Inhibitory Effect of Central Leptin on Hepatic Glucose Production in Streptozotocin (STZ) -Induced Diabetic Rats

by

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Abstract

Uncontrolled hepatic glucose production contributes significantly to hyperglycemia in type 2 diabetic patients. Leptin plays important roles in the regulation of blood glucose homeostasis. Recent studies have suggested that chronic central leptin administration can normalize blood glucose concentrations without increasing serum leptin and insulin concentrations. We hypothesize that leptin inhibits hepatic glucose output by inhibiting gluconeogenesis. This work sought to directly examine hepatic glucose output in leptin-treated diabetic rats via an isolated perfused liver system and to determine the glucose production rate in response to lactate and fructose. Leptin-treated rats had nearly a 4-fold lower glucose response to lactate gavage than did vehicle-treated rats, suggesting that hepatic gluconeogenesis from lactate is severely blunted in leptin-treated rats. However, the results of hepatic glucose production from fructose were different from the results from lactate. When fructose was used as the gluconeogenic precursor, there was no difference in the hepatic glucose production between leptin- and vehicle-treated rats. Additionally, gastric gavage of fructose resulted in a greater increase in blood glucose concentrations in leptin-treated rats than the gastric gavage of lactate in leptin-treated rats.

To further understand the effect of intracerebroventricular (ICV) leptin on the role of leptin in hepatic glucose metabolism, we isolated liver cells from leptin- and vehicle

treated diabetic rats, and compared the ability of these cells to produce glucose in the presence of a gluconeogenic precursor in the freshly prepared or cultured state. We found that compared with the control group, in the presence of different concentration of lactate (1mM, 2mM, 5mM and 10Mm) the production of glucose by the primary cultured hepatocytes in leptin-treated group was significantly reduced 41 %, 44 %, 53 % and 58 %, respectively. However, glucose production in the freshly prepared liver cells was not different between cells derived from leptin-treated rats as compared with vehicle-treated rats. Therefore, leptin is capable of reducing glucose production from lactate in isolated hepatocytes.

In conclusion, chronic central leptin inhibits hepatic gluconeogenesis to help control whole body glucose levels preventing the overproduction of glucose in diabetic rats. These results indicate that uncontrolled diabetes can be rescued without insulin by leptin to eliminate hepatic overproduction of glucose. Determining the mechanism by which this occurs may lead to new therapeutic agents that help control diabetes, independent of or in conjunction with insulin.

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Chapter 1

Introduction

The incidence of diabetes is increasing worldwide. At least 171 million people currently have diabetes, and this figure is likely to more than double to 366 million by 2030. The top 10 countries, in numbers of people with diabetes, are currently India, China, United States, Indonesia, Japan, Pakistan, Russia, Brazil, Italy, and Bangladesh [1]. As for the United States, diabetes has become epidemic. According to the American Diabetes Association, diabetes affects nearly 24 million Americans. Diabetes is the sixth leading cause of death in the United States, affecting about 7.8% of the population [2]. Many health complications result from diabetes including cardiovascular disease and stroke, kidney disease, blindness, non-traumatic amputation and many others [3].

Not only are more people getting this disease, but they are developing it at younger ages. Acquiring diabetes at a younger age increases the likelihood that one or more of the diabetes-related complications will develop sometime in the person's lifetime. This will put a greater burden on the health care system. The national cost of diabetes in the U.S. in 2007 exceeded $174 billion. This estimate includes $116 billion in excess medical expenditures attributed to diabetes, as well as $58 billion in reduced national productivity. People with diagnosed diabetes, on average, have medical expenditures that are approximately 2.3 times higher than the expenditures would be in the absence of diabetes. Already 1 out of every 10 healthcare dollars is spent on diabetes

and its complications [4]. As for Alabama, it leads the nation in number of diagnosed

cases of diabetes per capita with more than 440,000 citizens diagnosed with the disease,

or about 10% of the population. The growth rate for new cases is 30 percent above the

national average. It is estimated that diabetes costs Alabama businesses more than $3

billion annually [5]. Type 2 is the most prevalent form of diabetes, accounting for

approximately 90 - 95% of all cases of diabetes, and an increase in this form of the

disease accounts for diabetes' increased incidence. Type 2 diabetes has a particularly

strong association with weight gain irrespective of ethnicity or gender [6-7]. Type 2

diabetes usually stems from insulin resistance, when the body does not effectively

respond to the glucoregulatory mechanisms of insulin and progressive β-cell failure. Both

type 2 diabetes and obesity represent dysregulated glucometabolic syndrome conditions

in humans and experimental models [8].

The reason for the association between obesity and type 2 diabetes is not fully

understood. Interestingly, a decrease in leptin signaling in the brain leads to both obesity

and insulin resistance, a hallmark of type 2 diabetes. Type 2 diabetes is characterized by

peripheral insulin resistance with an insulin-secretory defect that varies in severity. In the

progression from normal glucose tolerance to abnormal glucose tolerance, postprandial

glucose levels first increase. Eventually, fasting hyperglycemia develops as inhibition of

hepatic gluconeogenesis declines. Insulin resistance in obesity and type 2 diabetes is

manifested by decreased insulin-stimulated glucose transport and metabolism in skeletal

muscle and adipocytes by impaired suppression of hepatic glucose output [9]. When the

adipose depot is expanded, as in obesity, plasma free fatty acids (FFAs) become elevated,

due, most likely, to increased release from the expanded adipose mass and probably also

to impaired hepatic metabolism. Elevated FFAs impair the ability of insulin to suppress hepatic glucose output and to stimulate glucose uptake into skeletal muscle, as well as to inhibit insulin secretion from pancreatic β cells [10]. The increased hepatic glucose output is a major contributor to elevated glucose levels in patients with type 2 diabetes, causing many of the complications of this disease. One way to control diabetes is to inhibit this upregulated hepatic glucose production, specifically by inhibition of gluconeogenesis.

Leptin, the product of the *obese (ob)* gene, is produced primarily by white adipose tissue and serves as a peripheral signal to the central nervous system of nutritional status [11-12]. Leptin plays an important role in regulating energy stores and in the choice of fuels to be used under various nutritional conditions [13]. Genetic leptin deficiency in rodents and humans results in hyperphagia and marked obesity, as well as insulin resistance or diabetes. Numerous studies have shown that leptin circulates in proportion to body fat mass in humans [12, 14].

Evidence indicates that leptin may play especially important roles in the regulation of glucose homeostasis, and has lead to the suggestion that leptin can be used as a therapeutic agent of diabetes, either alone or as an adjunct to insulin therapy [15-37]. Our laboratory has extended this finding by demonstrating that chronic central leptin administration in the brain of diabetic rats can normalize blood glucose concentrations independent of food intake and insulin level [13]. By using various gluconeogeneic diets, we also found that leptin-treated diabetic rats have a decreased ability to convert certain gluconeogeneic precursors into glucose; specifically, those precursors that enter the

gluconeogenic pathway between dihydroacetone phosphate/glyceraldehyde 3-phosphate and pyurvate.

In the present study, we employed the streptozotocin (STZ)-induced diabetic rat model, which can be used to investigate leptin action without the effects of endogenous leptin and insulin. The aim of our study was to employ the isolated perfused rat liver model (IPRL) to evaluate the effects of central leptin administration on hepatic glucose production in response to two gluconeogenic precursors, lactate and fructose. The IPRL model has been used to explore the physiology and pathophysiology of the rat liver for many years. This *in situ* model provides the opportunity to assess liver function in the whole organ in an isolated setting directly. The IPRL model also allows repeated sampling of the perfusate, permits easy exposure to the liver to different concentrations of test substances, and can be done independent of the influence of other organ system, plasma constituents and neural-hormonal effect [38-40]. In a second study, we examined the gluconeogenic response of isolated hepatocytes derived from diabetic rats chronically treated with ICV leptin or vehicle.

We hypothesize that chronic central leptin administration greatly decreases hepatic glucose output in response to lactate in an isolated perfused liver system and isolated hepatocytes. Hepatic glucose production from lactate was severely blunted in leptin-treated rats as compared to rats that were vehicle-treated in the isolated perfused liver system, but no significant difference in hepatic glucose production between leptin- and vehicle-treated rats was found with fructose. Compared with the control group, in the presence of different concentration of lactate (1, 2, 5 and 10 mM) the production of glucose by the primary cultured hepatocytes in leptin-treated group was significantly

reduced 41 %, 44 %, 53 % and 58 %, respectively. However, glucose production in the freshly prepared liver cells was signficantly higher in cells derived from leptin-treated rats as compared with vehicle-treated rats treated with higher lactate doses. The significant difference in hepatic glucose production between leptin- and vehicle-treated rats indicates that central leptin administration inhibits hepatic gluconeogenesis. The reason for the difference in hepatic glucose production between leptin- and vehicle-treated rats in cultured and freshly prepared hepatocytes is not known and requires further investigation.

Chapter 2

Literature Review

2.1. Obesity, Insulin Resistance and Type 2 Diabetes

Type 2 diabetes, accounting for 90-95% of those with diabetes, has become a major global health problem, currently affecting 5% to 10% of most populations [41]. By the year 2025, it is estimated that approximately 300 million people worldwide will have this condition [42]. It is generally accepted that the prevalence of type 2 diabetes increases with the increasing prevalence of overweight and obesity among different racial and ethnic groups. The risk of developing diabetes starts to increase even with modest weight gain. It has been reported that a period of gradual weight gain usually precedes the onset of type 2 diabetes [43]. Complications of diabetes affect many tissues and organs. Having too much sugar in the blood for long periods of time can cause serious health problems if it's not treated. Hyperglycemia can cause damage to the vessels that supply blood to vital organs, which can increase the risk of heart disease and stroke, kidney disease, vision problems, and nerve problems in people with diabetes. Among the many potential chronic complications of type 2 diabetes, cardiovascular disease accounts for more than 80% of adverse outcomes among patients and is ultimately responsible for a considerable proportion of diabetic mortality [44]. There are two fundamental abnormalities accounting for the pathogenesis of type 2 diabetes. First, pancreatic β-cell

dysfunction is a critical component in the pathogenesis of type 2 diabetes. Inadequate insulin secretion from the pancreatic β cells, including a blunted first phase insulin response to a glucose load and a disruption of insulin pulsatility can lead to a specific degree of insulin insufficiency [45]. The second is resistance to insulin action in glucose and lipid metabolism, concomitantly with or subsequent to the disturbance in insulin secretion [46] . Insulin resistance is a major cause of type 2 diabetes, which is determined by impaired sensitivity to insulin of its main target organs, i.e. adipose tissue, liver and muscle. In adipose tissue, insulin reduces free fatty acid efflux from adipocytes to decrease lipolysis; in liver, insulin reduces key enzyme activities to inhibit gluconeogenesis and in skeletal muscle, insulin stimulates the translocation of the GLUT4 to the plasma member to induce glucose uptake. During insulin resistance conditions, the circulating free fatty acid concentration elevates and ectopic fat accumulates to impede insulin-mediated glucose uptake in skeletal muscle, while hepatic glucose output increases [47].

2.1.1. The Pathogenesis of Obesity-Related Insulin Resistance

The prevalence of diabetes worldwide is increasing rapidly in association with the increase in obesity. Overweight and obesity are the most common nutritional disorders in the United States, affecting the majority of adults in the country. Obesity, particularly central obesity, is a well established risk factor for insulin resistance and results in type 2 diabetes mellitus and other features of the metabolic syndrome such as dyslipidemia, cardiovascular disease and hypertension [47]. Now a growing number of children are being diagnosed with obesity-related type 2 diabetes. Over 75% of type 2 diabetic patients are overweight or obese, indicating the strong relationship between obesity and

type 2 diabetes. Obesity may well have a significant role to play in the pathogenesis of diabetes [16]. Impaired insulin release and insulin resistance are principal factors for the development of type 2 diabetes [48]. Wang and Beydoun (2007) searched the studies published between 1990 and 2006 and found that among adults, obesity prevalence increased from 13% to 32% between the 1960s and 2004, 66% of adults are overweight or obese, 16% of children and adolescents are overweight and 34% are at risk of overweight [48]. If current trends continue, by 2015 75% of adults will be overweight or obese, and 41% will be obese [49].

2.1.2. Candidate Mediators of Obesity-Related Insulin Resistance

Adipose tissue is now regarded as a major endocrine organ and a variety of factors released by adipocytes potentially mediate insulin resistance. Adipose tissue regulates energy metabolism by releasing non-esterified fatty acids (NEFAs) and glycerol, adipokines, such as leptin and adiponectin, and proinflammatory cytokines [50-52]. Increased release of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), retinal-binding protein-4 (RBP4), resistin, monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8) and additional products of macrophages and other cells that populate adipose tissue may play an important role in the development of obesity-induced insulin resistance [51, 53-54]. These negative mediators modulate insulin action via various pathways leading to insulin resistance. They influence their respective signal modulators via deactiving pathways such as tyrosine phosphatases, serine kinases, lipid phosphatase and degradation pathways, leading to an inhibition of insulin receptor substrate (IRS)

protein activity or other proteins further downstream of the IRS proteins, like Akt, interfering with insulin signaling [55].

TNF-α and IL-6 have adverse effects on energy metabolism and insulin sensitivity in peripheral tissue through classical receptor-mediated processes to stimulate both the c-Jun aminoterminal kinase (JNK) and the IκB kinase-β (NF-κB) pathways, resulting in the upregulation of potential regulators of inflammation and subsequently impairing insulin signaling through direct activation of a serine kinase to increase serine phophorylation of IRS-1 and IRS-2 [55]. Moreover, TNF-α alters the gene expression of the insulin receptor, GLUT4, adiponectin, and PPARγ to affect insulin sensitivity [56]. TNF-α has been shown to reduce insulin-stimulated glucose uptake in adipose tissue in both *in vitro* and *in vivo* studies [55]. The lack of TNF-α or its action significantly restored insulin sensitivity in high-fat-induced obesity mice or *ob/ob* mice. High-fat fed, mice lacking TNF-α (TNF-α$^{-/-}$) exhibited an improved signaling capacity of the insulin receptor in adipose tissue and skeletal muscle compared with TNF-α $^{+/+}$ mice [57].

Resistin, as the name implied, is a small inflammatory molecule with hyperglycemic action by reducing glucose uptake in muscles and fat tissue. Resistin also works through inhibiting hepatic AMPK activity, which results in elevated expression of gluconeogenic enzymes, thereby causing uncontrolled hepatic glucose production from gluconeogenesis, which contributes to the development of hepatic insulin resistant [58].

RBP4 induces insulin resistance through reduced phophatidylinositol-3-OH kinase (PI3K) signaling in muscle and enhanced expression of the gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (PEPCK) in the liver through a retinol-dependent mechanism [53].

MCP-1 and IL-8 may contribute to insulin resistance either by attracting macrophages, particularly into adipose tissue, which become the major source of inflammatory mediators, or by decreasing insulin-stimulated glucose uptake and insulin receptor phosphorylation [59].

Increased NEFAs are associated with the insulin resistance observed in obesity and insulin resistance. Increased intracellular NEFAs might compete with glucose for substrate oxidation and this process leads to the suppression of glycolysis through the inhibition of the key enzymes activities, including pyruvate dehydrogenase, phosphofructokinase, and hexokinase II. An increased mass of stored triglyceride, especially in visceral or deep subcutaneous adipose depots, exacerbates the insulin resistance syndrome and further in turn leads to increased circulating levels of NEFA and glycerol, both of which contribute to insulin resistance to stimulate hepatic gluconeogenesis and inhibit insulin-stimulated glucose metabolism in skeletal muscle [60-61]. NEFAs activate protein kinase C in both liver and muscle to silence downstream insulin-signaling by promoting serine phosphorylation of IRS-1 and IRS-2, which reduces tyrosine phosphorylation and the ability to activate PI3K [55, 62-64].

Obesity is also associated with mechanical stress, excess lipid accumulation, and abnormalities in energy metabolism and availability of nutrients. Obesity-induced endoplasmic reticulum (ER) stress in peripheral tissues is perhaps a core mechanism involved in triggering insulin resistance and type 2 diabetes. ER stress promotes a JNK-dependent serine phosphorylation of IRS-1, which in turn inhibits insulin receptor signaling in insulin target cells. It was also reported that expression levels of several ER stress markers are increased in dietary (high-fat diet-induced) and genetic (*ob/ob*) models

of obesity. Suppression of the JNK pathway enhances insulin signaling, which leads to a decrease in gluconeogenesis and amelioration of glucose intolerance. Similar effects were observed in high-fat/high-sucrose diet-induced diabetic mice and *db/db* mice. In addition, ER stress activates the JNK pathway, leading to induction of apoptosis in various cells, such as pancreatic β cells [65-66].

2.2. Glucose Homeostasis and Diabetes

2.2.1. Glucose Homeostasis

In order to ensure euglycemia, which is necessary for metabolic processes in the cells, glucose levels are carefully regulated to around 90 mg/dl. The process of maintaining blood glucose at a steady-state level is called glucose homeostasis [67-68]. Glucose homeostasis, maintaining blood glucose level within a narrow range despite wide fluctuations in the demand (e.g. vigorous exercise) and supply (e.g. large carbohydrate meals) of glucose, protects the body from the adverse effects of both hypoglycemia and hyperglycemia. Plasma glucose concentration is the balance between the rate of glucose entering the circulation and the rate of glucose removal from the circulation. Healthy adults maintain a physiological balance between glucose appearance and glucose disappearance. Under fasting conditions, the fasting glucose level is tightly controlled within a narrow range from 70 to 110 mg/dl because the rate of endogenous glucose output from the liver are well matched with the rate of glucose taken up by peripheral tissues. Under the fed state, serum glucose comes from absorption from intestine and release from liver. The latter occurs in the liver to produce glucose by breakdown of preformed glycogen (glycogenolysis) as well as by de novo synthesis of

glucose primarily from noncarbohydrate precursors such as lactate, pyruvate, glycerol, and alanine (gluconeogenesis). An increased influx of glucose stimulates pancreatic β-cells to secrete insulin to control postprandial glucose through two major mechanisms. One is to stimulate the uptake of glucose into insulin-sensitive peripheral tissues and another is to inhibit hepatic glucose output by direct and indirect effects on the liver, including inhibition of glucagon secretion from pancreatic α-cells. [69] Glucose is removed from the system by uptake into almost all cell types, but most importantly into muscle and adipose tissue. Recent evidence suggests that the CNS can also sense glucose and act to affect systemic glycemia, at least in part by regulating gluconeogenesis [70].

Glucose homeostasis requires the coordinated actions of various organs. The liver plays a critical role in glucose homeostasis by maintaining a balance between the uptake and storage of glucose and the release of glucose. Greater than 90% of endogenous glucose production is derived from liver and around 40% of alimentary glucose is taken up by liver for storage as glycogen. A progressive loss in these liver functions is associated with the deterioration of glycemic control and eventually develops diabetes [71]. One site of control is the activity of the opposing enzymes glucokinase and glucose 6-phosphatase (G-6-Pase). Net hepatic glucose flux in and out of the liver is the balance between the rate of glucose phosphorylation catalyzed by glucokinase (GK), the first step of hepatic glucose utilization, and the rate of glucose-6-phosphate (G-6-P) dephosphorylation catalyzed by G-6-Pase, the last step of hepatic glucose production. In nondiabetic rats, glucose-induced suppression of net hepatic glucose production is associated with increased glucose phosphorylation and GK activity is required to suppress hepatic glucose production. On the other hand, impairment of GK activity and

increased glucose-6-phosphatase activity may contribute to elevated hepatic glucose production in experimental and human diabetes [71-73]. In liver, insulin is a major regulator of both the gene expression and activities of these glucose metabolism enzymes. At the cellular level, increased flux through G-6-Pase and/or decreased flux through GK lead to an inappropriate hepatic glucose output. The imbalance in the expression levels of G-6-Pase and GK may contribute to the loss of control of hepatic glucose output in the diabetes and the phenotype of the insulin resistance. It is generally thought that the three rate-controlling enzymes in gluconeogenesis are G-6-Pase, fructose-1, 6-bisphosphatase (FBPase) and phosphoenolpyruvate carboxykinase (PEPCK). Activities of these enzymes are significantly involved in the action of some hormones on hepatic gluconeogenesis and the gene expression of these enzymes are tightly controlled at the transcriptional level via the action of several hormones such as insulin, glucagon, and glucocorticoids [71, 74-77].

Under fasting conditions, glucose homeostasis is achieved by activating the expression of gluconeogenic genes in response to glucagon and glucocorticoids to keep a relatively constant glucose level. Hepatic gluconeogenesis is regulated by inhibitory (insulin) signaling pathways and opposing stimulatory (cAMP) pathways. The transcription factor cAMP-Response Element Binding Protein (CREB) plays a critical role in the induction of gluconeogenic genes. Insulin, in part, helps reduce hepatic gluconeogenesis by decreasing cAMP signaling through the phosphorylation of one of the coactivators of CREB, CREB binding protein (CBP) at Ser436. This disrupts the CREB-CBP complex, reducing cAMP signaling [78]. Another coactivator of CREB, Transducer of Regulated CREB Activity 2 (TORC2), is phosphorylated, via an Akt-

mediated, SIK2-dependent pathway, at Ser171 and sequestered in the cytoplasm under

feeding conditions. In response to fasting, TORC2 is dephosphorylated and transported to

the nucleus where it enhances CREB-dependent transcription [79-80]. PPAR gamma co-

activator 1 (PGC-1α) is a transcriptional target of CREB and functions as a central

regulator of gluconeogenesis to coactivate a variety of transcription factors such as

hepatic nuclear factor-4α (HNF-4α), glucocorticoid receptor, and forkhead box O1

(FOXO1) to bind to the promoter regions of several genes encoding key gluconeogenic

enzymes such as PEPCK and G-6-Pase to enhance their transcription [81]. Mice

deficient in PGC-1α display fasting hypoglycemia with reduced expression of

gluconeogenic genes [80-83]. In addition, PGC-1α suppresses insulin secretion from

pancreas by inhibiting GLUT 2 activities [84].

2.2.2. Impaired Glucose Metabolism during Type 2 Diabetes

Circulating glucose levels reflect a balance between glucose production by the

liver and glucose utilization by skeletal muscle. Under fasting conditions, this balance is

largely maintained by decreasing glucose uptake into muscle and adipose tissue, and

increasing glucose output from the liver via gluconeogenesis, which accounts for the

majority of glucose output. Hyperglycemia, the hallmark metabolic abnormality

associated with type 2 diabetes, is the result of combined effects of unrestrained elevated

hepatic glucose output and impaired peripheral glucose utilization. Uncontrolled hepatic

glucose output is a combined result of increased gluconeogenesis, increased glycogen

breakdown and impaired glycogen storage. Decreased peripheral utilization is mainly a

consequence of impaired uptake of glucose by the metabolically active tissues muscle

and fat. In type 2 diabetes, insulin resistance in liver contributes to the excessive hepatic

glucose output. Suppression of hepatic glucose output by insulin is impaired, along with decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle [77].

