood glucose concentration 15 minutes after the injection. Rats treated with central leptin had increased

peripheral insulin sensitivity. In addition, blood glucose concentrations in diabetic rats were normalized by the 4th day of receiving central leptin administration. Central insulin administration did not affect insulin sensitivity or normalize blood glucose concentrations. Compared to control diabetic rats, diabetic rats receiving central leptin administration, but not diabetic rats receiving central insulin administration, had higher circulating adiponectin concentrations and lower serum and muscle triglyceride concentrations. Therefore, central leptin, but not central insulin, enhances peripheral insulin sensitivity. Adiponectin may be a down-stream target for central leptin signaling.

In the second study, we examined the hypothesis that the sympathetic nervous system is involved in mediating the ability of central leptin to normalize blood glucose concentrations in diabetic rats. A group of rats were chemically sympathectomized with guanethidine (100 mg/kg body weight). Rats were cannulated in the lateral ventricle and then made diabetic with STZ treatment. After establishment of diabetes, rats were given daily injections of leptin (10 μg/day) or vehicle. Leptin decreased blood glucose concentrations equally in both guanethidine-treated and vehicle-treated diabetic rats. This appears to suggest that an intact sympathetic nervous system is not required for central leptin to enhance peripheral insulin sensitivity. However, sympathetic activity was not completely blocked in some tissues (spleen and brown adipose tissue) and not blocked at all in other tissues (liver and white adipose tissue). Therefore, further study is

needed to determine the role of the sympathetic nervous system in mediating central leptin's effect on peripheral insulin sensitivity in diabetic rat.

# ACKNOWLEDGMENTS

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TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF FIGURES

Figure

# CHAPTER I

# INTRODUCTION

More than 170 million people in this world have diabetes mellitus. Among all the diabetic people, type 2 diabetes accounts for about 90% (1). Impaired glucose tolerance is a pre-condition of diabetes. Approximate 7% people with impaired glucose tolerance will development to diabetes each year (1). Nearly 25% of obese children and 21% of obese adolescents have impaired glucose tolerance. Four percent of obese adolescents have type 2 diabetes (2). Insulin resistance and β-cell dysfunction are the major etiologies of the development of type 2 diabetes (3). Insulin resistance refers to the state in which the physiologic effect of insulin is less than expected, especially for increasing glucose uptake in skeletal muscle and suppressed glucose output in liver (1, 3-4). Obesity is the primary risk factor for type 2 diabetes and has a strong positive relationship with the occurrence of type 2 diabetes (4). During 1999-2002, the prevalence of overweight or obesity was 85.2% and the prevalence of obesity was 54.8% for adults with diagnosed diabetes (5).

Leptin is a cytokine and produced mainly by adipose tissues. Leptin plays an important role in regulation of energy expenditure, food intake, and body weight. Leptin also has a function in increasing insulin sensitivity. Cerebrospinal fluid leptin concentration is less elevated than serum leptin concentration in people with increasing

body mass index (BMI) (6). Streptozotocin (STZ) is drug that destroys islet β-cells and is used to induce diabetes in rats. STZ-induced rats have the following features: markedly decreased serum leptin concentration, hypoinsulinemia, and insulin resistance (6, 7). Intracerebroventricular leptin administration can normalize blood glucose concentrations and greatly increase peripheral insulin sensitivity in STZ-induced diabetic rats. This effect of central leptin is independent of circulating concentration of leptin (8). Therefore, central leptin contributes to peripheral insulin sensitivity, but the mechanism in which it does this is not clear (8). Hypothalamic insulin may also contribute to peripheral insulin sensitivity (9). Adiponectin is another adipocytokine, which is produced exclusively by adipose tissue. Adiponectin has a positive relationship with insulin sensitivity. Its serum concentration decreases in obese and diabetic rats, diabetic monkeys, and diabetic humans (10).

The purpose of this study include the following aspects: 1) to examine the mechanisms of central leptin on peripheral insulin sensitivity; 2) to investigate central insulin effects on peripheral insulin sensitivity; 3) to find possible connections among central leptin, central insulin, and adiponectin. Intracerebroventricular leptin or insulin was administered to STZ-induced diabetic rats. Chronic insulin, leptin, or vehicle was administered for 10 days in STZ-treated rats or control rats. After one week of injection, in vivo insulin sensitivity was measured. Blood glucose concentrations were decreased from 4th day in diabetic rats receiving intracerebroventricular leptin administration, but were not changed in diabetic rats receiving intracerebroventricular insulin administration. Diabetic rats receiving intracerebroventricular leptin administration also had higher circulating adiponectin concentrations than other diabetic rats and reduced serum and

muscle triglyceride concentrations. This data suggested that decreased central leptin, but not central insulin, may contribute to insulin resistance. Adiponectin may be a down-stream target for central leptin signaling. Leptin ability to reduce serum and muscle triglyceride concentration may also be related improving insulin sensitivity.

# CHAPTER II

# REVIEW OF LITERTURE

## Diabetes Mellitus

Diabetes mellitus is a group of diseases characterized by hyperglycemia, which is caused by absolute or relative insufficiency of insulin secretion and defects in insulin action. Typical symptoms of diabetes include polyuria, polydipsia, polyphagia, and weight loss (11).

According to the new criteria for the diagnosis of diabetes mellitus, three distinct methods to diagnose diabetes are used, and each must be verified, on a subsequent day, by any one of these three methods to ensure the diagnosis. These three methods include: 1) symptoms of diabetes plus casual plasma glucose concentration ≥ 200 mg/dl (11.1 mmol/l); 2) fasting plasma glucose (FPG) ≥ 126 mg/dl (7.0 mmol/l); 3) 2-h plasma glucose ≥ 200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test (OGTT) (11).

Acute complications of diabetes are diabetic ketoacidosis, hyperosmolar nonketotic coma, lactic acidosis, and hyperglycemia. The consequence of acute complications could be fatal. Long-term hyperglycemia can cause damage to many organs, such as the eyes, kidneys, blood vessels, heart, nerves, etc. The reason for these long-term complications is that prolonged hyperglycemia will change the structures of microvascular, macrovascular, and neural cells, then affect the functions of the tissues and cells.

Diabetes doubles the risk of death in humans. In 2000, it caused more than 210,000 deaths in the United States. Diabetes is the leading cause of blindness, treated end-stage renal disease, and non-traumatic lower-limb amputation. At the same time, it is the major risk factor for heart disease, stroke, and high blood pressure (12).

In 1999, American Diabetes Association (ADA) modified the classification of diabetes mellitus. In this updated document, diabetes mellitus was divided into four subgroups: 1) Type 1: The feature of type 1 diabetes is immune-induced destruction of β-cells, so a type 1 diabetics has some extent of and different rate of β-cells destruction. In the person with genetic tendency to be diabetic, exposure to some environmental factors, such as infectious agents, component of food and toxins, can trigger the occurrence of diabetes. Several antibodies, including islet cell antibodies (ICA), insulin autoantibodies (IAA), antibodies against glutamine acid decarboxylase (GAD65) and the transmembrane tyrosine phosphatase IA-2, could be used clinically as markers to predict the occurrence of type 1 diabetes (13). 2) Type 2: In this type of diabetes, patients have insulin resistance with relative insulin deficiency, without autoimmune destruction of β-cells. Insulin treatment is not necessarily needed for all type 2 diabetes patients, but some type 2 diabetic patient may need it at some stages (13). Obesity is a major risk factor for type 2 diabetes. About 60-90% of type 2 diabetics are obese. Impaired insulin release and insulin resistance are principal factors for the development of type 2 diabetes (14). 3) Gestational diabetes mellitus (GDM): GDM refers to carbohydrate intolerance with the onset or first recognition during pregnancy (11). 4) Other specific types: This category includes diabetes caused by many different etiologies except type 1, type 2 and

gestational diabetes mellitus. As we increase our understanding of the etiology of diabetes, more patients will belong to this class.

Compared to the old diagnosis and classification criteria of 1979 (15), several important changes had been made with the new criteria: 1) the expert committee terminated the terms insulin-dependent diabetes mellitus, non-insulin-dependent diabetes mellitus and their acronyms, IDDM and NIDDM, because all these terms were based on treatment, not on etiology, and thus were confusing. Clinically, both type 1 and type 2 diabetes mellitus patients need insulin treatment in some etiologic stages; 2) the term of malnutrition-related diabetes mellitus was not retained, because there was not sufficient evidence to support it (11).

In 2002, about 18.2 million people had diabetes, 13 million diagnosed and 5.2 undiagnosed in the United States. Among these diabetics, type 2 diabetes accounted for 90% to 95% of all diagnosed cases (12). The state of Alabama has the greatest incidence of this disease per capita. The average prevalence rate of diabetes in the United States of America increased from 0.4% in 1935, to 0.9% in 1960, to 3% in 1993 (16). Some research shows an increase in the incidence of type 2 diabetes in youth as well. If current trends hold, one of every three children born in the year 2000 will develop diabetes in future (17). The major causes for the increased incidence of type 2 diabetes are an increased rate of obesity and a more sedentary lifestyle. Over 50% of adults in the United States are overweight (body mass index, BMI $\geq$ 25 kg/m$^2$) and about 20% adults are considered obese (body mass index, BMI $\geq$ 30kg/m$^2$) (7). A similar tendency was observed in children. The prevalence of overweight of children in the United States of

American was 15.5% for 12-19 years olds, 15.3% for 6-11 years olds, and 10.4% for 2-5 years olds (18).

Because most diabetic patients need treatment for their whole life span, the medical-related expenses for them are huge. In 2002, total expenses for the treatment of diabetes in the United States were $132 billion (12). Thus, preventing and improving the treatment of diabetes is not only beneficial to diabetics in terms of human suffering, but also to the United States society as a whole in terms of impact on the health care system.

## Insulin Signal Transduction and Insulin Resistance

### Insulin Signal Transduction

After release by β-cells, insulin must be delivered though blood to target cells to induce a series of reactions (Figure 1). Binding to insulin receptors is required for insulin action. The insulin receptor is a large trans-membrane glycoprotein, which is composed of 2 α and 2 β subunits (19). Disulfide bonds are used to link the two α subunits and between α and β subunits (20). α subunits are completely extracellular, while the β subunits are transmembrane proteins. The α subunit has a high affinity for insulin binding and the binding of insulin to α subunit will lead autophosphorylation of β subunit of the insulin receptor 1 to 15 minutes after insulin stimulation (21). Two types of autophosphorylation are included in this process: Cis-autophosphorylation, which refers to each β subunit phosphorylating itself, and trans-autophosphorylation, which refers to one β subunit phosphorylating the other β subunit. In 1992, Frattali et al. showed that insulin increases β subunit cis-autophosphorylation only 3-fold, but it increased β subunit

trans-autophosphorylation about 20- to 30-fold. These data suggested that insulin mainly increases β subunit trans-autophosphorylation, which may be important for insulin signal transduction (22). Phosphorylation of β subunit will increase its tyrosine kinase activity to downstream targets, such as insulin receptor substrate (IRS) and an adaptor protein with homology with Src and collagen (Shc). Therefore, insulin signal can phosphorylate multiple tyrosine residues in IRS and Shc (23).

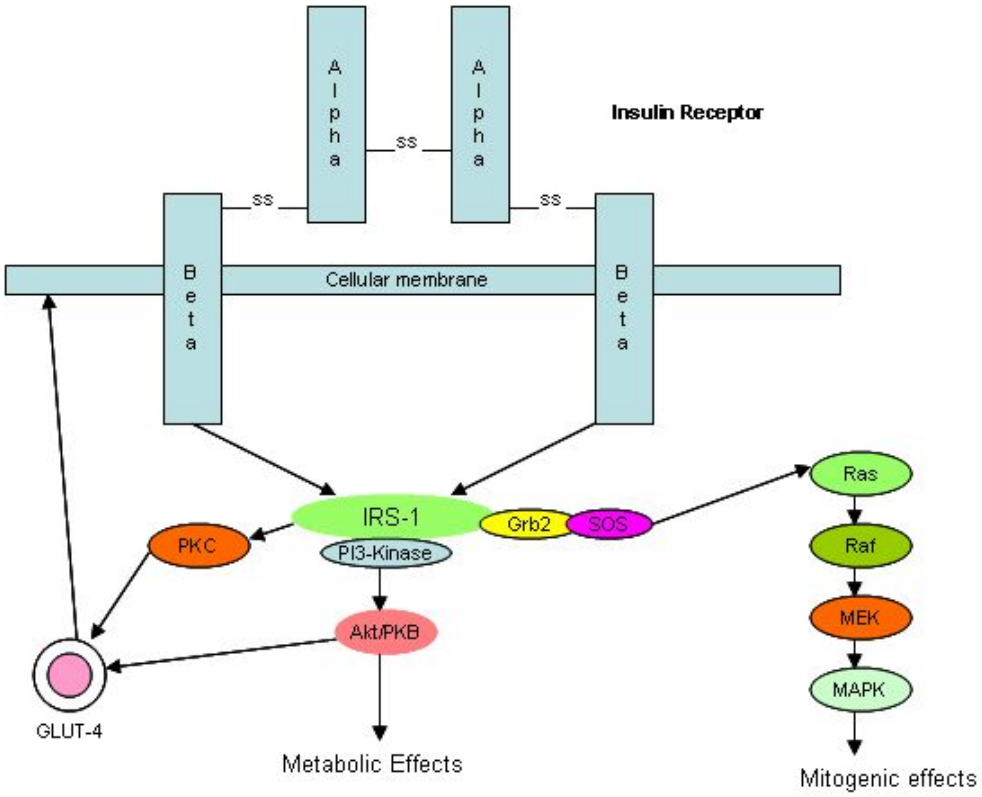


Figure 1. Insulin Signal Transduction. IRS-1: insulin receptor substrate-1; PI-3 Kinase: phosphatidylinositol 3 kinase; PKC: protein kinase C; PKB: protein kinase B; GLUT-4: glucose transport protein 4; Grb2: growth factor receptor bound 2; SOS: mammalian homologue of Drosophila son-of-sevenless protein; Ras: rat sarcoma protein; MEK: mitogen-activated protein kinase kinase; MAPK: mitogen activated protein kinase.

During the course of insulin signal transduction, IRS proteins work as insulin receptor specific docking protein to propagate the insulin signal. Multiple tyrosine phosphorylations will provide special binding site for downstream signaling molecules which contain src homology 2 (SH2) domains, including phosphatidylinositol 3-kinase (PI 3-Kinase), and growth factor receptor bound 2 (Grb-2) (23).

PI 3-kinase catalyzes phosphorylation of phosphatidylinositol, phosphatidylinositol 4-phosphate [PI(4)P], and phosphatidylinositol 4,5,-biphosphate [PI(4,5)$P_2$] to produce phosphatidylinositol 3-phosphate [PI(3)P], phosphatidylinositol 3, 4–bisphosphate and phosphatidylinositol trisphosphate (24). The PI 3-kinase is very important in many insulin-regulated metabolic actions, including glucose transport, glycogen synthesis, and protein synthesis (25, 26). In isolated rat adipocytes, insulin (200 nM) increases the activity of PI 3-kinase and this effect is inhibited by norepinephrine (27). In intact 3T3-L1 cells, a specific inhibitor of PI-3 kinase could effectively inhibit insulin-stimulated glucose transport and DNA synthesis, but did not influence insulin-stimulated mitogenic kinases. Therefore, beside being a crucial part of the insulin signal cascade, PI-3 kinase is also a critical divergent point for this complicated network (26). Insulin also activates PI-3 kinase in rat and human isolated skeletal muscle (24, 28). PI-3 kinase inhibitors, LY294002 and wortmannin, can inhibit insulin-stimulated protein kinase B (PKB) phosphorylation, which is related to glucose transportation (28). In intact mouse soleus muscle, wortmannin inhibits insulin's effect on glucose uptake, activation of glycogen synthase and amino acid uptake. In isolated rat adipocytes, wortmannin blocks lipogenesis. However, wortmannin does not influence muscle deoxyglucose uptake which is induced by okadaic acid or protein kinase C activator tumor promoting agent

(11). Therefore, PI 3-kinase is required for insulin-stimulated glucose uptake, but not necessary for other factors to induced adequate glucose intake in muscles and adipocytes.

The insulin-sensitive serine/threonine kinase Akt (protein kinase B, PKB) is a direct downstream target of PI-3 kinase. PKB is activated by insulin and plays a role in the translocation of glucose transporter 4 (GLUT4) in adipocytes (30). In 3T3-L1 cells, constitutively active Akt stimulates glucose uptake into adipocytes without insulin. It also increases synthesis of GLUT 1 (31). Insulin also activates Akt-1 and Akt-2 in rat adipocytes. In rat adipocytes, Akt-2 (PKB-β) exists in considerable amounts in cytosol and membrane fractions and has a similar distribution in vesicles with GLUT 4 (32). Overexpression of Akt can increase translocation of GLUT 4 even without the stimulation from insulin, but overexpression of Akt–K179A (kinase–inactive mutant) decreases insulin-stimulated GLUT 4 translocation (33). So Akt is at least one, if not the only, crucial component for PI-3 kinase-stimulated glucose intake.

Protein kinase C is another kinase that plays a role in insulin-induced glucose transportation. PKC has three subgroups: the $Ca^{2+}$-dependent or conventional PKCs (cPKCs), the $Ca^{2+}$-independent or novel PKCs (nPKCs), and atypical PKCs (aPKCs). The $Ca^{2+}$-dependent group includes α, βI, βII and γ and depends on both $Ca^{2+}$ and diacylglycerol (DAG) for their activation; the $Ca^{2+}$-independent group includes δ, ε, η and θ and depends on DAG (34); atypical PKCs include λ and ζ. Atypical PKCs are not activated by diacylglycerol or $Ca^{2+}$ (35). In 1997, Bandyopadhyay et al. suggested that insulin will increase PKC- α, β and ζ activity in 3T3/L1 cells. Overexpression of wild-type PKC- ζ, but not PKC- α, β, increased basal and insulin-stimulated glucose transport in fibroblasts and adipocytes. At the same time, a dominant-negative mutant form of

PKC-ζ, not PKC- α, β, decreased basal and insulin–stimulated glucose transport (36). The same year, Standaert et al. made a similar suggestion: insulin enhances PKC-ζ through PI-3 kinase and PKC-ζ may play a role in insulin-induced glucose transport (37). The other member of atypical PKCs, PKC-λ, may also be important for insulin-induced glucose transporter translocation. PI-3 kinase has at least two downstream effectors to stimulate GLUT-4 translocation, Akt/PKB and PKC. The dominant negative mutants of PKC-λ could inhibit insulin-induced glucose intake up to 50 to 60%, while this mutants had no effect on insulin-induced activation of Akt (38). Therefore, PKC looks like it plays a part in insulin-induced GLUT-4 translocation.

Insulin Resistance

Insulin resistance refers to a loss of the ability to some extent to normally respond to insulin stimulation. It is an early characteristic of the progress to type 2 diabetes**.** The typical symptoms of insulin resistance include glucose intolerance, decreased insulin-stimulated glucose uptake, hyperinsulinemia, hypertension, and dyslipidemia. Skeletal muscle, liver, adipose tissue, brain, and pancreas are the major organs related more or less to insulin sensitivity. Different organs may have different degree of insulin resistance, but it is difficult to measure insulin sensitivity in these individual organs in humans in vivo.

Many methods are used to measure insulin sensitivity, such as oral glucose tolerance test (OGTT), glucose or insulin area under the curve after ingestion of glucose, hyperinsulinemic-euglycemic clamp, frequently sampled intravenous glucose tolerance test, cellular and molecular studies of insulin signaling. OGTT has been used for many

decades to evaluate insulin sensitivity and has reliable results (39, 40). In OGTT test, blood glucose concentrations are tested at 0, 30, 60, 90, and 120 minutes after a 75g glucose load and C-peptide should be measured at the same time to calculate insulin secretion. OGTT is simpler than other methods and useful to evaluate β-cell function and insulin sensitivity (40). The hyperinsulinemic-euglycemic clamp is used to assess insulin sensitivity, as well as β-cell function. With the hyperinsulinemic-euglycemic clamp, a standardized dose of insulin is stably infused into blood. Glucose concentration is monitored and the glucose infusion rate is changed to achieve a normal blood glucose concentration. The mean glucose infusion rate is collected to assess insulin sensitivity. Compared to other methods, the hyperinsulinemic-euglycemic clamp provides more precise data about insulin sensitivity and β-cell function. Therefore, it is the gold standard method (40, 41).

After secretion, insulin will be delivered to target tissues and induces a series of change. During diabetes, glucose transportation is the most important. Theoretically, any problem in one or more points from insulin crossing blood vessels to glucose transportation could potentailly cause insulin resistance.

In 1987, Lillioja et al. found fasting plasma glucose and insulin concentrations were negatively related to capillary density. Thus, they suggested that diffusion distance from capillary to muscle cells and muscle types may contribute insulin resistance (42). In normal dog, transcapillary insulin transport may be a limiting factor for insulin effect on glucose utilization (43). In obese humans, the blood flow of insulin-sensitive tissues decreases and may contribute to decreased insulin sensitivity (44). But many researchers believe that impairments after insulin binding to its receptor, other than impaired delivery

of insulin from blood to target tissues, are responsible for insulin resistance (45-49). In both normal and diabetic patients, muscle glycogen synthesis represents the predominant consequence of glucose disposal. In type 2 diabetes, impaired glycogen synthesis is the major issue in insulin resistance (46). Normal people with a similar insulin and blood glucose concentration had significantly higher glycogen synthesis rate than diabetic people (183 ± 39 vs. 78 ± 28 μmol-glycosyl units/kilogram muscle weight/minute, $P < 0.05$). Compared to normal people, the mean glucose uptake speed was considerably lower in diabetic patients (30 ± 4 vs. 51 ± 3 μmol/kilogram/minute, $P < 0.005$). The average rate of nonoxidative glucose metabolism was also far lower in diabetic patients (22 ± 4 vs. 42 ± 4 μmol/kilogram/minute, $P < 0.005$) (46). After being transported into cells, glucose will be conversed into glucose-6-phosphate, which will be used to synthesize glycogen. Thus, any problem from glucose transport to glycogen synthesis could induce insulin resistance. Rothman et al. (1992) tried to find what induces insulin resistance by using $^{31}P$ nuclear magnetic resonance (NMR) to measure glucose 6-phosphate concentration in gastrocnemius muscle (47). In their study, six normal persons and six type 2 diabetic patients were chosen as subjects. Glucose metabolism was examined by a hyperglycemic-hyperinsulinemic clamp. The beginning glucose concentrations were 5.5 ± 0.1 mmol/liter for normal subjects and 14.1 ± 1.4 mmol/liter for diabetes subjects. But during the hyperglycemic-hyperinsulinemic clamp, all subjects have similar steady-state plasma glucose and insulin concentrations (11 mmol/liter and 450 pmol/liter, respectively). Data of whole body oxidative and nonoxidative glucose metabolism also were collected. Under the hyperglycemic-hyperinsulinemic condition, the total whole-body glucose metabolism in normal subjects is much higher than their

diabetic opponent (50 ± 6 vs. 23 ±3 μmmol/kg body weight /min, $P < 0.01$). A similar situation was found for oxidative and nonoxidative glucose metabolism (14 ± 1 vs. 9 ±1 μmmol/kg body weight /min, $P < 0.01$ for oxidative glucose metabolism and 31 ± 7 vs. 13 ±3 μmmol/kg body weight /min, $P<0.05$ for nonoxidative glucose metabolism). Glucose 6-phosphate concentrations, which can be used as an indicator for the efficiency of glucose transport and/or hexokinase, were measured under basal condition and during the hyperglycemic-hyperinsulinemic clamp. The basal glucose 6-phosphate concentrations were not different between these two groups (0.12 ± 0.01 mmol/ kg muscle for normal group and 0.13 ± 0.01mmmol/ kg muscle for diabetic group). During the hyperglycemic-hyperinsulinemic clamp, the mean glucose 6-phosphate concentrations in the normal group was greater than that in the diabetic group (0.24 ± 0.01 mmol/kg muscle vs. 0.17 ± 0.01 mmol/kg muscle, $P < 0.01$). Increasing plasma insulin concentration to 8,480 ± 1,260 pmol/l caused the average glucose 6-phosphate concentrations to enhance to 0.22 ± 0.03 mmol/kg muscle, which is close to the glucose 6-phosphate concentrations of the normal subjects with low-dosage insulin (456 pmol/liter) stimulation. Therefore, a defect of glucose transport or glucokinase or both caused the decrease of muscle glycogen synthesis, which is the major pathway for glucose disposal. The impaired muscle glucose transport/phosphorylation is not only in diabetics, but also in the lean, non-diabetics people who have at least one parent with type 2 diabetes. Compared to normal subjects, offspring of parents with type 2 diabetes showed 50% decrease in total glucose metabolism, 70% reduction ($P < 0.005$) in the rate of muscle glycogen synthesis and a 40% decrease ($P < 0.003$) in muscle glucose-6 phosphate concentration, even with normal glucose concentration (48). Thus, impaired

muscle glucose transport/phosphorylation may be related to the genetic background and play a crucial role in the development of type 2 diabetes.