For individuals with diabetes, in the fasting state, under the effect of glucagon, hepatic gluconeogensis is promoted by means of cAMP, which also stimulates the breakdown of stored liver glycogen. At the same time, because of insulin deficiency in the portal circulation and/or the failure of the liver to respond appropriately to insulin, both peripheral glucose utilization and hepatic glucose metabolism (gluconeogenesis and glycogenolysis) cannot be regulated properly and effectively. Increased amounts of gluconeogenic precursors, such as lactate, pyruvate, and glucogenic amino acids, accumulate in the blood stream and are utilized by the liver to make glucose. Thus, gluconeogenesis runs at an exaggerated rate and concomitant with the stimulation of G-6-Pase and inhibition of GK, accounts for an increased endogenous glucose output rate. The imbalance in the expression levels of G-6-Pase and GK may contribute to the loss of control of hepatic glucose output in the diabetes and the phenotype of the insulin resistance. On the other hand, reduced glucose utilization in the peripheral tissues such as skeletal muscle exaggerates the degree of hyperglycemia [75, 77].

Under fed conditions, PGC-1α is expressed at very low levels in the liver. Insulin sequesters TORC2 in the cytoplasm and silences hepatic PGC-1α expression and transcriptional activity via Akt-mediated phosphorylation [80, 85-86]. Insulin also sequesters FOXO1 in the cytoplasm, preventing its nuclear interaction with PGC-1α [83]. For individuals with diabetes in the fed state, exogenous insulin is ineffective in suppressing postprandial glucagon secretion through the physiological paracrine route,

resulting in an abnormally high glucagon-to-insulin ratio which favors elevated hepatic glucose production and thus promoting hyperglycemia that would further exacerbate the insulin-resistant phenotype and ultimately result in type 2 diabetes.

2.2.3. Hormone Effects on Glucose Homeostasis (Regulation of Plasma Glucose)

Plasma glucose concentration is the net balance between the rate of glucose entering the circulation from the endogenous glucose production and/or absorption from the gut and the rate of glucose removal from the circulation due to glucose utilization by different peripheral tissues. To avoid postprandial hyperglycemia, which is an uncontrolled increased in blood glucose concentrations following meals, and fasting hypoglycemia, which is decreased blood glucose concentrations during periods of fasting, the body regulates glucose levels by secreting two hormones, insulin and glucagon, which have opposite functions to each other regarding their effect on the blood glucose homeostasis. Insulin reduces endogenous glucose output through inhibiting the process of glycogenolysis and gluconeogenesis and stimulates glucose uptake through promoting GLUT 4 translocation to the cell membrane. Glucose uptake is the rate-limiting step in glucose utilization. Besides insulin and glucagon, there are a variety of regulatory hormones involved in glucose metabolism including, amylin, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), epinephrine, cortisol, and growth hormone. Of these glucoregulatory hormones, insulin and amylin are derived from pancreatic β-cells, glucagon from pancreatic α-cells and GLP-1 and GIP from the L-cells of the intestine. These regulatory hormones are designed to maintain circulating glucose concentrations in a relatively narrow range [87].

Glucagon

Glucagon, a 29 amino-acid peptide, is a key catabolic hormone of glucose metabolism. It plays a major role in maintaining plasma glucose during fasting conditions by promoting hepatic gluconeogenesis by means of cAMP and stimulating hepatic glycogenolysis to increase the hepatic glucose production and maintain normal blood glucose concentrations. Glucagon is an important counter-regulatory hormone to prevent hypoglycemia due to the effect of insulin after glucose ingestion. Glucagon promotes hepatic glucose production by activating the transcription of gluconeogenic genes via phosphorylation of CREB protein, resulting in recruitment of the coactivators CREB-CBP as mentioned above and subsequent activation of hepatic gluconeogenesis as well as fatty acid oxidation [78, 86]. In addition to glucagon, catecholamines, growth hormone, and cortisol also act to increase blood glucose concentrations preventing hypoglycemia [88].

Insulin

In contrast, insulin signaling leads to phosphorylation of CBP at serine 436, disrupting the activation of the CREB–CBP complex. This inhibits the recruitment of other coactivators and fails to activate gene transcription through their intrinsic histone acetyltransferase activity, ultimately leading to an inhibition of hepatic gluconeogenesis. Insulin has anabolic actions on glucose metabolism, stimulates the utilization and storage of glucose as lipid and glycogen, and reduces glucose synthesis and release. This is accomplished through the coordinated regulation of enzyme synthesis and activity. Insulin inhibits the transcription of the genes encoding gluconeogenic enzymes such as G-6-Pase, FBPase and PEPCK, while stimulating the transcription of those encoding glycolytic enzymes including GK, phosphofructokinase and pyruvate kinase, as well as

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lipogenic enzymes, such as fatty acid synthase and acetyl CoA carboxylase. The rate of glucose entry into muscle can be modified significantly by circulating concentrations of insulin. Insulin stimulates glucose uptake by activating the glucose transporter GLUT4, which is predominantly expressed in mature skeletal muscle and fat tissues. Insulin increases cell surface GLUT4 levels by the dose-dependent translocation of GLUT4 to the plasma membrane [89-90]. These effects mentioned above are mediated by a series of transcription factors and co-factors, including sterol regulatory element-binding protein (SREBP)-1, hepatic nuclear factor (HNF)-4α, FOXO1 and PGC1α [91-94]. One mechanism by which insulin signaling antagonizes gluconeogenesis is through phosphorylation of FOXO1, the subsequent exclusion of FOXO1 from the nucleus, and increasing its susceptibility to degradation. On the other hand, the expression of PGC-1 α is inhibited by insulin as several animal models of deficiency in insulin signaling show a rise in hepatic PGC-1α expression. In addition, insulin regulates the activities of some other enzymes, including glycogen synthase and citrate lyase, through alternating their phosphorylation state. All these actions lead to a direct hypoglycemic effect of insulin. Impaired insulin signaling coupled with elevated glucagon levels result in hyperglycemia and subsequent type 2 diabetes mellitus [95-97].

Amylin

Amylin, a 37–amino acid peptide first reported in the literature in 1987, is a neuroendocrine hormone co-expressed and co-secreted with insulin by pancreatic β-cells in response to nutrient stimuli. Preclinical findings indicate that amylin works with insulin to help coordinate the rate of glucose appearance and disappearance in the circulation, thereby preventing an abnormal rise in glucose concentrations. Amylin exerts

these actions primarily through the central nervous system. Type 1 diabetes is an amylin-deficient state, whereas amylin levels are often elevated in patients with impaired glucose tolerance, insulin-resistant obesity, and type 2 diabetes [98-99]. In the fed state, amylin suppresses postprandial glucagon secretion centrally via efferent vagal signals, while also slowing the rate of gastric emptying. In animal models, amylin has been shown to dose-dependently reduce food intake and body weight. Therefore, amylin regulates the rate of glucose appearance from both endogenous and exogenous sources, and with insulin regulates the rate of glucose utilization [100-106]. The regulation of food intake and the ability to control plasma substrate levels for energy production involve complex mechanisms that ensure a constant and adequate supply of metabolites both in the fasting and in the fed state.

A number of hormonal peptides released from the gastrointestinal (GI) tract in response to the ingestion of food have been shown to play a critical role in the postprandial control of glucose homeostasis. They are known to act through three main mechanisms of action: 1) by stimulating insulin secretion from pancreatic β cells, 2) by inhibiting hepatic gluconeogenesis through the suppression of glucagon secretion, and 3) by inhibiting GI motility [107].

Glucagon-like peptide-1

Glucagon-like peptide-1 (GLP-1), a potent incretin hormone, has ability to stimulate glucose-dependent insulin secretion, while suppressing postprandial glucagon secretion from the pancreas [107-109]. Animal studies have demonstrated that the action of GLP-1 occurs directly through activation of GLP-1 receptors on the pancreatic β-cells and indirectly through sensory nerves [108, 110]. GLP-1 may promote pancreatic β-cells

proliferation and enhance functional β-cells mass [111]. Usually people with type 2 diabetes often have inappropriately elevated levels of glucagon and the levels and actions of GLP-1 appear to be deficient in many people with type 2 diabetes [112]. Repeated daily injections or continuous subcutaneous administration of native GLP-1 lowers blood glucose in subjects with type 2 diabetes [113-115]. Acute infusion or subcutaneous administration of native GLP-1 amide lowers meal-related glucose excursions in human subjects via inhibition of gastric emptying and glucagon secretion, and potentiation of glucose-dependent insulin secretion [108, 116-117]. Administration of GLP-1 in short-term studies of normal and diabetic subjects has been found to enhance satiety, reduce food intake, and promote weight loss [116, 118].

2.2.4. Adipokines as regulators of glucose homeostasis

The view that adipose tissue serves as a crucial integrator of glucose homeostasis is already widely accepted. Over the last two decades, numerous adipose-secreted proteins have been shown to behave as hormones and to control glucose metabolism. These fat-derived proteins and peptides are termed "adipokines", which play a central role in whole body glucose homeostasis by influencing a variety of biological and physiological processes [54, 119-120].

Leptin

Leptin is a multifunctional protein secreted primarily by adipocytes in response to hyperinsulinemia after a meal. In addition to its well-described role in energy metabolism, leptin has notable effects on glucose homeostasis. Leptin has been suggested as a good candidate to replace insulin or combine with insulin therapy to treat diabetes [32, 34, 121-122]. Exogenous leptin reverses hyperglycemia in *ob/ob* mice before body

weight is corrected. Further, pair feeding these rats does not improve glucose tolerance like leptin does, suggesting that leptin has an effect on blood glucose independent of its ability to inhibit feeding. Leptin also improves glucose homeostasis in lipodystrophic mice and humans with lipodystophy or congenital leptin deficiency [123-126]. When insulin-deficient rodents with uncontrolled diabetes due to autoimmune or chemical destruction of β-cells are made hyperleptinemic by adenoviral transfer of the leptin gene, leptin reverses the catabolic consequences of a total lack of insulin. This may be due to the potential suppression of glucagon action on liver and enhancing the insulin mimetic actions of IGF-1 on skeletal muscle [56]. Gluconeogenesis is inhibited by suppression of hyperglucagonemia, which is mediated by reductions in hepatic CREB, PEPCK, and PGC-1α [27, 127]. Up-regulation of insulin-like growth factor 1 (IGF-1) expression and plasma levels and increasing IGF-1 receptor phosphorylation in muscle may explain the increased insulin receptor substrate 1, PI3K, and extracellular signal-regulated kinases (ERK) phosphorylation in skeletal muscle [27, 32]. Leptin activates whole-body glucose metabolism, targeting the liver and peripheral tissues to increase insulin sensitivity. Leptin regulates glucose metabolism in the liver both indirectly, through the central nervous system and directly, via hepatic leptin receptors [14, 128].

Acute leptin infusion significantly stimulates insulin-inhibition actions on hepatic glucose production via decreased glycogenolysis in rats with hepatic insulin resistance. Hyperinsulinemic-euglycemic clamp studies showed that leptin-treated MKR mice have an approximate 4-fold increase in glucose infusion rate compared with control mice, suggesting that leptin either greatly reduces hepatic glucose output or increases glucose disposal rate [122]. Chronic leptin treatment increases glucose uptake across the hindlimb

by 47% under the presence of insulin and by 27% under the basal state, compared with the controls [29]. In addition, in soleus muscle, leptin per se also exerts a direct and acute insulin-like effect on glycogen synthesis, lactate formation, and glucose oxidation [18, 21].

Adiponectin

Adiponectin is an adipocyte-derived plasma protein, which acts as an insulin sensitizer, and stimulates fatty acid oxidation in an AMP-activated protein kinase (AMPK) and peroxisome proliferator activated receptor-α (PPAR-α)-dependent manner [52, 129-130]. Adiponectin signals through two receptors, AdipoR1 and AdipoR2, which are expressed mainly in skeletal muscle and liver, respectively. Activation of these receptors induces AMPK and PPARα activity and increase fatty acid oxidation and glucose uptake. Plasma adiponectin levels are inversely correlated with the degree of insulin resistance. Reduced expression of adiponectin has been associated with metabolic syndrome and type 2 diabetes and its expression was also found to be reduced in obese mice and humans. Adiponectin activates glucose utilization by muscle and suppresses hepatic glucose production. The stimulatory effects of adiponectin on insulin-stimulated glucose uptake and fatty acid oxidation are impaired in skeletal muscle in obese subject and type 2 diabetics [130-132]. Globular adiponectin stimulates glucose transport in skeletal muscle of type 2 diabetic patients [133]. The metabolic and insulin-sensitizing effects of adiponectin can be at least partially explained by its direct activation on the energy-sensing kinase – AMPK, through its suppression of the activities of G-6-Pase and PEPCK, without increasing insulin secretion [134].

Visfatin and Omentin

Besides leptin and adiponectin, visfatin and omentin also have positive effects on glucose uptake. Both peptides are secreted from visceral fat. Visfatin is an important factor in insulin-receptor signaling and mediates glucose uptake by direct binding and activation of the insulin receptor. However, the significance of visfatin of its role is still questionable [70]. Omentin is a newly identified secretory protein that has an endocrine role in modulating insulin sensitivity. *In vitro* studies have shown that omentin increases insulin sensitivity by activating Akt and enhancing insulin-stimulated glucose transport in isolated human adipocytes. Treatment with recombinant omentin-1 enhances insulin-stimulated glucose uptake in human subcutaneous and omental adipocytes. Furthermore, omentin plasma levels and omentin gene expression in visceral adipose are decreased in obesity [135].

Tumor Nerosis Factor alpha (TNF- α) and Resistin

By contrast, some adipokines have pro-hyperglycemic effects. TNF- α was the first secreted adipose protein shown to have effects on glucose homeostasis. Its plasma concentration is elevated in obesity and insulin-resistant conditions. TNF- α reduces insulin action, and blocking the actions of TNF- α restores insulin sensitivity *in vivo* and *in vitro* only in animal models but not in humans. Resistin, a small inflammatory molecule with hyperglycemic effects, induces glucose intolerance and reduces insulin sensitivity [136-138]. Resistin may inhibit hepatic AMPK activity, which results in elevated expression of the gluconeogenic enzymes, G-6-Pase and PEPCK, thereby affecting glucose metabolism and leading to decreased glycogen storage that contributes to the development of hepatic insulin resistance [58]. But conflicting evidence exists which suggests that central resistin has both insulin-sensitizing and insulin-resistant-

promoting effects. Park et al. (2008) demonstrated that central resistin nullifies central leptin action and its signaling increases glucose-induced insulin secretion and β-cell mass and induces hyperinsulinemia and prevents obesity [139]. However, Singhal et al. (2007) found that central resistin induces hepatic insulin resistance via NPY [140].

Retinol binding Protein 4 (RBP4)

Retinol binding protein 4, as the name indicates, was once thought only to function in the delivery of retinol to tissues. However, it is now considered a new adipokine linking glucose uptake in adipocytes with insulin sensitivity. In muscle, RBP4 impairs insulin action by decreasing the activity of the enzyme PI-3 kinase and the phosphorylation of insulin receptor substrate-1. In liver, increasing RBP4 does not alter PI-3 kinase activity, but upregulates expression of hepatic gluconeogenic enzyme PEPCK. RBP4 regulates glucose metabolism in skeletal muscle and liver via the control of the expression of GLUT4 in adipose tissue. Yang et al. (2005) found that the decrease in GLUT4 expression that occurs in the fatty tissue of obese animals is accompanied by increased expression and secretion of RBP4. Decreased glucose influx into adipocytes through GLUT4 and increased secretion of RBP4 leads to the secretion of other adipokines—including adiponectin, TNF-α and resistin, which are also altered in obesity and type 2 diabetes. These factors also may contribute to the regulation of blood glucose concentration in these disorders [53, 119].

2.3. Leptin

2.3.1. Physiological functions and mechanisms of leptin

Leptin was discovered in 1994 by Jeffrey Friedman and colleagues at Rockefeller University. It had been known since the 1960s that *ob/ob* mice could not produce a circulating satiety factor, but could respond to it. Using positional cloning, Friedman showed that the *ob* gene coded for a secreted protein derived from fat, and that affected *ob/ob* mice had a base-pair substitution that formed a stop codon, resulting in the production of a truncated, nonfunctional protein [12]. This *ob* gene product, later called leptin, circulates as a 16 kDa protein in mouse and human plasma and plays a key role in the regulation of body weight via its central actions on energy expenditure and food intake. Many factors have been associated with an increase in leptin secretion, (i.e., overfeeding, glucose concentration, amino acid levels, insulin, glucocortiocids and estrogens), while others have been associated with a decrease in leptin (i.e., fasting, free fatty acids and other lipid metabolites, androgens, thyroid hormones, catecholamines, inflammatory cytokines, and agonists of PPARγ). However, the crucial factors in regulating serum leptin concentrations seem to be short-term caloric intake and the amount of energy stored in adipocytes [141]. Leptin is an important mediator in a feedback regulatory loop between the brain and fat tissue to communicate the amount of body fat in the body. Increases in feeding or decreases in energy expenditure lead to an increase in body fat. Serum leptin concentrations are positively correlated with the amount of body fat and also reflect the nutritional status [20, 121, 141]. Stimulation of leptin receptors found in the hypothamamus of the brain inhibits feeding and enhances energy expenditure and promotes a loss of body fat [11, 142]. Fasting decreases plasma

leptin levels and refeeding fasted rats can restore leptin mRNA levels within 4 hours to the levels of fed animals. Therefore, leptin appears to act like a sensor monitoring the level of energy stores in the body and transmitting this information to the brain.

Leptin signaling pathways

Leptin is transported into the brain via a saturable transport system, which is located at both the endothelium and choroids plexus. After crossing the blood-brain barrier (BBB), leptin exerts its action on energy intake and expenditure through binding to the long form of the leptin receptor (ObRb), the only isoform with clearly demonstrated signaling capability in the Janus-Kinase (JAK)-signal transducer and activator of transcription-3 (STAT3) pathway [143]. Upon leptin binding to the receptor, a conformational change leads to the activation of JAK2 by transphosphorylation and subsequent phosphorylation of tyrosine residues in the receptor in the cytoplasmic region. Phosphorylation of Tyr1138 allows association of STATs, which then become substrates of receptor-associated JAKs. Phosphorylation of STATs leads to their dissociation from the receptor and the formation of active dimers. Then the phosopho-STAT3 dimerizes translocates to the nucleus, where it binds to the promoter regions of target genes and induces Suppressor of Cytokine Signaling-3 (SOCS3) and POMC (pro-opiomelanocortin) expression, while repressing AgRP (agouti-related peptide) [144]. Leptin signaling occurs typically through leptin receptor-mediated activation of JAK-STAT3 pathway, but leptin can also act through some of the components of the insulin-signaling cascade. [145-146]. Leptin can enhance the phosphorylation of IRS-1 or IRS-2 via JAK2 activation [144, 147]. Recent data suggest that leptin is also involved in the activation of IRS-phosphoinositide 3-kinase (PI3K) pathway, like insulin, to exert several different

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effects of the hormone at multiple sites [144, 148]. For example, leptin controls food

intake and body weight by increasing the activities of hypothalamic PI3K and

phosphodiesterase 3B (PDE3B), leading to a decrease in cAMP concentration and CREB

activities [149]. In liver, leptin upregulates glycogen synthesis via PI3K pathway to

activate glycogen synthase by inhibiting the phosphorylation of glycogen synthase

kinase-3 by Akt [23].

Leptin resistance and leptin (receptor) deficiency

Most overweight humans have higher circulating levels of leptin, corresponding

to their increased amount of body fat. For people who are chronically overweight, the

effects of leptin to control appetite and energy expenditure are inhibited. This is termed

leptin resistance. Leptin resistance has been suggested to be caused by defects at the level

of leptin transport across the blood-brain barrier into the central nervous system (CNS)

and/or disruption of leptin signal transduction and post-receptor signaling in

hypothalamic targets [150-153]. Impaired leptin transportation in the brain and decreased

ability of leptin to activate JAK-STAT3 signaling pathway in the arcuate nucleus of the

hypothalamus have been reported to contribute to leptin resistance. Bjorbaek et al.(1999)

found that leptin treatment to cells expressing the long-form of leptin receptor (ObRb)

will induce leptin resistance. The mechanism of leptin resistance is caused by induction

of SOCS-3 expression by leptin. Therefore, SOCS-3 is considered to have a role in the

development of such resistance [150-153]. This up-regulates expression of SOCS3 and

protein tyrosine phosphatase 1B (PTP1B), which are negative regulators of leptin

signaling, to block phosphorylation of JAK2 and also impair insulin signaling, and thus

favoring weight gain, insulin resistance and the development of obesity and type 2

diabetes [154-155]. However, some studies has suggested that diet-induced and age-related obesity could change the protein levels of hypothalamic leptin receptors which would lead to impaired leptin transport across the BBB and impaired leptin signaling [156-158]. Deficiencies of either leptin or leptin receptors result in severe obesity and type 2 diabetes [12, 159-160]. Animals or humans that either cannot produce functional leptin or that develop a resistance to circulating leptin concentrations develop obesity. In either case, obesity appears to be related to a relative decrease in leptin signaling in the brain [12, 161-162]. Interestingly, all the known animal models with low leptin signaling in the brain (*ob/ob, db/db, fa/fa, lipodystrophy, streptozotocin-induced diabetes)* demonstrate insulin resistance or diabetes [13, 15, 35, 163-164]. Obese (*ob/ob)* mice lacking functional leptin are grossly overweight and hyperphagic, particularly at young ages, and develop severe insulin resistance. Leptin deficiency also manifests changes in immune function, bone structure, the cardiovascular system including angiogenesis, supportive tissue function, malignancies, and reproductive function. Chronic administration of recombinant murine or human leptin reduces adipose mass of *ob/ob* mice through effects on food intake and energy expenditure, but has no effect on *db/db* mice, which has a genetic defect in the long form of the leptin receptor (ObRb). Transgenic overexpression of the leptin gene leads to increased insulin sensitivity in normal mice and delays the onset of impaired glucose metabolism in lethal yellow KKA^y mice [24-25, 32]. The *db/db* mouse is hyperleptinemic and develops obesity and severe type 2 diabetes due to a functional defect in the long-form leptin receptor in the brain [159, 165].