The answer of what is the primary defect step for the reducing muscle glycogen synthesis in diabetes, glucose transport or hexokinase, was found in 1999 (45). Cline GW et al. utilized $^{13}C$ and $^{31}P$ nuclear magnetic resonance to measure intracellular glucose, glucose 6-phosphate, and glycogen concentration in the gastrocnemius muscle of type 2 diabetes patients and normal people under hyperglycemic-hyperinsulinemic condition. When using a specific dose of insulin (40 mU/square meter of body-surface area/minute to keep glucose concentration around 180 mg/dl) with the hyperglycemic-hyperinsulinemic clamp, the mean glucose infusion rate of the diabetic group was only about 20% of that of normal subjects (3.2 ± 1.7 mg/kg/min and 15.8 ± 3.4 mg/kg/min, respectively). While the mean glycogen synthesis rate and the incremental glucose 6-phosphate concentrations in diabetic patients were also about 20% of that of normal subjects. So the difference of mean glycogen synthesis rate and the incremental glucose 6-phosphate concentration between diabetic and health subjects are proportional to the difference of the mean glucose infusion rate between two groups. The average intracellular glucose concentration was 2.0 ± 8.2 mg/dl in normal subjects, compared to 4.3 ± 4.9 mg/dl in diabetic patients. The average intracellular glucose concentration in diabetic patients equaled to 1/25 of the calculated value if the hexokinase was the primary rate-limiting step of glycogen synthesis. When using higher dosage insulin (400 mU/square meter of body-surface area/minute to keeping glucose concentration around 180 mg/dl) hyperglycemic-hyperinsulinemic clamp in diabetic patients, the mean glucose infusion rate during clamp of the diabetic patients increased to 11.2 ± 0.8 mg/kg/min and

glycogen-synthesis rate increased four times as well. However, in these patients glucose-6-phosphate concentration increased only about two times and intracellular glucose concentration went down a little. If the main reason for insulin resistance is impaired hexokinase, the big increase of glucose infusion rate (from 3.2 ± 1.7 mg/kg/min to 11.2 ± 0.8 mg/kg/min) would induce a huge accumulation of intracellular glucose. Therefore, impaired insulin-stimulated glucose transport, other than impaired hexokinase, is the main cause for the decrease of insulin-stimulated muscle glycogen synthesis in type 2 diabetes patients (45). Similar results were found in adipose cells from type 2 diabetes patients. In the adipose cell from type 2 diabetes patients, the effect of insulin-stimulated glucose transport was only 40% of that in normal people (49).

From insulin receptor to GLUT-4 translocation, many potential points in this cascade may induce insulin resistance. Mutations of insulin receptors can induce severe insulin resistance, but this is only responsible for less than 1% of the insulin resistance cases in type 2 diabetic patients (50, 51). Compare to healthy subjects, lean to moderately obese type 2 diabetic patients have similar IRS-1 protein content in vastus lateralis muscle. But after infusion of high concentration of insulin (5.5 nmol/kg/min) to reach hyperinsulinemia (638 ± 44 and 729 ± 90 nmol/l for control and type 2 subjects, respectively), tyrosine phosphorylated IRS-1 in muscle increased to near 600% of basal state in control subjects, but there was no significant change in type 2 diabetes subjects (52). Differing from skeletal muscle, adipocytes from type 2 diabetes have a decreased content of IRS-1 protein and a smaller maximal response to the stimulation of high insulin concentrations. These adipocytes had only 10% to 50% IRS-1 content compared with those adipocytes from normal subjects (53). These changes of IRS-1 are not only in

type 2 diabetic patients, but also in normoglycemic persons with a strong family history of type 2 diabetes. Compared to normoglycemic subjects without family history of type 2 diabetes, subjects who had normal blood glucose concentrations and at least 2 first-degree relatives with type 2 diabetes, had normal basal and insulin-stimulated insulin receptor tyrosine phosphorylation, but significantly decreased basal and insulin-stimulated IRS-1 tyrosine Phosphorylation (46). Insulin (55), okadaic acid (56) and PI3-kinase (57) can promote serine phosphorylation of IRS-1 to modulate IRS-1 activity. Aspirin may also increase insulin sensitivity, because aspirin can prevent serine phosphorylation of IRS-1 by other kinases (58). In 3T3-L1 adipocytes, insulin induces the phosphorylation of IRS-1 at serine 307, 612 and 632. Insulin also causes IRS-1 phosphorylation on serine 307 and 632 in skeletal muscle and white adipose in vivo. All these phosphorylations, which involve PI-3 kinase and MAP kinase pathways, may be a part of insulin signaling regulation system. Overactivity of these pathways may also induce to insulin resistance (55-57).

PI3-kinase is important for glucose metabolism (28, 59). Insulin stimulation of PI-3 kinase pathway was significantly decreased in type 2 diabetic subjects. Compared to lean subjects, obese nondiabetic and type 2 diabetic subject have a significantly decreased PI-3 kinase activity ($P < 0.05$) (59). GLUT-4 is mainly found in skeletal muscle, adipose tissue, and cardiac muscle and helps to move glucose into the cytoplasm (60). There is no significant difference of the content of GLUT-4 protein in the plasma membrane fraction of muscles during basal condition between healthy people and type 2 diabetes patients (61). The diabetic state may increase the phosphorylation of GLUT-4, which contributes to insulin resistance (62).

Metabolic and Dietary Factors Inducing Insulin Resistance

Many factors could induce insulin resistance. Hyperglycemia is the major characteristic of diabetes. At the same time, hyperglycemia per se can induce insulin resistance. Skeletal muscle is the major tissue responsible for glucose uptake. Adipose tissue, skeletal muscle, and liver are the major sites for insulin resistance. Compared to healthy subjects, leg muscle glucose uptake is decreased significantly in type 1 and type 2 diabetes subjects (63, 64). One possible mechanism for hyperglycemia-induced insulin resistance is the overactivity of the hexosamine pathway (65). Hexosamine pathway is a "glucose sensing pathway", which utilizes a small fraction of glucose through several steps to synthesis the end product UDP-N-acetylglucosamine (UDP-GlcNAc), a substrate for O-linked GlcNAc transferase (OGT). O-linked glycosylation by GlcNAc can modify the serine and threonine residues of cytosolic and nuclear proteins and change the functions of these proteins (65, 66). Modest overexpression of O-linked GlcNAc transferase in muscle and adipose tissue will induce insulin resistance (66). In this pathway, Glutamine: fructose-6-phoshate amidotransferase (GFAT) catalyzes the conversion of fructose-6-phosphate to glucosamine (GLcN)-6-phosphate, which is first and rate-limiting step of the hexosamine pathway. In the GFAT transgenic mice, GFAT was overexpressed in skeletal muscle and adipose tissue. GFAT activity in transgenic mice increased to 2.4 times over the control group. During the hyperinsulinemic-euglycemic clamp, the insulin-stimulated glucose disposal rate of transgenic mice was significantly less than that of the control mice (68.5 ± 3.5 vs. 129.4 ±.9.4 mg/kg per min, $P < 0.001$) (67). In the liver of transgenic mice, overexpression of GFAT about 1.6 times that of controls will bring about similar results. UDP-GlcNAc, the end product of the

hexosamine pathway is also enhanced to 1.2-fold in the transgenic mice ($P < 0.001$). At 6 months of age, transgenic mice have normal insulin sensitivity, but increased hepatic glycogen content, serum free fatty acids, triglycerides, and activity of glycogen synthase. But at the age of 8 months, the transgenic mice have significant insulin resistant and show a decrease in the glucose disposal rate (68). Analogous results were observed from human subjects (69, 70). GFAT is found in the skeletal muscle biopsies in normal and diabetic humans, whose activity is completely inhibited by UDP-GlcNAc. GFAT activity significantly rose in type 2 diabetes patients compared with healthy subjects ($9.5 \pm 1.3$ vs. $6.5 \pm 1.2$ pmol, $P < 0.05$), who also show a decrease in whole-body glucose uptake ($20 \pm 3$ vs. $47 \pm 4$ mmol/min, $P < 0.001$) (69). In cultured human skeletal muscle cells, which were taken from type 2 diabetic patients and health controls, higher glucose treatment (20 mM vs. 5mM) increased GFAT activity by 22% ($P < 0.05$), higher insulin treatment (30 μM vs. 22 pM) boosted GFAT activity by 43% ($P < 0.005$). Meanwhile both higher glucose and insulin produced a 61% raise of GFAT activity. Although in this research, there were no distinctive results between the cultured muscle cells from control and diabetes subjects for GFAT activity and its regulation by glucose and insulin, this may be due to the influence of in vitro condition. Cultured muscle cells from both control and diabetes subjects show the same positive relationship between GFAT activity and glucose and insulin stimulation (70). Therefore, the hexosamine pathway may play a crucial role in hyperglycemia-induced insulin resistance.

Overactivation of PKC is another proposed hypothesis for hyperglycemia-induced insulin resistance. In overexpressing PKC, Chinese hamster ovary (CHO) cells, which express PKC about 7 times as much as controls, 12-O-tetradecanoylphorbol-13-acetate

(TPA) activates PKC and inhibits insulin-stimulated tyrosine phosphorylation of IRS-1 by approximately 75% and insulin-stimulated PI-3 kinase activity by 72% (71). In 1996, Danielsent *et al.* showed that increase activity of PKC α would inhibit human insulin receptor signaling (72). Compared to euglycemia (5 mM glucose), hyperglycemia (25 mM glucose) decreased insulin–stimulated insulin receptor β-subunit tyrosine autophosphorylation by 34%, insulin-stimulated IRS-1 phosphorylation by 72%, and insulin-stimulated PI3 kinase activity by 85% (72). These inhibitory effects of high glucose were completely prevented by an inhibitor of PKC. Because PKC is part of the insulin signaling cascade, overactivation of PKC may be a pathophysiologic reaction to prolonged hyperglycemia and hyperinsulinemia (73).

Obesity is an independent, strong factor contributing to pathogenesis of insulin resistance and type 2 diabetes (74-75). Adipose tissues in humans can be classified as visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) (74-75). Both VAT and SAT influence insulin resistance. VAT has stronger relationship with insulin resistance than SAT (75). Abdominal adiposity is a strong marker and maybe the primary determinant of insulin resistance ($r = -0.89$, $P < 0.001$) (76). Obesity increases free fatty acid concentrations (77) and diabetic patients have a defect in insulin's ability to suppress plasma free fatty acid concentrations (78), while weight loss can significantly ameliorate insulin resistance and decrease free fatty acid concentrations (79). Free fatty acids inhibit insulin-stimulated glucose uptake through inhibiting glycogen synthesis and carbohydrates oxidation (80). Obese humans, offspring of type 2 diabetic patients, and type 2 diabetic patients have an increase in intramyocellular lipid. Women with previous gestational diabetes and insulin resistant patients have enhanced hepatocellular lipids

(81). Elevated free fatty acid concentrations in obese people may trigger the abnormal lipids deposition in insulin-sensitive tissues, such as skeletal muscle and adipose tissue, and finally promote the progression of insulin resistance.

All blood glucose comes from carbohydrates, proteins, and lipids in food, either directly or indirectly. Therefore, it is very crucial to elucidate the relationship between food components and hyperglycemia and insulin resistance. In human diets, carbohydrates include digestible carbohydrates and nondigestible carbohydrates. Digestible carbohydrates are composed of monosaccharide (glucose, fructose and galactose), disaccharide (sucrose, maltose and lactose), and polysaccharide (amylose, amylopectin). Nondigestible carbohydrates are the major component of dietary fibers. The effects of total dietary carbohydrates on insulin action are not clear (82). A decrease in glucose tolerance is associated with aging and may be due to insulin resistance and β-cell dysfunction as part of the process of aging. Chan et al. (1988) found that compared with ad libitum diets, a diet with 85% of its energy derived from carbohydrate can significantly improve the insulin sensitivity index and glucose disappearance rate in elderly men (83). In a 6-year study of nearly 36,000 Iowa women, who had no diabetes at the beginning of this study, there was no linkage between total carbohydrates intake, refined grains, fruit, and vegetable and incidence of diabetes (84). In healthy people, the higher carbohydrate diet (> 50% of energy as carbohydrate and < 30% of energy as fat) and higher fat diet (> 45 % of energy as fat and < 40% of energy as carbohydrate) had no effect on fasting blood glucose, serum insulin concentrations, and mean whole body glucose uptake during a hyperinsulinemic-euglycemic clamp (85). Hughes *et al.* (1995) also demonstrated similar results. They found that a diet composed of 60% carbohydrate

and 20% fat did not change fasting glucose or insulin concentrations in 20 subjects with impaired glucose tolerance (86). Combination of high-monounsaturated fat and low–carbohydrate (carbohydrate 40%; fat 40%; protein 20%) diet reduces postprandial glucose and plasma insulin concentrations (87). All the controversial results about the effects of dietary carbohydrates may be due to the percentage and types of carbohydrate and other components in diets or the specific duration the diets are fed.

Sucrose is a disaccharide composed of one glucose and one fructose connected by an α1-4 glycosidic bond. Sucrose accounts for 30% of dietary carbohydrates consumed. In rats, a high-sucrose diet (68% of energy as sucrose, 20% of energy as protein and 12% of energy as fat) can induce whole-body insulin resistance, hyperglycemia, and impaired insulin secretion (88-90). All this may be the result of changing insulin signaling (91). Fructose comes from caloric sweeteners (mainly sucrose and high-fructose corn syrup), vegetables, and fruits. In the United States, high-fructose corn syrup is the major source of caloric sweeteners in beverages, baked goods, and processed foods. In 1997, the average caloric sweeteners consumption of each American was 154 pounds per year, which is equaled to 0.42 pound or 53 teaspoons per day in 1997. Also in 1997, use of high-fructose corn syrup per person was 62.4 pounds, of which about 72% of was used in beverages (92). The United States is the major user of high-fructose corn syrup in the world, which is made by the catalytic isomerization of glucose to fructose. There are two major types of high-fructose corn syrup: HFCS-42 and HFCS-55, which contain 42% and 55% fructose, respectively (93). The metabolism of fructose is different from that of glucose. Fructose is metabolized mainly in liver. In liver, fructose is first converted to fructose 1-phosphate, and then it is decomposed into dihydroxyacetone phosphate and

glyceraldehyde. These compounds are converted to pyruvate, which may be transformed to lactate, glucose, glycogen or acetyl-CoA. Fructolysis bypasses the phospofructo-1 kinase step, which is the major regulatory step in glycolysis and highly regulated. Therefore larger amounts of fructose will provide an extra, but less regulated source of carbons for hepatic lipogenesis and gluconeogenesis. In addition to its effect of enhancing lipogenesis, fructose does not stimulate insulin secretion from β-cell (94) or increase leptin secretion from adipose tissue (93). Therefore, increased overconsumption of high-fructose corn syrup may contribute to the epidemic of obesity and insulin resistance. In rats, high-fructose diets increase triacylglycerol levels and induce insulin resistance (95). Fructose may be the primary nutrient mediator of sucrose-induced insulin resistance in rats (96). In humans, fructose has acute and chronic effects on lipid profiles (97-99). Non-diabetic subjects and type 2 diabetic subjects were given a meal containing 1g fat /kg body weight with 0.75 g fructose/kg body weight or 0.75 g starch /kg body weight. All subjects had higher plasma glucose and insulin concentrations after the starch containing meal than after fructose containing meal. Plasma non-esterified fatty acid concentrations were lower 1-2 h after meal in the fructose group than in starch group, but higher 4-6 h after the meal in the fructose group. Plasma triacylglycerol concentration has a similar change (97). Dietary fructose acutely decreases plasma concentrations of leptin and insulin, as well as increases ghrelin and triglycerides concentrations in women (98). Leptin, insulin, and ghrelin play a role in the long-term regulation of energy balance. Therefore, increased consumption of fructose may cause an increase of caloric intake, weight gain, and dyslipidemia. In healthy people, the response to a long-term fructose diet may differ by gender (99). Twenty-four healthy adults were chosen as subjects and

received randomized, balanced crossover diets. One diets contained 17% of energy as fructose, the other contained similar amount glucose and was almost fructose free. All the other components of these two diets, including carbohydrate, protein, lipid, fiber, were not different in amount or type. Each diet was fed for 6 weeks. Compared to the glucose diet, the diet containing fructose induced higher fasting, postprandial, and day long plasma triacylglycerol concentrations with statistic significance in men. However, the fructose diet was not different from the glucose diet in women with respect to the changes of fasting and postprandial triacylglycerol concentrations (99). Overall, increased consumption of fructose plays an important role in the epidemic of obesity and insulin resistance in the United States. Fructose enhances hepatic lipogenesis and triacylglycerol synthesis and interferes with glucose metabolism. Meanwhile, fructose disturbs the secretion of insulin and leptin. Therefore, the intake of large amounts of fructose may contribute to the development of obesity and insulin resistance (93-99).

If fructose can induce insulin resistance through increasing the production of hepatic triacylglycerol and lipogenesis, lipids from the diet may also have an influence on insulin resistance. Many studies have verified the connection between high fat intake and insulin resistance (100-103). A high-fat diet brings about impaired glucose tolerance and type 2 diabetes in C57BL/6J mice, which can be used as a dietary model of type 2 diabetes. In this type of mouse, a high-fat diet increases glucose concentrations and impairs insulin response in just 1 week and will last at least 12 months (100). In female Sprague-Dawley rats, 8 weeks on a high fat diet (61% energy as fat and 24% as carbohydrate) significantly increases plasma glucose and triacylglycerol concentrations, and the hepatic glucose output rate as compared to rats fed a high-carbohydrate diet (66% energy as carbohydrate

and 12 % as fat) (101). Similar results have been shown by others (102). In this study, a high-fat diet that contained 32% lard, 18% corn oil, 27% sucrose, and 23% casein by calories, markedly decreased insulin-stimulated glucose transport in both the epitrochlearis and soleus muscles. Substituting corn oil with fish oil provided more omega-3 fatty acids and attenuates the effects of the high-fat diet on insulin sensitivity (102). A high-fat diet containing 59% of total energy as high-oleic sunflower oil (mainly n-6 polyunsaturated lipid) also caused insulin resistance in adipocytes (103). A low-fat diet (17% of total energy as fat) had a higher capability to improve high-fat-induced insulin resistance in rats than did energy restriction (70% of ad labium food intake of the high-fat diet) (104). Based on the survey of 1317 subjects, high-fat, low–carbohydrate diets are related to the onset of type2 diabetes in human (105). High energy and energy-adjusted saturated fat intake also increase glycosylated hemoglobin (Hb $A_{1C}$) concentrations (106). Reducing dietary fat alone can improve body weight and glucose tolerance over a 2 to 3 years period (107). Intake of high-fat diet for several weeks reduces insulin sensitivity in healthy African-American and Caucasian woman (108). The mechanism in which a high-fat diet induces insulin resistance are not clear. It may be achieved via decreasing sympathetic tone (109), altering posttransport steps (110), activation of PKC θ (111), or increasing serine phosphorylation of IRS-1, which leads to a decrease in IRS-1 tyrosine phosphorylation and attenuated insulin signaling (111,112). Overexpression of lipoprotein lipase would considerably decrease plasma triacylglycerol and non-esterified fatty acids and improve insulin resistance (113).

Another factor related to insulin resistance is caffeine. The content of caffeine is 27 to 120 mg/100 ml for coffee, 16 to 33 mg/100 ml for tea, and 10 mg/100 ml for classic

Coca-Cola. The average daily intake of caffeine in the United States can reach 210 to 238 mg. The major action of caffeine is binding to adenosine receptors where it acts as an adenosine receptor antagonist (114). Acute intake of caffeine (5mg/kg body weight) increases insulin, proinsulin, and C-peptide concentrations, but it significantly impairs insulin sensitivity during an OGTT test in type 2 diabetic patients (115) and in healthy obese people (116). This effect of caffeine is independent of weight loss (116). In adipose cells, this inhibitory effect of caffeine may through inhibiting insulin-induced activation of Akt, but without changing PI3 kinase or PKC-zeta (PKCζ) or through antagonism of adenosine receptors (117). In humans, caffeine (3 mg/kg body weight) following a continuous infusion of caffeine (0.6mg/kg/h) significantly reduces insulin sensitivity and elevates plasma free fatty acids, norepinephrine concentrations, and blood pressure. The adenosine reuptake inhibitor dipyridamole did not block the effect of caffeine on insulin sensitivity. Therefore, the acute inhibitory effect of caffeine on insulin sensitivity may be via, at least partially, increasing plasma epinephrine concentrations (118). Further research about the long-term effects of caffeine on insulin sensitivity is needed.

In conclusion, insulin stimulates glucose uptake mainly in skeletal muscle and adipose tissue and decreases hepatic glucose output. Insulin resistance is a state in which a defined amount of insulin induces a weaker than normal action of glucose uptake in peripheral tissues and attenuated inhibition of hepatic glucose output. According to new National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III), metabolic syndrome or insulin resistance is diagnosed when any three of the following criteria are met (119). Abdominal obesity: waist circumference > 88 cm (women) or 102 cm (men); triglycerides ≥ 1.7 mmol; HDL cholesterol < 1.16 mmol (women) or < 0.91

mmol (men); blood pressure ≥ 130/85 mm Hg; fasting plasma glucose ≥ 6.1 mmol. In the United States of American, about 24% of Caucasians, 22% of African Americans, 32% of Mexican Americans, and 20% of other racial and ethnic groups combined may be diagnosed as having insulin resistance syndrome (120). Therefore, further research is necessary to understanding the mechanisms of insulin resistance, which will be helpful in clarifying the etiology of diabetes and improving treatments of type 2 diabetes for a considerable diabetic population in the United States.

## Leptin

### Leptin Receptor and Leptin Signal transduction

Leptin is the product of the obese (ob) gene. The research of leptin began in 1994 (121, 122). Leptin is synthesized mainly by white adipose tissue, but other tissues such as placenta, skeletal muscle, stomach fundus, osteoblasts, also express and secrete it (123, 124). Leptin secretion is regulated by distinct factors in different tissues (123). In adipose tissue, stimulators include, but not limit to overfeeding, obesity (except ob mutation), insulin, glucocorticoids, and acute infection. On the other hand, fasting, cold exposure, and testosterone inhibit leptin expression. Hypoxia enhances leptin secretion in placenta and smoking reduces it.

Leptin is a 146-amino-acid polypeptide with a four-helix bundle secondary structure (125). Leptin concentration in blood is proportional to total body fat mass. After secreted into blood, leptin circulates as a 16 kD protein and is partially bound to plasma proteins. Leptin performs its function mainly via binding to specific receptors (123). Thus, leptin

can act as a hormone. However, it also plays a role as a paracrine agent. For example, local leptin secreted by human osteoblasts may directly contribute to osteoblastic cell growth and bone mineralization (124). Leptin has a wide tissue distribution and the small intestine may have the highest concentration (126). About 80% of all leptin is cleared by kidney (127).

Leptin receptors (OB-R) are the products of the diabetic gene (db) (123). There are six isoforms of leptin receptors (Ob-Ra-Ob-Rf), which have the same extracellular ligand-binding and transmembrane domains. An exception is Ob-Re which is without the transmembrane domain. Ob-Rb (the long form of leptin receptor) is the only leptin receptor capable of activating the janus kinase (Jak)-signal transducers and activators of transcription (STAT) signal transduction pathway (123, 128). Therefore, Ob-Rb plays a main role in leptin signal transduction. Mutations of the db gene induce severe early-onset obesity, and extreme insulin resistance in mice (129). Ob-R is distributed in both the nervous system and peripheral tissues (130). Ob-Ra has a ubiquitous expression with higher concentrations in lung and kidney. Ob-Ra also has higher density at the choroid plexus and brain microvessels, both sites related to different blood barriers (130, 131). Hence, Ob-Ra may play a role in leptin crossing these blood barriers. Ob-Rb is expressed in the arcuate, ventromedial, and lateral hypothalamic nuclei, which are sites related to the regulation of food intake and body weight (130). Ob-Re is the soluble isoform of the leptin receptor. In the leptin nonresponsive Zucker diabetic fatty (ZDF) rat, which has a single amino acid mutation in the leptin receptor and increased leptin mRNA and protein levels, the concentration of the soluble leptin receptor is increased at least 20-fold. Overexpression of Ob-Re does not change leptin expression, but induces a significant

increase in circulating leptin concentrations and a further decrease of food intake and body weight. Therefore, the soluble leptin receptor (Ob-Re) may delay the clearance of leptin and play a role in controlling plasma leptin concentrations (131). The functions of Ob-Rc, Ob-Rd, and Ob-Rf are not clear. Ob-Rc may intervene in leptin binding and internalization (122). Ob-Rf is mainly expressed in liver and spleen, but the function of this type receptor is not clear (128).

Leptin participates not only in food intake and weight regulation, but also sexual development, reproduction, and other endocrine and metabolic aspects (133). Ob-Rb plays a major role in leptin signal transduction. Leptin signaling pathways include the Janus tyrosine kinase/ signal transducer and activator of transcription (Jak/STAT) pathway, the mitogen-activated protein kinase (MAPK) pathway, suppressors of cytokine signaling (SOCS), PI3 kinase, IRS-1, PKC, and nitric oxide (NO) (134-146). Jak/STAT pathway is the main pathway for leptin signal transduction. Leptin receptors mediate leptin-stimulated tyrosine phosphorylation of Jak2 and STAT 3 (134-136). STAT3 signaling is essential for leptin to regulate food intake and energy balance (135). Leptin also stimulates the phosphorylation of MAP kinase, which induces cell proliferation (137, 138). The SOCS family of proteins includes SOCS1-SOCS7 and cytokine-inducible Src-homology 2 (SH2) containing protein (CIS). Every SOCS proteins contain a central SH2 domain. SOCS proteins play a vital role in suppressing cytokine signaling (140-142). SOCS3 may substantially contribute to fetal liver erythropoiesis regulation (139). Activation of the long-form of the leptin receptor (Ob-Rb) stimulates SOCS3 mRNA and protein expression. The pretreatment of cells with leptin causes leptin resistance, which is caused by SOCS3 inhibiting leptin-stimulated tyrosine phosphorylation of Jak2. SOCS3

binds to Jak2 via a leptin-dependent manner (140). Therefore, SOCS3 may be a part of the negative feedback system of leptin signaling and overactivity of SOCS3 may play a role in leptin resistance (140-142). IRS-1 and PKC are part of the insulin signal cascade, but they are also affected by leptin (143-145). Therefore, leptin may influences insulin sensitivity. Leptin enhances NO synthesis in a dose–dependent manner. NO can decrease blood pressure. Because leptin also stimulates sympathetic activity, leptin may contribute to blood pressure regulation (146).

Leptin and Insulin Resistance

Plasma leptin concentrations are significant higher in females than males (147-149). In obese adults without diabetes, leptin concentrations are significantly higher than in lean subjects (26.9 ± 3.9 vs. 5.9 ± 0.7 μg/l). In these nondiabetic adults, leptin levels have a significant positive relationship with BMI and homeostasis model assessment ratio (HOMA-R), which is used to evaluate insulin resistance (149). In lean people with type 2 diabetes, leptin levels are lower than in people with matched BMI (150, 151). Obese type 2 diabetic patients (BMI 33.0 ± 1.0 $kg/m^2$), and obese patients with impaired fasting glucose patients (BMI 33.3 ± 1.5 $kg/m^2$) have higher leptin concentrations than nonobese, nondiabetic subjects. However, obese type 2 diabetic patients have lower leptin concentrations than BMI-matched nondiabetic subjects (152). Therefore, the leptin concentration might be determined by age, gender, BMI, the degree of insulin resistance, and insulin secretion. Leptin concentrations have a significant relationship with insulin resistance in both diabetics and non diabetics patients (147-149, 153-154), which is independent of age, body mass index, fat mass, and lipids (148, 153).

Both central and peripheral leptin can increase insulin sensitivity (8, 155). Leptin does not affect glucose- or glucagon-like peptide-1-induced insulin secretion from islets of ob/ob or lean mice. Leptin has a negative impact on the phospholipase C (PLC)/PKC-induced insulin secretory pathway in the islets from ob/ob mice, but no effect on insulin secretion from islets of lean mouse. Because ob/ob mice do not produce functional leptin, leptin may have a function in constraining oversecretion of insulin (145). In vivo, leptin stimulates a distinct pathway from the insulin cascade at insulin sensitive sites, such as the liver and adipose tissue, but this pathway partially overlaps with the insulin signaling pathway (154). Leptin can stimulate both insulin synthesis and secretion in HIT-T-15 cells, which is a hamster beta cell line (156). Chronic central leptin can normalize blood glucose concentrations and significantly increase insulin sensitivity in diabetic rats (8). Lin et al. (2002) injected streptozotocin (STZ) to induce diabetes in rats and then administrated ICV leptin (10 μg) daily into the lateral ventricle. In diabetic rats that received ICV leptin, the elevated blood glucose concentration (406 ± 25.2 mg/dl) was returned to normal by the 4th day after central leptin injection. Central injections of leptin do not change the plasma leptin concentration. Therefore, inadequate central leptin signaling may contribute to insulin resistance. However, the mechanism by which central leptin improves peripheral insulin sensitivity is not clear (8). Leptin may increase insulin sensitivity in the liver through decreasing hepatic glucose output (155). Before leptin binds to its receptor — Ob-Rb in brain, plasma leptin must cross the blood-cerebrospinal fluid (CSF) barrier at the choroid plexus and the blood-brain barrier (BBB) at the cerebral endothelium (157-159). Leptin travels across blood-brain barrier via a saturable uptake system (158). It also traverses the blood-cerebrospinal fluid barriers via non-saturable

mechanisms (159). The high affinity leptin transporters in the hypothalamus and choroid plexus are mainly related to leptin transportation into the central nervous system and blood-cerebrospinal fluid under physiologic conditions. The low affinity transporters at the blood-brain barrier outside the hypothalamus play a role in leptin transportation under pharmacological or pathophysiological conditions (157). Therefore, either defects in leptin transport or impaired central leptin signaling, or both may contribute to insulin resistance. Levin et al. (2004) used diet-induced obese (DIO) rats to examine alterations in leptin transport and impaired central leptin signaling. The DIO rat is a good model for human obesity, because it develops obesity with many typical aspects of the metabolic syndrome. DIO rats have reduced central leptin signaling at the age of 4-5 weeks. This defect occurs before the onset of obesity and is not caused by deficient transportation of leptin across the blood-brain barrier, because at this age, leptin transportation is not different between DIO rats and control rats. Therefore, impaired leptin signaling may be an innate characteristic, while impaired leptin transport may be an acquired feature. Both contribute to leptin resistance and obesity (160). Leptin is a sensitizer for insulin. On the other hand, insulin also can stimulate leptin secretion (161, 162). Insulin can increase leptin secretion and production in the white adipose tissue (161). Laferrere et al. (2002) suggested that for healthy, non-smoker, non-obese humans, a single pulse of insulin administration can act synergistically with dexamethasone to elevate plasma leptin levels. Increasing insulin concentrations can prevent the drop in leptin concentrations and maintain the basal leptin levels during fasting.

Hence, under physiological conditions, leptin plays a crucial role in increasing insulin sensitivity (Figure 2). The effect of insulin on the body directly depends on the

insulin concentration and insulin sensitivity. When insulin sensitivity decreased, insulin concentration would increase to compensate for this decrease. Insulin is also a stimulator for leptin secretion and in turn, increases insulin sensitivity. It appears the balance between insulin secretion and insulin sensitivity maintains a normal effect of insulin on the body.

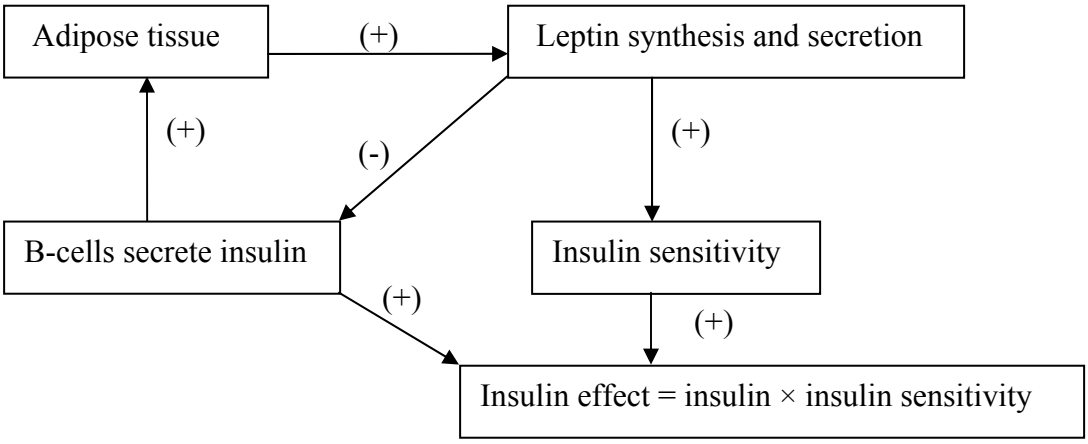


Figure 2: The relationship among leptin, insulin, and insulin sensitivity under physiological conditions. +: increase; -: decrease.

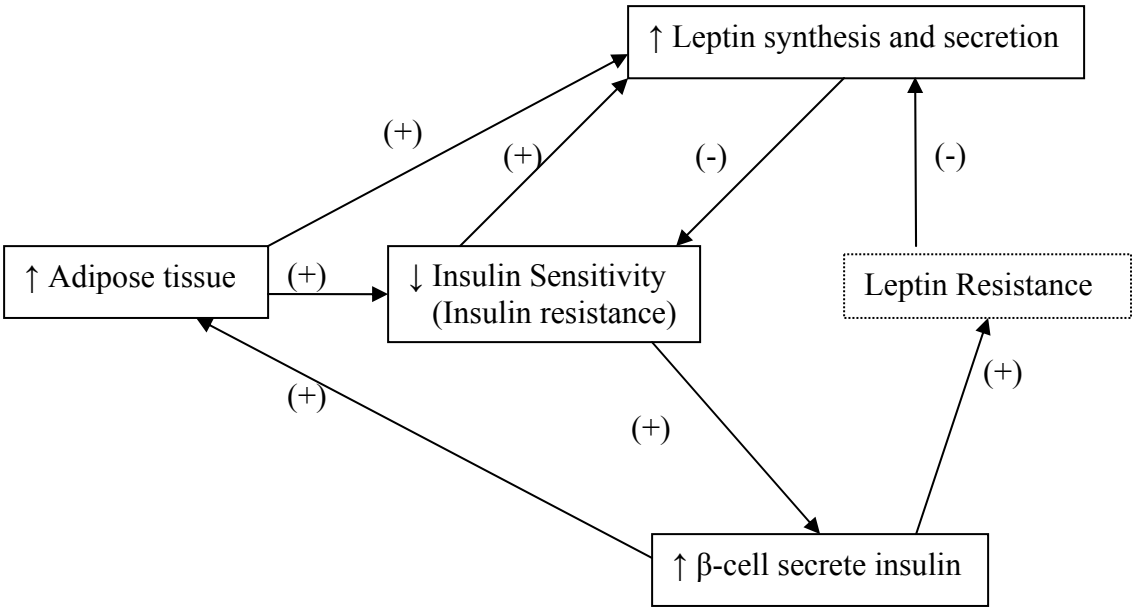


Figure 3: The relationship among leptin, insulin, and insulin sensitivity under insulin resistance and leptin resistance conditions. +: increase, -: decrease.

As in figure 3, obesity is associated with the decrease of insulin sensitivity (163). At the same time, it enhances leptin synthesis and secretion (149). Because of leptin resistance, the increased leptin concentration cannot completely improve insulin sensitivity. Decreased insulin sensitivity (insulin resistance) would stimulate insulin synthesis and secretion from β-cell, as well as be associated with hyperleptinemia (164). Hyperinsulinemia magnify the existing leptin resistance (165). In adipose tissue, the antilipolytic effect of insulin is much less influenced under insulin resistance conditions. Thus the enhanced basal insulin concentration induces further lipid storage and obesity (166), and finally further decreases insulin sensitivity.

Leptin and AMPK

AMP-activated protein kinase (AMPK) is a very conserved enzyme, which can be found probably in all eukaryotes (167). The research on AMPK started in 1973 (168, 169). Early work showed that a protein kinase could decrease the activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limit enzyme for cholesterol synthesis (168). It was thought that another kinase phosphorylates and reduces the activity of acetyl-CoA carboxylase, the vital enzyme controlling fatty acid synthesis (169). It was later found that these two kinases are the same (170). Besides HMG-CoA reductase, acetyl-CoA carboxylase, the substrates for AMPK include hormone-sensitive lipase (HSL) (171), glycogen synthase, phosphorylase kinase (172), glycerol phosphate acyl transferase (GPAT), cystic fibrosis transmembrane regulator (CFTR), and IRS-1 (173). AMPK is a heterotrimeric complex composed of a catalytic subunit ($\alpha$) and two regulatory subunits ($\beta$ and $\gamma$). Two isoforms of the catalytic subunit ($\alpha1$ and $\alpha2$) have been identified. These two isoforms have different tissue distributions; $\alpha1$ has a ubiquitous distribution; most of $\alpha2$ locates in skeletal muscle, heart, and liver (174). The $\beta$ subunit has two isoforms, $\beta1$ and $\beta2$. The $\gamma$ subunit has three isoforms, $\gamma1$, $\gamma2$ and $\gamma3$. All twelve different combinations of isoforms appear to be able to form complexes (173).

AMPK is phosphorylated and activated by an upstream protein kinase (AMP-activated protein kinase kinase, AMPKK). In mammals, AMPKK is the counterpart of Pak1p, Tos3p, and Elm1p in yeast (175). As the crucial allosterical regulator for AMPK, AMP has at least four effects on the AMPK system: (a) its an allosteric activator of AMPK; (2) it makes dephospho-AMPK a better substrate for AMPKK by binding to

dephospho-AMPK; (3) it binds to phospho-AMPK and makes it a worse substrate for protein phosphatase 2C (PP2C), which dephosphorylates and inactivates AMPK; (4) it directly activates AMPKK (167). Although AMP has many effects, the AMPK system is regulated by the AMP/ATP ratio, not by AMP per se (167). A decrease in this ratio is a strong stimulator for activation of AMPK.

The AMPK system originally developed as a stress-response system against the effects of nutritional or environmental pressure (167). To test this idea, high concentrations of fructose (20 mM) was used to treat intact hepatocytes. Fructose causes a large, but temporary increase in the AMP/ATP ratio because fructose is rapidly phosphorylated and consumes ATP inside the cell, but is only slowly metabolized. It also induces activation of AMPK, and inhibits acetyl-CoA carboxylase (176). Because AMPK is responsive to ATP depletion or low energy status, activation of AMPK turns on pathways that synthesize ATP and turns off pathways that utilize ATP. Activation of AMPK inhibits fatty acid and sterol synthesis under conditions where the cellular energy is low. Both pathways utilize NADPH and ATP (167). NADPH is the product of pentose-phosphate pathway, which produces less ATP from glucose than does glycolysis. The enzymes of the pentose-phosphate pathway are located in the cytosol, which is the location of fatty acid synthesis. Fatty acid synthesis depends on NADPH to proceed. When there is excess carbohydrate from the diet, the major purpose of fatty acid synthesis is to convert carbohydrates to fat for long-term energy storage. Increased AMPK activity will inhibit acetyl-CoA carboxylase, decreasing the concentration of the product of acetyl-CoA carboxylase, malonyl-CoA. Decreased levels of malonyl-CoA increase the rate of fatty acid oxidation (177, 178). The important regulated step of fatty

acid oxidation is the movement of fatty acid into mitochondria. This step is catalyzed by two carnitine: palmitoyl–CoA acyltransferases, CPT I and CPT II. CPT I is located in the outer mitochondrial membran and is inhibited by malonyl-CoA (178, 179). So the inhibition of acetyl-CoA carboxylase by AMPK will activate fatty acid oxidation and inhibit fatty acid synthesis at the same time. This decreases ATP utilization by inhibiting the biosynthetic pathway and creates ATP by activating the catabolic pathway. Heart and skeletal muscle have a different isoform of CPT I, which is much more sensitive to the concentration of malonyl-CoA than the liver isoforms (180-182). Heart and skeletal muscle do not play a role in fatty acid synthesis, but express acetyl-CoA carboxylase. Therefore, acetyl-CoA carboxylase in these tissues may play a regulatory role in fat oxidation, glycolysis, and fatty acid elongation (183). When the energy status is adequate in these tissues, the ATP/AMP ratio is high and this inhibits AMPK. Without the inhibition from AMPK, acetyl-CoA carboxylase is in its active form and produces more malonyl-CoA, which will inhibit CPT and fatty acid oxidation. Under stress or low-energy situations, the ratio of AMP/ATP decreases, which activates AMPK. This lowers the activity of acetyl-CoA carboxylase and decreases the concentration of malonyl-CoA. The decline of malonyl-CoA lifts the inhibition on fatty acid oxidation. So fatty acids move into mitochondria and become the major energy source for ATP production. Glucose transporter 4 (GLUT-4) is another important downstream target of AMPK. GLUT-4 is responsive to insulin stimulation. Insulin-mediated translocation of GLUT-4 requires phosphatidylinositol 3-kinase (PI3K) activation and is inhibited by the PI3K inhibitor, wortmannin (184). Skeletal muscle contraction also causes a decrease in acetyl CoA carboxylase activity and an increase in free AMP and AMPK activity (185, 188). In

1999, Bergeron et al. suggested that 5-aminoimidazole-4-carboxyamide-1-β-D-ribofuranoside (AICAR), an AMPK agonist, increases glucose uptake in skeletal muscle, which is not inhibited by wortmannin, or 8-(p-sulfophenyl)-theophylline, an adenosine receptor inhibitor (186). In this study, activation of AMPK by AICAR (40 mg/kg bolus and 7.5 mg/kg/min continue infusion) enhances glucose uptake more than two times that of controls. This effect of AICAR on AMPK raises skeletal muscle glucose transport both in vivo and in vitro (186). *In vivo* infusion of AICAR activates AMPK and stimulates muscle glucose transportation through increasing translocation of GLUT-4. Preincubation of muscles with the kinase inhibitor iodotubercidin or adenine 9-β-d arabinofuranoside (araA), a precursor of aria-ATP (a competitive inhibitor of AMPK), inhibits AICAR- and cyanide-stimulated glucose uptake but does not impair basal or insulin-stimulated glucose uptake (187). The combination of a maximal dose of AICAR and maximal dose of insulin has an additive effect on skeletal muscle glucose transport. However, the combination of maximal dose of AICAR and muscle contraction does not have a cumulative effect on glucose transport (188). Therefore, muscle contraction and AICAR stimulation will activate AMPK and enhance glucose uptake via a pathway that is independent of the insulin signal cascade (185-188).

As a fuel sensor (189), AMPK regulates additional aspects of energy expenditure and energy intake than those have been discussed previously. For example, AMPK can inhibit protein synthesis (190). Further research is needed to investigate the complete role of AMPK in energy metabolism.