Physiology of leptin in the brain

Most of the effects of leptin are achieved via leptin receptors in the central nervous system (CNS). The brain is implicated as a key target for the glucose-lowing action of leptin. The hypothalamus is the principal target of leptin in its regulation of energy metabolism and the arcuate nucleus (ARC) is well characterized in terms of its role in the central effects of leptin. The ARC contains two distinct subsets of hypothalamic neurons, which produce different types of neuropeptides. Leptin binds to its receptors on these two subsets of neurons. The neuropeptides produced by these neurons potently exert opposite effects on food intake and energy expenditure. One subset of neurons synthesizes and releases two orexigenic neuropeptides: neuropeptide Y (NPY) and AgRP. The other subset of neurons produce the anorexigenic peptides α-melanocyte-stimulating hormone (α-MSH), which is derived from POMC and cocaine and amphetamine-related transcript (CART). Both sets of neurons project to second-order melanocortin receptor (MCR) expressing neurons within the hypothalamus, which includes the paraventricular (PVH), ventromedial (VMH), dorsomedial (DMH), and lateral hypothalamus, as well as to other brain regions, such as the brain stem [166]. Leptin binding to its receptor activates α-MSH and CART neurons and lead to an inhibition of food intake and an increase in energy expenditure. On the contrary, leptin binding to its receptor inhibits NPY and AgRP neurons, preventing an increase in food intake and a decrease in energy expenditure [26]. Thus, leptin is able to both stimulate anorexigenic pathways and inhibit orexigenic pathways. Intracerebroventricular (ICV) administration of leptin can restore insulin sensitivity and reverse the diabetic phenotype of lipodystrophic mice at doses that are ineffective when administered peripherally [167]. Centrally administered leptin has been shown to increase insulin-stimulated glucose

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utilization and to favor the expression of uncoupling proteins (UCPs), which may play a role in the increased energy dissipation caused by leptin [168].

2.3.2. Leptin effect on peripheral tissues

The antihyperglycemic actions of leptin on peripheral tissues, including liver, skeletal muscle, adipose tissue and pancreatic β-cells, are mediated by leptin binding to receptors in hypothalamus through central neural pathway and/or directly binding to receptors on peripheral tissues. Leptin improves insulin sensitivity in peripheral tissues by improving glucose and lipid metabolism through both central and peripheral administration.

<u>Central leptin effects on pheripheral target tissues</u>

Leptin receptors in the brain mediate glucose metabolism in certain peripheral tissues. Microinjection of leptin into the VMH increases glucose uptake in skeletal muscle, brown adipose tissue (BAT), and heart, whereas leptin injections into the ARC increase glucose uptake in BAT, while injections into the DMH or PVH have no effect [166]. Chronic leptin treatment significantly stimulates glucose uptake across the hindlimb by 47% in the presence of insulin and by 27% under the basal state [29]. Leptin restores euglycemia in streptozotocin-induced diabetic rats without increasing serum leptin and insulin concentrations. Interestingly, the dose of leptin that normalizes blood glucose levels when injected centrally has no effect when injected systemically. In addition, the upregulated hepatic G-6-Pase, GLUT2 are reduced to normal levels after central chronic leptin treatment in STZ-induced diabetic rats [13, 15]. A reduction of both gluconeogenesis and the efflux of glucose from liver could lead to the decreased hepatic glucose output after leptin treatment. Restoration of leptin receptor expression (ObRb) in

the ARC and the VMH of the ObRb–mutated Koletsky rat by adenovirus- or adeno-associated virus–mediated gene transfer improved peripheral insulin sensitivity and reduced plasma glucose concentration [166]. Leptin receptors in the ARC and the VMH, as well as the brain melanocortin pathway is thus involved in the regulation of glucose uptake in peripheral tissues as well as in energy metabolism [166]. However, a controversial finding showed that an acute ICV recombinant leptin infusion antagonizes the action of insulin on the hepatic gene expression of PEPCK, resulting in marked changes in the intrahepatic flux of glucose metabolism with increased gluconeogenesis and a concurrent decrease in glycogenolysis [169]. Similar evidence is provided by Gutierrez-Juarez et al. (2004) who examined the ability of the melanocortin system to regulate the effect of leptin on hepatic glucose metabolism. Acute ICV leptin resulted in increased gluconeogenesis and decreased glycogenolysis. They also found that blocking the melanocortin pathway with MCR4 receptor antagonist inhibited leptin's ability to increased PEPCK and G-6-Pase expression. On the contrary, the MCR4 receptor antagonist had no effect on the leptin-mediated change in glycogenolysis, and thus this effect of leptin appears to be melanocortin-independent [27]. Leptin administration is also associated with a marked decrease in the hepatic abundance of glucokinase mRNA and with several-fold increases in the hepatic abundance of PEPCK and glucose-6-phosphatase mRNAs. The latter effects of leptin on the hepatic gene expression of key glycolytic and gluconeogenic enzymes are consistent with a study in hepatoma cell lines, in which the inhibitory action of insulin on the gene expression of the gluconeogenic enzyme PEPCK is diminished by leptin [170-171]. These apparently contradictory

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observations on the relationship between leptin and hepatic insulin signaling may suggest that this peptide has both insulin-like and anti-insulin actions in the liver.

Unlike insulin which favors lipid storage as triglyceride, leptin induces changes in the partitioning of fatty acids in muscle, stimulates fatty acid oxidation directly in muscle and decreases adipose tissue mass. Leptin appears to attenuate insulin actions on lipogenesis on muscle fatty acid metabolism without inhibiting insulin-stimulated glucose disposal [172]. Leptin stimulates lipid oxidation via two distinct mechanisms, one is through direct activation of AMPK, independent of the sympathetic nervous system, and the other is through central effects, dependent on the α-adrenergic sympathetic pathway [173]. AMPK functions as a principal mediator of the effects of leptin on fatty-acid metabolism in muscle. Leptin activates AMPK leading to phosphorylation and inhibition of acetyl CoA carboxylase (ACC). This decreases the concentration of the product of ACC, malonyl CoA, and thereby allows fatty acids to enter the mitochondria where they are oxidized [174]. The infusion of leptin into the brain inhibits white adipose tissue lipogenesis independent of signal transducer and activator of STAT3 signaling. Whereas the effects of central leptin on the regulation of food intake, liver glucose production and luteinizing hormone secretion are all STAT3-dependent. However, when hypothalamic PI3K signaling is prevented or when sympathetic denervation of adipose tissue is performed, the effect of central leptin administration on adipose tissue lipogenesis is lost [175].

Direct actions of leptin on peripheral target tissues

The long-form of the leptin receptor is not only expressed in the brain, but also is widely distributed in other tissues including liver, skeletal muscle, adipose tissue and

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kidney [14, 128]. Several studies have demonstrated that leptin exerts effects on metabolism through direct actions on peripheral tissues. The application of leptin (6.25–100 nM) for 1–2 h to pancreatic islets isolated from ob/ob mice reduces insulin secretion in a degree from about 13 to almost 80%. Leptin also significantly reduces insulin release from the perfused pancreas of *ob/ob* mice [176-177]. Expression of the functional leptin receptor mRNA in pancreatic islets suppresses glucose-induced insulin secretion through activation of ATP-sensitive K^+ channels in pancreatic islets [176-177]. Leptin constrains the enhancement of the phospholipase C-mediated regulatory component of the insulin secretory process in a dose-dependent manner in *ob/ob* mice. At physiological concentrations, leptin suppresses the second phase of insulin secretion and preproinsulin mRNA expression [178-179]. Ablation of leptin receptors from β-cells results in enhanced basal insulin secretion and fasting hypoglycemia [70]. GLP-1-induced phosphorylation of GLUT2 was abolished by leptin in INS-1 cells *in vitro* and *in vivo* in perfused rat pancreases [180-181]. In addition to the inhibitory effect of leptin on pancreatic GLUT2 expression, leptin also reduces glucose transport by 35% in isolated rat hepatocytes, which also express GLUT2, suggesting that leptin alters glucose transport in other glucose-responsive tissues, such as the liver and skeletal muscle [180]. Leptin stimulates lipolysis and counteract the metabolic actions of insulin in adipocytes *in vitro* [123, 182]. Leptin increases the partition of long-chain fatty acids toward oxidation in pancreatic islets and soleus muscles of normal rats [183-184]. In young diet-induced obese rats, the increased hyperleptinemia exerts a protective action by stimulating lipid oxidation in nonadipose tissues. The chronic lack of leptin action in the *fa/fa* ZDF rats leads to the enhancement of lipogenesis in nonadipose tissues and makes

these tissues susceptible to genetic lipotoxicity [185]. Leptin increases glucose transportation and utilization in brown adipose tissue, skeletal muscle and a myotube cell line *in vitro* [19, 28-29, 37]. Intravenous leptin administration results in increased glucose utilization in brown adipose tissue and muscle and UCPs expression in brown adipose tissue, but decreased glucose uptake in white adipose tissue [168]. Short-term leptin treatment in high fat-fed rats alters muscle fatty acid metabolism by increasing fatty acids uptake and oxidation in muscle [186]. Insulin facilitates triacylglycerol storage in the form of ectopic fat in muscle and the effect of leptin on lipid metabolism in muscle is opposite of that of insulin. The effect of leptin on lipid metabolism in muscle is consistent with data from whole-animal studies in which leptin promotes fatty acids oxidation and decreases adipose tissue mass [172, 184, 187-189]. Leptin, independent of either insulin or central administration, exerts a direct effect on muscle glucose handling.

Insulin-mimicking effect of leptin through PI3K signaling pathway

Numerous evidence support an insulin-sensitizing action of leptin both via central hypothalamic actions and through peripheral mechanisms. The signaling process that mediates leptin action interacts at many points with the insulin signal transduction pathway [127, 171, 190-193]. In fact, most insulin-dependent actions involve PI3K activation, making this a relevant point of cross-talk between the insulin and leptin signaling pathways [144]. Leptin exerts a direct and acute insulin-like effect to stimulate glucose uptake, glycogen synthesis, lactate formation and glucose oxidation in soleus muscle and C2C12 myotubes through a PI3K-dependent pathway. In C2C12 myotubes, leptin has been shown to induce tyrosine phosphorylation of IRS-2 leading to activation of PI3-kinase by activating JAK2 [18-19, 21]. In fact, PI3-kinase has a central role in

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glycogen synthesis and the signaling of GLUT4 translocation. Therefore, leptin-treated

mice show up to a twofold increase in GLUT4 expression in the isolated soleus muscle of

ob/ob mice, but no inhibitory effect in the *db/db* mice that lack the functional leptin

receptor [28, 194]. In addition, leptin exerts action on glucose oxidation by stimulating

the activities of pyruvate dehydrogenase and key enzymes in the Kreb's cycle. Leptin

induces an upregulation of the expression of UCP-3 mRNA in mitochondria in the

skeletal muscle of rodents, and thus indirectly activates pyruvate dehydrogenase and the

Kreb's cycle [21, 195-197]. Liver is a major site of glucose metabolism. Several lines of

evidence indicate that leptin mimics some of the anabolic actions of insulin in the liver.

Leptin, at physiological concentrations, suppresses glucagon-induced cAMP elevation in

a PI3K-dependent manner, an intracellular signaling pathway activated by insulin [127].

Short-term exposure to leptin enhances insulin's ability to inhibit hepatic glucose

production without affecting peripheral insulin action, and induces a redistribution of

intrahepatic glucose fluxes and changes in the gene expression of hepatic enzymes that

closely resemble those of fasting [198]. Chronic peripheral leptin administration through

subcutaneous osmotic pump restores euglycemia and substantially improved glucose

metabolic rates during the post-absorptive state in STZ-induced diabetic rats. It also

restores insulin sensitivities at the levels of the liver and the peripheral tissues during a

glucose clamp, which is associated with a decrease in the rate of glucose production

under the basal and hyperinsulinemic conditions and an increase in glucose utilization in

the whole body during insulin infusion [22]. Simila to insulin, acute leptin infusion

significantly inhibits hepatic glucose production via decreased glycogenolysis, even in

rats with insulin resistance [32, 122]. Leptin enhances the inhibitory effects of insulin on

glycogenolysis and hepatic glucose production in liver *in vivo* and increases glycogen

synthesis in perfused mouse liver [23, 30-31, 198]. Hyperinsulinemic-euglyemic clamps

yield an almost 50% inhibition of glucose production by suppression of glycogenolysis

after 6 hour leptin infusion in rats [198]. Moreover, hyperinsulinemic-euglycemic clamp

studies also demonstrate that leptin-treated MKR mice have an approximate 4-fold

increase in glucose infusion rate compared with control mice [32, 122].

However, literature describing the effects of leptin on hepatic glucose metabolism

in rodents is conflicting in that some describe leptin increasing, while others describe

leptin decreasing hepatic gluconeogenesis in rats. Infused leptin induces a direct marked

reduction in the rate of glucagon-stimulated glucose production in perfused rat liver and

is also capable of reducing glucose production from different gluconeogenic precursors in

isolated hepatocytes [30, 199]. Similar findings were observed in a portal vein leptin

injection study, in which glucose production from lactate was inhibited by leptin by

39~66 % and PEPCK activity was reduced by 22~52 %. Leptin both alone and in

combination with insulin reduces hepatic gluconeogenesis and decreases the synthesis of

key enzymes of gluconeogenesis [17]. However, controversial evidence was shown in the

perfused rat liver and other *in vivo* studies. Leptin directly affects hepatic glucose

metabolism consisting of an insulin-mimicking effect on glycogenolysis and a glucagon-

like effect on gluconeogenesis, favoring the gluconeogenic pathway to increase PEPCK

gene expression [30, 37]. Administration of recombinant murine leptin directly on human

hepatoma cells (HepG2) or *in vivo* to mouse livers increases hepatic insulin sensitivity by

increasing phosphorylation of IR with a concomitant increases in PTP1B expression in

hepatocytes to counter-regulate insulin action and to maintain insulin signaling in proper balance. [200].

2.3.3. Leptin Therapy for Diabetes

States of congenital leptin deficiency because of mutations in the leptin gene have been associated with severe obesity, glucose intolerance, and insulin resistance in humans. These disturbances can be completely reversed by leptin administration [121]. Lipodystrophic syndromes, the conditions of complete or partial lack of adipose tissue, have also been associated with low leptin concentrations and milder insulin resistance and a milder metabolic syndrome. Studies in such patients and similar animal models have shown that long-term leptin administration and transgenic overexpression of leptin significantly improve glycemia, dyslipidemia, hepatic steatosis, and insulin resistance. It also improves lipidemia and insulin resistance in HIV positive patients with partial lipoatrophy [120, 125-126, 132, 201-203]. Meanwhile, fat accumulation in both liver and skeletal muscle of the congenital lipodystrophic mice, which has been shown to contribute to insulin resistance, is reduced by exogenous leptin administration [124-125, 204-205]. Leptin increases fatty acid oxidation in these tissues possibly reversing the lipotoxicity associated with insulin resistance and improving glucose metabolism in these tissues [33, 206]. In addition, transgenic overexpression of leptin accelerates the recovery from diabetes in calorically-restricted lethal yellow agouti mice (KKA^y mice) [25].

People with type 1 diabetes mellitus have inappropriately increased blood glucose concentrations before and after food ingestion. Excessive postprandial glucose production occurs in the presence of decreased and delayed insulin secretion and uncontrolled glucagon release. These abnormalities in hormone secretion, coupled with the impaired

insulin-induced suppression of glucose production and insulin-stimulated glucose uptake, account largely for the excessive amounts of glucose accumulated in the systemic circulation for disposal by peripheral tissues following food ingestion. When insulin secretion is defective, lack of suppression of glucagon can lead to substantial hyperglycemia by enhancing rates of glucose production. Therefore, insulin is a key metabolic regulator vital to glucose and lipid homeostasis and used to treat people with type 1 diabetes mellitus. Since hypoglycemia, increased adiposity, hepatic steatosis, and adverse plasma lipoprotein profiles are the most serious side effects of insulin therapy, treatment approaches are needed to mimic the rapid responsiveness of endogenous insulin secretion and reduce the risk of hypoglycemia. Wang et al. (2010) suggest another hormone, leptin, may be a candidate to replace insulin or combine with insulin therapy to treat type 1 diabetes. Leptin mimics some of the anabolic actions of insulin in liver and other tissues [34]. Accordingly, leptin increases glucose uptake in skeletal muscle and brown adipose tissue *in vivo* and increases glucose uptake in a mytotube cell line *in vitro* [29, 37]. Recent studies suggest peripheral or central leptin treatment has beneficial effect because of its ability to normalize blood glucose level in insulin-deficient diabetic rodents and *ob/ob* mice [123-126]. Our previous study and studies from other research groups have demonstrated that chronic central leptin administration can restore euglycemia in STZ-induced diabetic rats [13, 15, 22, 35, 56]. Leptin also markedly enhances the inhibitory effects of insulin on glycogenolysis and hepatic glucose production *in vivo* and in hepatocytes *in vitro* [30, 198]. In addition, central leptin administration acutely rescues the hepatic insulin resistance induced by short-term hyperphagia [122].

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Before the discovery of insulin, type 1 diabetes was treated with severely restricting caloric intake to control hyperglycemia. Leptin is critical for control of appetite, body weight and energy homeostasis. Leptin may be a good candidate to normalize glycemia in insulin-deficient patients through its appetite-suppressant effects. However, reduction of food intake alone cannot explain the full effect of leptin to normalize glycemia in insulin-deficient model based on the pair-fed experiments [13, 34].

In fact, the majority of the effect of leptin on appetite, body weight and energy homeostasis is mediated through the hypothalamus. Leptin action in these neurons also regulates peripheral glucose metabolism and this is partly independent of its effect on food intake and adiposity. This may contribute to the efficacy of leptin treatment in insulin-deficient rodent models. Yu et al. (2008) tested the effect of subcutaneous leptin in nonobese diabetic mice, a standard model for type 1 diabetes, and found that compared with insulin-treated mice, leptin-treated mice showed similar reductions in blood sugar and had a similar metabolic fingerprint with regard to glucose and ketones in the urine. They also found that combining low doses of leptin and insulin led to steadier long-term blood glucose control than high doses of insulin alone, suggesting that leptin could be used as an adjunct to conventional insulin therapy [56].

Wang et al. (2010) have proposed the hypothesis that leptin may control glycemia by inhibiting the secretion of glucagon, which stimulates the liver to produce glucose and contributes to the development of hyperglycemia in insulin-deficient mice. Through the central nervous system, leptin reduces plasma glucagon levels to a similar degree as insulin [34]. Yu et al. (2008) employed various insulin-deficient type 1 diabetic models to examine leptin action on glucagon action on hepatic glucose fluxes. In Nonobese

Diabetic (NOD) mice, hyperglucagonemia is suppressed by hyperleptinemia. In STZ-induced diabetic rats, hyperleptinemia inhibited hepatic glucagon action by reducing the level of phosphorylation of CREB, and PGC-1α mRNA and PEPCK, a key gluconeogeneic enzyme that is stimulated by glucagon.[56] In an in vitro study, Zhao et al. (2000) found that leptin induces an insulin-like, PI3K-dependent, intracellular signaling pathway, to suppress glucagon-induced cAMP in hepatocytes [127]. In contrast, Ahrén et al. (1999) have reported that leptin increases circulating glucagon concentrations in fasted mice via sympathetic neural activation [207]. In our previous study, we found the serum glucagon was increased in STZ-induced diabetic rats, however, there is no significant difference in serum glucagon concentrations between leptin-treated and vehicle-treated diabetic rats. A greater glucagon/insulin ratio, and higher PEPCK gene expression and protein levels were found in leptin-treated diabetic rats. Kulkarni's team (2008) reported that pancreas-specific leptin receptor (ObRb) knockout mice had higher insulin levels than control mice and that altered leptin activity contributes to type 2 diabetes. They hypothesized that the antidiabetic effects of leptin are a consequence of the hormone's action on pancreatic α cells, which secrete glucagon. However, there is no evidence of leptin receptors in pancreatic α cells, so the effects on glucagon could result from leptin action on other tissues [208-209]. Therefore, whether leptin's action on glucose homeostasis is dependent on inhibiting glucagon secretion and/or glucagon action has yet to be determined.

An alternative mechanism was proposed recently. Leptin was found to regulate insulin-like growth factor-binding protein 2 (IGFBP2) expressions in liver, and pharmacologic IGFBP2 levels reversed both type 1 and type 2 diabetes in mice.

However, whether leptin effect on normalizing hyperglycemia is dependent on IGFBP2 induction is unknown [36].

Despite the uncertainty about the mechanism by which leptin is able to improve glucose concentrations in type 1 diabetes, there are phase II clinical trials currently underway that are using leptin or its analog as a theraputetic agent. A phase II trial of leptin receptor agonists as an adjunct to insulin therapy is under the way [210]. Because of an overlap between signal-transducing pathways of leptin and insulin, a common pathogenesis of leptin and insulin resistance has also been suggested and would offer a variety of new approaches for novel therapies. This area remains a significant area of research [121, 211]. In addition, a phase II clinical trial has been completed that examined the antiobesity actions of a combination of leptin agonist (metreleptin) and an amylin agonist (pramlintide) as therapeutic agents against diet-induced obesity [212].

2.4. Research Hypothesis

We have previously found that chronic central leptin treatment normalizes blood glucose level in STZ-induced diabetic rats and fasting diabetic or nondiabetic leptin-treated rats cannot maintain normal blood glucose levels. A preliminary study in our laboratory, in which leptin-treated diabetic rats were fed various gluconeogeneic diets, suggested that leptin may inhibit gluconeogenesis between the entry point of fructose (dihydroxyacetone phosphate/glyceraldehyde 3-phosphate) and amino acids (pyruvate, TCA cycle intermediates) into the gluconeogeneic pathway. However, this interpretation is complicated by any potential leptin-induced change in peripheral glucose utilization. Therefore, the objectives of this study were to 1) determine whether chronic central leptin

administration inhibits hepatic gluconeogenesis in STZ-induced diabetic rats and if so, 2) determine the enzymatic site of the inhibition. To examine hepatic gluconeogenesis, independent of glucose utilization, we employed the isolated perfused liver model and isolated hepatocytes.

We hypothesized that chronic central leptin administration into the brain of diabetic rats inhibits hepatic gluconeogenesis, leading to a decrease in hepatic glucose output, and, thus contributing to normalization of blood glucose concentration in leptin-treated diabetic rats.

References

1. http://emedicine.medscape.com/article/766143-overview.

2. Laditka, S.B., M.P. Mastanduno, and J.N. Laditka, *Health care use of individuals with diabetes in an employer-based insurance population.* Arch Intern Med, 2001. **161**(10): p. 1301-8.

3. http://www.diabetes.org/diabetes-basics/diabetes-statistics/.