Insulin resistance is a major problem for diabetes. Insulin-stimulated GLUT-4 translocation is impaired in people with type 2 diabetes. However, exercise in type 2

diabetic patients brings about a normal raise in GLUT4 translocation and glucose uptake (191). Type 2 diabetic subjects (average body mass index 26 ± 1 kg/m$^2$) and age and BMI-matched control subjects exercised on a cycle ergometer for 45 min at a workload corresponding to 70% of maximum workload. Muscle samples were taken before exercise, at 20 and 45 min during exercise, and at 30 min postexercise. Forty-five minutes of exercise reduces the blood glucose concentration from 7.6 to 4.8 mmol/l in the diabetic subjects, but does not change glucose concentrations in the control group. Beside the effect on blood glucose, exercise also significantly increases AMPK α2 activity in both groups, without any change in AMPK α1 activity (191). Further research was done by Hojlund et al. in 2004. They showed that the expression of all AMPK subunit isoforms (α1, α2, β1, β2, γ1, γ2, γ3) and AMPK activity are not different between the obese (average body mass index 29.6 ± 0.9 kg/m$^2$) diabetic subjects and matched obese control subjects (192). Therefore, AMPK system is intact and could be a potential target to improve insulin sensitivity in diabetic patients.

Metformin is a widely used antidiabetic medicine. It does not increase insulin secretion. Metformin has no significant effects on the secretion of glucagon, cortisol, or growth hormone. Metformin lowers blood glucose concentrations primarily through decreasing hepatic glucose production and ameliorating insulin sensitivity in skeletal muscle and adipose tissue. It also improves the lipid profile and induces weight loss. The molecular mechanism of the metformin effect is not clear (193). Thiazolidinediones (TZDs) are another class of antihyperglycemic agents, which are selective agonists for nuclear peroxisome proliferation receptor-gamma (PPARγ). These drugs include rosiglitazone and pioglitazone (195). TZDs can improve insulin sensitivity. The most

consequential reason for using TZDs is that it can preserve or improve pancreatic β-cell function (194). In 2002, Fryer et al. suggested that the anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways (196). In this research, incubating muscle cells with rosiglitazone increases the AMP/ATP ratio and activates AMPK in muscle cells. Hyperosmotic stress and metformin also activate AMPK in muscle cells by inducing phosphorylation of AMPK at threonine 172 in α-subunit. However, neither hyperosmotic stress nor metformin trigger any changes in the AMP/ATP ratio. All these data suggested that AMPK can be activated by at least two distinct signaling mechanisms. Recently, Leclerc et al. (2004) found that metformin can inhibit insulin secretion in human islets and clonal β-cells by activating AMPK (197). Because hyperinsulinemia promotes leptin resistance (165), the inhibitory effect of metformin on insulin secretion may provide another mechanism for its antidiabetic effect.

In conclusion, a decrease in the AMP/ATP ratio will activate AMPK, which is followed by activating the catabolic pathways producing ATP and inhibiting the anabolic pathways that utilize ATP. Activation of AMPK increases glucose uptake into skeletal muscle and adipocytes (186), fatty acid oxidation (178) and improves insulin sensitivity (196). Type 2 diabetic patients have normal AMPK expression and activity, while exercise induces AMPK alpha2 activity (191, 192). Therefore, pharmacological agonists of AMPK may be potential agents in the treatment of type 2 diabetes.

Besides leptin decreasing the lipogenic effects of insulin without impairing insulin-stimulated glucose metabolism (198), leptin also has acute effect on skeletal muscle. This effect may be mediated by AMPK. Data from Yasuhiko *et al.* (2002) supports this view

(199). Both intrahypothalamic and intravenous injections of leptin increase the activity of α2 subunit of AMPK in skeletal muscle. Leptin may have an indirectly effect through activation of the hypothalamic-sympathetic nervous system axis, and also a direct effect on the activity skeletal muscle AMPK. Accompanying its activation of AMPK, leptin suppresses the activity of acetyl CoA carboxylase. Inhibiting AMPK blocks the effect of leptin on acetyl CoA carboxylase (199). In the same year, another study showed different results about the relationship between leptin and AMPK. In this research, leptin significantly enhances fatty acid oxidation and reduces triacylglycerol content in cardiac muscle without changing glucose oxidation rates. During this process, leptin does not have a significant influence on AMPK activity, AMPK phosphorylation state, acetyl CoA carboxylase activity, or malonyl-CoA concentration (200). Therefore, AMPK-acetyl CoA carboxylase-malonyl-CoA axis could be one signal pathway for the effect on leptin on fatty acid utilization, but not the only one. Blocking the activation of AMPK α2 does not influence exercise tolerance or induce abnormal cardiac function during a peak exercise (201).

In summary, leptin is an adipocytokine produced mainly in adipose tissue. The blood level of leptin is proportional to the size of the body fat mass. Leptin plays a critical role in the regulation of energy balance, weight control, and reproduction. AMPK is an important downstream target of the leptin signal cascade and contributes to the regulation of energy metabolism. Agonists of AMPK are potential candidates for the treatment of diabetes.

## Adiponectin

Adiponectin and Adiponectin Receptor

Adiponectin is a recently discovered cytokine produced exclusively by adipocytes (202). Adiponectin was discovered by different research groups in 1995-1996 (202-206). Therefore, it has different names: adipocyte complement-related protein of 30 kD (Acrp 30) (202), adipoQ (204), Adipose Most Abundant Gene Transcript 1 (apM1) (204), gelatin-binding protein of 28 kD (GBP28) (205), and adiponectin. Adiponectin is the most common name for this adipocytokine. Human adiponectin is a 30 kD protein containing 247 amino acids. Adiponectin is the product of the gene apM1, the transcript of which is the most abundant in human adipose tissue (204, 206). Adiponectin has four main domains: a signal sequence at the cleaved amino terminus, a region without homology to any known protein, a collagenous domain, and a globular domain (203, 206). Endogenous adiponectin secreted by adipocytes has eight different isoforms after post-translational modification. Among these eight isoforms, six are hydroxylated and glycosylated at four lysines (residues 68, 71, 80, 104). The hydroxylation and glycosylation of these four lysines in the collagenous domain are crucial for adiponectin to inhibit hepatic glucose production (207). Full–length adiponectin produced by mammalian cells has a strong effect to enhance the effects of insulin in liver. This is not true of adiponectin produced by bacteria (207).

Adiponectin is expressed and secreted only from white adipose tissue (201, 202) and brown adipose tissue (208). Its gene expression is regulated by hormones and other factors (209-213). Adiponectin production is inhibited by β-adrenergic agonists (209),

tumor necrosis factor (TNF) α and dexamethasone (210), type 2 diabetes (211), gestational diabetes (212), and obesity (213). The factors increasing adiponectin include adrenalectomy (214), type 1 diabetes (215), and weight loss (211). The effect of insulin on adiponectin secretion is conflicting (202, 210). One study showed that insulin has a negative effect on adiponectin gene expression in 3T3-L1 adipocytes (210). Another study, which was also done with 3T3-L1 adipocytes, suggested that insulin enhances the secretion of adiponectin (202). The controversy about insulin's effect may due to the differences in insulin concentration and exposure time of the studies. Further *in vivo* studies are needed to clarify the relationship between insulin and adiponectin secretion.

Adiponectin receptors include adiponectin receptor 1 and 2 (AdipoR1 and AdipoR2). AdipoR1 is expressed primarily in skeletal muscle and AdipoR2 is expressed mainly in the liver. These receptors have high affinity for globular and full-length adiponectin (216). Until now, the signaling cascade of adiponectin was not very clear. However, the downstream targets appear to include AMPK (216-219). In myocytes, globular and full-length adiponectin increase phosphorylation and activation of AMPK and glucose uptake, stimulate phosphorylation of acetyl coenzyme A carboxylase, and decrease fatty oxidation (217). In vivo injection of globular adiponectin enhances AMPK activity and phosphorylation of acetyl coenzyme A carboxylase in the gastrocnemius muscle (218). Only full-length adiponectin can stimulate hepatocyte AMPK and inhibit the production of molecules involved in gluconeogenesis in the liver (217).

Adiponectin makes up 0.01% of total human plasma protein with a varied concentration between 5-30 μg/ml. This concentration is about 1000 times greater than other hormones, such as leptin and insulin (206). Adiponectin may play a role in

inhibiting inflammatory pathways, enhancing insulin sensitivity, and preventing atherosclerosis (206, 219).

Factors Influencing adiponectin Concentration

Adiponectin expression is greater in lean humans than obese humans and greater in women than BMI-matched men (220-222). There is a significant inverse relationship between adiponectin and BMI ($R = -0.55$ and $P < 0.01$). When males and females have matched BMI (< 30 $kg/m^2$), females have 65% higher adiponectin concentrations (14.2 ± 1.6 μg/ml vs. 8.6 ± 1.1 μg/ml, $P < 0.02$) (220). Another study also showed that in nearly 360 non-diabetic adult women, with a wide range of BMI values from 14.8 to 36.3 $kg/m^2$, adiponectin concentration is not only negatively correlated with BMI and body fat mass, but also with serum leptin concentration, fasting immunoreactive insulin, and calculated insulin resistance (221). On the other hand, weight reduction can significantly increase adiponectin concentrations (222). In 22 obese patients who undertook gastric partition surgery, a 21% drop in BMI induced a 46% elevation in mean plasma adiponectin concentrations. The increase of adiponectin concentration is also significantly inversely correlated with the decrease of BMI ($r = -0.5$, $P < 0.01$), waist ($r = -0.04$, $P = 0.04$) and hip ($r = -0.6$, $P < 0.0007$) circumferences (223). Subcutaneous adipose tissue may also play a role in plasma adiponectin concentrations, because adiponectin gene expression is 33% lower in visceral adipose tissue than in subcutaneous adipose tissue in lean humans, and 28% lower in obese humans (224). Anorexia nervosa is an eating disorder, which is characterized by chronic self-starving and severe weight loss. This is associated with a remarkable reduction of adipose tissue and lean body mass. In most anorexia nervosa

patients, plasma adiponectin concentration significantly increases (225, 226). But when it develops to a life-threatening stage, adiponectin levels will drop to a low level (227). Leptin levels decrease in anorexia nervosa patients due to the dramatic reduction of fatty tissue. However, results about the insulin sensitivity in anorexia nervosa patients have been conflicting (225, 226). This may be due to the different methods they used to assess insulin sensitivity or to differences in stage of the disease of these patients. Exercise can significantly improve insulin sensitivity, but it does not cause any change in adiponectin concentration in healthy people. Therefore, adiponectin may be not related to exercise-related improvements in insulin sensitivity (228).

In type 1 diabetic patients with normal BMI, adiponectin concentrations are significantly greater in both males and females (13.6 ± 1.8 μg/ml and 16.1 ± 1.6 μg/ml, respectively) than that of correspondently healthy subjects ( 6.9 ± 0.7 μg/ml and 10.0 ± 0.8 μg/ml, respectively). Insulin therapy does not significantly change adiponectin concentrations in type 1 diabetes (215). On the contrary, type 2 diabetic patients have lower adiponectin concentrations than normoglycemic lean and obese people (229-231). Diabetic patients with coronary artery disease have lower adiponectin concentrations than diabetic patients without coronary artery disease (231). The Pima Indians of Arizona in the United States are a special population that has the highest incidence of type 2 diabetes known in all populations and a high tendency to be obese (232). Pima Indians patients who develop type 2 diabetes have lower adiponectin concentrations than healthy controls. Another study using nearly 28,000 Europeans suggested a similar conclusion (233). In this study, high concentrations of adiponectin are correlated with a reduced tendency of being type 2 diabetes. This relationship between adiponectin and type 2 diabetes is

independent of age, sex, waist-to-hip ratio, body mass index, smoking, exercise, alcohol intake, education, and glycosylated hemoglobin $A_{1C}$ (233).

Therefore, the plasma adiponectin concentration is correlated with many factors. It is also an independent factor and may play a primary role in the development of type 2 diabetes.

Adiponectin and Insulin Resistance

Insulin resistance is the major characteristic of type 2 diabetes. Adiponectin is a major factor correlated with insulin sensitivity in lean or obese, diabetic and non-diabetic humans (215, 220-222). Hypoadiponectinemia has a closer relationship with the degree of insulin resistance and hyperinsulinemia than with the degree of adiposity and glucose intolerance (234). Many studies have explored the relationship between adiponectin and insulin resistance (235-238). In mice, acute administration of the globular domain of adiponectin can significantly reduce the increased free fatty acid concentration induced by a high fat meal or by i.v. injection of Intralipid. A low dose (5 μg/day) of the globular domain of adiponectin significantly reduces body weight in mice fed a high-fat/sucrose diet, but does not change food intake (235). Adiponectin–knockout mice have a significantly lower level of fatty-acid transport protein 1 (FATP-1) in skeletal muscle, but no change in FATP-1 in liver or white adipose tissue. These mice have normal levels of GLUT-4 and uncoupling protein 2 in muscle, hepatic glucose-6-phosphatase, leptin, peroxisome proliferator-activated receptor-γ (PPAR-γ) in white and brown adipose tissue, and IRS-1, IRS-2, GLUT-1 and GLUT-4 in white adipose tissue. But the mRNA levels and plasma concentration of tumor necrosis factor-α (TNF-α) are higher in adipose tissue

of these knockout mice. Adiponectin–knockout mice also have a delayed free fatty acid clearance. After consuming a high-fat/ high-sucrose diet for two weeks, these knockout mice have significantly higher plasma glucose, insulin, and plasma free fatty acid concentrations than wild-type mice, although there are no significant differences in body weight and adiposity between the two groups. Adenoviral-mediated overproduction of adiponectin can remarkably decrease glucose, insulin, adipose TNF-α mRNA expression, and free fatty acid concentrations, and reverse the decrease in FATP-1 mRNA and insulin sensitivity observed in adiponectin–knockout mice fed a high-fat/high-sucrose diet (236). Even in ob/ob and streptozotocin-treated mice, a single injection of adiponectin can temporarily reverse hyperglycemia (237). Changes of adiponectin receptor gene expression may also play a role in the insulin resistance state. Compared to lean mice, AdipoR1 expression is reduced 36% in type 2 diabetic obese (ob/ob) mice, while no significant change in AdipoR2 gene expression is found in obese mice or streptozotocin-induced diabetic mice (238).

The effect of adiponectin to improve insulin sensitivity in mice may be through inhibition of the expression of hepatic gluconeogenic enzymes and endogenous glucose production (239), and increasing fatty acid oxidation in muscle (235). Similar results were found in humans (240, 241). In healthy and diabetic Pima Indians, plasma adiponectin concentrations are negatively and independently correlated with endogenous glucose production in liver (240). At the molecular level, adiponectin may activate AMPK, which then phosphorylates related enzymes to increase fatty acid oxidation and glucose uptake in skeletal muscle and decrease gluconeogensis in liver (217, 218, 239).

Regulation of skeletal muscle IR tyrosine kinase activity in human skeletal muscle may also contribute to adiponectin improving insulin sensitivity in humans (241).

Thiazolidinediones are a relatively new type of antidiabetic, insulin-sensitizers, which were first approved for the treatment of type 2 diabetes in the United States in 1997 as troglitazone. The other two thiazolidinediones, rosiglitazone and pioglitazone, were permitted in the United States in 1999. Then troglitazone was withdrawn for its toxicity in liver. As an insulin-sensitizer, thiazolidinediones can decrease blood glucose and free fatty acid concentrations. PPARγ is the target of thiazolidinediones. PPARγ is distributed mainly in adipose tissue, as well as pancreatic β cells, vascular endothelium and macrophages. By binding to PPARγ, thiazolidinediones increase adiponectin concentrations in adipose tissue and trigger other changes (242-247). In the A-ZIP/F-1 (fatless) mice, insulin-stimulated glucose uptake and IRS-1-associated PI-3 kinase activity in skeletal muscle decrease 53% and 66%, respectively. This type of mice also has hepatic insulin resistance. Three weeks of treatment with rosiglitazone neutralizes the insulin resistance in skeletal muscle, but exacerbates insulin resistance in liver (244). The possible explanation for this is that without white adipose tissue, thiazolidinediones cannot increase production of adiponectin to improve insulin resistance in liver. Moreover, thiazolidinediones may have some effects that are independent of adiponectin, in reducing triglyceride content and enhancing insulin sensitivity in skeletal muscle. Metformin or PPARα agonists do not induce any changes in adiponectin concentrations in type 2 diabetic mice. Fourteen days of treatment with rosiglitazone triggers a 130% increase in adiponectin levels in normal humans (245). Troglitazone (200 mg/day) also increases adiponectin concentrations in glucose intolerant humans (3.3 ± 0.6 vs. 5.9 ±1.4

μg/ml, $P < 0.01$), while 400 mg/day troglitazone induces a stronger effect on adiponectin concentrations (3.0 ± 0.3 vs. 8.8 ± 2.0 μg/ml, $P < 0.001$) (246). Troglitazone (600 mg/day) increases adiponectin concentrations in lean, obese, and obese diabetic humans and significantly decreases fasting plasma glucose concentrations in diabetic patients after 12 weeks of treatment (11.1 ± 0.9 mmol/l vs. 9.1 ± 0.9 mmol/l, $P < 0.005$) (247).

In a summary, adiponectin is an adipocytokine produced exclusively in adipose tissue and the only known adipocytokine that has a negative relationship with the mass of adipose tissue. Adiponectin can increase insulin sensitivity in skeletal muscle and liver. These effects of adiponectin are, at least partially, dependent on AMPK. As an insulin sensitizer, thiazolidinediones have been used to treat type 2 diabetics for a few years. Increasing the production of adiponectin may account for a part of the mechanisms of the effects of thiazolidinediones on insulin sensitivity. Analogs of adiponectin or new agonists of adiponectin may contribute to type 2 diabetes treatments in the future.

**Research Objectives**

We have previously found that central leptin administration greatly increases peripheral insulin sensitivity and normalizes blood glucose concentrations in diabetic rats. For study 1, we compared central leptin administration with that of insulin to affect peripheral insulin sensitivity and normalize blood glucose concentrations in diabetic rat. We also examined the possible role of changes in serum adiponectin concentrations and changes in serum and muscle triglyceride levels mediate the effects on insulin sensitity.

For study 2, we hypothesized that sympathetic nervous system is required to mediate the effects of central leptin to enhance insulin sensitivity in diabetic rats. We used

guanethidine treatment to chemically denervate the sympathetic nervous system and then examined the ability of central leptin administration to decrease blood glucose concentrations in diabetic rats.

# CHAPTER III

# CENTRAL LEPTIN, BUT NOT CENTRAL INSULIN, ATTENUATES THE DECREASE OF ADIPONECTIN CONCENTRATIONS AND INCREASES INSULIN SENSITIVITY IN STREPTOZOTOCIN (STZ)-INDUCED DIABETIC RATS


## Abstract

This study examined the effect of intracerebroventricular leptin and insulin on insulin sensitivity and adiponectin concentration in streptozotocin (STZ)-induced diabetics rats. Male Wistar rats were cannulated in the lateral ventricle. After recovery, intravenous STZ was injected to induce diabetes. Chronic insulin (10 mU/day), leptin (10 μg/day), or vehicle was administered for 10 days beginning at 4 days after establishment of hyperglycemia in STZ-treated rats. After one week of injection, in vivo insulin sensitivity was determined. Blood glucose concentrations were normalized from the 4th day in diabetic rats receiving intracerebroventricular leptin administration, but were not changed in diabetic rats receiving intracerebroventricular insulin administration. Compared to diabetic-control and diabetic-insulin rats, diabetic rats receiving intracerebroventricular leptin had enhanced insulin sensitivity and circulating adiponectin concentrations, as well as reduced serum and muscle triglyceride concentrations ($P < 0.05$). These data indicate that leptin acting centrally has a role in regulating peripheral insulin sensitivity and suggest the involvement of adiponectin as a potential down-stream mediator.

## Introduction

In 2002, about 18.2 million people had diabetes in the United States, with type 2 diabetes accounted for 90% to 95% of all diagnosed diabetes cases (12). The major pathology of type 2 diabetes is insulin resistance and relative insulin insufficiency (11). Therefore, improving insulin sensitivity could benefit at least 16 million people in the United States.

Leptin is a hormone derived primarily from white adipose tissue (121, 122). Leptin not only participates in food intake and body weight regulation, but also sexual development, reproduction, and other endocrine and metabolic aspects (133). Administration of leptin, either peripherally or centrally, can increase insulin sensitivity (8, 155, 248). Subcutaneous infusion of relatively high doses (4 mg/kg) of leptin can normalize blood glucose concentrations and restore insulin sensitivity in the liver of STZ-induced diabetic rats. This effect of leptin is independent of a decrease in food intake or a decrease in blood glucose concentrations (248). Daily bolus injections of leptin (10ug) into the lateral ventricle of the brain normalize blood glucose concentrations and increase whole body insulin sensitivity in STZ-induced diabetic rats. This effect was independent of a decrease in food intake. Serum leptin concentrations did not increase after central leptin administration, suggesting that leptin was acting centrally. How leptin acting centrally improves peripheral insulin sensitivity is not clear (8). In addition, central insulin may also play a role in the regulation of insulin sensitivity. The infusion of either insulin or an insulin mimetic into the third ventricle inhibits glucose production (9). This effect is independent of the serum insulin concentration (9). Thus it appears that both leptin and insulin acting centrally may affect peripheral insulin sensitivity. Since leptin

and insulin share part of their signal transduction pathway (249), it is speculated that this shared pathway is involved in the regulation of insulin sensitivity.

Adiponectin is a cytokine expressed and secreted only in white adipose tissue (201, 202) and brown adipose tissue (208). The effect of leptin and insulin on adiponectin secretion is not clear (202, 210). Adiponectin may play a role in enhancing insulin sensitivity (206, 219). In obese (ob/ob) mice and streptozotocin-induced diabetic mice, a single injection of adiponectin can temporarily normalize hyperglycemia without any changes in insulin levels (237). In mice, acute administration of the globular domain of adiponectin can significantly reduce the increased free fatty acid concentration, which is associated with increased insulin sensitivity (235).

Triglycerides concentrations have also been suggested to have a role on the regulation of insulin sensitivity. Increased intramyocellular triglyceride has a strong negative relationship with whole body insulin sensitivity (251). Serum triglyceride is positively correlated with insulin resistance (253) and negatively correlated with adiponectin concentration (252).

This study compared central administration of leptin with that of insulin on blood glucose concentrations and insulin sensitivity in diabetic rats. In addition, the potential involvement of changes in serum adiponectin concentrations and muscle and serum triglyceride concentrations were examined.

## Materials and Methods

*Animals***.** Thirty-six male Wistar rats (260g to 280g) were housed in individual hanging wire cages. All cages were kept in a temperature-controlled room with a 12:12 hour light-dark cycle. Rats had free access to powdered chow (Prolab RMH 300 Meal, Purina Mills, Richmond, IN) and water. All experimental protocols were approved by Auburn University's Institutional Animal Care and Use Committee.

*Experimental design*. Thirty-six rats were implanted with an intracerebroventricular cannula directed into the lateral ventricle (See Cannula placement). After recovery, sixteen rats were administered streptozotocin (STZ) to induce diabetes (see induction of diabetes); the rest of the rats were given vehicle as a control. After hyperglycemia was verified, about one third of the rats in each group (diabetic vs. control) were given daily ICV injections of either leptin (10 µg, Calbiochem, San Diego, CA), insulin (10 mU, Invitrogen, Carlsbad, CA, or phosphate-buffered saline (control injection) in a volume of 10 µl. Therefore, rats were divided into one of six groups: control-leptin, control-insulin, control-vehicle, diabetic-leptin, diabetic-insulin, and diabetic-vehicle. Body weight and food intake were measured every day and blood glucose concentration was monitored every other day. After 6 days of intracerebroventricular injections, an in vivo insulin sensitivity test was performed. Briefly, insulin (25 mU/kg) was administrated via the tail vein and blood glucose concentrations were determined at 0, 15, 30, 60, 90, and 120 minutes after the injection of insulin. All rats were sacrificed on day 10. Soleus muscle was collected to determine

triglyceride content and blood was collected to determine serum adiponectin, insulin, and triglycerides concentrations.

*Cannula placement*. Rats were anesthetized by an intraperitoneal injection of ketamine-xylazine (100 mg/kg and 1 mg/kg, respectively) and placed in a stereotaxic apparatus. A 22-gauge, stainless steel guide cannula (Plactic One, Roanoke, VA) was implanted into the lateral ventricle (0.8 mm posterior and 1.4 mm lateral to bregma, 3.5 mm deep from the surface of skull). Four stainless steel screws and dental cement were used to fix the guide cannula to the skull. A "dummy" cannula 1 mm longer than the guide cannula was placed into the guide cannula. The rats were housed in individual cages and recovered for 4 days. Angiotensin II (40 ng in a total volume of 6 μl) was used to verify the placement of cannula. Rats that drank less than 5 ml of water within 15 min after administration of angiotensin II were excluded from the experiment. After the drinking test, 32 rats were used in this study.

*Induction of diabetes*. Diabetes was induced with a single intravenous injection via the tail vein of freshly prepared STZ (50 mg/kg; Sigma-Aldrich Corporation, St Louis, MO) in 0.05 M citrate buffer (pH 4.5). Control rats were injected with citrate buffer only. All STZ-injected rats had blood glucose concentrations greater than 350 mg/dl.

*Hormone and metabolite analysis*. Serum insulin concentrations were determined by a sensitive RIA specific for rat insulin (Linco Research, St. Charles, MO), with the lowest limit of detection at 0.02 ng/ml. Serum leptin was measured by a RIA for rat leptin (Linco Research, St. Charles, MO). The limit of detection for this assay is 1.0 ng/ml. Blood glucose concentrations were determined with an Accu-Check simplicity

glucometer (Boehringer Mannheim Corporation, Indianapolis, IN). Triglyceride contents in muscle and serum concentrations were determined with a commercial kit (Sigma-Aldrich Corporation, St. Louis, MO). Adiponectin concentrations were determined with a mouse adiponectin RIA kit (Linco Research, St. Charles, MO). The lowest level of detection of this assay is 0.5 ng/ml.

*Statistical analysis*. All results were presented as means ± standard error of the mean (SE). Statistical analyses were performed by using SSPS 12.0 and SAS 8.02. Two-way analysis of variance (ANOVA) was utilized to analyze STZ treatment and ICV injections on food intake, body weight, and serum hormone and metabolite concentrations. Statistical significance among the groups was determined with a one-way analysis of variance (ANOVA), followed by a Duncan's multiple range test. Simple linear regression was performed between plasma triglyceride concentrations and insulin sensitivity. A difference of $P < 0.05$ was considered statistically significant.

**Results**

*Food intake and Body weight.* Basal body weights and food intakes were measured on the day before leptin, insulin, or vehicle administration. Although STZ-induced diabetic rats had a higher basal food intake than non-diabetic rats, the difference was not statistically significant (25.7 ± 0.9 vs. 23.2 ± 0.6 grams/day, $P = 0.55$). However, the STZ-induced diabetic rats had significant lower body weights ($P < 0.05$) and higher blood glucose concentrations ($P < 0.001$) than the non-diabetic rats (Table 1). STZ treatment and leptin decreased body weight ($F_{1, 506} = 85.4$, $P < 0.001$ and $F_{2, 506} = 19.4$, $P < 0.001$, respectively). Rats treated with leptin (control-leptin and STZ-leptin groups)

had significantly lower body weights than the other four groups ($P < 0.05$). Among these four groups, control-insulin rats had greater body weighs than STZ-control and STZ-insulin rats ($P < 0.05$), but not different from control-control rats (Fig. 4). STZ treatment also increased food intake ($F_{1, 410} = 86.1$, $P < 0.001$), while leptin treatment significantly decreased food intake ($F_{2, 410} = 101.2$, $P < 0.001$). Because of the diabetic states, STZ-control and STZ-insulin groups had greater daily food intakes than the other four groups ($P < 0.05$). There was no significant difference in daily food intake between control-leptin and STZ-leptin group, as well as between control-control and control-insulin groups (Fig.5). As for the cumulative food intake (Fig. 6), leptin-treated rats (control-leptin and STZ-leptin groups) had less cumulative food intake than the other four groups. Rats in STZ-control and STZ-insulin groups had greater cumulative food intake than other four groups ($P < 0.05$) and STZ-control groups had the highest cumulative food intake. There was no difference in cumulative food intake between rats in control-control and control-insulin groups.

*Effect of central leptin and insulin on blood glucose concentrations.* Before leptin (10 μg)**,** insulin (10 mU) or vehicle injections, diabetic rats had much greater blood glucose concentrations than control rats ($P < 0.001$) (Table 1). After administration of leptin, the blood glucose concentrations of rats in the STZ-leptin group began to decrease and returned to the normal range from day 3 through the end of the study (Fig. 7). Blood glucose concentrations of the STZ-insulin group were not different from the STZ-control group from day -1 to day 3 ($P < 0.05$). However, at day 5, the blood glucose concentrations of the STZ-insulin group was significantly lower than the STZ-control group ($382.8 \pm 22.7$ vs. $474.5 \pm 14.8$ mg %, $P < 0.05$), but still significantly higher than

the control-insulin group and the STZ-leptin group (382.8 ± 22.7 vs. 116.8 ± 1.6 and 116.2 ± 6.4 mg %, respectively, $P < 0.001$). STZ treatment significantly increased blood glucose concentrations ($F_{1, 90} = 497.8$, $P < 0.001$), while leptin treatment significantly decreased blood glucose concentrations ($F_{2, 90} = 59.6$, $P < 0.001$). ICV leptin and insulin administration did not change blood glucose concentrations in control rats.

*Insulin sensitivity test.* Fifteen minutes after IV injection of insulin (25 mU/kg), blood glucose concentrations decreased in all groups. However, the decrease in the control-leptin and diabetic-leptin groups was greater than that of the other four groups (39.6 ± 2.7% vs. 14.7 ± 2.0%) (Fig. 8). This indicates that rats in the control-leptin and diabetic-leptin groups had significantly greater sensitivity to insulin than the other fours groups ($P < 0.05$) (Fig. 9). STZ treatment had no effect on insulin. There was no difference in insulin sensitivity among the control-insulin, control-vehicle, diabetic-insulin, and diabetic-vehicle groups (Fig. 9).

*Concentrations of serum adiponectin and insulin.* Adiponectin concentrations decreased in all groups over time (Fig. 10). On day 0 and day 2, adiponectin concentrations were not different among the STZ-control group, the STZ-insulin group, and the STZ-leptin group. However, on day 4, adiponectin concentrations in STZ-leptin group were significantly greater than those of the STZ-control group, and the STZ-insulin group ($P < 0.05$). Leptin or insulin treatment did not cause any change of adiponectin concentrations in non-diabetic rats (Fig. 10). Although all the groups showed a decrease in adiponectin concentrations over time, STZ-leptin rats showed less of a decrease than the other two STZ-treated groups (- 36.6 ± 3.2% of STZ-leptin vs. - 65.3 ± 5.3% of STZ-control and -63.3 ± 3.7 % of STZ-insulin, $P < 0.05$) (Fig. 11).

Insulin concentrations were lower in diabetic rats than that in control rats. However, control-leptin rats had a much lower insulin concentration than control-control or control-insulin rats (0.16 ± 0.04 ng/ml vs. 2.46 ±1 ng/ml and 3.65 ± 1.26 ng/ml, respectively, $P< 0.05$). In the diabetic rats, STZ-leptin rats had a lower insulin concentration, but there was no significant difference with the other two diabetic groups (Fig. 12).

*Concentrations of serum triglycerides and contents of muscle triglycerides*. Leptin treatment significantly decreased serum triglyceride concentrations ($F_{2, 24} = 13.42$, $P < 0.001$) and muscle triglyceride contents ($F_{2, 24} = 5.36$, $P < 0.001$). This was true for both control and diabetic rats. (Fig. 13 and Fig. 14). STZ treatment had no effects on serum triglyceride concentrations ($F_{1, 24} = 0.79$, $P = 0.38$) or muscle triglyceride content ($F_{1, 24} = 2.33$, $P = 0.14$). Insulin injection significantly decreased serum triglyceride concentrations in STZ-treated rats ($P < 0.05$, Fig.13), but had no effect in control rats (Fig. 13).

*Relationship between serum triglycerides and insulin sensitivity*. Serum triglyceride concentrations had a significant inverse relationship with insulin sensitivity ($R^2 = 0.48$, $P < 0.0001$ (Fig. 15). When serum triglyceride concentrations increased, insulin sensitivity decreased. Control-leptin and STZ-leptin rats had the lowest serum triglyceride concentrations (0.16 ± 0.04 ng/ml and 0.26 ± 0.12 ng/ml, respectively, Fig. 13) and the highest insulin sensitivity index (38.8 ± 4.6 % and 40.4 ± 3.4%, respectively, Fig. 9).

## Discussion

In the present study, we evaluated the chronic effects of leptin and insulin administered centrally on blood glucose concentrations and peripheral insulin sensitivity

in diabetic and nondiabetic rats. Chronic leptin administration, but not chronic insulin administration, normalized blood glucose concentrations and increased peripheral insulin sensitivity in diabetic rats. Interestingly, leptin also increased insulin sensitivity in non-diabetic rats even though blood glucose concentrations remaining unchanged. This appears to be due to the fact that leptin also greatly reduced serum insulin concentrations in control rats to the levels observed in the diabetic rats. Because central leptin administration at the dose we used does not change peripheral leptin concentrations (8), leptin appears to be acting centrally to enhance peripheral insulin sensitivity. Leptin did not increase circulation insulin concentrations, as mentioned above, but leptin greatly decreased insulin concentrations in non-diabetic rats.

Our results are consistent with those of Lin et al. (2002) in that central leptin administration increased peripheral insulin sensitivity equally in diabetic rats and control rats (8). However, Lin et al. (2002) (8) found that diabetic, vehicle-treated rats have a decrease in insulin sensitivity as compared to non-diabetic, vehicle-treated rats. This decrease was not observed in the present study. This difference may be due to the different methods used to measure insulin sensitivity in the two studies. The previous study used the steady-state plasma glucose (SSPG) method to determine insulin sensitivity, whereas the present study used an *in vivo* insulin sensitivity test.

We did not find any significant change in peripheral insulin sensitivity in either non-diabetic or diabetic rats due to central insulin administration. There was a slight decrease in the blood glucose concentrations of insulin-treated diabetic rats by day 4 of treatment, but the magnitude of the decrease was much less than that of leptin. These results appear to conflict with the results of Obici et al. (2002) (9). In that study, central administration

of insulin or an insulin mimetic decreased hepatic glucose output and central antagonism of the insulin cascade weakened the hypoglycemic effect of circulating insulin. This difference may be due to the difference in methodology and duration time. Obici et al. (2002) used a hyperinsulemic-euglycemic clamp to determine insulin sensitivity and looked at short-term insulin exposure (hours). In the present study, we used an *in vivo* insulin sensitivity test and looked at long-term exposure to insulin (days). Since we obtained samples daily, it is possibly that we missed a potential insulin-induced temporary decrease in the blood glucose concentration. However, this seems unlikely. On day 4, we examined blood glucose concentrations 4 hours after insulin injection and did not observe a decrease. It is also possible that the dose of insulin was too small to cause an effect on insulin sensitivity. However, the dose we used was great enough to significantly decrease food intake and serum triglyceride concentrations in diabetic rats. The effect of insulin on food intake was consistent with previous studies that suggest a lack of central insulin signaling contributes to the hyperphagia in diabetic rats (254).

It appears that the leptin-induced increase in insulin sensitivity is coordinated with the leptin-induced decrease in serum insulin concentrations. This suggests that the two effects share a common mechanism. Leptin is known to increases sympathetic nerve activity via its receptors in the hypothalamus. Phosphoinositide-3 kinase (PI3-K) may mediate the leptin-induced sympathetic outflow (255). Increased sympathetic nerve activity has been found to inhibit the production of insulin (256). Interestingly, insulin is also known to activity the PI3-K system. Since insulin did not have the same effect as leptin on insulin sensitivity, this would suggest that the activation of PI3-K alone is not sufficient to increase insulin sensitivity.

As a fuel sensor, AMP-activated protein kinase (AMPK) regulates glucose and lipid metabolism (189). Intrahypothalamic injections of leptin increase the activity of AMPK in skeletal muscle apparently through activation of the sympathetic nervous system (199). Accompanying the activation of AMPK, leptin suppresses the activity of acetyl CoA carboxylase, decreasing fatty acid synthesis and increasing fatty acid oxidation in the muscle (199). This would tend to decrease muscle triglyceride content. This is consistent with our finding that central leptin decreased muscle triglyceride content in both diabetic and non-diabetic rats.

As an insulin sensitizer (228), adiponectin concentrations have a positive relationship with insulin sensitivity (211-213). In adiponectin knockout mice, two weeks of high-fat/ high-sucrose diet induces significantly higher plasma glucose, insulin, and free fatty acid concentrations than in wild-type mice, although there are no significant differences in body weight and adiposity between two groups. Adenoviral-mediated overproduction of adiponectin can remarkably decrease glucose, insulin, and free fatty acid concentrations, and enhanced insulin sensitivity in these adiponectin–knockout mice treated with a high-fat/high-sucrose diet (236). Our study is the first to observe that serum adiponectin concentrations were decreased in STZ-induced diabetic rats as compared to non-diabetic rats. Central leptin attenuated the decrease of adiponectin concentrations in STZ-induced diabetic rats. After 4 days of central leptin administration, the adiponectin concentrations of diabetic rats were not different from non-diabetic rats. The attenuated decrease of adiponectin concentrations in diabetes rats may contribute to the increase of insulin sensitivity and normalization of blood glucose concentrations. AMPK is a downstream target of adiponectin (217, 218). In myocytes, globular and full-length adiponectin

decrease fatty oxidation and increase activation of AMPK, glucose uptake, and phosphorylation of acetyl CoA carboxylase. In vivo injection of globular adiponectin enhances AMPK activity and phosphorylation of acetyl CoA carboxylase in gastrocnemius muscle (218). Therefore, activation of AMPK, perhaps through activation of the sympathetic nervous system, may play a crucial role in leptin enhancing peripheral insulin sensitivity.

Circulating insulin decreases not only blood glucose concentrations, but also serum triglyceride concentrations (78), Therefore, it is not surprising that diabetic-control rats had higher serum triglyceride concentrations than their non-diabetic counterparts. Central insulin administration did not change the circulating insulin concentration and had no effect on serum triglyceride concentrations in non-diabetic rats, but it significantly decreased serum triglyceride concentrations in diabetic rats. This may due to the decreased food intake in insulin-treated diabetic rats as compared to control diabetic rats. Thus, central insulin signaling may play a role in regulating peripheral triglyceride concentrations during diabetes. Insulin sensitivity was inversely correlated with serum triglyceride concentrations. This has been observed previously (253). Muscle triglyceride content was also inversely related to insulin sensitivity, though in this study the relationship was not as strong. This has also been observed in humans. In sedentary men, higher muscle triglyceride content is associated with lower insulin sensitivity (251). Central insulin did not change muscle triglyceride concentrations, but central leptin decreased muscle triglyceride concentrations in both control and diabetic rats. Leptin treatment in both control and diabetic rats was related to an increase in insulin sensitivity.

The role the decrease in serum or muscle triglycerides play in the leptin-induced increase in insulin sensitivity remains to be addressed.

In summary, we showed that chronic central leptin administration, but not insulin, greatly increased peripheral insulin sensitivity. In diabetic rats, the increase in peripheral insulin sensitivity was associated with a normalization of blood glucose concentrations. Central leptin administration also attenuated an STZ-induced decrease in adiponectin concentrations, and reduced serum and muscle triglyceride levels. Given that adiponectin is associated with increased insulin sensitivity (252), it is possible the some of the effects of central leptin to enhance insulin sensitivity in diabetic rats may be mediated through adiponectin. Further studies are needed to determine the relationship among central leptin, adiponectin, and peripheral insulin sensitivity.

Table 1. Basal food intake, body weight, and blood glucose concentrations of control and diabetic rats

| | Control (n = 16) | STZ (n =16) |
|---|---|---|
| Food intake, g | 23.2 ± 0.6 | 25.7 ± 0.9 |
| Body weight, g | 299.2 ± 5.5 | 273.5 ± 4.3* |
| Blood glucose, Mg/dl | 116.2 ± 3.0 | 409.6 ± 20.8** |

Values are means ± SE. Basal food intake, body weight, and blood glucose concentrations were measured 1 day before leptin (10 μg), insulin (10 mU) or vehicle injection. * $P < 0.05$ vs. control. ** $P < 0.001$ vs. control.

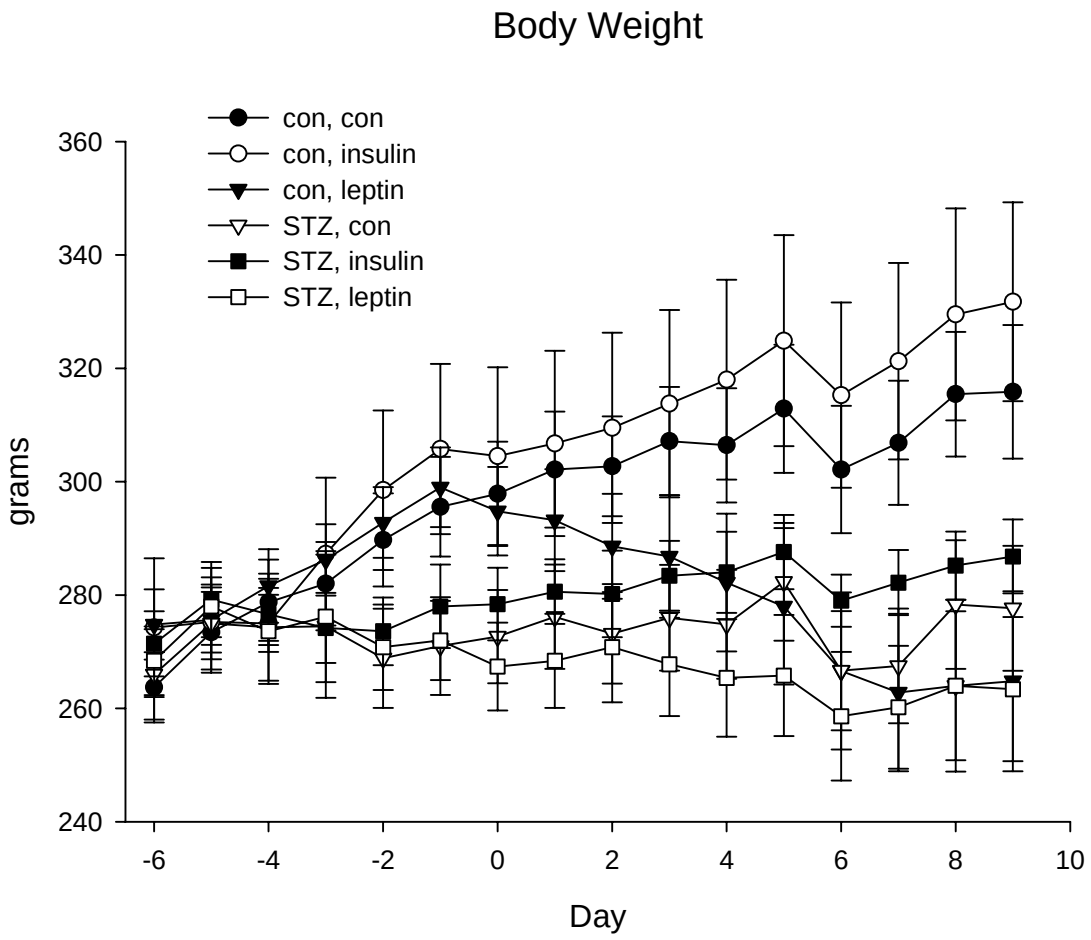


Figure 4. Effect of icv leptin (10 µg), insulin (10 mU), or vehicle on body weight in control rats and diabetic rats. Values are means ± SE. Day -3 is the first day after streptozoticin (STZ) injection; Day 0 refers to the starting for leptin, insulin, or control injection

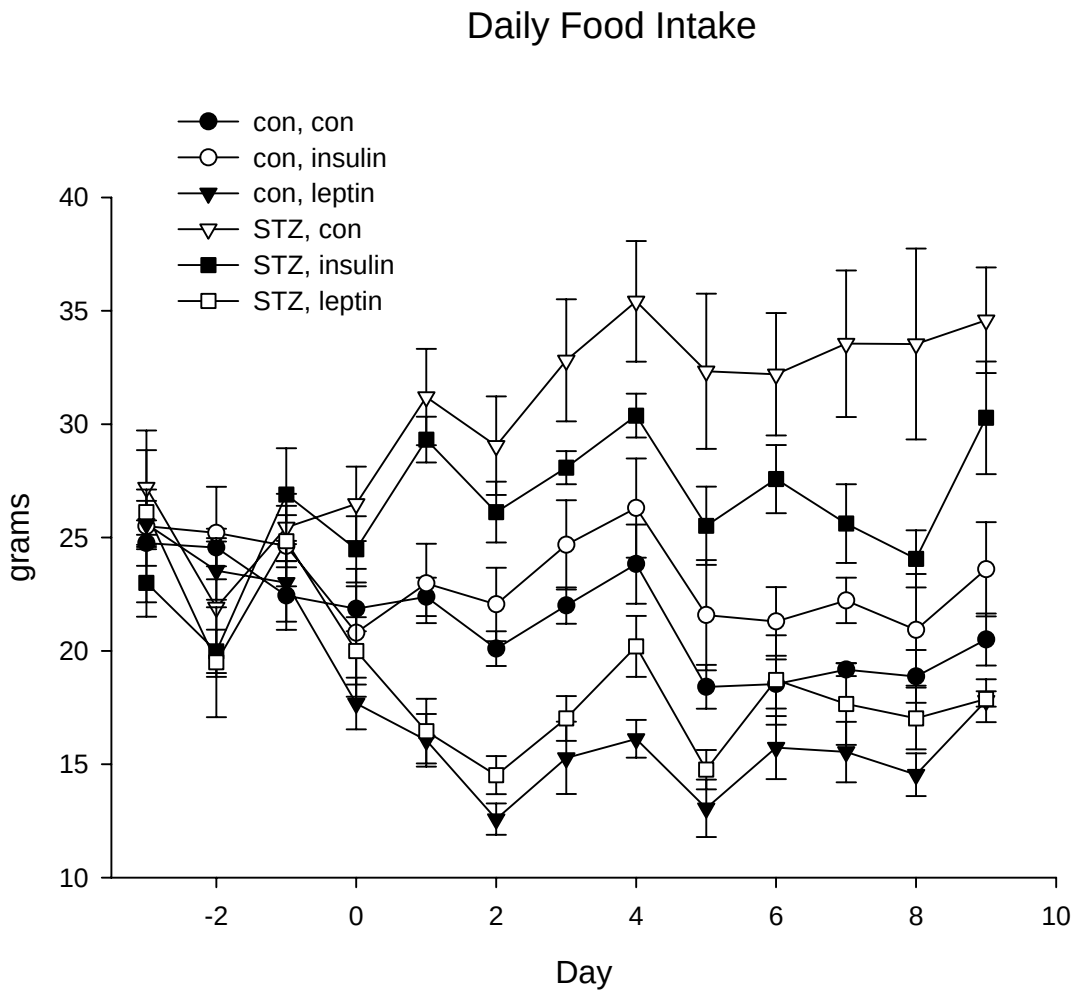


Figure 5. Effect of icv leptin (10 μg) or insulin (10 mU) or vehicle on daily food intake in control rats and diabetic rats. Values are means ± SE. Day -3 is the first day after streptozoticin (STZ) injection; Day 0 refers to the starting for leptin, insulin or control injection.