4. http://www.health.state.ny.us/diseases/conditions/diabetes/.

5. http://www.aces.edu/extcomm/impact/Diabetes.pdf.

6. Colditz GA, W.W., Rotnitzky A, Manson JE, *Weight gain as a risk factor for clinical diabetes mellitus in women.* Ann Intern Med 1995. **122**: p. 481-486.

7. Chan, J.M., et al., *Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men.* Diabetes Care, 1994. **17**(9): p. 961-9.

8. Burkemper, K.M. and D.R. Garris, *Influences of obese (ob/ob) and diabetes (db/db) genotype mutations on lumber vertebral radiological and morphometric indices: skeletal deformation associated with dysregulated systemic glucometabolism.* BMC Musculoskelet Disord, 2006. **7**: p. 10.

9. Reaven, G.M., *Pathophysiology of insulin resistance in human disease.* Physiol Rev, 1995. **75**(3): p. 473-86.

10. Kahn, B.B. and J.S. Flier, *Obesity and insulin resistance.* J Clin Invest, 2000. **106**(4): p. 473-81.

11. Halaas, J.L., et al., *Weight-reducing effects of the plasma protein encoded by the obese gene.* Science, 1995. **269**(5223): p. 543-6.

12. Zhang, Y., et al., *Positional cloning of the mouse obese gene and its human homologue.* Nature, 1994. **372**(6505): p. 425-32.

13. Lin, C.Y., et al. *Central leptin increases insulin sensitivity in streptozotocin-induced diabetic rats*. Am J Physiol Endocrinol Metab 2002 May [cited 282 5]; E1084-91].

14. Lee, G.H., et al., *Abnormal splicing of the leptin receptor in diabetic mice.* Nature, 1996. **379**(6566): p. 632-5.

15.   Hidaka, S., et al., *Chronic central leptin infusion restores hyperglycemia independent of food intake and insulin level in streptozotocin-induced diabetic rats.* Faseb J, 2002. **16**(6): p. 509-18.

16.   Al-Daghri, N., et al., *Role of leptin in glucose metabolism in type 2 diabetes.* Diabetes Obes Metab, 2002. **4**(3): p. 147-55.

17.   Anderwald, C., et al., *Short-term leptin-dependent inhibition of hepatic gluconeogenesis is mediated by insulin receptor substrate-2.* Mol Endocrinol, 2002. **16**(7): p. 1612-28.

18.   Ceddia, R.B., W.N. William, Jr., and R. Curi, *Leptin increases glucose transport and utilization in skeletal muscle in vitro.* Gen Pharmacol, 1998. **31**(5): p. 799-801.

19.   Berti, L., et al., *Leptin stimulates glucose transport and glycogen synthesis in C2C12 myotubes: evidence for a P13-kinase mediated effect.* Diabetologia, 1997. **40**(5): p. 606-9.

20.   Brennan, A.M. and C.S. Mantzoros, *Leptin and adiponectin: their role in diabetes.* Curr Diab Rep, 2007. **7**(1): p. 1-2.

21.   Ceddia, R.B., W.N. William, Jr., and R. Curi, *Comparing effects of leptin and insulin on glucose metabolism in skeletal muscle: evidence for an effect of leptin on glucose uptake and decarboxylation.* Int J Obes Relat Metab Disord, 1999. **23**(1): p. 75-82.

22.   Chinookoswong, N., J.L. Wang, and Z.Q. Shi, *Leptin restores euglycemia and normalizes glucose turnover in insulin-deficient diabetes in the rat.* Diabetes, 1999. **48**(7): p. 1487-92.

23.   Cohen, S.M., J.G. Werrmann, and M.R. Tota, *13C NMR study of the effects of leptin treatment on kinetics of hepatic intermediary metabolism.* Proc Natl Acad Sci U S A, 1998. **95**(13): p. 7385-90.

24.   Ogawa, Y., et al., *Increased glucose metabolism and insulin sensitivity in transgenic skinny mice overexpressing leptin.* Diabetes, 1999. **48**(9): p. 1822-9.

25.   Masuzaki, H., et al., *Glucose metabolism and insulin sensitivity in transgenic mice overexpressing leptin with lethal yellow agouti mutation: usefulness of leptin for the treatment of obesity-associated diabetes.* Diabetes, 1999. **48**(8): p. 1615-22.

26.   Morton, G.J., *Hypothalamic leptin regulation of energy homeostasis and glucose metabolism.* J Physiol, 2007. **583**(Pt 2): p. 437-43.

27.   Gutierrez-Juarez, R., S. Obici, and L. Rossetti, *Melanocortin-independent effects of leptin on hepatic glucose fluxes.* J Biol Chem, 2004. **279**(48): p. 49704-15.

28.   Liu, Y.L., V. Emilsson, and M.A. Cawthorne, *Leptin inhibits glycogen synthesis in the isolated soleus muscle of obese (ob/ob) mice.* FEBS Lett, 1997. **411**(2-3): p. 351-5.

29.   Yaspelkis, B.B., 3rd, et al., *Chronic leptin administration increases insulin-stimulated skeletal muscle glucose uptake and transport.* Metabolism, 1999. **48**(5): p. 671-6.

30.   Nemecz, M., et al., *Acute effect of leptin on hepatic glycogenolysis and gluconeogenesis in perfused rat liver.* Hepatology, 1999. **29**(1): p. 166-72.

31.   O'Doherty, R.M., et al., *Sparing effect of leptin on liver glycogen stores in rats during the fed-to-fasted transition.* Am J Physiol, 1999. **277**(3 Pt 1): p. E544-50.

32.   Toyoshima, Y., et al., *Leptin improves insulin resistance and hyperglycemia in a mouse model of type 2 diabetes.* Endocrinology, 2005. **146**(9): p. 4024-35.

33.   Yildiz, B.O. and I.C. Haznedaroglu, *Rethinking leptin and insulin action: therapeutic opportunities for diabetes.* Int J Biochem Cell Biol, 2006. **38**(5-6): p. 820-30.

34.   Wang, M.Y., et al., *Leptin therapy in insulin-deficient type I diabetes.* Proc Natl Acad Sci U S A, 2010. **107**(11): p. 4813-9.

35.   Miyanaga, F., et al., *Leptin as an adjunct of insulin therapy in insulin-deficient diabetes.* Diabetologia, 2003. **46**(10): p. 1329-37.

36.   Hedbacker, K., et al., *Antidiabetic effects of IGFBP2, a leptin-regulated gene.* Cell Metab, 2010. **11**(1): p. 11-22.

37.   Kamohara, S., et al., *Acute stimulation of glucose metabolism in mice by leptin treatment.* Nature, 1997. **389**(6649): p. 374-7.

38.   Bessems M, t.H.N., Tolba R, Doorschodt BM, Leuvenink HG, Ploeg RJ, Minor T, van Gulik TM, *The isolated perfused rat liver: standardization of a time-honoured model.* Laboratory Animals 2006. **40**(3): p. 236-246.

39.   Gores GJ, K.L., LaRusso NF, *The isolated perfused rat liver: conceptual and practical considerations.* Hepatology, 1986. **6**(3): p. 511-517.

40.   Adams MD, R.P., Judd RL., *Comparative effects of englitazone and glyburide on gluconeogenesis and glycolysis in the isolated perfused rat liver.* Biochem Pharmacol, 1998. **55**(11): p. 1915-1920.

41.   Zimmet, P., K.G. Alberti, and J. Shaw, *Global and societal implications of the diabetes epidemic.* Nature, 2001. **414**(6865): p. 782-7.

42.   Fujimoto, W.Y., *The importance of insulin resistance in the pathogenesis of type 2 diabetes mellitus.* Am J Med, 2000. **108 Suppl 6a**: p. 9S-14S.

43.   Mantzoros, C.S., *Obesity and Diabetes* 1ed. 2006, Totowa, NJ: Humana Press.

44.   Goldstein, B.J., *Insulin resistance: from benign to type 2 diabetes mellitus.* Rev Cardiovasc Med, 2003. **4 Suppl 6**: p. S3-10.

45.   Weyer, C., et al., *The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus.* J Clin Invest, 1999. **104**(6): p. 787-94.

46.   Saltiel, A.R. and C.R. Kahn, *Insulin signalling and the regulation of glucose and lipid metabolism.* Nature, 2001. **414**(6865): p. 799-806.

47.   Zeyda, M. and T.M. Stulnig, *Obesity, inflammation, and insulin resistance--a mini-review.* Gerontology, 2009. **55**(4): p. 379-86.

48.   Felber, J.P. and A. Golay, *Pathways from obesity to diabetes.* Int J Obes Relat Metab Disord, 2002. **26 Suppl 2**: p. S39-45.

49.   Wang, Y. and M.A. Beydoun, *The obesity epidemic in the United States--gender, age, socioeconomic, racial/ethnic, and geographic characteristics: a systematic review and meta-regression analysis.* Epidemiol Rev, 2007. **29**: p. 6-28.

50.   Shoelson, S.E., J. Lee, and A.B. Goldfine, *Inflammation and insulin resistance.* J Clin Invest, 2006. **116**(7): p. 1793-801.

51.   Wellen, K.E. and G.S. Hotamisligil, *Inflammation, stress, and diabetes.* J Clin Invest, 2005. **115**(5): p. 1111-9.

52.   Scherer, P.E., *Adipose tissue: from lipid storage compartment to endocrine organ.* Diabetes, 2006. **55**(6): p. 1537-45.

53.   Yang, Q., et al., *Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes.* Nature, 2005. **436**(7049): p. 356-62.

54.   Fain, J.N., et al., *Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans.* Endocrinology, 2004. **145**(5): p. 2273-82.

55.   Barry J. Goldstein, D.M.-W., *Type 2 Diabetes: Principles and Practice* 2ed. 2007, New York, NY: Informa Healthcare.

56.   Yu, X., et al., *Making insulin-deficient type 1 diabetic rodents thrive without insulin.* Proc Natl Acad Sci U S A, 2008. **105**(37): p. 14070-5.

57.   Avogaro, A. and A. Tiengo, *Alcohol, glucose metabolism and diabetes.* Diabetes Metab Rev, 1993. **9**(2): p. 129-46.

58.   Li, F.P., et al., *Effects of resistin expression on glucose metabolism and hepatic insulin resistance.* Endocrine, 2009. **35**(2): p. 243-51.

59.   Trøseida M, L.K., Damåsd JK, Lars Mørkride TC, Brendbergf R and Mollnesg TE, *Exercise reduces plasma levels of the chemokines MCP-1 and IL-8 in subjects with the metabolic syndrome.* European Heart Journal, 2003. **25**(4): p. 349-355.

60.   Randle, P.J., et al., *The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus.* Lancet, 1963. **1**(7285): p. 785-9.

61.   Masur K, T.F., Zänker KS, *Pathophysiology of diabetes mellitus type2: roles of obesity , insulin resistance and β cell dysfunction.* Diabetes and Cancer. Epidemiological Evidence and Molecular Links. Front Diabetes., 2008. **19**: p. 1-18.

62.   Boden, G., *Role of fatty acids in the pathogenesis of insulin resistance and NIDDM.* Diabetes, 1997. **46**(1): p. 3-10.

63.   Boden, G. and G.I. Shulman, *Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction.* Eur J Clin Invest, 2002. **32 Suppl 3**: p. 14-23.

64.   Shulman, G.I., *Cellular mechanisms of insulin resistance.* J Clin Invest, 2000. **106**(2): p. 171-6.

65.   Kaneto, H., Y. Nakatani, and M. Matsuhisa, *ER stress and the JNK pathway in insulin resistance.* Gene Therapy and Molecular Biology, 2004. **8**: p. 515-522.

66.   Ozcan, U., et al., *Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes.* Science, 2004. **306**(5695): p. 457-61.

67.   Tortora GJ , G.S., ed. *Principles of Anatomy and Physiology*. 10 ed. 2003, John Wiley & Sons: New York.

68.   Guyton AC, H.J., ed. *Textbook of Medical Physiology*. 10 ed. 2000, W.B. Saunders Company: Philadelphia PA.

69.   Gerich, J.E., *Control of glycaemia.* Baillieres Clin Endocrinol Metab, 1993. **7**(3): p. 551-86.

70.   Rosen, E.D. and B.M. Spiegelman, *Adipocytes as regulators of energy balance and glucose homeostasis.* Nature, 2006. **444**(7121): p. 847-53.

71.   Torres, T.P., et al., *Restoration of hepatic glucokinase expression corrects hepatic glucose flux and normalizes plasma glucose in zucker diabetic fatty rats.* Diabetes, 2009. **58**(1): p. 78-86.

72.   Liu, Z., et al., *Effect of acute diabetes on rat hepatic glucose-6-phosphatase activity and its messenger RNA level.* Biochem Biophys Res Commun, 1994. **205**(1): p. 680-6.

73.   Barzilai, N., et al., *Glucosamine-induced inhibition of liver glucokinase impairs the ability of hyperglycemia to suppress endogenous glucose production.* Diabetes, 1996. **45**(10): p. 1329-35.

74.   Shao, J., et al., *Chronic hyperglycemia enhances PEPCK gene expression and hepatocellular glucose production via elevated liver activating protein/liver inhibitory protein ratio.* Diabetes, 2005. **54**(4): p. 976-84.

75.   Hawkins, M., et al., *Glycemic control determines hepatic and peripheral glucose effectiveness in type 2 diabetic subjects.* Diabetes, 2002. **51**(7): p. 2179-89.

76.   Hawkins, M., et al., *Fructose improves the ability of hyperglycemia per se to regulate glucose production in type 2 diabetes.* Diabetes, 2002. **51**(3): p. 606-14.

77.   Wu, C., et al., *Increasing fructose 2,6-bisphosphate overcomes hepatic insulin resistance of type 2 diabetes.* Am J Physiol Endocrinol Metab, 2002. **282**(1): p. E38-45.

78.   Zhou, X.Y., et al., *Insulin regulation of hepatic gluconeogenesis through phosphorylation of CREB-binding protein.* Nat Med, 2004. **10**(6): p. 633-7.

79.   Ryu, D., et al., *TORC2 regulates hepatic insulin signaling via a mammalian phosphatidic acid phosphatase, LIPIN1.* Cell Metab, 2009. **9**(3): p. 240-51.

80.   Koo, S.H., et al., *The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism.* Nature, 2005. **437**(7062): p. 1109-11.

81.   Yoon, J.C., et al., *Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1.* Nature, 2001. **413**(6852): p. 131-8.

82.   Herzig, S., et al., *CREB regulates hepatic gluconeogenesis through the coactivator PGC-1.* Nature, 2001. **413**(6852): p. 179-83.

83.   Puigserver, P., et al., *Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction.* Nature, 2003. **423**(6939): p. 550-5.

84.   Aronoff SL, B.K., *Glucose Metabolism and Regulation: Beyond Insulin and Glucagon.* diaspect., 2004. **17**(3): p. 183-190.

85.   Liang, H. and W.F. Ward, *PGC-1alpha: a key regulator of energy metabolism.* Adv Physiol Educ, 2006. **30**(4): p. 145-51.

86.   Unger, R.H., *Glucagon physiology and pathophysiology.* N Engl J Med, 1971. **285**(8): p. 443-9.

87.   He, L., et al., *Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein.* Cell, 2009. **137**(4): p. 635-46.

88.   Wardlaw GM, K.M., ed. *Perspectives in Nutrition.* 5 ed. 2002, McGraw-Hill: New York.

89.   Furtado, L.M., et al., *Activation of the glucose transporter GLUT4 by insulin.* Biochem Cell Biol, 2002. **80**(5): p. 569-78.

90.   Govers, R., A.C. Coster, and D.E. James, *Insulin increases cell surface GLUT4 levels by dose dependently discharging GLUT4 into a cell surface recycling pathway.* Mol Cell Biol, 2004. **24**(14): p. 6456-66.

91.   Azzout-Marniche, D., et al., *Insulin effects on sterol regulatory-element-binding protein-1c (SREBP-1c) transcriptional activity in rat hepatocytes.* Biochem J, 2000. **350 Pt 2**: p. 389-93.

92.   Stoffel, M. and S.A. Duncan, *The maturity-onset diabetes of the young (MODY1) transcription factor HNF4alpha regulates expression of genes required for glucose transport and metabolism.* Proc Natl Acad Sci U S A, 1997. **94**(24): p. 13209-14.

93.   Barthel, A., D. Schmoll, and T.G. Unterman, *FoxO proteins in insulin action and metabolism.* Trends Endocrinol Metab, 2005. **16**(4): p. 183-9.

94.   Matiello, R., et al., *Differential regulation of PGC-1alpha expression in rat liver and skeletal muscle in response to voluntary running.* Nutr Metab (Lond), 2010. **7**: p. 36.

95.   Baron, A.D., et al., *Role of hyperglucagonemia in maintenance of increased rates of hepatic glucose output in type II diabetics.* Diabetes, 1987. **36**(3): p. 274-83.

96.   Dunning, B.E. and J.E. Gerich, *The role of alpha-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications.* Endocr Rev, 2007. **28**(3): p. 253-83.

97.   Wang, Y., et al., *The CREB coactivator CRTC2 links hepatic ER stress and fasting gluconeogenesis.* Nature, 2009. **460**(7254): p. 534-7.

98.   Hartter, E., et al., *Basal and stimulated plasma levels of pancreatic amylin indicate its co-secretion with insulin in humans.* Diabetologia, 1991. **34**(1): p. 52-4.

99.   Sanke, T., et al., *Plasma islet amyloid polypeptide (Amylin) levels and their responses to oral glucose in type 2 (non-insulin-dependent) diabetic patients.* Diabetologia, 1991. **34**(2): p. 129-32.

100.   Moore, C.X. and G.J. Cooper, *Co-secretion of amylin and insulin from cultured islet beta-cells: modulation by nutrient secretagogues, islet hormones and hypoglycemic agents.* Biochem Biophys Res Commun, 1991. **179**(1): p. 1-9.

101.   Koda, J.E., et al., *Amylin concentrations and glucose control.* Lancet, 1992. **339**(8802): p. 1179-80.

102.   Young, A., *Amylin's physiology and its role in diabetes.* Curr Opin Endocrinol Diab 1997. **4**: p. 282-290.

103.   Kruger, D.F., P.M. Gatcomb, and S.K. Owen, *Clinical implications of amylin and amylin deficiency.* Diabetes Educ, 1999. **25**(3): p. 389-97; quiz 398.

104.   Gedulin, B.R., T.J. Rink, and A.A. Young, *Dose-response for glucagonostatic effect of amylin in rats.* Metabolism, 1997. **46**(1): p. 67-70.

105.   Rushing, P.A., et al., *Inhibition of central amylin signaling increases food intake and body adiposity in rats.* Endocrinology, 2001. **142**(11): p. 5035.

106.   Rushing, P.A., et al., *Amylin: a novel action in the brain to reduce body weight.* Endocrinology, 2000. **141**(2): p. 850-3.

107.   Perfetti, R., et al., *Control of glucose homeostasis by incretin hormones.* Diabetes Technol Ther, 1999. **1**(3): p. 297-305.

108.   Drucker, D.J., *Enhancing incretin action for the treatment of type 2 diabetes.* Diabetes Care, 2003. **26**(10): p. 2929-40.

109.   Matsuyama, T., et al., *Glucagon-like peptide-1 (7-36 amide): a potent glucagonostatic and insulinotropic hormone.* Diabetes Res Clin Pract, 1988. **5**(4): p. 281-4.

110.   Drucker, D.J., *Glucagon-like peptides: regulators of cell proliferation, differentiation, and apoptosis.* Mol Endocrinol, 2003. **17**(2): p. 161-71.

111.   Perfetti, R. and P. Merkel, *Glucagon-like peptide-1: a major regulator of pancreatic beta-cell function.* Eur J Endocrinol, 2000. **143**(6): p. 717-25.

112.   Toft-Nielsen, M.B., et al., *Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients.* J Clin Endocrinol Metab, 2001. **86**(8): p. 3717-23.

113.   Todd, J.F., et al., *Subcutaneous glucagon-like peptide-1 improves postprandial glycaemic control over a 3-week period in patients with early type 2 diabetes.* Clin Sci (Lond), 1998. **95**(3): p. 325-9.

114.   Zander, M., et al., *Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study.* Lancet, 2002. **359**(9309): p. 824-30.

115.   Meneilly, G.S., et al., *Effects of 3 months of continuous subcutaneous administration of glucagon-like peptide 1 in elderly patients with type 2 diabetes.* Diabetes Care, 2003. **26**(10): p. 2835-41.

116.   Deacon, C.F., *Therapeutic strategies based on glucagon-like peptide 1.* Diabetes, 2004. **53**(9): p. 2181-9.

117.   Elliott, R.M., et al., *Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns.* J Endocrinol, 1993. **138**(1): p. 159-66.

118.   Nauck, M.A., et al., *Glucagon-like peptide 1 (GLP-1) as a new therapeutic approach for type 2-diabetes.* Exp Clin Endocrinol Diabetes, 1997. **105**(4): p. 187-95.

119.   Muoio, D.M. and C.B. Newgard, *Metabolism: A is for adipokine.* Nature, 2005. **436**(7049): p. 337-8.

120.   Sweeney, L.L., A.M. Brennan, and C.S. Mantzoros, *The role of adipokines in relation to HIV lipodystrophy.* Aids, 2007. **21**(8): p. 895-904.

121.   Brennan, A.M. and C.S. Mantzoros, *Drug Insight: the role of leptin in human physiology and pathophysiology--emerging clinical applications.* Nat Clin Pract Endocrinol Metab, 2006. **2**(6): p. 318-27.

122.   Pocai, A., et al., *Central leptin acutely reverses diet-induced hepatic insulin resistance.* Diabetes, 2005. **54**(11): p. 3182-9.

123.   Fruhbeck, G., M. Aguado, and J.A. Martinez, *In vitro lipolytic effect of leptin on mouse adipocytes: evidence for a possible autocrine/paracrine role of leptin.* Biochem Biophys Res Commun, 1997. **240**(3): p. 590-4.

124.   Shimomura, I., et al., *Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy.* Nature, 1999. **401**(6748): p. 73-6.

125.   Ebihara, K., et al., *Transgenic overexpression of leptin rescues insulin resistance and diabetes in a mouse model of lipoatrophic diabetes.* Diabetes, 2001. **50**(6): p. 1440-8.

126.   Lee, J.H., et al., *Recombinant methionyl human leptin therapy in replacement doses improves insulin resistance and metabolic profile in patients with lipoatrophy and metabolic syndrome induced by the highly active antiretroviral therapy.* J Clin Endocrinol Metab, 2006. **91**(7): p. 2605-11.

127.   Zhao, A.Z., et al., *Leptin induces insulin-like signaling that antagonizes cAMP elevation by glucagon in hepatocytes.* J Biol Chem, 2000. **275**(15): p. 11348-54.

128.   Tartaglia, L.A., et al., *Identification and expression cloning of a leptin receptor, OB-R.* Cell, 1995. **83**(7): p. 1263-71.

129.   Kadowaki, T., et al., *Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome.* J Clin Invest, 2006. **116**(7): p. 1784-92.

130.   Chen, M.B., et al., *Impaired activation of AMP-kinase and fatty acid oxidation by globular adiponectin in cultured human skeletal muscle of obese type 2 diabetics.* J Clin Endocrinol Metab, 2005. **90**(6): p. 3665-72.

131.   Bruce, C.R., et al., *The stimulatory effect of globular adiponectin on insulin-stimulated glucose uptake and fatty acid oxidation is impaired in skeletal muscle from obese subjects.* Diabetes, 2005. **54**(11): p. 3154-60.

132. Tsiodras, S. and C. Mantzoros, *Leptin and Adiponectin in the HIV Associated Metabolic Syndrome: Physiologic and Therapeutic Implications.* Am J Infect Dis, 2006. **2**(3): p. 141-152.

133. Kuoppamaa, H., et al., *Globular adiponectin stimulates glucose transport in type 2 diabetic muscle.* Diabetes Metab Res Rev, 2008. **24**(7): p. 554-62.

134. Kumar, S. and S. O'Rahilly, *Insulin resistance: insulin action and its disturbances in disease.* 2005, Chichester, England: John Wiley & Sons.

135. de Souza Batista, C.M., et al., *Omentin plasma levels and gene expression are decreased in obesity.* Diabetes, 2007. **56**(6): p. 1655-61.

136. Banerjee, R.R., et al., *Regulation of fasted blood glucose by resistin.* Science, 2004. **303**(5661): p. 1195-8.

137. Steppan, C.M. and M.A. Lazar, *The current biology of resistin.* J Intern Med, 2004. **255**(4): p. 439-47.

138. Kim, K.H., et al., *Dominant inhibitory adipocyte-specific secretory factor (ADSF)/resistin enhances adipogenesis and improves insulin sensitivity.* Proc Natl Acad Sci U S A, 2004. **101**(17): p. 6780-5.

139. Park, S., et al., *Long-term effects of central leptin and resistin on body weight, insulin resistance, and beta-cell function and mass by the modulation of hypothalamic leptin and insulin signaling.* Endocrinology, 2008. **149**(2): p. 445-54.

140. Singhal, N.S., M.A. Lazar, and R.S. Ahima, *Central resistin induces hepatic insulin resistance via neuropeptide Y.* J Neurosci, 2007. **27**(47): p. 12924-32.

141. Bluher, S. and C.S. Mantzoros, *Leptin in reproduction.* Curr Opin Endocrinol Diabetes Obes, 2007. **14**(6): p. 458-64.

142. Campfield, L.A., et al., *Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks.* Science, 1995. **269**(5223): p. 546-9.

143. Pocai A, M.K., Buettner C, Gutierrez-Juarez R, Obici S, Rossetti L., *Central leptin acutely reverses diet-induced hepatic insulin resistance.* Diabetes, 2005. **54**(11): p. 3182-3189.

144. Fruhbeck, G., *Intracellular signalling pathways activated by leptin.* Biochem J, 2006. **393**(Pt 1): p. 7-20.

145. Howard, J.K. and J.S. Flier, *Attenuation of leptin and insulin signaling by SOCS proteins.* Trends Endocrinol Metab, 2006. **17**(9): p. 365-71.

146. Schwartz, M.W. and D. Porte, Jr., *Diabetes, obesity, and the brain.* Science, 2005. **307**(5708): p. 375-9.

147. Bjorbaek, C., et al., *Divergent signaling capacities of the long and short isoforms of the leptin receptor.* J Biol Chem, 1997. **272**(51): p. 32686-95.

148. Niswender, K.D., et al., *Intracellular signalling. Key enzyme in leptin-induced anorexia.* Nature, 2001. **413**(6858): p. 794-5.

149. Zhao, A.Z., et al., *A phosphatidylinositol 3-kinase phosphodiesterase 3B-cyclic AMP pathway in hypothalamic action of leptin on feeding.* Nat Neurosci, 2002. **5**(8): p. 727-8.

150. Kastin, A.J. and W. Pan, *Dynamic regulation of leptin entry into brain by the blood-brain barrier.* Regul Pept, 2000. **92**(1-3): p. 37-43.

151. Banks, W.A., C.R. DiPalma, and C.L. Farrell, *Impaired transport of leptin across the blood-brain barrier in obesity.* Peptides, 1999. **20**(11): p. 1341-5.

152. Burguera, B., et al., *Obesity is associated with a decreased leptin transport across the blood-brain barrier in rats.* Diabetes, 2000. **49**(7): p. 1219-23.

153. Morash, B., et al., *Leptin gene expression in the brain and pituitary gland.* Endocrinology, 1999. **140**(12): p. 5995-8.

154. El-Haschimi, K., et al., *Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity.* J Clin Invest, 2000. **105**(12): p. 1827-32.

155. Munzberg, H., J.S. Flier, and C. Bjorbaek, *Region-specific leptin resistance within the hypothalamus of diet-induced obese mice.* Endocrinology, 2004. **145**(11): p. 4880-9.

156. Scarpace, P.J., M. Matheny, and N. Tumer, *Hypothalamic leptin resistance is associated with impaired leptin signal transduction in aged obese rats.* Neuroscience, 2001. **104**(4): p. 1111-7.

157. Wilsey, J. and P.J. Scarpace, *Caloric restriction reverses the deficits in leptin receptor protein and leptin signaling capacity associated with diet-induced obesity: role of leptin in the regulation of hypothalamic long-form leptin receptor expression.* J Endocrinol, 2004. **181**(2): p. 297-306.

158. Madiehe, A.M., et al., *Differential expression of leptin receptor in high- and low-fat-fed Osborne-Mendel and S5B/Pl rats.* Obes Res, 2000. **8**(6): p. 467-74.

159. Chen, H., et al., *Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice.* Cell, 1996. **84**(3): p. 491-5.

160. Chua, S.C., Jr., et al., *Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor.* Science, 1996. **271**(5251): p. 994-6.

161. Maffei, M., et al., *Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects.* Nat Med, 1995. **1**(11): p. 1155-61.

162. Halaas, J.L., et al., *Physiological response to long-term peripheral and central leptin infusion in lean and obese mice.* Proc Natl Acad Sci U S A, 1997. **94**(16): p. 8878-83.

163. Bray, G.A., .York,D.A., *Genetically transmitted obesity in rodents.* Physiol Rev. , 1971. **51**: p. 598-646.

164. Seip, M. and O. Trygstad, *Generalized lipodystrophy, congenital and acquired (lipoatrophy).* Acta Paediatr Suppl, 1996. **413**: p. 2-28.

165. Sharma, K., P. McCue, and S.R. Dunn, *Diabetic kidney disease in the db/db mouse.* Am J Physiol Renal Physiol, 2003. **284**(6): p. F1138-44.

166. Toda, C., et al., *Distinct effects of leptin and a melanocortin receptor agonist injected into medial hypothalamic nuclei on glucose uptake in peripheral tissues.* Diabetes, 2009. **58**(12): p. 2757-65.

167. Asilmaz, E., et al., *Site and mechanism of leptin action in a rodent form of congenital lipodystrophy.* J Clin Invest, 2004. **113**(3): p. 414-24.

168. Rouru, J., et al., *Effects of intravenously infused leptin on insulin sensitivity and on the expression of uncoupling proteins in brown adipose tissue.* Endocrinology, 1999. **140**(8): p. 3688-92.

169. Liu, L., et al., *Intracerebroventricular leptin regulates hepatic but not peripheral glucose fluxes.* J Biol Chem, 1998. **273**(47): p. 31160-7.

170. Barzilai, N., et al., *Leptin selectively decreases visceral adiposity and enhances insulin action.* J Clin Invest, 1997. **100**(12): p. 3105-10.

171.   Cohen, B., D. Novick, and M. Rubinstein, *Modulation of insulin activities by leptin.* Science, 1996. **274**(5290): p. 1185-8.

172.   Muoio, D.M., et al., *Leptin opposes insulin's effects on fatty acid partitioning in muscles isolated from obese ob/ob mice.* Am J Physiol, 1999. **276**(5 Pt 1): p. E913-21.

173.   Prieur, X., et al., *Leptin regulates peripheral lipid metabolism primarily through central effects on food intake.* Endocrinology, 2008. **149**(11): p. 5432-9.

174.   Minokoshi, Y., et al., *Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase.* Nature, 2002. **415**(6869): p. 339-43.

175.   Buettner, C., et al., *Leptin controls adipose tissue lipogenesis via central, STAT3-independent mechanisms.* Nat Med, 2008. **14**(6): p. 667-75.

176.   Emilsson, V., et al., *Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion.* Diabetes, 1997. **46**(2): p. 313-6.

177.   Kieffer, T.J., et al., *Leptin suppression of insulin secretion by the activation of ATP-sensitive K+ channels in pancreatic beta-cells.* Diabetes, 1997. **46**(6): p. 1087-93.

178.   Ookuma, M., K. Ookuma, and D.A. York, *Effects of leptin on insulin secretion from isolated rat pancreatic islets.* Diabetes, 1998. **47**(2): p. 219-23.

179.   Ishida, K., et al., *Leptin suppresses basal insulin secretion from rat pancreatic islets.* Regul Pept, 1997. **70**(2-3): p. 179-82.

180.   Lam, N.T., et al., *Leptin reduces glucose transport and cellular ATP levels in INS-1 beta-cells.* J Mol Endocrinol, 2004. **32**(2): p. 415-24.

181.   Fehmann, H.C., et al., *Interaction of GLP-I and leptin at rat pancreatic B-cells: effects on insulin secretion and signal transduction.* Horm Metab Res, 1997. **29**(11): p. 572-6.

182.   Muller, G., et al., *Leptin impairs metabolic actions of insulin in isolated rat adipocytes.* J Biol Chem, 1997. **272**(16): p. 10585-93.

183.   Shimabukuro, M., et al., *Direct antidiabetic effect of leptin through triglyceride depletion of tissues.* Proc Natl Acad Sci U S A, 1997. **94**(9): p. 4637-41.

184.   Muoio, D.M., et al., *Leptin directly alters lipid partitioning in skeletal muscle.* Diabetes, 1997. **46**(8): p. 1360-3.

185.   Jequier, E., *Leptin signaling, adiposity, and energy balance.* Ann N Y Acad Sci, 2002. **967**: p. 379-88.

186.   Todd, M.K., B.B. Yaspelkis, 3rd, and L.P. Turcotte, *Short-term leptin treatment increases fatty acids uptake and oxidation in muscle of high fat-fed rats.* Metabolism, 2005. **54**(9): p. 1218-24.

187.   Fruhbeck, G., S.A. Jebb, and A.M. Prentice, *Leptin: physiology and pathophysiology.* Clin Physiol, 1998. **18**(5): p. 399-419.

188.   Pelleymounter, M.A., et al., *Effects of the obese gene product on body weight regulation in ob/ob mice.* Science, 1995. **269**(5223): p. 540-3.

189.   Levin, N., et al., *Decreased food intake does not completely account for adiposity reduction after ob protein infusion.* Proc Natl Acad Sci U S A, 1996. **93**(4): p. 1726-30.

190.   Szanto, I. and C.R. Kahn, *Selective interaction between leptin and insulin signaling pathways in a hepatic cell line.* Proc Natl Acad Sci U S A, 2000. **97**(5): p. 2355-60.

191.   Wang, Y., et al., *Leptin receptor action in hepatic cells.* J Biol Chem, 1997. **272**(26): p. 16216-23.

192.   Kim, Y.B., et al., *In vivo administration of leptin activates signal transduction directly in insulin-sensitive tissues: overlapping but distinct pathways from insulin.* Endocrinology, 2000. **141**(7): p. 2328-39.

193.   Carvalheira, J.B., et al., *Interaction between leptin and insulin signaling pathways differentially affects JAK-STAT and PI 3-kinase-mediated signaling in rat liver.* Biol Chem, 2003. **384**(1): p. 151-9.

194.   Sarmiento, U., et al., *Morphologic and molecular changes induced by recombinant human leptin in the white and brown adipose tissues of C57BL/6 mice.* Lab Invest, 1997. **77**(3): p. 243-56.

195.   Gong, D.W., et al., *Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin.* J Biol Chem, 1997. **272**(39): p. 24129-32.

196.   Gomez-Ambrosi, J., G. Fruhbeck, and J.A. Martinez, *Leptin, but not a beta 3-adrenergic agonist, upregulates muscle uncoupling protein-3 messenger RNA expression: short-term thermogenic interactions.* Cell Mol Life Sci, 1999. **55**(6-7): p. 992-7.

197. Sivitz, W.I., B.D. Fink, and P.A. Donohoue, *Fasting and leptin modulate adipose and muscle uncoupling protein: divergent effects between messenger ribonucleic acid and protein expression.* Endocrinology, 1999. **140**(4): p. 1511-9.

198. Rossetti, L., et al., *Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action.* J Biol Chem, 1997. **272**(44): p. 27758-63.

199. Ceddia, R.B., et al., *Acute effects of leptin on glucose metabolism of in situ rat perfused livers and isolated hepatocytes.* Int J Obes Relat Metab Disord, 1999. **23**(11): p. 1207-12.

200. Lam, N.T., et al., *Leptin increases hepatic insulin sensitivity and protein tyrosine phosphatase 1B expression.* Mol Endocrinol, 2004. **18**(6): p. 1333-45.

201. Chan, J.L., et al., *Recombinant methionyl human leptin administration to achieve high physiologic or pharmacologic leptin levels does not alter circulating inflammatory marker levels in humans with leptin sufficiency or excess.* J Clin Endocrinol Metab, 2005. **90**(3): p. 1618-24.

202. Oral, E.A., et al., *Leptin-replacement therapy for lipodystrophy.* N Engl J Med, 2002. **346**(8): p. 570-8.

203. Javor, E.D., et al., *Long-term efficacy of leptin replacement in patients with generalized lipodystrophy.* Diabetes, 2005. **54**(7): p. 1994-2002.

204. Lewis, G.F., et al., *Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes.* Endocr Rev, 2002. **23**(2): p. 201-29.

205. Kelley, D.E., et al., *Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance.* Am J Physiol Endocrinol Metab, 2003. **285**(4): p. E906-16.

206. Petersen, K.F., et al., *Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes.* Diabetes, 2005. **54**(3): p. 603-8.

207. Ahrén B, H.P., *leptin increases circulating glucose, insulin and glucagon via sympathetic neural activation in fasted mice.* Int J Obes Relat Metab Disord, 1999. **23**(6): p. 660-665.

208. Morioka, T., et al., *Disruption of leptin receptor expression in the pancreas directly affects beta cell growth and function in mice.* J Clin Invest, 2007. **117**(10): p. 2860-8.

209. Morioka T, A.E., Hu J, Dishinger JF, Kurpad AJ, Elias CF, Li H, Elmquist JK, Kennedy RT, Kulkarni RN., *Disruption of leptin receptor expression in the pancreas directly affects β cell growth and function in mice.* J Clin Invest, 2007. **117**(10): p. 2860-2868.

210. http://www.nature.com/scibx/journal/v3/n10/full/scibx.2010.296.html.

211. Matarese, G., C. Mantzoros, and A. La Cava, *Leptin and adipocytokines: bridging the gap between immunity and atherosclerosis.* Curr Pharm Des, 2007. **13**(36): p. 3676-80.

212. Roth, J.D., et al., *Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies.* Proc Natl Acad Sci U S A, 2008. **105**(20): p. 7257-62.

Chapter 3

Blunted hepatic glucose production from lactate, but not fructose, in leptin-treated streptozotocin (STZ)- induced diabetic rats

Abstract Uncontrolled hepatic glucose production contributes significantly to hyperglycemia in type 2 diabetic patients. Leptin plays important roles in the regulation of blood glucose homeostasis. We hypothesize that leptin inhibits hepatic glucose output by inhibiting gluconeogenesis. This work sought to directly examine hepatic glucose output in leptin-treated diabetic rats via an isolated perfused liver system and to determine the glucose production rate in response to lactate and fructose. Wistar rats were implanted with an intracerebroventricular (ICV) cannula directed into the lateral ventricle. Following recovery, rats were made diabetic with an IV injection of streptozotocin (STZ). After confirmation of hyperglycemia, rats were administrated a daily ICV bolus injection of either leptin (5 microgram/day) or vehicle. Blood glucose concentrations were determined daily. After the blood glucose concentration of the leptin-treated rats returned to normal, each rat was fasted (vehicle-treated rats for 16 hours, leptin-treated rats for 6 hours) and baseline fasting blood glucose concentrations were determined. Gluconeogenic precursors (either lactate (16.6 mmol/kg) or fructose (8.3 mmol/kg)) and saline were gavaged into the stomach and blood glucose concentrations were determined every 15 minutes for 2 hours. After several days, the treatment groups were reversed and the proceeding repeated. After several days of

recovery, rats were fasted again as described previously, anesthetized with pentobarbital, and their livers were prepared for perfusion. Livers were perfused with oxygenated Krebs-Henseleit buffer at a flow rate of 35 ml/min. After 30 minutes of basal perfusion, a gluconeogenic precursor (either lactate or fructose) was added to the buffer (2 mM final concentration) and perfused for an additional hour. The perfusate was collected in 30-second fractions and the glucose concentrations in selected fractions were determined enzymatically. Leptin-treated rats had nearly a 4-fold lower glucose response to lactate gavage than did vehicle-treated rats. This was consistent with the finding that hepatic glucose production from the gastric gavage of lactate was severely blunted in leptin-treated rats as compared to rats that were vehicle-treated. However, the results of hepatic glucose production from fructose were different from the results from lactate. When fructose was used as the gluconeogenic precursor, there was no difference in the hepatic glucose production between leptin- and vehicle-treated rats. Additionally, gastric gavage of fructose resulted in a greater increase in blood glucose concentrations in leptin-treated rats than the gastric gavage of lactate in leptin-treated rats. These data suggest that chronic leptin administration leads to an inhibition of gluconeogenesis, which may explain why blood glucose concentrations are normalized in leptin-treated diabetic rats under fed conditions and decreased in leptin-treated diabetic rats under fasting conditions. It also suggests the site of the leptin-induced impairment in gluconeogenesis is prior to the gluconeogenic enzymes, fructose 1, 6-bisphosphatase.

3.1 Introduction

Many studies have shown a strong association between type 2 diabetes and obesity. Interestingly, a lack of leptin signaling in the brain leads to both obesity and insulin resistance, a hallmark of type 2 diabetes. Type 2 diabetes is characterized by insulin resistance coupled with a relative insufficient secretion of insulin. This leads to increased glucose production by the liver, which accounts for most of the excess glucose in the blood and decreased glucose uptake by insulin-sensitive tissues, like muscle and fat. One potential way to control diabetes is to inhibit glucose production by the liver, through the inhibition of the biochemical pathway gluconeogenesis [1-2].

Leptin, a circulating satiety factor produced by the fat cells, mediates its effect on body weight through its receptors in the hypothalamus. Through its interaction with the long form of the leptin receptor (ObRB), leptin inhibits feeding and increases energy expenditure. This shifts energy balance toward weight loss. Most obese people are thought to be resistant to the effects of leptin, leading to positive energy balance and weight gain [3-6]. Evidence also indicates that leptin may play especially important roles in the regulation of blood glucose [7-12]. Chronic leptin administrated peripherally appears to increase the rate of glucose utilization and decrease hepatic glucose production during insulin clamp studies [13-14]. Other studies have suggested that leptin alters glucose flux within the liver. Leptin has been shown to increase gluconeogenesis, but concomitantly decrease glycogenolysis such that there is either no change in hepatic glucose output [14] or a decrease [15]. Earlier work from our laboratory demonstrated that chronic leptin administered into the brains of diabetic rats leads to the normalization of blood glucose concentrations independent of food intake and insulin level [11]. This

finding has been also by reported by others [10, 16]. Blood glucose concentrations of leptin-treated diabetic and nondiabetic rats decrease by about 50% during a short-term fast (six hours)[11]. Since an increase in gluconeogenesis contributes to both the hyperglycemia of type 1 diabetic rats and the maintenance of blood glucose during a fast, we hypothesized that chronic central leptin administration leads to a decrease in gluconeogenesis. Support for this comes from a preliminary study in our laboratory in which leptin-treated, streptozotocin-induced diabetic rats were fed various gluconeogeneic diets and the blood glucose response was compared to fasted rats. Leptin-treated diabetic rats were unable to increase their blood glucose concentrations after eating albumin (amino acids), whereas they were able to increase their blood glucose concentration after eating fructose. This suggests that chronic leptin treatment inhibits gluconeogenesis between the entry points of fructose (dihydroacetone phosphate and glyceraldehyde 3-phosphate) and amino acids (pyruvate, TCA cycle intermediates) into the gluconeogeneic pathway.

Since changes in blood glucose concentrations are also affected by potential changes in the glucose disposal rate, we sought to examine the effect of chronic leptin treatment on hepatic glucose output, independent of potential changes in peripheral glucose utilization. Therefore, the aim of this study was to use the isolated perfused rat liver model to evaluate the effects of chronically administrated leptin on hepatic gluconeogensis in STZ-induced diabetic rats. We hypothesized that chronic leptin administration into the brain of diabetic rat decreases gluconeogenesis in the liver, which contributes to the normalization of blood glucose concentrations in leptin-treated diabetic rats.

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3.2 Materials and methods

Animals   Male, Wistar rats (225-300g; Harlan, Indianapolis,IN) were housed in

individual wire mesh cages before and during experiments. The room temperature was

held constant (23°C ± 3) with a 12:12 h light: dark cycle. Animals were provided

standard pellet rat chow and water ad libitum. Experimental protocols were approved by

Auburn University's Institutional Animal Care and Use Committee prior to initiation of

the experiments.