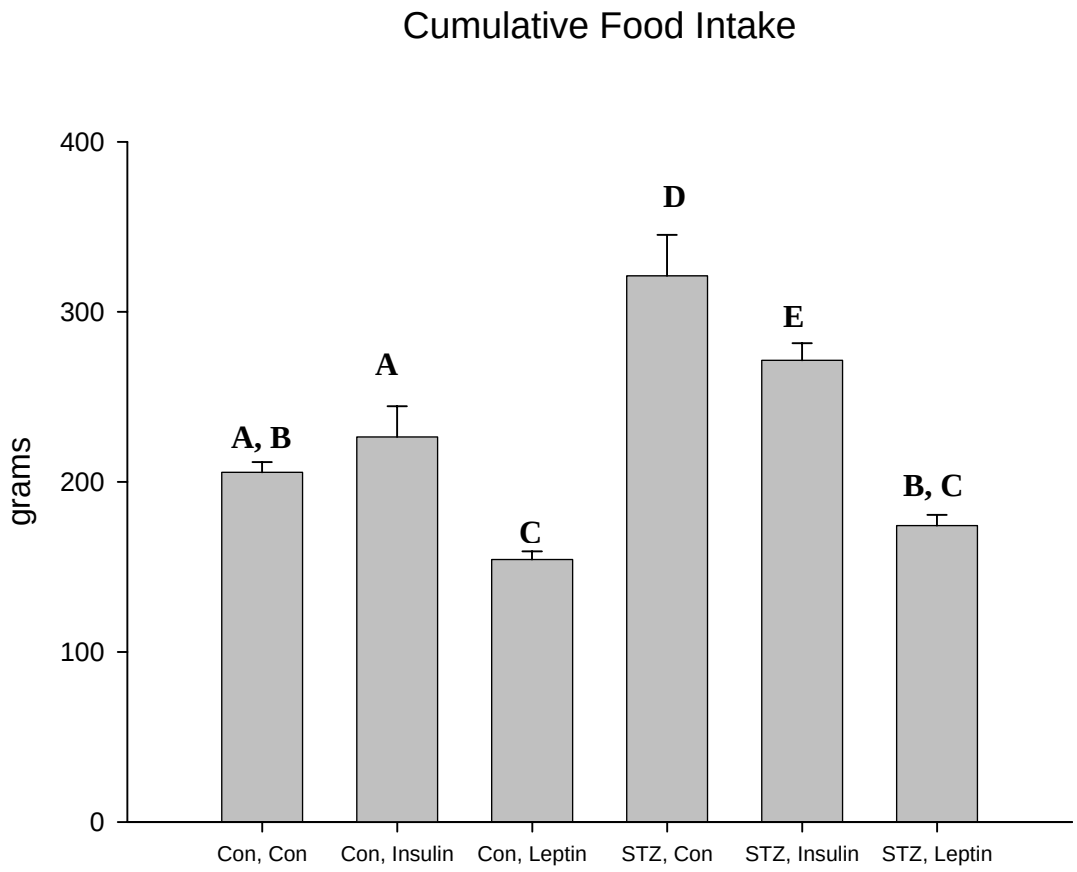


Figure 6. Effect of icv leptin (10 μg) or insulin (10 mU) or vehicle on cumulative food intake in control rats and diabetic rats. Values are means ± SE. Means with different letters are statistically different.

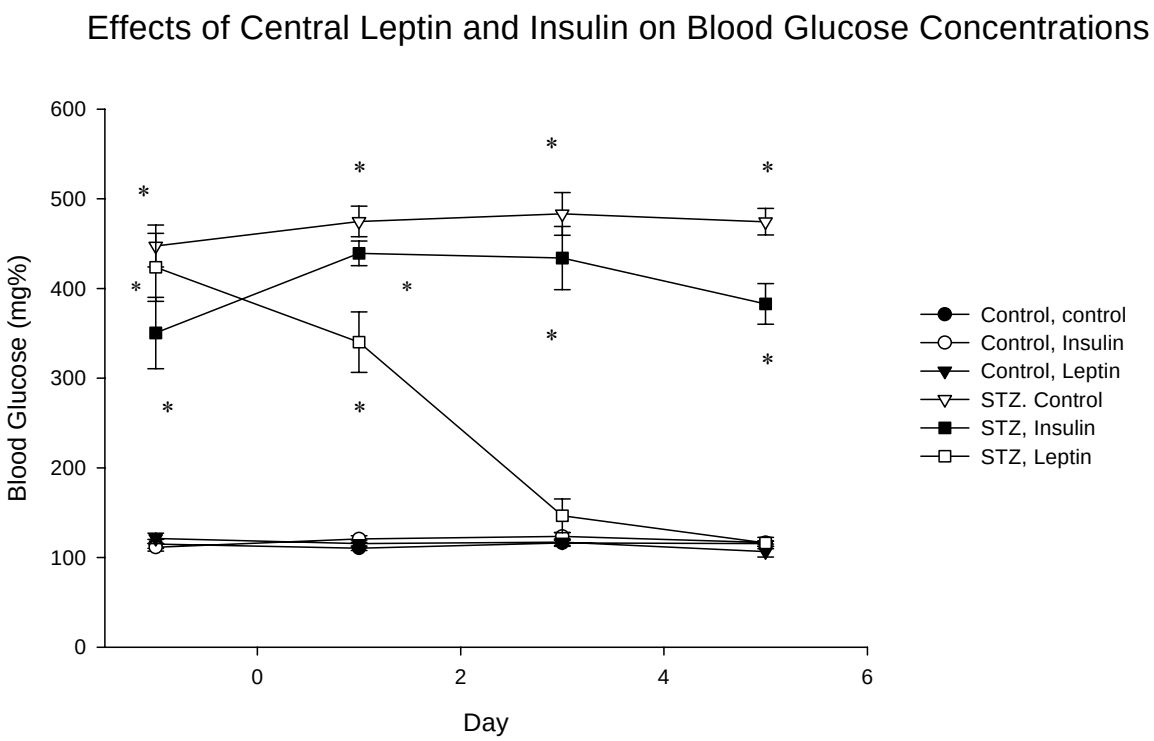


Figure 7. Effect of icv leptin (10 μg) or insulin (10 mU) or vehicle on blood glucose concentration in control rats and diabetic rats. Values are means ± SE. * $P < 0.05$ vs. control.

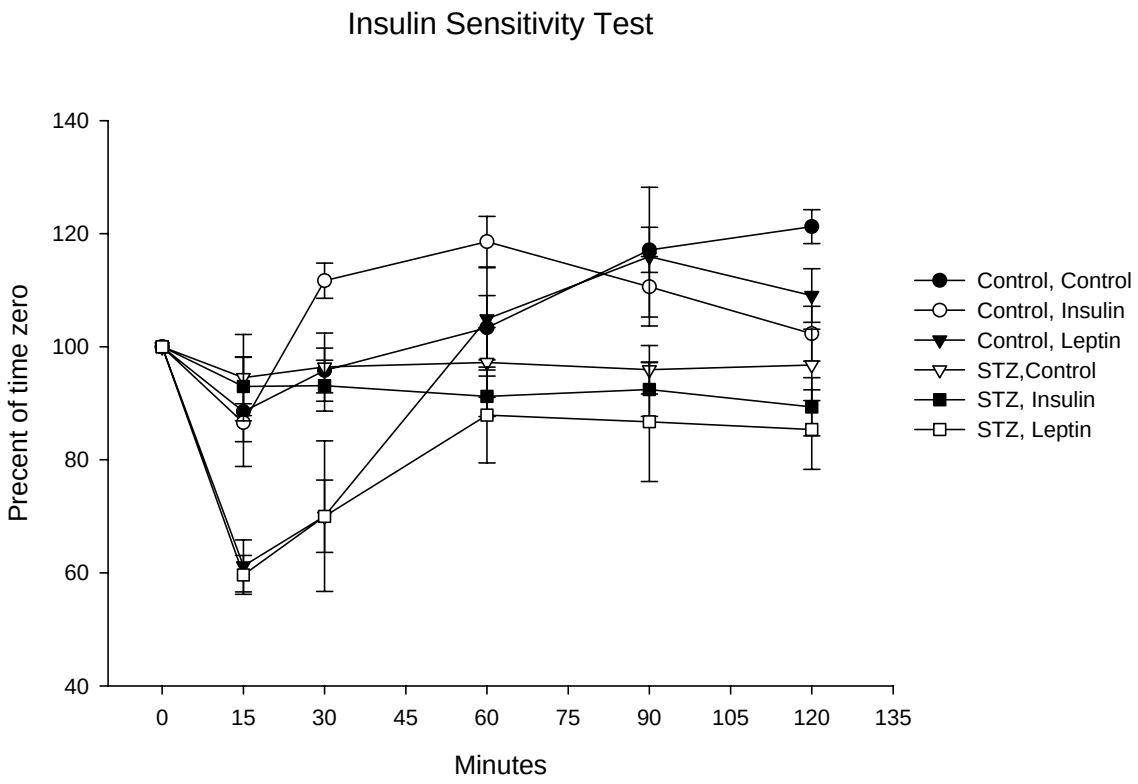


Figure 8. Insulin sensitivity test in the leptin (10 μg), insulin (10 mU) or vehicle treatment of control rats and diabetic rats. Values are expressed as means ± SE.

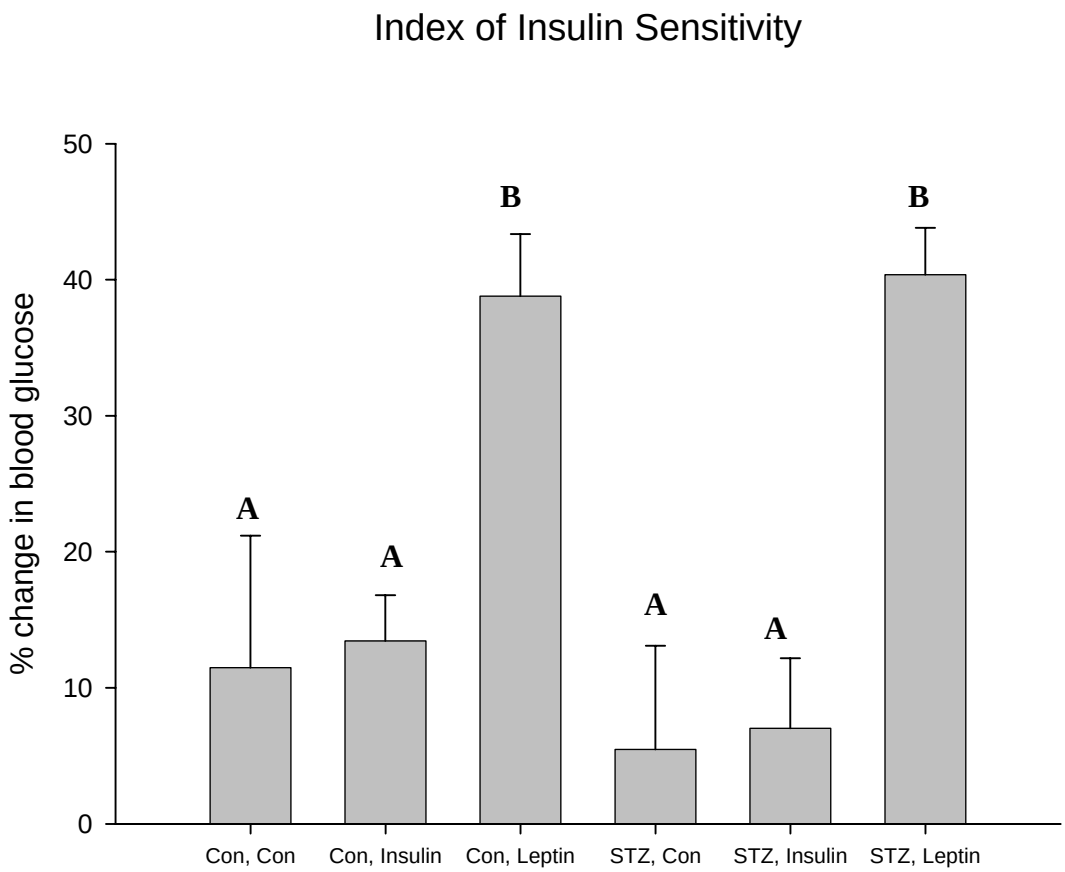


Figure 9. Index of insulin sensitivity. Values are means ± SE. Means with different letters are statistically different.

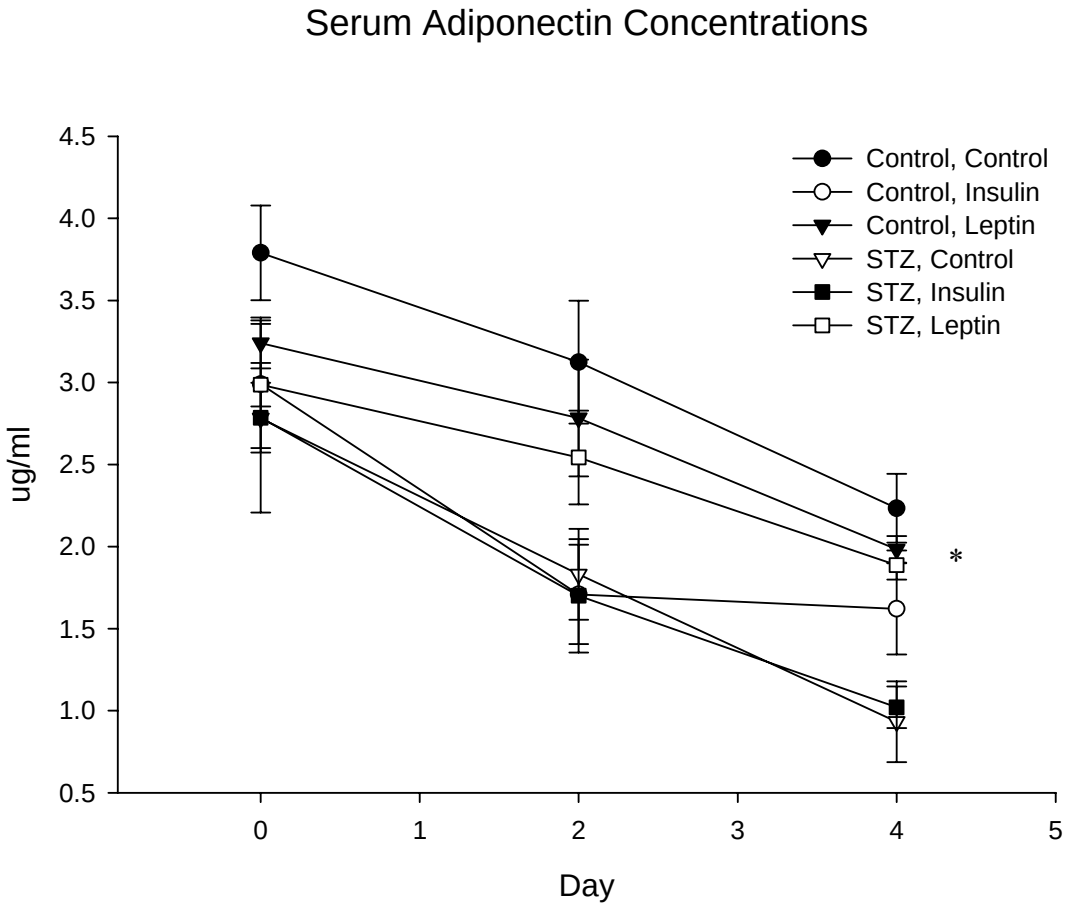


Figure 10. Serum adiponectin concentrations in rats. Values are means ± SE. *: $P < 0.05$, STZ-leptin group vs. STZ-control and STZ-insulin group, respectively.

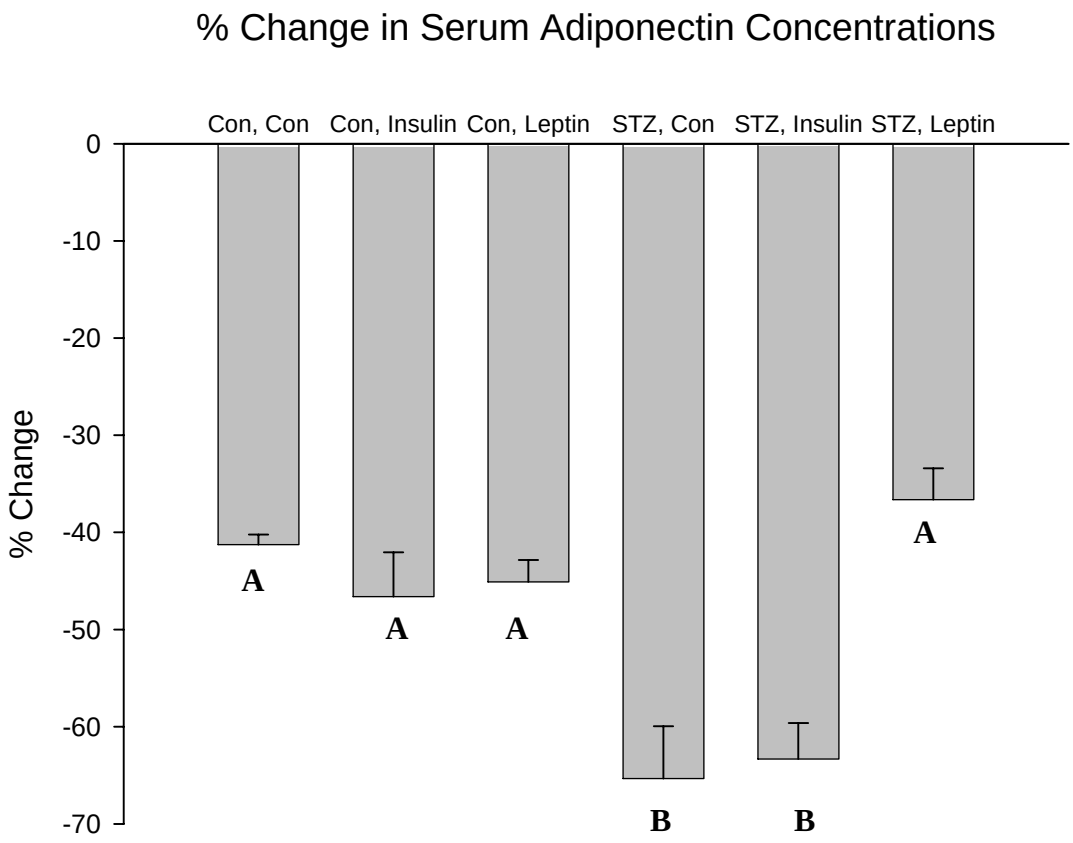


Figure 11. Change in serum adiponectin concentrations in rats. Values are means ± SE. Means with different letters are statistically different.

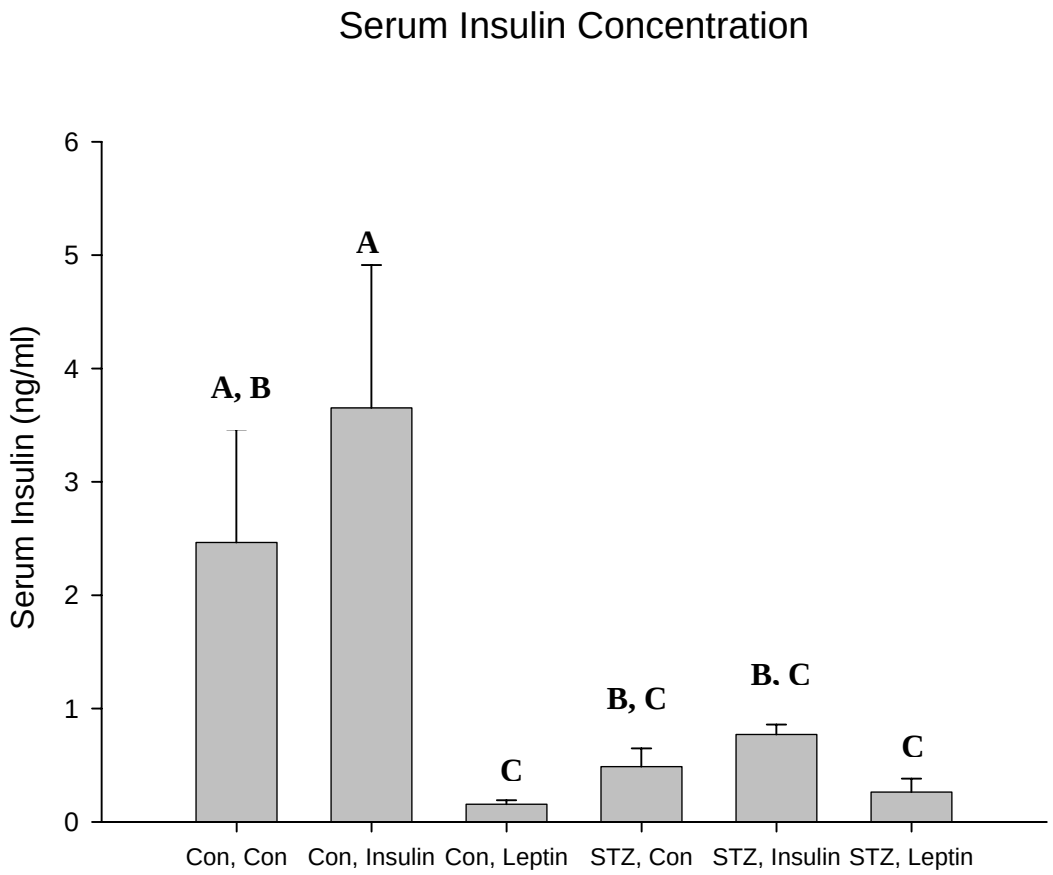


Figure 12. Serum insulin concentrations in rats. Values are means ± SE. Means with different letters are statistically different.