Experimental design   Rats were implanted with an intracerebroventicular (ICV) cannula

in the lateral ventricle of the brain under ketamine anesthesia. All rats were made diabetic

with an intravenous injection of streptozotocin (STZ) (50mg/kg). After hyperglycemia of

the rats was confirmed, half of the rats were given daily ICV bolus injections of leptin (5

microgram/day). The other half were given daily ICV injections of vehicle. Blood

glucose concentrations were determined daily. After the blood glucose concentration of

the leptin-treated rats returned to normal, each rat was fasted (vehicle-treated rats for 16

hours, leptin-treated rats for 6 hours) and baseline fasting blood glucose concentrations

were determined. (Leptin-treated rats were fasted for less time to prevent severe

hypoglycemia. Previously, we found that a 6-hour fast caused a 50% decrease in the

blood glucose concentration [11]). Gluconeogenic precursors (either lactate (16.6

mmol/kg) or fructose (8.3 mmol/kg)) and saline were gavaged into the stomach and blood

glucose concentrations were determined every 15 minutes for 2 hours. After several days,

the treatment groups were reversed and the proceeding repeated. After several days of

recovery, rats were fasted again as described previously, anesthetized with pentobarbital,

and their livers prepared for perfusion. Livers were perfused with oxygenated Krebs-Henseleit buffer (KHB) buffer at a flow rate of 35 ml/min. After 30 minutes of basal perfusion, a gluconeogenic precursor (either lactate or fructose, 2 mM final concentration was infused into the livers with a syringe pump at a point just before the hepatic portal vein cannula and perfused for an additional hour. The perfusate was collected in 30-second fractions and the glucose concentrations in selected fractions were determined enzymatically with a spectrophotometer.

Cannula implantation   After rats were anesthetized with an intraperitoneal injection of ketamine-xylazine (100 mg/kg ketamine and 1 mg/kg xylazine), they were placed in a stereotaxic apparatus, and implanted with a 22-gauge, stainless steel guide cannula(Plastic One, Roanoke,VA) directed into the lateral ventricle of the brain as described by Lin *et al*. using the following coordinates: 0.8 mm posterior to bregma, 1.4 mm lateral to the midline, and 3.5 nm ventral to the surface of the skull. The guide cannula was secured to the skull with four stainless steel screws and dental cement. A removable "dummy" cannula that extends 1 mm beyond the guide cannula was inserted to prevent clogging of the guide cannula. The animals were placed in individual cages and allowed to recover for 4 days. Cannulation placement was confirmed by a positive drinking response after administration of angiotensin II (40 ng/6 μl). Animals that did not drink at least 3 ml of water within 15 min after treatment were included in the vehicle group in the experiment [11].

Induction of diabetes   Insulin-deficient diabetes was produced by an intravenous

injection of freshly prepared streptozotocin (STZ) (50 mg/kg; Sigma, St. Louis, MO) in

0.05 M citrate buffer (pH 4.5). STZ- treated rats whose blood glucose concentrations

were > 400 mg/dl in a fed state were considered to be diabetic.

Liver perfusion   The technique of liver perfusion used was the non-recirculating

perfusion system described by Thurman and Scholz (1973), Miller et al. (1951) and

Adams et al. (1998) [17-19]. STZ-treated rats were fasted (vehicle-treated rats for 16

hours, leptin-treated rats for 6 hours), but allowed free access to water and anesthetized

with pentobarbital sodium (50 mg/kg) prior to liver perfusion. The abdomen was opened

through a longitudinal incision. The portal vein was cannulated with a 16 gauge stainless

steel catheter and a 14 gauge catheter was inserted into the thoracic inferior vena cava

The hemoglobin-free perfusion medium Krebs-Henseleit bicarbonate buffer (KHBC) was

freshly made and maintained at 37°C (pH=7.4). The buffer saturated with 95% oxygen

and 5% carbon dioxide was perfused via the hepatic portal vein at a constant flow rate of

35 mL/min. Successful perfusion is characterized by a liver that immediately changes

from maroon to a homogeneously tan color, which signals complete removal of blood

and its subsequent replacement with perfusion buffer. After 30 minutes of basal

perfusion, a gluconeogenic precursor (either lactate or fructose) was infused into the liver

with a syringe pump at a point before the hepatic portal vein cannula (2 mM final

concentration). The perfusate was collected in 30-second fractions for an additional hour.

Gastric gavage *test*   *In vivo* gluconeogenesis was examined by the gastric gavage of two

different gluconeogenic precursors, lactate and fructose. These precursors enter the

pathway of gluconeogenesis at different points. Diabetic rats were randomly assigned to one of the two treatments (i.e., gluconeogenic precursor or saline). Before the gastric gavage test, rats were fasted (vehicle-treated rats for 16 hours, leptin-treated rats for 6 hours). A blood sample was taken, and then the gluconeogenic precursor (either lactate (16.6 mmol/kg) or fructose (8.3 mmol/kg) 3 ml) or saline was administered through a stainless steel gavage tube placed into the rat's stomach. Blood glucose concentrations were determined every 15 minutes for 2 hours. After several days, the treatment groups (i.e. gluconeogenic precursor or saline) were reversed and the proceeding repeated. The change in blood glucose due to the gavage of the gluconeogenic precursor was determined by subtracting the blood glucose concentration in response to the gavage saline from the blood glucose concentration in response to the gavage of the gluconeogenic precursor (i.e., lactate or fructose).

Blood glucose concentration determination Blood was sampled from the tail vein. Blood glucose concentrations were monitored using an Accu-Chek Active glucometer (Roche Diagnostics).

Glucose assay Glucose concentrations of perfusates from the isolated liver perfusion experiments were determined using the hexokinase-glucose-6-phosphate dehydrogenase enzymatic assay, and expressed as μmol of glucose released per gram of liver per hour [20]. To determine the gluconeogenic rate from lactate or fructose, the hepatic glucose production rate was measured in the absence and presence of the gluconeogenic precursors.

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Statistical analysis   Data were presented as means ± SEM. Statistical analyses were performed with the use of SAS statistical software (version 9.1, SAS Institute). Multiple comparisons of time-dependent changes in lactate or fructose gavage were performed by two-way ANOVA for repeated measures and Tukey's studentized range test. Area under the curve (AUC) was determined by using the baseline glucose value as a cutoff line and under the curve or by taking the positive incremental area under the curve into the account. Trapezoid method was performed to calculate area under a curve [21]. Statistical significant differences between the leptin- and vehicle-treated rats for the mean values in AUC were assessed by Student's *t* test. A *P* value of 0.05 or less was considered statistically significant.

3.3 Results

Chronic leptin administration restored blood glucose concentration to normal level
Basal blood glucose level was determined one day after all rats were injected with STZ. Blood glucose concentrations from injected animals in a fed state were all greater than 400 mg/dl, therefore, they were all considered diabetic. The non-fasting blood glucose concentrations of diabetic rats averaged 451 ± 21 mg/dl before the daily treatment with central leptin began. Figure 3.1 shows the daily blood glucose concentration of STZ-induced diabetic rats given chronic ICV leptin (5 microgram/day) or vehicle. The blood glucose concentration of the leptin-treated diabetic rats decreased dramatically after the 4th day of ICV leptin injection (*P* < 0.05) as compared to vehicle-treated rats and remained close to normal level (175 ± 3 mg/dl) throughout the remained of the study .

Lactate gavage

The gastric gavage of lactate increased blood glucose concentrations in diabetic rats that received daily ICV vehicle injections compared to those same rats that were gavaged with saline. This leads to a significant change in the blood glucose concentration of over 115 mg/dl (Figure 3.2). However, the lactate-induced change in blood glucose concentrations in diabetic rats receiving daily ICV injections of leptin was severely blunted (Figure 3.2). Expressing the change in blood glucose concentration as the area under the curve showed that diabetic rats that had been chronically treated with leptin had nearly a 4-fold lower change in blood glucose response to lactate gavage than did vehicle-treated rats (Figure 3.2, $P < 0.05$). This suggests that leptin-treated diabetic rats have an impairment of gluconeogenesis when lactate is the gluconeogenic precursor. The fasting glucose levels of leptin-treated STZ rats were lower than the vehicle-treated STZ rats (260 ± 70 mg/dl vs. 78 ± 12 mg/dl).

Fructose Gavage

When fructose was used as the gluconeogenic precursor, there was no difference in the hepatic glucose production between leptin- and vehicle-treated rats (Figure 3.3). Additionally, gastric gavage of fructose resulted in a greater increase in blood glucose concentrations in leptin-treated rats than the gastric gavage of lactate in leptin-treated rats (Figure 3.2, 3.3).

Blunted hepatic glucose production from lactate in leptin-treated STZ diabetic rats in IPRL model.

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To determine whether chronic central leptin treatment specifically affected hepatic gluconeogenesis, we examined the glucose production from lactate and fructose in isolated perfused livers from leptin- and vehicle-treated rats. Hepatic glucose production from lactate was severely blunted in leptin-treated rats as compared with that of vehicle-treated rats (Figure 3.4). However, hepatic glucose production from fructose was not different between leptin-and vehicle-treated rats (Figure 3.5). The difference of hepatic glucose production in leptin-and vehicle-treated rats in the presence of fructose and lactate was illustrated by the Figure 3.6. There was a significant decrease ($P < 0.01$) in hepatic glucose production with lactate infusion in leptin-treated rats compared to vehicle-treated rats, however there was no difference in the hepatic glucose production from leptin-treated and vehicle-treated rats when infused with fructose. In addition, since lactate and fructose are 3-carbon and 6-carbon precursors, respectively, hepatic glucose production from both leptin and vehicle- treated rats in the presence of fructose is significantly higher than in the presence of lactate with the same amount of fructose ($P < 0.05$ and $P < 0.0001$, respectively).

3.4 Discussion

The STZ-model is widely used in medical research to produce an animal model for type 1 diabetes. Insulin is an important physiological regulator of leptin. Dose-dependent increases in leptin mRNA are observed in response to insulin in 3T3 cells [22-23] and cultured rat adipocytes [24]. *In vivo*, insulin increases leptin mRNA expression [25-28] and plasma leptin concentrations [29] within a few hours of administration. Also leptin mRNA expression increases in abdominal subcutaneous adipose tissue in humans when

treated with insulin [30]. Therefore, insulin regulates expression and production of leptin

and marked and rapid decreases of circulating leptin in STZ diabetic rats can be reversed

by insulin [31]. So the STZ-model is not only a type 1 diabetes model, it also a model of

leptin-deficiency model. By using such a model, we can study the effect of exogenous

leptin without the complicating effects of endogenous leptin and insulin.

In this study, we demonstrated that chronic leptin administration leads to an

inhibition of gluconeogenesis from lactate both *in vivo* the gastric gavage test and *in vitro*

the isolated perfused liver model. Usually changes in blood glucose concentration are the

result of changes in glucose production, including absorption from GI tract, hepatic

glycogenolysis, and gluconeogenesis, as well as changes in glucose utilization by the

body's tissues. Our laboratory and others have shown that chronic central leptin

administration normalizes blood glucose concentrations in diabetic rats [11]. To

determine whether chronic ICV leptin treatment specifically affects hepatic

gluconeogenesis, we determined the hepatic glucose output of glucose after a fast (no

glucose from GI tract and deletion of liver glycogen) in leptin-and vehicle-treated

diabetic rats by using an IPRL model, which has been used to explore the physiology and

pathophysiology of the rat liver for many years. This *in vitro* model provides the

opportunity to assess hepatic glucose production in an isolated setting directly, in contrast

to *in vivo* model. The IPRL model allows repeated sampling of the perfusate, permits

easy exposure to the liver to different concentrations of test substances and can be done

independent of the influence of other organ system, plasma constituents and neural-

hormonal effect [19, 32-33]. Study has shown that infused leptin induces a direct marked

reduction in the rate of glucagon-stimulated glucose production in perfused rat liver and

is also capable of reducing glucose production from different gluconeogenic precursors in isolated hepatocytes [34-35]. Similar findings were observed in a portal vein leptin injection study, in which glucose production from lactate was inhibited by leptin by 39~66 % [36]. However, controversial evidence was shown in the perfused rat liver and other in vivo studies. Leptin directly affects hepatic glucose metabolism consisting of an insulin-mimicking effect on glycogenolysis and a glucagon-like effect on gluconeogenesis, favoring the gluconeogenic pathway to increase phosphoenolpyruvate carboxykinase (PEPCK) gene expression [34, 37].

Previous *in vivo* studies have shown that leptin treatment profoundly improves hyperglycemia by restoring normal the rates of glucose turnover (production and utilization) [12, 15, 38]. Research has also demonstrated that leptin enhances insulin's stimulation of glucose uptake in the brown adipose tissue and skeletal muscle [15, 37, 39]. The results obtained from the present study using the insulin-deficient model of diabetes demonstrated that leptin inhibits hepatic gluconeogenesis from lactate, which may contribute to the ability of leptin to correct hyperglycemia in diabetic rats, independent with insulin. Another intriguing finding of chronic central leptin administration is that it leads to a decreased in blood glucose concentrations in both diabetic and nondiabetic rats under fasting conditions [11]. Since blood glucose concentration during a fast is supported by an increase in hepatic gluconeogenesis, the finding that chronic ICV leptin administration impaired hepatic gluconeogenesis, suggests that this may also be related to a fact that the fasting body glucose levels of leptin-treated STZ rats are lower than the vehicle-treated STZ rats.

The inhibition in glucose production with lactate suggests that leptin treatment

impaired gluconeogensis. This finding coupled the lack of a difference in glucose

production between leptin- and vehicle-treated rats with fructose helps define the site of

the leptin-induced impairment in gluconeogenesis. Fructose enters gluconeogenesis as

dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. To convert these

intermediates into glucose requires the gluconeogenic enzymes, fructose 1, 6-

bisphosphate and glucose 6-phosphate. Therefore, it appears that the site of the leptin-

induced impairment in gluconeogenesis is prior to the gluconeogenic enzymes, fructose

1, 6-bisphosphatase. To convert lactate into glucose also requires these same enzymes,

plus PEPCK. The current results with lactate would then appear to suggest that leptin

inhibits PEPCK. Several lines of evidence have implicated PEPCK to be the rate-

determining step in hepatic gluconeogenesis [40-43]. However, previous results have

shown that gene expression and protein level of PEPCK is up-regulated by leptin [43-44],

we have found similar results in our laboratory. In a study conducted in isolated rat

hepatocytes, both pyruvate carboxylase (PC) and pyruvate kinase were shown to play a

greater role than PEPCK in determining the gluconeogenic flux. Similar results were

obtained that PC rather than PEPCK was shown to be the primary rate-determining step

for gluconeogenesis [45-48]. It has been reported that the hepatic PC activity in the STZ-

induced rats is increased 2-fold over that of control rats and that a high plasma

glucagon/insulin ratio may account for the increase in this enzyme activity [49-50].

Making STZ-induced diabetic rat hyperleptinemic with an adenovirus inhibits hepatic

glucagon concentrations [50]. Therefore, it's tempting to speculate that leptin impairs

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gluconeogenesis at a site distal to PEPCK, like PC, and that this may be related to leptin-induced changes in the secretion level or the activity of glucagon.

In summary, hepatic glucose production from lactate was severely blunted in leptin-treated rats as compared to rats that were vehicle-treated, which was consistent with the finding from the lactate gavage test. However, when fructose was used as the gluconeogenic precursor, there was no difference in the hepatic glucose production between leptin- and vehicle-treated rats. This was also consistent with the result of the gastric gavage of fructose. Overall, the results suggest that chronic leptin administration leads to an inhibition of gluconeogenesis and the site of the leptin- induced impairment in gluconeogenesis is prior to the gluconeogenic enzyme, fructose 1, 6-bisphosphatase. These results may help explain why blood glucose concentrations are normalized in leptin-treated diabetic rats under fed conditions and decreased in leptin-treated diabetic rats under fasting conditions.
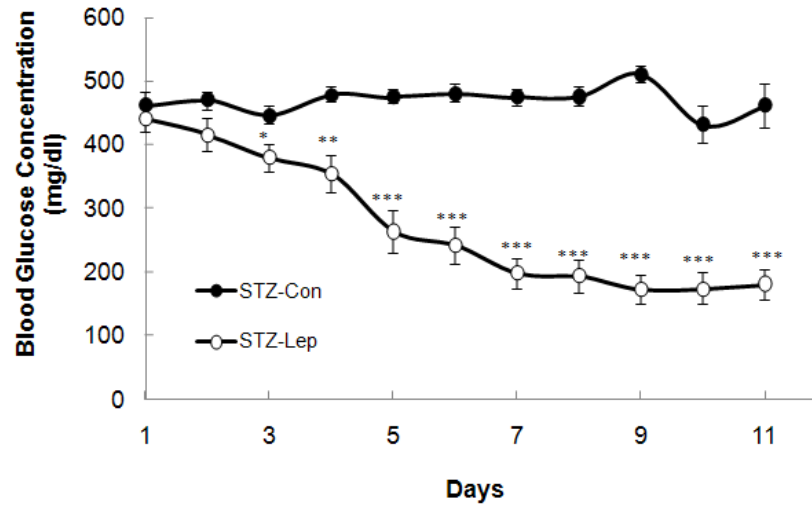
Figure 3.1. Effect of daily ICV leptin (○) or vehicle (●) on blood glucose concentration of diabetic rats. 1st day of ICV leptin (or vehicle) injection. Blood glucose concentration in diabetic rats was significantly decreased after 3 days of ICV leptin injection. Value are means ± SEM (leptin-treated n=18, vehicle-treated n=15). *P<0.05, ** P<0.01, *** P<0.001 vs. STZ-lep
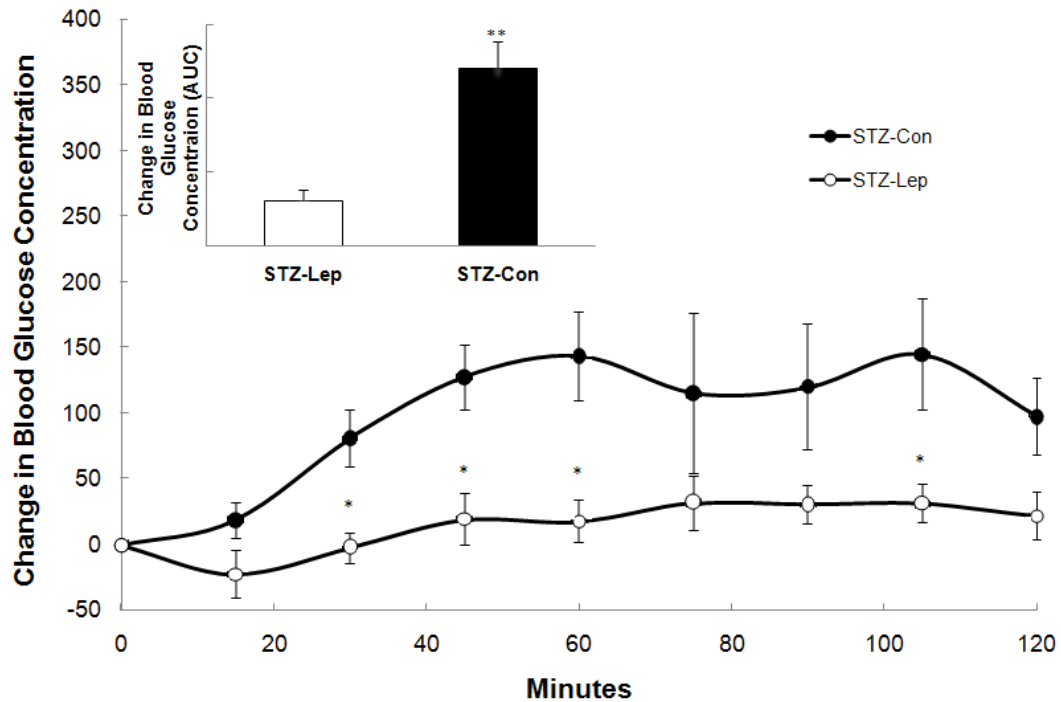
Figure 3.2. Gastric gavage of gluconeogenic precursor in fasted leptin-(○) and vehicle-treated (●) diabetic rats. Inset shows lactate-treated (16.6 mmol/kg) AUC was determined by taking the positive incremental area under the curve into the account (negative incremental area is negligible). Values are means ± SEM (leptin-treated n=18, vehicle-treated n=15). *$P < 0.05$, vs. STZ-lep
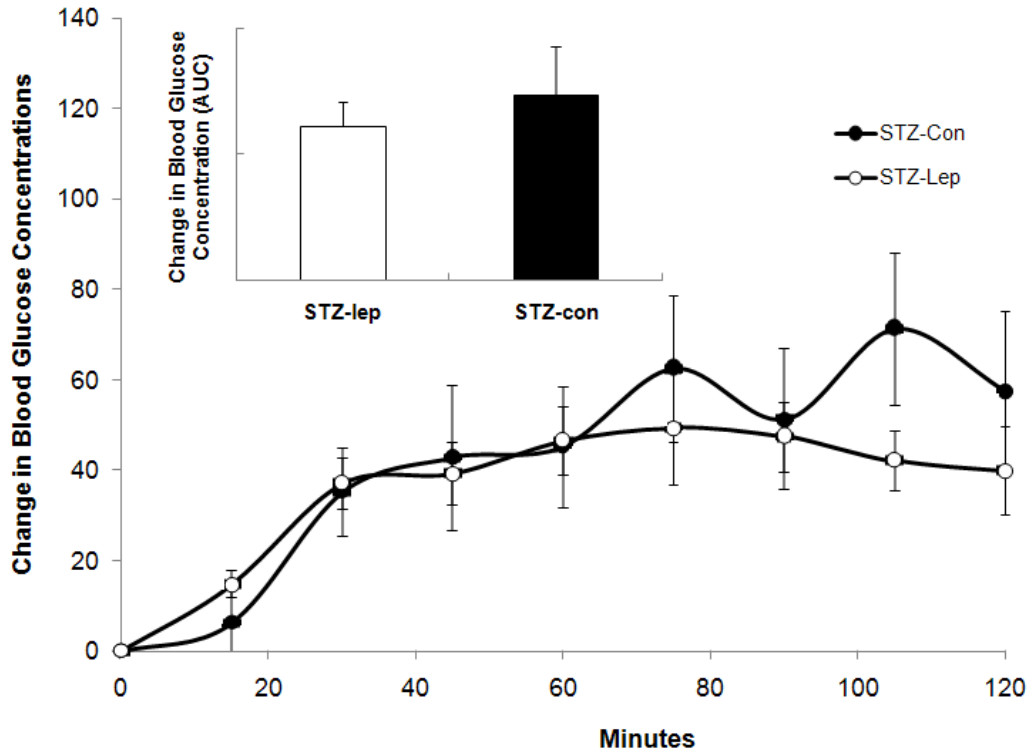
Figure 3.3. Gastric gavage of gluconeogenic precursor in fasted leptin-(○) and vehicle-treated (●) diabetic rats. Inset shows fructose-treated (8.3 mmol/kg) AUC was determined by taking the positive incremental area under the curve into the account (negative incremental area is negligible). Values are means ± SEM (leptin-treated n=5, vehicle-treated n=5).
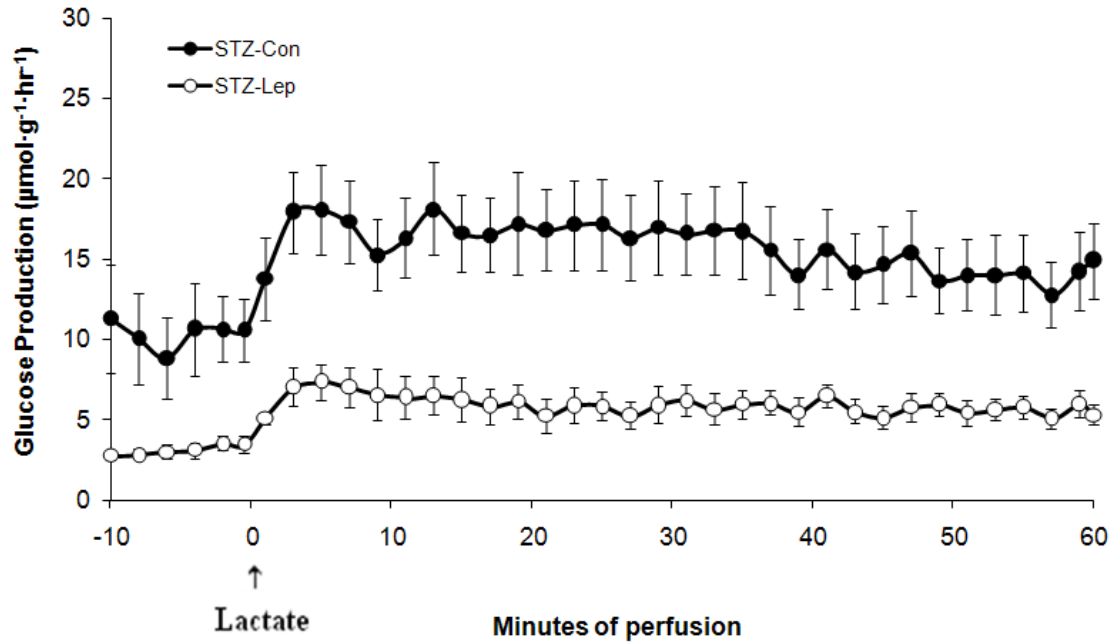
Figure 3.4. Hepatic glucose production from lactate (2mM) from isolated perfused livers of fasted leptin-(○) and vehicle-treated (●) diabetic rats. Leptin treatment severely blunted glucose production from lactate. Values are means ± SEM (leptin treated n=9, vehicle treated n=9).
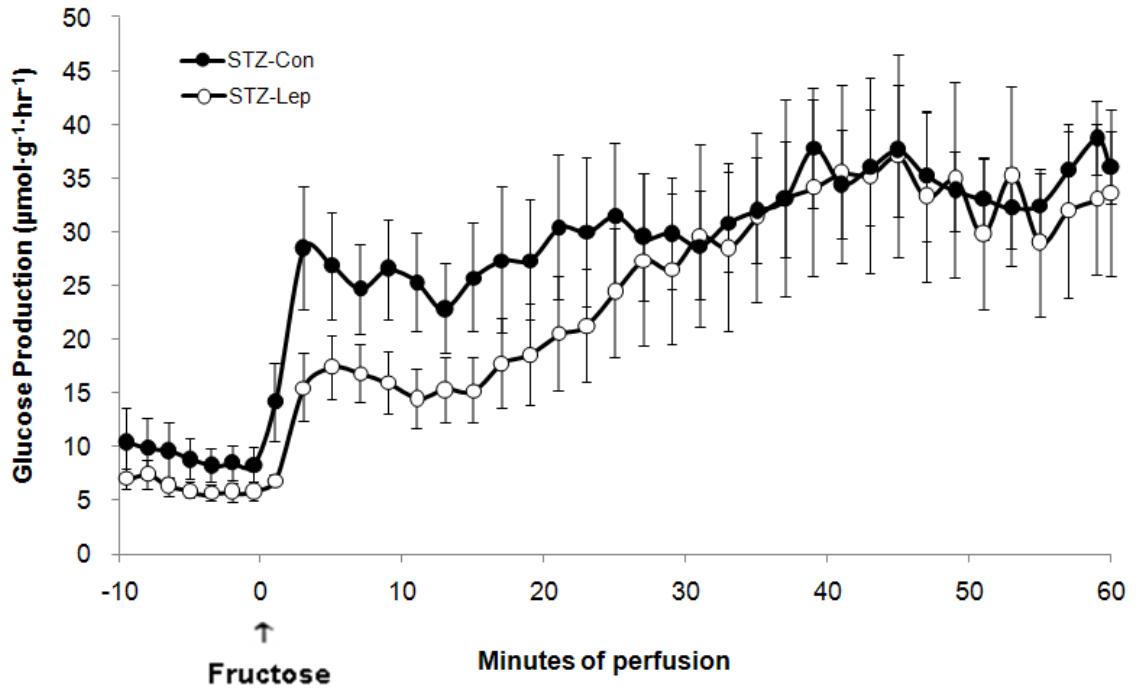
Figure 3.5. Hepatic glucose production from fructose (2mM) from isolated perfused livers of fasted leptin-($\circ$) and vehicle-treated ($\bullet$) diabetic rats. There was no difference in the hepatic glucose production between leptin-and vehicle-treated rats. Values are means ± SEM (leptin treated n=6, vehicle treated n=9).
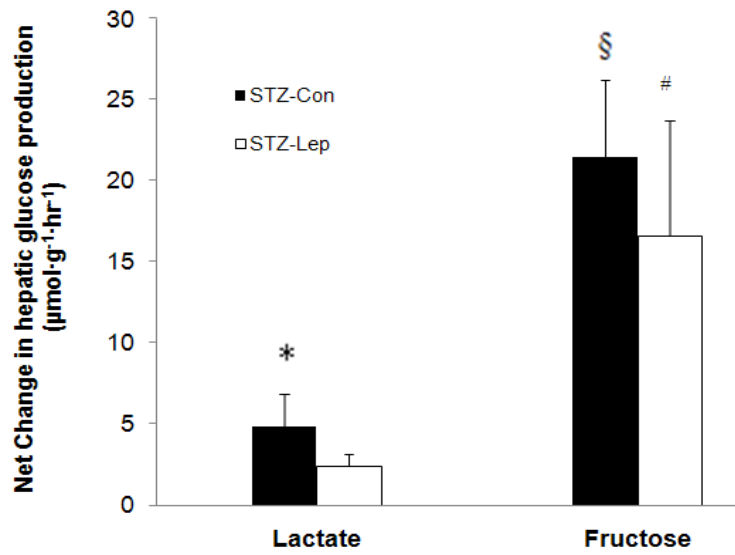
Figure 3.6. Changes in hepatic glucose production in response to lactate and fructose in isolated rat liver perfusion model. Values are means ± SEM. *$P < 0.01$, STZ-Lep vs. STZ-Con. § $P < 0.05$, Fructose vs. Lactate (STZ-Lep). # $P < 0.0001$, Fructose vs. Lactate (STZ-Con).