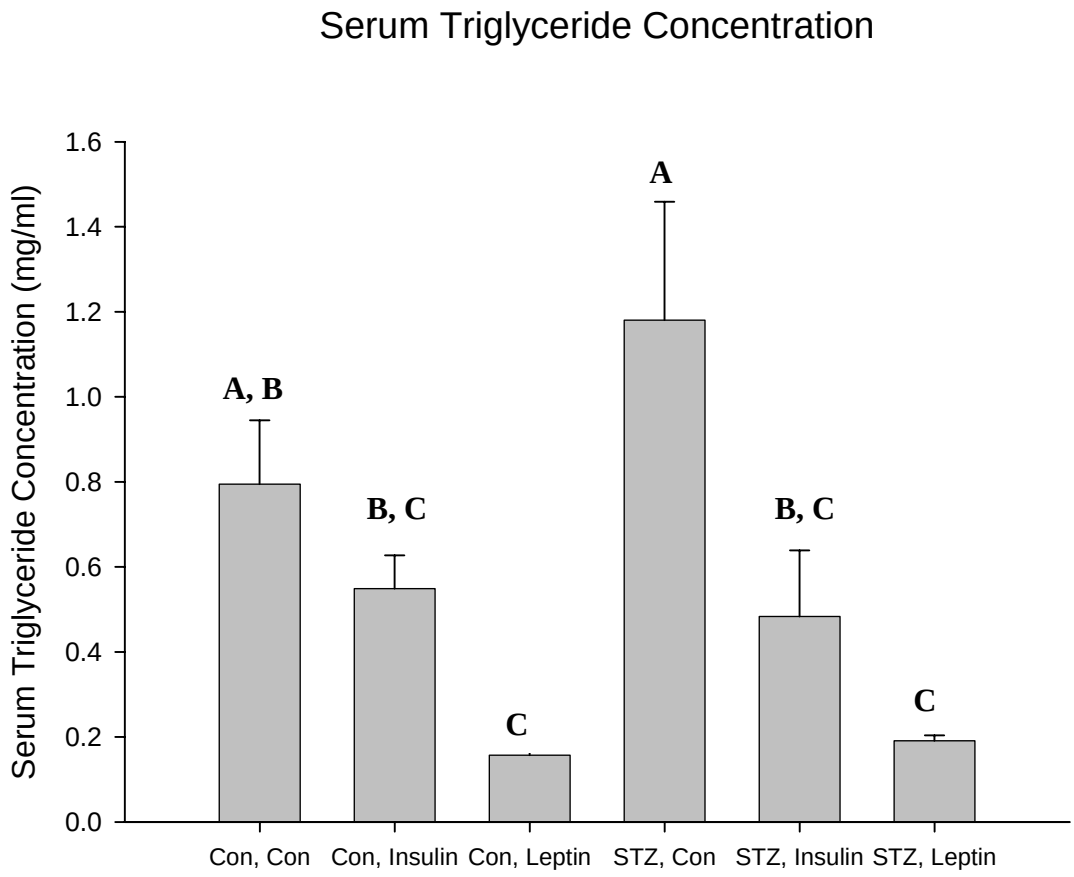


Figure 13. Serum triglyceride concentrations in rats. Values are means ± SE. Means with different letters are statistically different.

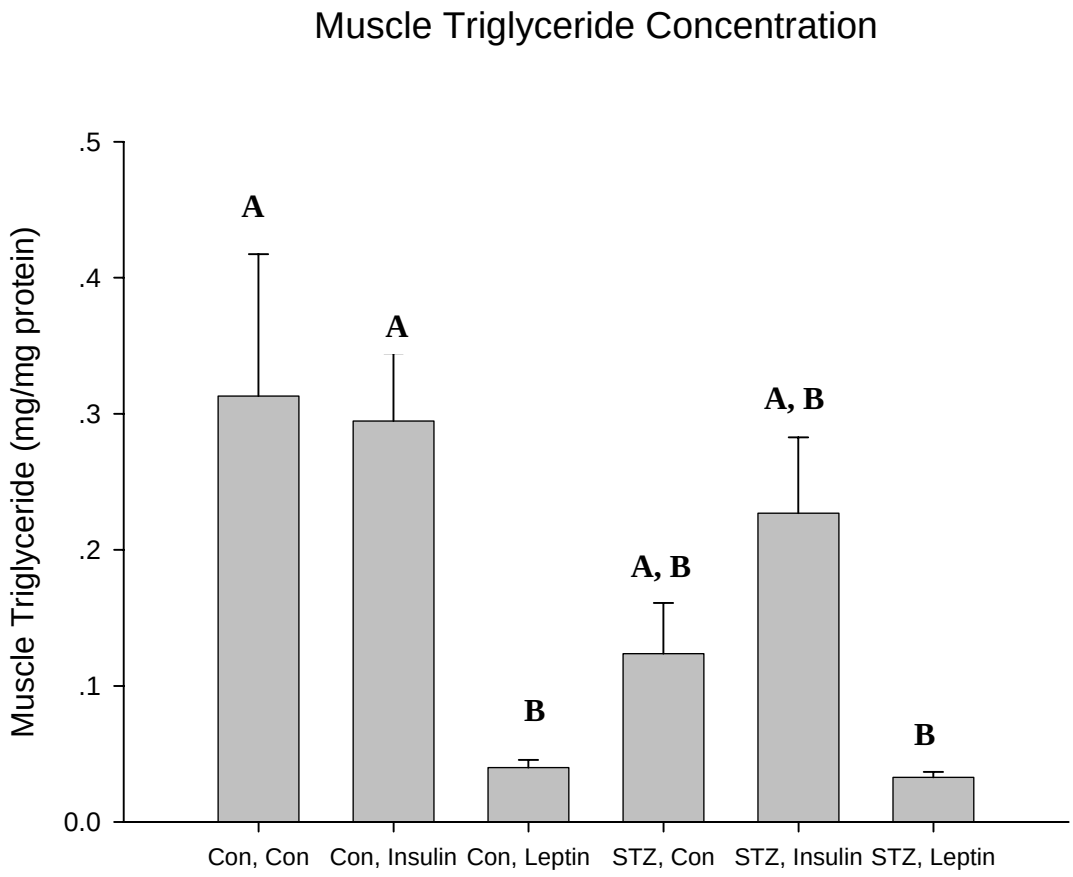


Figure 14. Muscle triglyceride concentrations in rats. Values are means ± SE. Means with different letters are statistically different.

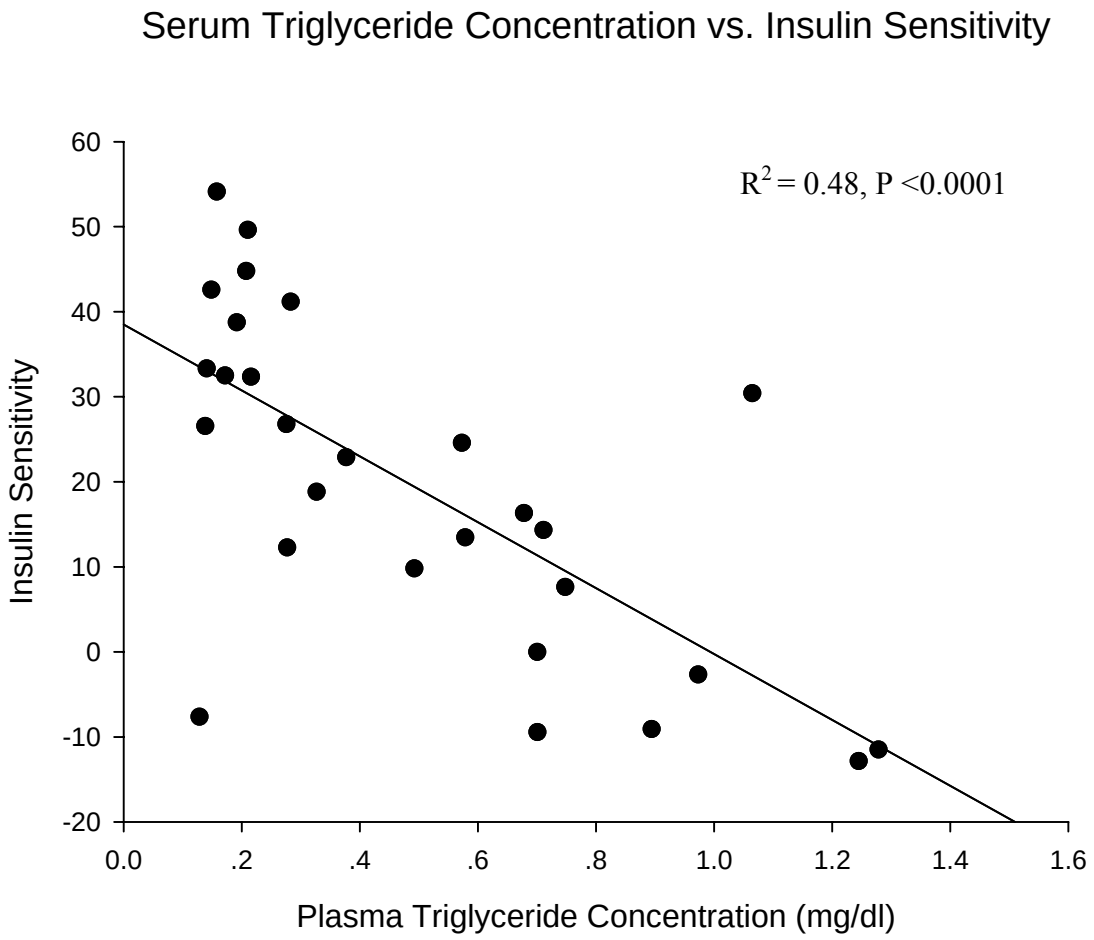


Figure 15. Serum triglyceride concentration vs. insulin sensitivity.

# CHAPTER IV

# CENTRAL LEPTIN DECREASES BLOOD GLUCOSE CONCENTRATIONS IN GUANETHIDINE-TREATED STREPTOZOTOCIN-INDUCED DIABETIC RATS


## Abstract

This study examined the effect of intracerebroventricular leptin on insulin sensitivity in guanethidine-treated, streptozotocin (STZ)-induced diabetics rats. Young male Wistar rats were divided into two groups. One group was treated every other day with guanethidine monosulfate (100 mg/kg body weight) for two weeks to denervate the sympathetic nervous system. The other group received control injections. After a one-week rest period, rats had an ICV cannula placed in lateral ventricle. After recovery, all rats were administered intravenous STZ to induce diabetes. ICV leptin (10μg/day), or vehicle was administered daily for 5 days. Blood glucose concentrations were decreased from the second day in both guanethidine-treated and control diabetic rats receiving leptin administration. Rats receiving guanethidine-treatment also had decreased body weights and norepinephrine content in the spleen and brown adipose tissue. Guanethidine did not decrease norepinephrine content in the liver or white adipose tissue. While these data do not support a role for the sympathetic nervous system in mediating the ability of central leptin to normalize blood glucose concentrations in diabetic rats, further investigation is warranted.

## Introduction

Chronic central leptin can normalize blood glucose concentrations and increase insulin sensitivity in diabetic rats (8). Lin et al. (2002) observed that chronic administration of leptin into the lateral ventricle corrects the hyperglycemia found in diabetic rats. This normalization of blood glucose concentrations was accompanied by a large increase in peripheral insulin sensitivity. Chronic central injections of leptin do not change the serum leptin concentration, suggesting that leptin is acting centrally to increase peripheral insulin sensitivity. The mechanism with which central leptin increases peripheral insulin sensitivity is not clear (8). Intrahypothalamic injections of leptin increase the activity of AMP-activated protein kinase (AMPK) in skeletal muscle (199). Accompanying the activation of AMPK, leptin suppresses the activity of acetyl CoA carboxylase, decreasing fatty acid synthesis and increasing fatty acid oxidation in the muscle (199). Activation of AMPK also can increase glucose uptake (187).

Central leptin increases sympathetic nerve activity via its receptors in the hypothalamus (255). Phosphoinositol-3 kinase (PI3-kinase) may mediate the leptin-induced sympathetic outflow, because pretreatment of a PI3-kinase inhibitor blocks the regulation of renal sympathetic nerve activity by intracerebroventricular administration of leptin (255). AMPK α2 knockout mice have an increase in sympathetic tone and a decrease in insulin sensitivity in vivo (189). Therefore, the sympathetic nervous system may be a mediator between central leptin signaling and the activation of AMPK. Guanethidine is a drug used to chemical denervate the peripheral sympathetic system. Chronic injections of guanethidine reduce norepinephrine content in various tissues (257-259).

To determine the role of the sympathetic nervous system in mediating the ability of central leptin to normalize blood glucose levels in diabetic rats, a group of rats had their sympathetic nervous system chemically denervated by the administration of guanethidine. Another group of rats were given control injections. All rats were made diabetic and received chronic ICV injections of either leptin or vehicle. Blood glucose concentrations and norepinephrine content of various tissues were determined.

**Materials and Methods**

*Animals***.** Male Wistar rats were housed in individual hanging wire cages. Rats were kept in a temperature controlled room with a 12:12 hour light-dark cycle. Rats had free access to chow (Prolab RMH 300 meal, Purina Mills, Richmond, IN) and water. All experimental protocols were approved by Auburn University's Institutional Animal Care and Use Committee before starting of the experiment.

*Experimental design*. A group of rats weighing approximately 75 grams each was subcutaneously administered guanthedine monosulfate (100 mg/kg, Sigma, St. Louis, MO) every other day for 2 weeks. Another group of rats received control injections. Rats were allowed to recover for one week. All rats were implanted individually with an intracerebroventricular cannula directed into the lateral ventricle (See Cannula placement). All rats were made diabetic by the administration of streptozotocin (STZ) (see induction of diabetes). After hyperglycemia was verified, all rats were administrated either ICV leptin (10μg) (Calbiochem, San Diego, CA), or vehicle (15 mM HCl: 7.5 mM NaOH in a 5:3 ratio) daily. Therefore, all rats were divided into one of four groups: control-vehicle, control-leptin, guanethidine-vehicle, and guanethidine-leptin. Blood

glucose concentrations were determined daily by an AccuCheck Active glucometer. After 6 days of intracerebroventricular injections, all rats were sacrificed. Spleen, brown adipose tissue, white adipose tissue, and liver were collected to measure norepinephrine content within each tissue. This was used as an indicator of how well guanethidine treatment decreased sympathetic activity.

*Cannula placement*. Rats were anesthetized by intraperitoneal injection of ketamine-xylazine (100 mg/kg ketamine and 1 mg/kg xylazine) and placed in a sterotaxic apparatus. A 22-gauge, stainless steel guide cannula (Plactic One, Roanoke, VA) was implanted into the lateral ventricle by use of the following criteria: 0.8mm posterior to bregma, 1.4 mm lateral to bregma, and 3.5 mm deep from the surface of skull. Four stainless steel screws and dental cement were used to fix the guide cannula to the skull. A "dummy" cannula, which was 1 mm longer than the guide cannula, was placed into the guide cannula to avoid clogging the inside of the guide cannula. The rats were placed in individual cages and recovered for 4 days. Angiotensin II (40 ng in a volume of 6 μl) was used to verify the placement of cannula. Rats that drank less than 5 ml of water within 15 min after administration of angiotensin II were excluded from the experiment. After the drinking test, 31 rats were used in this study.

*Induction of diabetes*. Diabetes was induced with a single intravenous injection of freshly prepared STZ (50mg/kg; Sigma) in 0.05 M citrate buffer (pH 4.5) via the tail vein. Control rats were injected with the citrate buffer only. All STZ injected rats had blood glucose concentrations greater than 350 mg/dl.

*Determination of tissue norepinephrine and protein content.* Norepinephrine concentrations were measured by HPLC. Tissues were crushed under liquid nitrogen with

a mortar and pestle. About 100 mg of sample was placed in pre-weighed microcentrifuge tubes. A cold solution containing 0.2 M perchloric acid and 1 ng/ml ascorbic acid (PCA/AA, 790 µl) was added and this mixture was sonicated 3 times (10-30 seconds each time) on ice. Ten µl of an internal standard, 3, 4-dihydroxybenzylamine hydrobromide (DHBA) was added. The solution (800 µl) was mixed and centrifuged at 10,000 rpm for 10min. The supernatant (600 µl) was added to a tube containing 200 mg of alumina, which was then mixed with 1 ml of 0.5 M Tris (pH 8.6), and centrifuged at 10,000 rpm for 1 min. The supernatant was removed and discarded, and the alumina was washed two times by mixing with 1 ml of water followed by centrifugation for 1 min at 10,000 rpm. After removing all the supernatant, catecholamines were eluted from the alumina by mixing with 300 µl of PCA/AA followed by centrifugation for 1 min at 10,000 rpm. The PCA/AA supernatant (about 300 µl), which contained the catecholamines, was transferred to vials suitable for HPLC analysis. The tissue extracts and standards (10 µl) were processed by HPLC using a Gynkotek HPLC pump (P580) and HPLC autosampler (Model GINA 50) attached to a Hewlett-Packard Zorbax SB-C18 Stable Analytical Column (4.6 × 250mm, 5-micron) pre-equilibrated with citrate buffer (21.01 g citrate, 245 mg sodium octyl sulfate per liter, 10% ethanol, pH 4) at 0.7 ml/min. The samples were analyzed at 50 nA for 15 minutes with a Decade digital electrochemical amperometric detector and VT-03 electrochemical flow cell (both from Gynkotek). The data were collected using the Chromeleon chromatography information management system (Gynkotek) and a Gateway PC computer. Protein concentrations were determined by the Bradford assay. Norepinephrine content was expressed as ng per mg protein.

*Statistical analysis*: All results were presented as means ± SE. Statistical analyses were performed by using SSPS 12.0 and SAS 8.02. Two-way analyses of variance (ANOVA) were utilized to test the main effects of guanethidine and leptin treatment on blood glucose concentrations and norepinephrine content with the various tissues. A difference of $P \leq 0.05$ was considered statistically significant.

**Results**

*Effect of guanethidine and central leptin on blood glucose concentration and body weight.* Before central leptin administration, there was no difference in blood glucose concentrations among the four groups (Fig. 16). After central administration of leptin, the blood glucose concentrations of guanethidine-treated and control rats decreased (Fig. 16). Blood glucose concentrations of the guanethidine-leptin group were lower than the guanethidine-control group from day 1 to day 3 ($P < 0.05$). Guanethidine treatment itself had no effect in blood glucose concentrations. The interaction between guanethidine and central leptin was also significant ($F_{1, 53} = 10.9$, $P < 0.05$). It appeared that leptin was slightly more effective at lowering blood glucose concentrations in guanethidine-treated rats.

Guanethidine, but not central leptin administration, had significant effect to decrease body weight ($F_{1, 36} = 13.26$, $P < 0.05$). Thus, control-leptin and control-control groups had a significantly higher body weight than the guanethidine-control and guanethidine-leptin groups ($P < 0.05$) (Fig. 17).

*Norepinephrine content tissues*. Guanethidine significantly decreased norepinephrine content in spleen ($F_{1, 27} = 35.02$, $P < 0.001$), brown adipose tissue ($F_{1, 36} =$

5.661, $P < 0.05$), but had no effect on norepinephrine content in liver or white adipose tissue. Central leptin treatment increased norepinephrine content in liver ($P < 0.05$), but no effect in spleen, brown adipose tissue, and white adipose tissue. There was also no significantly interaction between guanethidine and central leptin treatment on norepinephrine content in each of the tissues examined. In spleen, the two guanethidine-treated groups had a lower norepinephrine content than the two control groups ($P < 0.05$), and there was no significant difference of norepinephrine content between the guanethidine-control and guanethidine-leptin groups (Fig. 18). Results of norepinephrine in brown adipose tissue were a little different. Although the two guanethidine-treated groups had a lower norepinephrine content than the two control groups, the norepinephrine concentration of control-leptin rats had no significant difference with either guanethidine-control or guanethidine-leptin groups (Fig. 19). In liver, guanethidine-leptin groups had higher norepinephrine content than other three groups ($P < 0.05$) (Fig. 20). There was no difference of norepinephrine concentrations in the white adipose tissues of all groups (Fig. 21). This may be due to the absence of adipose tissue of some leptin-treated rats.

**Discussion**

In the present study, we evaluated the chronic effect of central leptin administration on blood glucose concentrations in guanethidine-treated rats. We demonstrated that at the dose we used, guanethidine treatment did not alter the effect of central leptin on blood glucose concentrations in diabetic rats. Guanethidine-treatment decreased body weight

gain and norepinephrine contents in spleen and brown adipose tissue, but not in liver or white adipose tissue.

Guanethidine treatment caused a reduction in body weight of the rats. This is consistent with the finding that guanethidine treatment has a negative effect on protein balance. Guanethidine treatment decreases norepinephrine contents and increases protein turnover in skeletal muscle (260). Guanethidine also inhibits protein synthesis in the liver and spleen (260). As other studies, we found that chemical sympathectomy with guanethidine, significantly decreased norepinephrine contents in spleen and brown adipose tissue, but did not abolish it (259). Furthermore, in this study, guanethidine treatment did not decrease norepinephrine content in liver or white adipose tissue. Thus, it appears that various tissues differed in their degree of susceptibility to chemical sympathectomy by guanethidine.