References

1.   Sloop, K.W., et al., Specific reduction of hepatic glucose 6-phosphate transporter-1 ameliorates diabetes while avoiding complications of glycogen storage disease. J Biol Chem, 2007. 282(26): p. 19113-21.

2.   Ohtsu, Y., et al., The novel gluconeogenesis inhibitor FR225654 that originates from Phoma sp. no. 00144. III. Structure determination. J Antibiot (Tokyo), 2005. 58(7): p. 479-82.

3.   Kastin, A.J. and W. Pan, Dynamic regulation of leptin entry into brain by the blood-brain barrier. Regul Pept, 2000. 92(1-3): p. 37-43.

4.   Banks, W.A., C.R. DiPalma, and C.L. Farrell, Impaired transport of leptin across the blood-brain barrier in obesity. Peptides, 1999. 20(11): p. 1341-5.

5.   Burguera, B., et al., Obesity is associated with a decreased leptin transport across the blood-brain barrier in rats. Diabetes, 2000. 49(7): p. 1219-23.

6.   Morash, B., et al., Leptin gene expression in the brain and pituitary gland. Endocrinology, 1999. 140(12): p. 5995-8.

7.   Shimizu, H., K. Inoue, and M. Mori, The leptin-dependent and -independent melanocortin signaling system: regulation of feeding and energy expenditure. J Endocrinol, 2007. 193(1): p. 1-9.

8.   Moran, O. and M. Phillip, Leptin: obesity, diabetes and other peripheral effects--a review. Pediatr Diabetes, 2003. 4(2): p. 101-9.

9.   Sahu, A., Minireview: A hypothalamic role in energy balance with special emphasis on leptin. Endocrinology, 2004. 145(6): p. 2613-20.

10.   Hidaka, S., et al., Chronic central leptin infusion restores hyperglycemia independent of food intake and insulin level in streptozotocin-induced diabetic rats. Faseb J, 2002. 16(6): p. 509-18.

11.   Lin, C.Y., et al. Central leptin increases insulin sensitivity in streptozotocin-induced diabetic rats. Am J Physiol Endocrinol Metab 2002 May [cited 282 5]; E1084-91].

12.   Chinookoswong, N., J.L. Wang, and Z.Q. Shi, Leptin restores euglycemia and normalizes glucose turnover in insulin-deficient diabetes in the rat. Diabetes, 1999. 48(7): p. 1487-92.

13.   Yaspelkis, B.B., 3rd, et al., Leptin administration improves skeletal muscle insulin responsiveness in diet-induced insulin-resistant rats. Am J Physiol Endocrinol Metab, 2001. 280(1): p. E130-42.

14.   Liu, L., et al., Intracerebroventricular leptin regulates hepatic but not peripheral glucose fluxes. J Biol Chem, 1998. 273(47): p. 31160-7.

15.   Barzilai, N., et al., Leptin selectively decreases visceral adiposity and enhances insulin action. J Clin Invest, 1997. 100(12): p. 3105-10.

16.   da Silva, A.A., et al., Chronic antidiabetic and cardiovascular actions of leptin: role of CNS and increased adrenergic activity. Am J Physiol Regul Integr Comp Physiol, 2006. 291(5): p. R1275-82.

17.   Thurman, R.G. and R. Scholz, Interaction of mixed-function oxidation with biosynthetic processes. 2. Inhibition of lipogenesis by aminopyrine in perfused rat liver. Eur J Biochem, 1973. 38(1): p. 73-8.

18.   Miller, L.L., et al., The dominant role of the liver in plasma protein synthesis; a direct study of the isolated perfused rat liver with the aid of lysine-epsilon-C14. J Exp Med, 1951. 94(5): p. 431-53.

19.   Adams, M.D., P. Raman, and R.L. Judd, Comparative effects of englitazone and glyburide on gluconeogenesis and glycolysis in the isolated perfused rat liver. Biochem Pharmacol, 1998. 55(11): p. 1915-20.

20.   Peterson, J.I. and D.S. Young, Evaluation of the hexokinase-glucose-6-phosphate dehydrogenase method of determination of glucose in urine. Anal Biochem, 1968. 23(2): p. 301-16.

21.   Allison, D.B., et al., The use of areas under curves in diabetes research. Diabetes Care, 1995. 18(2): p. 245-50.

22.   Leroy, P., et al., Expression of ob gene in adipose cells. Regulation by insulin. J Biol Chem, 1996. 271(5): p. 2365-8.

23.   Rentsch, J. and M. Chiesi, Regulation of ob gene mRNA levels in cultured adipocytes. FEBS Lett, 1996. 379(1): p. 55-9.

24.   Yoshida, T., et al., Regulation of obese mRNA expression by hormonal factors in primary cultures of rat adipocytes. Eur J Endocrinol, 1996. 135(5): p. 619-25.

25.   Saladin, R., et al., Transient increase in obese gene expression after food intake or insulin administration. Nature, 1995. 377(6549): p. 527-9.

26.   Mizuno, T.M., et al., Obese gene expression: reduction by fasting and stimulation by insulin and glucose in lean mice, and persistent elevation in acquired (diet-induced) and genetic (yellow agouti) obesity. Proc Natl Acad Sci U S A, 1996. 93(8): p. 3434-8.

27.   Cusin, I., et al., The ob gene and insulin. A relationship leading to clues to the understanding of obesity. Diabetes, 1995. 44(12): p. 1467-70.

28.   Zheng, D., et al., Differential expression of ob mRNA in rat adipose tissues in response to insulin. Biochem Biophys Res Commun, 1996. 218(2): p. 434-7.

29.   Hardie, L.J., et al., Circulating leptin levels are modulated by fasting, cold exposure and insulin administration in lean but not Zucker (fa/fa) rats as measured by ELISA. Biochem Biophys Res Commun, 1996. 223(3): p. 660-5.

30.   Pratley, R.E., et al., Insulin increases leptin mRNA expression in abdominal subcutaneous adipose tissue in humans. Mol Genet Metab, 2000. 70(1): p. 19-26.

31.   Havel, P.J., et al., Marked and rapid decreases of circulating leptin in streptozotocin diabetic rats: reversal by insulin. Am J Physiol, 1998. 274(5 Pt 2): p. R1482-91.

32.   Bessems, M., et al., The isolated perfused rat liver: standardization of a time-honoured model. Lab Anim, 2006. 40(3): p. 236-46.

33.   Gores, G.J., L.J. Kost, and N.F. LaRusso, The isolated perfused rat liver: conceptual and practical considerations. Hepatology, 1986. 6(3): p. 511-7.

34.   Nemecz, M., et al., Acute effect of leptin on hepatic glycogenolysis and gluconeogenesis in perfused rat liver. Hepatology, 1999. 29(1): p. 166-72.

35.   Ceddia, R.B., et al., Acute effects of leptin on glucose metabolism of in situ rat perfused livers and isolated hepatocytes. Int J Obes Relat Metab Disord, 1999. 23(11): p. 1207-12.

36.   Anderwald, C., et al., Short-term leptin-dependent inhibition of hepatic gluconeogenesis is mediated by insulin receptor substrate-2. Mol Endocrinol, 2002. 16(7): p. 1612-28.

37.   Kamohara, S., et al., Acute stimulation of glucose metabolism in mice by leptin treatment. Nature, 1997. 389(6649): p. 374-7.

38.   Rossetti, L., et al., Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action. J Biol Chem, 1997. 272(44): p. 27758-63.

39.   Sivitz, W.I., et al., Effects of leptin on insulin sensitivity in normal rats. Endocrinology, 1997. 138(8): p. 3395-401.

40.   Raman, P., S.S. Donkin, and M.E. Spurlock, Regulation of hepatic glucose metabolism by leptin in pig and rat primary hepatocyte cultures. Am J Physiol Regul Integr Comp Physiol, 2004. 286(1): p. R206-16.

41.   Cimbala, M.A., et al., Rapid changes in the concentration of phosphoenolpyruvate carboxykinase mRNA in rat liver and kidney. Effects of insulin and cyclic AMP. J Biol Chem, 1982. 257(13): p. 7629-36.

42.   Granner, D., et al., Inhibition of transcription of the phosphoenolpyruvate carboxykinase gene by insulin. Nature, 1983. 305(5934): p. 549-51.

43.   Burcelin, R., et al., Acute intravenous leptin infusion increases glucose turnover but not skeletal muscle glucose uptake in ob/ob mice. Diabetes, 1999. 48(6): p. 1264-9.

44.   Gutierrez-Juarez, R., S. Obici, and L. Rossetti, Melanocortin-independent effects of leptin on hepatic glucose fluxes. J Biol Chem, 2004. 279(48): p. 49704-15.

45.   Groen, A.K., et al., Control of gluconeogenesis in rat liver cells. I. Kinetics of the individual enzymes and the effect of glucagon. J Biol Chem, 1983. 258(23): p. 14346-53.

46.   She, P., et al., Phosphoenolpyruvate carboxykinase is necessary for the integration of hepatic energy metabolism. Mol Cell Biol, 2000. 20(17): p. 6508-17.

47.   Groen, A.K., et al., Control of gluconeogenesis in rat liver cells. Flux control coefficients of the enzymes in the gluconeogenic pathway in the absence and presence of glucagon. Biochem J, 1986. 237(2): p. 379-89.

48.   Exton, J.H. and C.R. Park, Control of gluconeogenesis in liver. 3. Effects of L-lactate, pyruvate, fructose, glucagon, epinephrine, and adenosine 3',5'-monophosphate on gluconeogenic intermediates in the perfused rat liver. J Biol Chem, 1969. 244(6): p. 1424-33.

49.   Jitrapakdee, S. and J.C. Wallace, Structure, function and regulation of pyruvate carboxylase. Biochem J, 1999. 340 (Pt 1): p. 1-16.

50.   Yu, X., et al., Making insulin-deficient type 1 diabetic rodents thrive without insulin. Proc Natl Acad Sci U S A, 2008. 105(37): p. 14070-5.

Chapter 4

Chronic effects of leptin on glucose production from lactate in isolated rat hepatocyte suspensions and primary cultures

Abstract   To further understand the effect of intracerebroventricular (ICV) leptin on the role of leptin in hepatic glucose metabolism, we isolated liver cells from leptin- and vehicle-treated diabetic rats, and compared the ability of these cells to produce glucose in the presence of a gluconeogenic precursor in the freshly prepared or cultured state. Wistar rats were implanted with an ICV cannula directed into the lateral ventricle. Following recovery, rats were made diabetic with an IV injection of streptozotocin (STZ). After confirmation of hyperglycemia, rats were administrated a daily ICV bolus injection of either leptin (5 microgram/day) or vehicle. Blood glucose concentrations were determined daily. After the blood glucose concentration of the leptin-treated rats returned to normal, each rat was fasted (vehicle-treated rats for 16 hours, leptin-treated rats for 6 hours) and baseline fasting blood glucose concentrations were determined and then anesthetized with pentobarbital. The portal vein was cannulated to allow the inflow of perfusion media. The thoracic inferior vena cava was cannulated to allow for the collection of the outflow from the liver. Livers were perfused with digestion media containing collgenase and hepatocytes isolated. Hepatocytes were incubated with various concentrations of lactate to determine the gluconeogenic capacity of cells derived from leptin- and vehicle treated rats. Some cells were incubated as cell suspensions

immediately after isolation. Other cells were cultured onto 60 mm collagen-coated plates and treated the next day. Both freshly prepared and primary cultured isolated hepatocytes were treated with different concentrations of lactate (0.5, 1, 2, 5, 10 mM, final concentration). Freshly prepared cells were incubated for 30 minutes, while cultured cells were incubated for 5 hrs. Media was then collected and glucose concentrations were determined enzymatically. We found that compared to the control group, in the presence of different concentration of lactate (1mM, 2mM, 5mM and 10mM) the production of glucose by the primary cultured hepatocytes in leptin-treated group was significantly reduced 41 %, 44 %, 53 % and 58 %, respectively. However, glucose production in the freshly prepared liver cells was significantly higher in cells derived from leptin-treated rats as compared with vehicle-treated rats treated with higher lactate doses. In conclusion, leptin is capable of reducing glucose production from lactate in isolated hepatocytes.

4.1 Introduction

Leptin, the product of the *obese (ob)* gene, is produced primarily in white adipose tissue and serves as a peripheral signal to the central nervous system of nutritional status [1-2]. Leptin plays an important role in regulating energy stores and in the choice of fuels to be used under various nutritional conditions [3]. Besides its role in the control of body weight and regulation of energy expenditure, leptin also influence glucose metabolism particularly in the liver, adipose tissue, heart and skeletal muscle [4-5].

Glucose homeostasis requires the coordinated actions of various organs. In particularly, the liver plays a vital role in blood glucose homeostasis by maintaining a

balance between the uptake and storage of glucose and also releasing glucose as fuel for various organs to use. About 90% of endogenous glucose production is derived from liver and as much as 40% of alimentary glucose is stored as glycogen in liver. A progressive loss in these liver functions is associated with the deterioration of glycemic control and the eventual development of diabetes [6].

Leptin regulates glucose metabolism via two distinct mechanisms, one is through the central nervous system and another is through leptin receptors widely distributed in peripheral tissues including liver, skeletal muscle, adipose tissue and kidney [4-5]. Our laboratory, as well as others, has shown that chronic ICV leptin injections normalize blood glucose concentrations without increasing serum leptin and insulin concentrations [3, 7-8]. Besides central effect of leptin action on glucose metabolism, it also has been reported leptin exerts a direct effect on glucose metabolism in skeletal muscle and also in isolated adipocytes [4-5]. However, both *in vivo* and *in vitro* studies have demonstrated conflicting results regarding the role of leptin in hepatic glucose metabolism. Chronic peripheral leptin administration restores blood glucose concentrations in STZ - treated diabetic rats [9]. In euglyceimc hyperinsulinemia clamp studies, an acute increase in plasma leptin enhances the inhibitory effect of insulin on hepatic glucose production [10]. In the presence of leptin (100 ng/ml) for 60 minutes, the glucose production by isolated hepatocytes of fasted rats in the presence of different gluconeogeneic precursors is significantly decreased [11-12]. However, *in vitro* studies with different cell lines such as human and rat hepatoma cells HepG2, HepG3 and H4II-E have demonstrated that leptin attenuates several insulin-induced effects [13]. Studies in rat H-35 and human HepG2 hepatoma cell lines have also reported that leptin did not cause any significant alteration

in insulin effects on these cells, therefore not contributing to the diabetic symptoms associated with obesity [14].

Freshly isolated and cultured hepatocytes, considered as a useful tool *in vitro* system for hepatic disease study, have been extensively used in basic research of liver disease, pathophysiology, pharmacology and other related area [15]. Since the role of leptin on hepatic gluconeogenesis is not yet clear, especially the central effect, the present study was aimed to delineate the central effect of leptin on hepatic glucose production, independent of glucose utilization. We isolated hepatocytes from leptin- and vehicle-treated diabetic rats, and compared the ability of these cells to produce glucose in the presence of lactate in freshly prepared or cultured state.

4.2 Materials and methods

Animals   Male, Wistar rats (225-300g; Harlan, Indianapolis,IN) were housed in individual wire mesh cages before and during experiments. The room temperature was held constant (23°C ± 3) with a 12:12 h light: dark cycle. Animals were provided standard pellet rat chow and water ad libitum. Experimental protocols were approved by Auburn University's Institutional Animal Care and Use Committee prior to initiation of the experiments.

Experimental design   Rats were implanted with an ICV cannula in the lateral ventricle of the brain under ketamine/xylazine anesthesia. All rats were made diabetic with an intravenous injection of STZ (50mg/kg). After hyperglycemia of the rats was confirmed, half of the rats were given daily ICV bolus injections of leptin (5 microgram/day). The

other half were given daily ICV injections of vehicle. Blood glucose concentrations were determined daily. After the blood glucose concentration of the leptin-treated rats returned to normal, each rat was fasted (vehicle-treated rats for 16 hours, leptin-treated rats for 6 hours) and baseline fasting blood glucose concentrations were determined. (Leptin-treated rats were fasted for less time to prevent severe hypoglycemia. Previously, we found a 6-hour fast to cause a 50% decrease in the blood glucose concentration [3].).