The results of glucose concentrations were consistent with previous studies (8). Central leptin administration decreased blood glucose concentrations in diabetic rats. Although the blood glucose concentrations in diabetic rats were not completely normalized by central leptin, blood glucose concentrations were significantly lower than in the control rats. Thus, our hypothesis that guanethidine treatment would block the effect of central leptin to decrease blood glucose concentrations was not supported by the present study. Guanethidine-treated diabetic rats were equally sensitive to the effects of central leptin as control rats. This may suggest that the sympathetic nervous system is not involved in leptin-mediated normalization of blood glucose concentrations in diabetic rats. However, there are also other possible reasons for the present results: 1) Partial sympathetic nervous system activity may be enough for central leptin to improving

peripheral insulin sensitivity. Guanethidine treatment reduced sympathetic activity in the spleen and brown adipose tissue to 35% and 39% of controls, respectively. Perhaps, this level of sympathetic activity is still sufficient to mediate the central effects of leptin to enhance insulin sensitivity. 2) Guanethidine treatment did not block sympathetic nervous system activity in liver and white adipose tissue. Insulin has important functions in liver and adipose tissue. It increases fuel storage in adipose tissue and inhibits hepatic glucose output to reduce blood glucose concentration (261). Therefore, if guanethidine treatment did not block sympathetic nervous system activity in liver and white adipose tissue, central leptin may still increase insulin sensitivity in liver and adipose tissue, resulting in normalization of blood glucose concentrations. 3) Blocking sympathetic nervous system per se may increase insulin sensitivity. Increased sympathetic function may contribute to the pathogenesis of metabolic syndrome, which includes insulin resistance (262). Some antihypertensive drugs (e.g. rilmenidine) can decrease blood pressure and sympathetic nervous activity. Meanwhile, these drugs also increase insulin sensitivity in patients with metabolic syndrome (263). Therefore, blocking sympathetic nervous system per se may have increased peripheral insulin sensitivity in diabetic rats. 4) Besides the sympathetic nervous system (264), there might be other down-stream targets for central leptin to improving insulin sensitivity. Thus, blocking the sympathetic nervous system may not be enough to alter the effect of central leptin. Presently there were not enough data in this study to support or refuse these possibilities.

In summary, we demonstrated that chemical sympathectomy with guanethidine (100 mg/kg body weight) did not block the effect of central leptin (10 μg/day) to enhance insulin sensitivity in diabetic rats. However, these results cannot exclude the sympathetic

nervous system from being a downstream target of central leptin. Further research is still needed to explore the possible relationship between central leptin, the sympathetic nervous system, and peripheral insulin sensitivity.

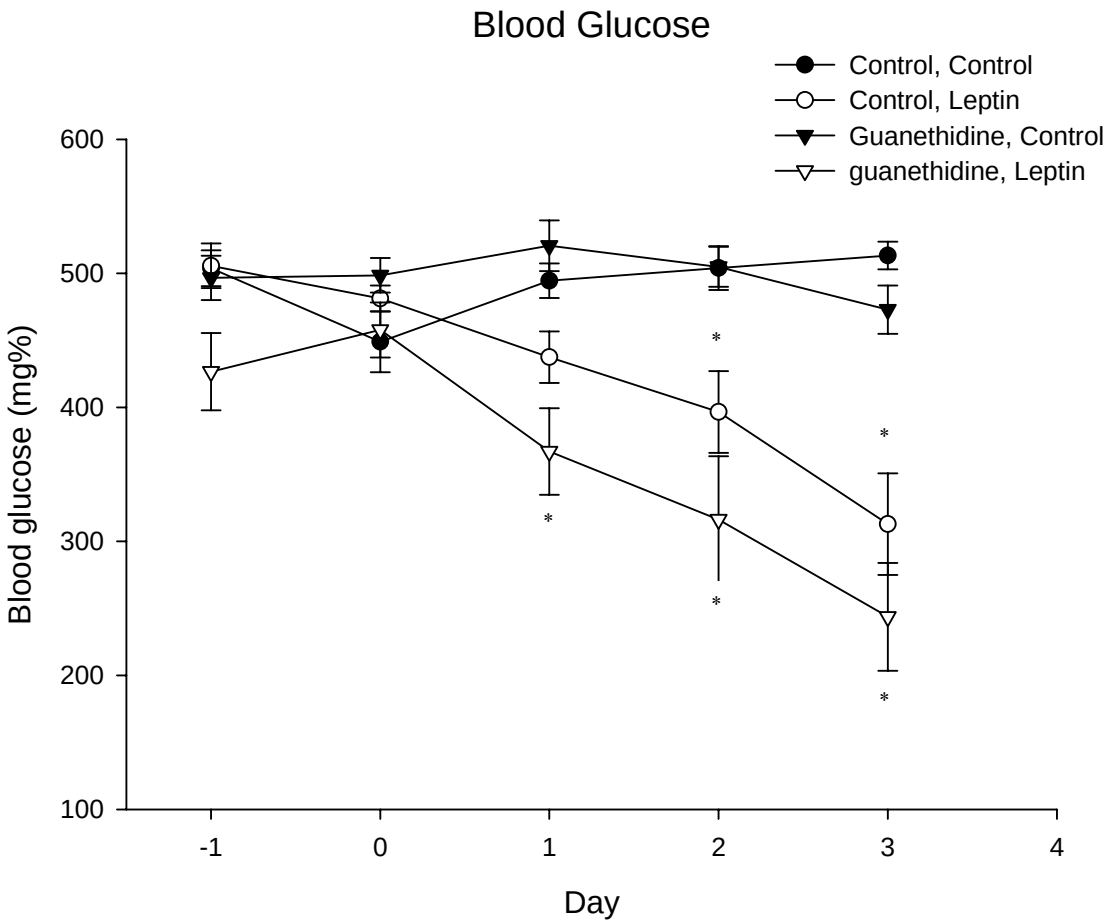


Figure 16. Effect of icv leptin (10 μg) or vehicle on blood glucose concentrations in control and guanethidine treated rats. Values are means ± SE. * $P < 0.05$ vs. control.

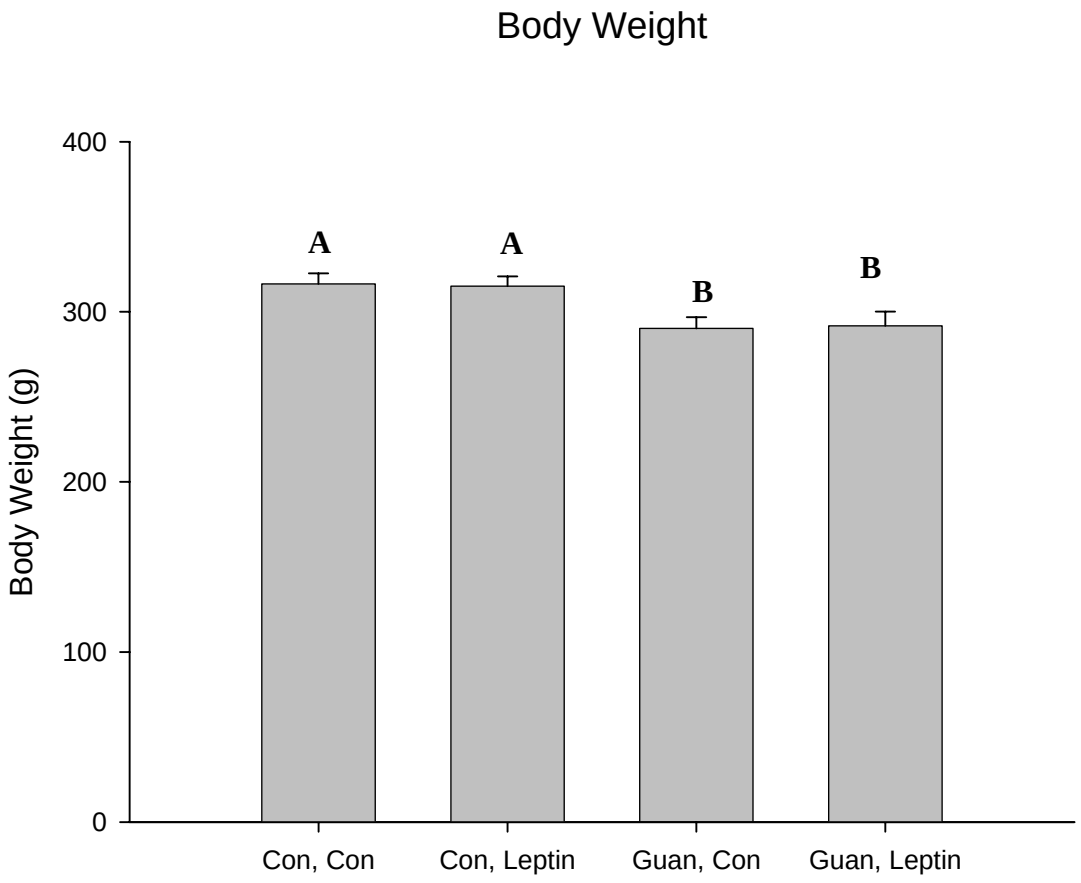


Figure 17. Effect of icv leptin (10 μg) or vehicle on body weight in control and guanethidine treated rats. Values are means ± SE. Means with different letters are statistically different.

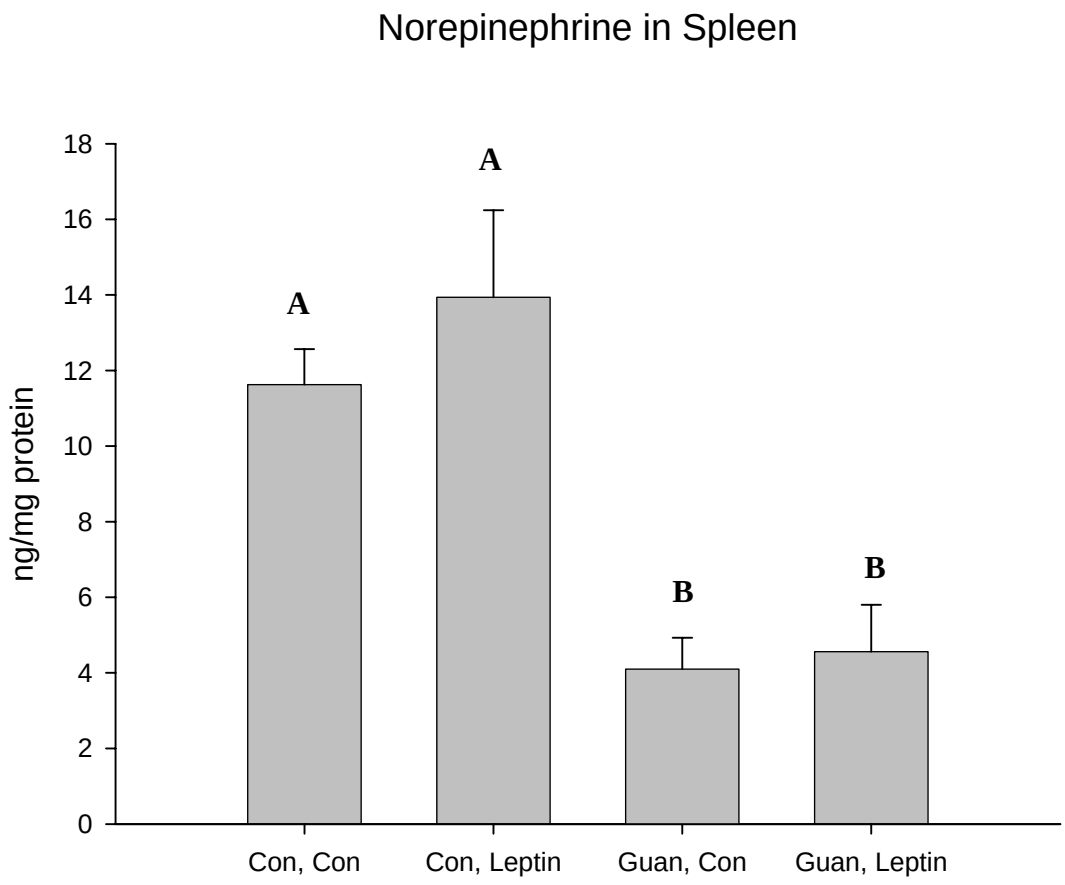


Figure 18. Effect of icv leptin (10 μg) or vehicle on norepinephrine contents in spleen of control and guanethidine treated rats. Values are means ± SE. Means with different letters are statistically different.

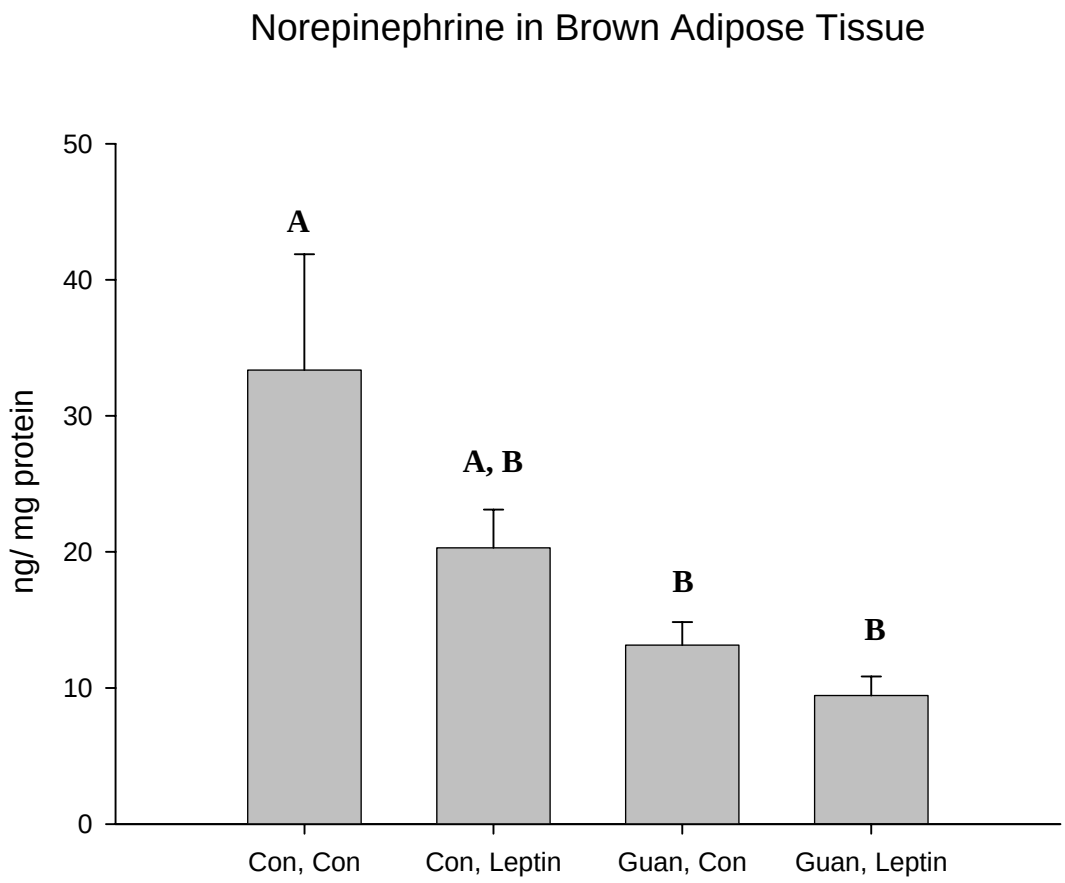


Figure 19. Effect of icv leptin (10 μg) or vehicle on norepinephrine contents in brown adipose tissue of control and guanethidine treated rats. Values are means ± SE. Means with different letters are statistically different.

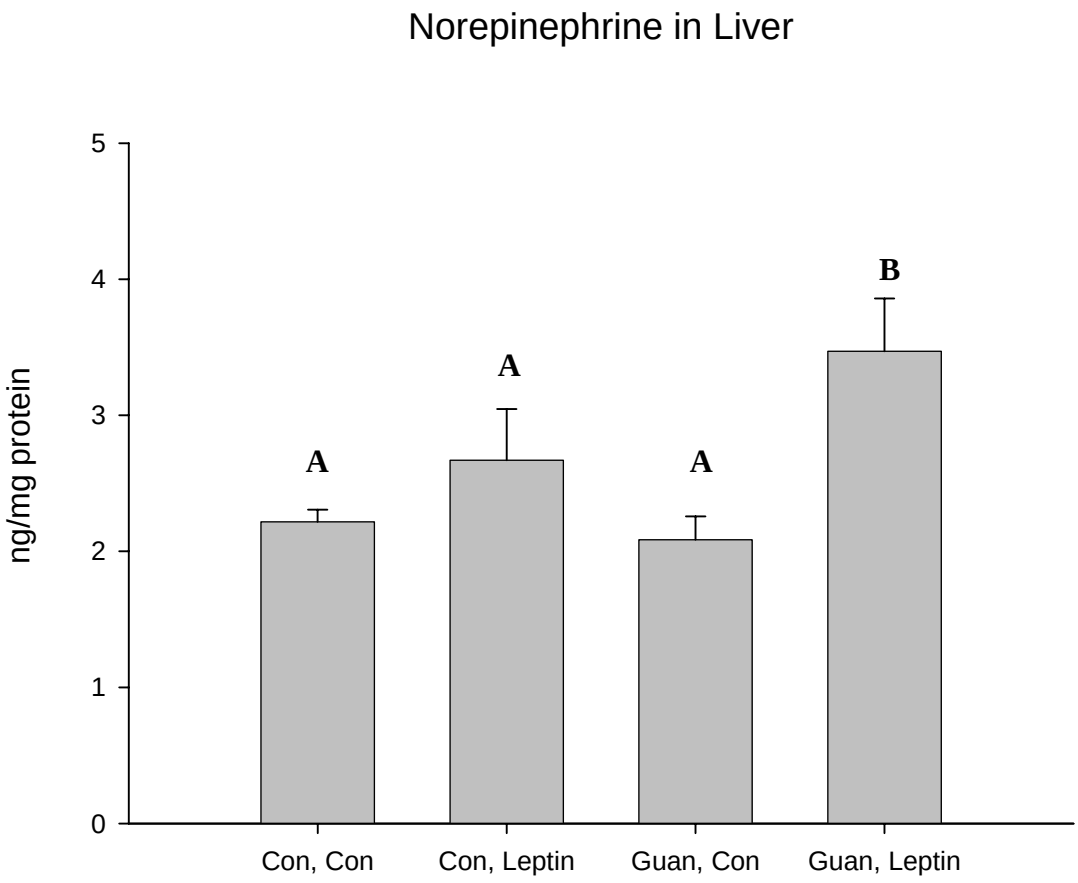


Figure 20. Effect of icv leptin (10 µg) or vehicle on norepinephrine contents in liver of control and guanethidine treated rats. Values are means ± SE. Means with different letters are statistically different.

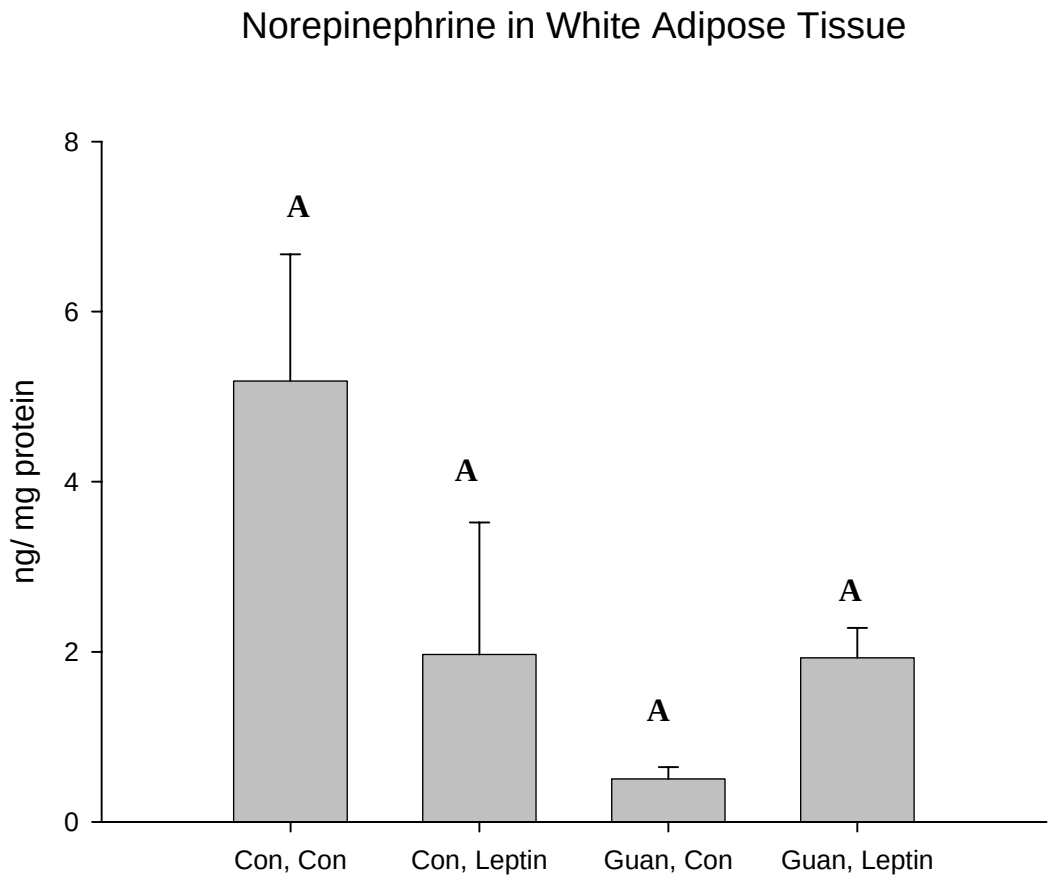


Figure 21. Effect of icv leptin (10 μg) or vehicle on norepinephrine contents in white adipose tissue of control and guanethidine treated rats. Values are means ± SE. Means with different letters are statistically different.

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# CHAPTER V

# CONCLUSION

Central leptin can increase peripheral insulin sensitivity in both diabetic and control rats. In the first study, all results suggested that central insulin can not change peripheral insulin sensitivity in both diabetic and control rats, although it can decrease serum triglyceride concentration in diabetic rats. Central insulin also did not affect blood glucose concentrations. Therefore, central insulin may not play a role in the regulation of peripheral insulin sensitivity.

Enhancing insulin sensitivity may be the most important physiological function for adiponectin. Because adiponectin levels decrease in type 2 diabetic patients, increases in adiponectin concentration may significantly increase insulin sensitivity in these patients. Similarly, diabetic rats had a lower adiponectin concentration than control rats. Central leptin attenuated the decrease of adiponectin concentrations in diabetic rats and increased peripheral insulin sensitivity. Therefore, adiponectin may be an important downstream mediator for central leptin signaling in regulating insulin sensitivity.

As in most of type 2 diabetic patients, serum triglyceride concentrations in diabetes rats in the first study was negatively correlated with peripheral insulin sensitivity. Central leptin can decrease serum and muscle triglyceride concentration in both diabetic and normal rats. This may also contribute to the improvement of peripheral insulin sensitivity,

since many studies have found a positive correlation between serum and muscle triglyceride and insulin resistance in animals and humans. The sympathetic nervous system may also be another down-stream mediator of central leptin. Although in the second study, there was no difference in the effect of central leptin in insulin sensitivity between control and sympathectomized diabetic rats. This may be due to incomplete blockade of the sympathetic nervous system.

Obesity is the major trigger for insulin resistance and type 2 diabetes, which are accompanied by increased free fatty acids, and triglyceride concentration. The results of these two studies further our understanding of insulin resistance. Both inadequate central leptin and declined peripheral adiponectin concentration may contribute insulin resistance in diabetes. Therefore, central leptin administration and enhancing peripheral adiponectin concentration could be two possible treatments of improving peripheral insulin sensitivity in the future.

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