Cannula implantation   After being anesthetized by an intraperitoneal injection of ketamine-xylazine (100 mg/kg ketamine and 1 mg/kg xylazine), animals were implanted with a 22-gauge, stainless steel guide cannula (Plastics One, Roanoke, VA) into the lateral ventricle of the brain using the following coordinates: 0.8 mm posterior to bregma, 1.4 mm lateral to the midline, and 3.5 mm ventral to the surface of the skull. After cannula implantation, the rats were placed individually in the hanging cages and allowed to recover for about 2 days. After that, cannula placement was confirmed by a positive drink test through Angiotensin II administration (40 ng/5 μl) [3].

Induction of diabetes   Insulin-deficient diabetes was produced by an intravenous injection of freshly prepared streptozotocin (STZ) (50 mg/kg; Sigma, St. Louis, MO) in 0.05 M citrate buffer (pH 4.5). STZ- treated rats whose blood glucose concentrations were > 400 mg/dl in a fed state were considered to be diabetic.

Blood glucose concentration determination   Blood was sampled from the tail vein. Blood glucose concentrations were monitored using an Accu-Chek Active glucometer

(Roche Diagnostics).

Isolation of rat liver cells   The technique of hepatocytes isolation used the liver
perfusion system described by Thurman and Scholz (1973), Miller et al. (1951) and
Adams et al. (1998) [16-18]. STZ-treated rats, fasted, but allowed free access to water
(vehicle-treated rats for 16 hours, leptin-treated rats for 6 hours), were anesthetized with
pentobarbital sodium (50 mg/kg) prior to liver perfusion. The abdomen was opened
through a longitudinal incision. The portal vein was cannulated with a 16-gauge stainless
steel catheter and a 14-gauge catheter was inserted into the thoracic inferior vena cava.
400 ml Oxygenated calcium-free perfusion buffer (g/L 8.3 NaCl, 0.5 KCl, 2.4 HEPES
and adjusted to pH 7.6 with 1 N NaOH) was perfused via the hepatic portal vein. The
effluent was collected via the thoracic inferior vena cava. Liver was perfused at a flow
rate of 35 ml/min for 10 minutes to flush the blood from the organ. Then the perfusion
medium was changed to 100 ml of digestion buffer (g/L 3.5 NaCl, 0.5 KCl, 0.7
$CaCl_2 \cdot 2H_2O$, 24 HEPES and adusted to pH 7.6 with1 N NaOH) first to flush the
perfusion buffer from the organ and then the media was changed to another 100 ml
digestion buffer containing collagenase (Liberase TM, Roche Applied Science (0.04
mg/ml). The effluent was recirculated to the reservoir after 2 minutes. The perfusion was
stopped after approximately 6 minutes of recirculation and the liver was carefully
transferred to a petri dish containing approximately 50 ml of the incubation buffer (g/L
6.7 NaCl, 0.44 KCl, 0.142 $MgSO_4$, 0.171 NaH_2PO_4, 0.191 $CaCl_2*2H_2O$, 4.77 HEPS, 2.1
$NaHCO_3$ adusted to pH 7.6 with 1 N NaOH). The connective capsule of the liver was
stripped and the liver was shaken to dissociate the cells into the medium. The incubation

media was brought up to a volume of 200 ml and the cells were incubated for 20 minutes at 37° C with gentle shaking. Cells were then be filtered through a polyethylene mesh. The filtered cell suspension was distributed equally into four 50 ml centrifuge tubes and centrifuged at 20 x g (4 °C) for five minutes. The supernatant was discarded and the cells were resuspended in wash buffer (g/L 8.3 NaCl, 0.5 KCl, 2.4 HEPES, 0.18 $CaCl_2*2H_2O$ adjust to pH 7.4 with 1 N NaOH) and centrifuged as described above. After three wash cycles, the cells were resuspended in 20 ml of incubation buffer. Cells numbers were determined with a hemocytometer and % viability was determined by trypan blue exclusion. The cell suspensions from each liver were then used to determine the glucose production from lactate in liver cells from cell suspensions and cell cultures.

Glucose production from cell suspension   Cells derived from each liver were dispensed into eighteen 25-ml erlenmeyer flasks (5 million cells/flask). A dose-response curve with various concentrations of lactate (0, 0.5, 1, 2, 5, 10 mM, final concentration) was run in triplicate to determine lactate-induced glucose production in hepatocytes from leptin- or vehicle-treated diabetic rats. Rubber stoppers were placed in the flasks and an atmosphere of 95% O_2 – 5% CO_2 was introduced. All flasks were placed in a metabolic incubator (37°C, 60 oscillations/min). After 30 minutes of incubation, the media were collected and glucose concentrations determined enzymatically (see below). Glucose production in the presence of lactate was considered total glucose production, while glucose production in the absence of lactate was considered glucose production derived from glycogenolysis. Glucose production via gluconeogenesis was calculated as total glucose production minus glucose production derived from glycogenolysis.

Glucose production from cell culture Some of the cells from each rat liver were cultured onto twelve 60 mm collagen-coated plates. Cells were diluted in Willison's E Plating buffer containing 10% fetal bovine serum (FBS) and plated at a density of 2 million cells/plate. Culture plates were placed in cell incubator at 37°C in a humidified atmosphere containing 95% O_2 and 5% CO_2. After 4 hours, the buffer were removed and replaced with fresh William's E Plating buffer containing 10% FBS. After an overnight incubation, the cells were washed in phosphate-buffered saline, and pretreated with serum free William's E media for 3 hours. Different concentrations of lactate (0, 0.5, 1, 2, 5, 10 mM, final concentration) in duplicate were then added to the cells and incubated for an additional 5 hours in incubation buffer (see above). Culture media was collected and glucose concentrations were determined enzymatically (see below). Total glucose production and glucose production from gluconeogenesis and glycogenolysis was determined as described above.

Glucose assay Glucose concentrations of in the cell media from isolated hepatocytes were determined using the hexokinase-glucose-6-phosphate dehydrogenase enzymatic assay as described previously [19], and expressed as μg glucose released per ml [19].

Statistical analysis All statistical analyses were performed with the SAS 9.1 (SAS Institute Inc., Chicago, IL, USA). Data were presented as means ± SEM. Two-way analysis of variance (ANOVA) with replication were utilized to test the main effects of leptin treatment, and various concentrations of lactate treatments on glucose output in the fresh isolated hepatocytes and primary-cultured hepatocytes experiments. Multiple

comparisons of means and two sample *t*-test were performed by the Bonferroni test to assess effects of various lactate concentrations on glucose production in the fresh isolated hepatocytes and primary-cultured hepatocytes experiments. Differences at $P \leq 0.05$ were considered significant.

4.3 Results

Chronic leptin administration restored blood glucose concentration to normal level

Basal blood glucose level was determined one day after STZ treatment. Blood glucose levels from animals higher than 400 mg/dl in a fed state were considered to be diabetic. The non-fasting blood glucose concentrations of diabetic rats averaged 451 ± 21 mg/dl before the daily treatment with central leptin began. Figure 4.1 shows the daily blood glucose concentration of STZ-induced diabetic rats given chronic ICV leptin (5 microgram/day) or vehicle. The blood glucose concentration of the leptin-treated diabetic rats decreased dramatically after the 3th day of ICV leptin injection ($P < 0.01$) as compared to vehicle-treated rats and remained close to normal level (161 ± 15 mg/dl) throughout the remained of the study.

Glucose production in freshly isolated hepatocytes

Chronic central leptin administration reduces the hyperglycemia in STZ-induced diabetic rats in a period of several days. In the previous study (chapter 3), we examined the inhibitory effect of leptin on hepatic glucose metabolism using the isolated rat liver perfusion system. Leptin's inhibitory effect on hepatic glucoenogenesis appears to account for at least part of leptin's action on body glucose concentrations. Firstly, to

investigate whether the central leptin-induced effects on the liver of the whole animal is also maintained in freshly isolated and cultured hepatocytes, we measured glucose production of isolated hepatocytes with various concentrations of lactate treatments. Since the animals were fasted prior to the collection of the liver cells, we expected glucose production from glycogenolysis to be low in both leptin- and vehicle-treated groups. Moreover, since glucose production in the absence of lactate is considered to be derived from glycogenolysis, we subtracted the glucose production of lactate-treated groups of the basal group (no lactate) to calculate the lactate-induced glucose production derived from gluconeogeneis. Since there is no significant difference between leptin- and vehicle-treated groups (13.434 ± 3.7 vs. 21.45 ± 5 µg glucose/ml), which represents no significant difference in the rate of glycogenolysis between hepatocytes derived from leptin- and vehicle-treated rats.), and the basal condition (without lactate) was set to zero. Therefore, the values of glucose production shown in the figures represent lactate-induced gluconeogenesis. Increasing the concentration of lactate caused an increase in the total glucose production from freshly prepared liver cells up to a concentration of 5 mM lactate. In the freshly prepared cells, no significant difference in total glucose production was found between cells derived from leptin- and vehicle-treated rats at any of the lactate concentrations. However, when lactate-induced gluconeogenesis was examined, glucose production was greater in hepatocytes derived from leptin-treated rats at the higher doses of lactate (2 mM, 5 mM and 10 mM), increased by 37 %, 23 % and 27 %, respectively (Figure 4.2).

Glucose production in primary cultured isolated hepatocytes

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Cultured hepatocytes were incubated in media with 10% fetal bovine serum. After exposure overnight to the media containing serum, the cells were serum-deprived for 3 hours before beginning the incubation with lactate. Significant differences in lactate-induced gluconeogenesis were found in the primary cultured hepatocytes derived from leptin-treated rats as compared with vehicle-treated rats. As shown in Fig. 4.3, compared to hepatocytes derived from vehicle-treated rats, the production of glucose in primary cultured hepatocytes derived from leptin-treated rats was significantly reduced in the presence of various concentration of lactate (1mM, 2mM, 5mM and 10Mm) by 41 %, 44 %, 53 % and 58 %, respectively (Figure 4.3).

4.4 Discussion

The present study demonstrates that the nutritional and metabolic state of the hepatocyte plays a key role in the degree of antigluconeogenic action of leptin. Our previous studies have shown that after a period of fasting (vehicle-treated rats for 16 hours, leptin-treated rats for 6 hours), the liver glycogen stores of the rat are almost completely depleted [18, 20]. Under such conditions of stimulated glycogen breakdown and negligible glycogen synthesis, the presence of gluconeogenic substrates results in the stimulation of the glucose production. We have examined in a preliminary study the blood glucose response of leptin-treated, streptozotocin-induced diabetic rats fed various gluconeogeneic diets. Results suggested that leptin may inhibit gluconeogenesis between the entry point of fructose (dihydroxyacetone phosphate/glyceraldehyde 3-phosphate) and amino acids (pyruvate, TCA cycle intermediates) into the gluconeogeneic pathway. However, this interpretation is complicated by any potential leptin-induced change in

peripheral glucose utilization. In the isolated perfused rat liver experiments (chapter 3), leptin blunted hepatic glucose production from lactate, but not fructose in STZ-induced diabetic rats. Therefore, we concluded that chronic central leptin administration inhibits hepatic gluconeogenesis in STZ-induced diabetic rats. To examine hepatic gluconeogenesis, independent of glucose utilization, we employed the isolated perfused liver model and isolated hepatocytes. When hepatocytes isolated from fasted diabetic leptin- or vehicle-treated rats were incubated with different concentrations of lactate, an inhibitory effect of leptin on hepatic glucose production (gluconeogensis) was observed in primary cultured hepatocytes. This finding suggests that central leptin administration has an inhibitory effect on hepatic glucose production independent of peripheral glucose utilization. However, this was not the finding in freshly prepared hepatocytes, in which lactate-induced glucose production was increased in leptin-treated rats compared to vehicle-treated rats at higher concentrations of lactate. What could be the difference between freshly prepared hepatocytes and cultured hepatocytes that could account for the different results with leptin treatment?

One possibility could be related to the fact that cultured hepatocytes were incubated with fetal bovine serum during the plating process. Serum contains basic components, such as hormones and growth factors. It is possible that the inhibitory effect of leptin on hepatic glucose production may be through inhibiting the action of some component in the serum. To exclude the possibility that residual insulin may mediate the effect of leptin action on hepatic glucose metabolism, serum insulin concentrations were measured in STZ-induced diabetic leptin-treated rats in a preliminary study. Compared with vehicle-treated normal rats, diabetic rats treated with vehicle or leptin treatment had

decreased and very low insulin levels [3, 21]. In addition, we have previously found that leptin reduces blood glucose concentration without increasing insulin sensitivity. This appears to argue against the possibility that leptin may exert its action on hepatic glucose metabolism though the potentiation of insulin. It is also possible that the leptin-mediated inhibitory effect on hepatic gluconeogenesis may be through the suppression of glucagon action.

Since glucagon is known to stimulate gluconeogenesis, hyperglucagonemia is present in the insulin-deficient state, and glucagon is largely responsibile for hepatic glucose overproduction in uncontrolled diabetes, it is possible that hepatocytes from leptin-treated rats are less responsive to glucagon as compared to vehicle-treated rats. This could be due to a reduction of serum glucagon concentrations or to an inhibition of glucagon signaling. Recent studies suggest leptin may control glycemia by inhibiting secretion of glucagon [22]. Yu et al. (2008) suggested that suppression of hyperglucagonemia contributes to the antidiabetic effect associated with leptin treatment in STZ-induced diabetic rats. Plasma glucagon concentrations significantly decline after the injection of Adv-leptin. Hyperleptinemia reduces hyperglucagonemia to normal levels and is associated with the suppression of p-CREB, PEPCK and PGC1α [21, 23]. However, under similar conditions as the present study, we did not find central leptin treatment to decrease serum glucagon levels in STZ-induced diabetic rats.

It is important to note that glucagon binds to the glucagon receptor, a G protein-coupled receptor located in the plasma membrane. So if in the freshly prepared hepatocytes, glucagon is depleted and leaves receptors unbounded located in the plasma membrane, leptin treatment may be unable to inhibit glucose production in hepatocytes in

the presence of lactate through an anti-glucagon effect. On the other hand, when hepatocytes were cultured with 10% fetal bovine serum, which included glucagon, ,hepatocytes derived from leptin-treated rats may have had a reduction in lactate-induced glucose production, compared with hepatocytes derived from vehicle-treated rats, due to anti-glucagon effect.

Our results suggest that chronic central leptin administration reduces hepatic glucose production in the cultured hepatocytes due to the inhibition of hepatic gluconeogenesis. However, this does not appear to be in agreement with the observation from our laboratory and those of others that leptin treatment increases PEPCK gene expression and PEPCK protein [10, 13]. It is well recognized that the expression of PEPCK gene is glucagon-dependent and the rate of transcription of the PEPCK gene is increased by glucagon (via cAMP) and glucocorticoids and is inhibited by insulin [25]. This suggests that the site of leptin-mediated inhibition of gluconeogenesis may be prior to PEPCK, like pyruvate carboxylase (PC). In a study conducted in isolated rat hepatocytes, both PC and pyruvate kinase were shown to play a greater role than PEPCK in determining the gluconeogenic flux. Similar results were obtained that PC rather than PEPCK was shown to be the primary rate-determining step for gluconeogenesis [26-29]. It has been reported that the hepatic PC activity in the STZ-induced rats is increased 2-fold over that of control rats and that a high plasma glucagon/insulin ratio may account for the increase in this enzyme activity (43). Our data are consistent with the possibility that central leptin treatment leads to an impairment of gluconeogenesis at the level of PC, even though PEPCK protein may be elevated.

Whatever the mechanisms are, these results indicate that central leptin administration can rescue uncontrolled diabetes at least in part by inhibiting hepatic gluconeogenesis. A determination of the mechanism of how this works will provide insights into potential new agents to replace or supplement insulin treatment of type 1 diabetes.
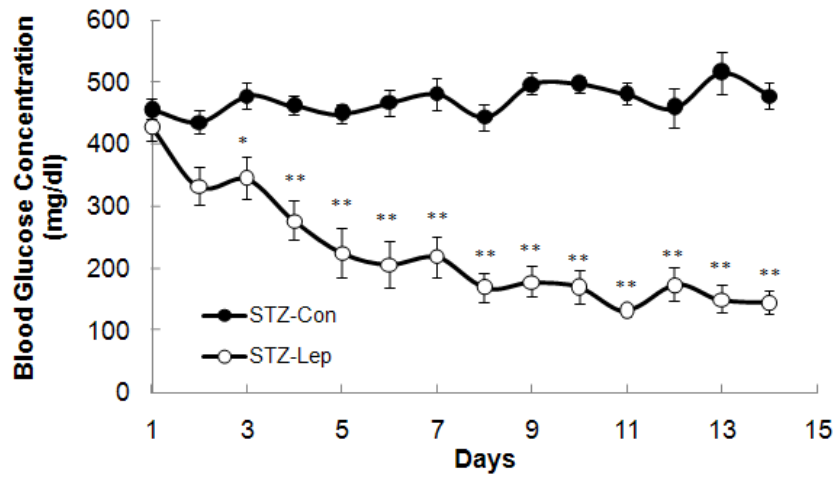
Figure 4.1. Effect of daily ICV leptin (○) or vehicle (●) on blood glucose concentration of diabetic rats. 1st day of ICV leptin (or vehicle) injection. Blood glucose concentration in diabetic rats was significantly decreased after 3 days of ICV leptin injection. Value are means ± SEM (leptin-treated n=11, vehicle-treated n=14). *$P<0.01$, ** $P<0.001$ vs. STZ-lep

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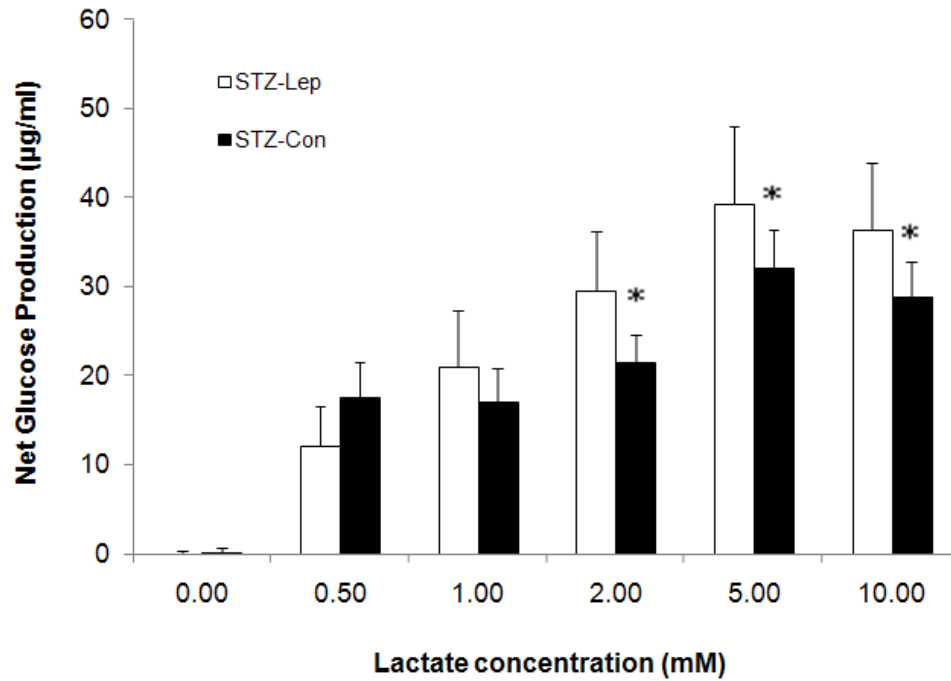
Figure 4.2. Effect of leptin on the net glucose production (gluconeogenesis) from freshly isolated hepatocytes derived from leptin- and vehicle-treated rats. Hepatocytes were incubated in the presence and absence of various concentrations of lactate. The hepatocytes were isolated from fasted rats. Data expressed as means ± s.e.m. (control group n=8, leptin-treated group n=7). *$P < 0.05$ vs. leptin
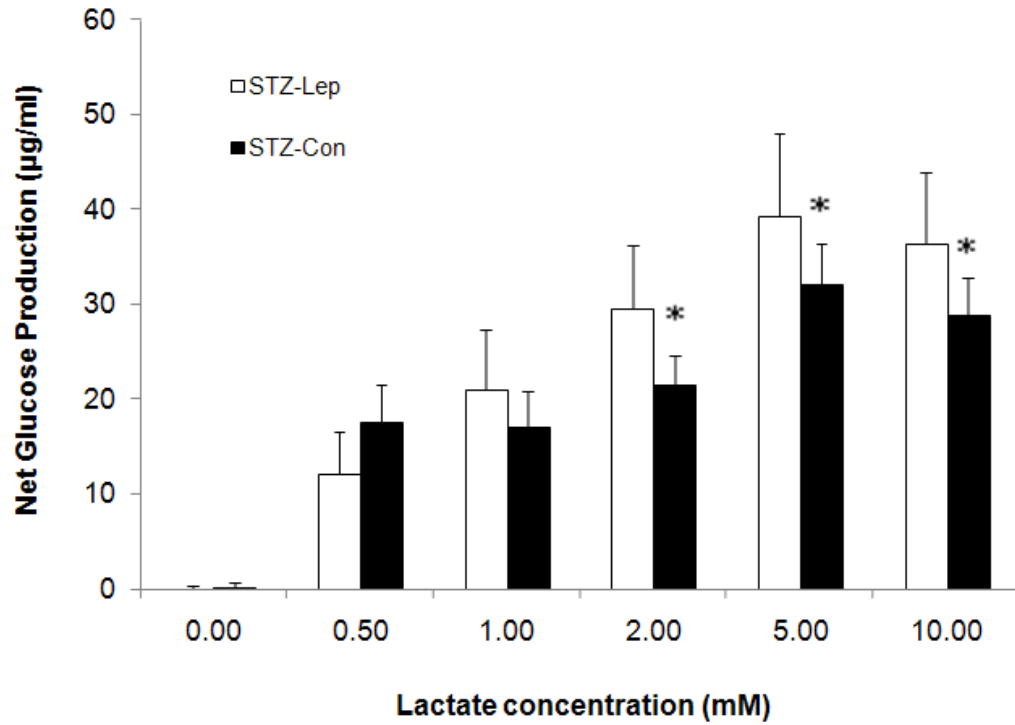
Figure 4.3. Effect of leptin on the net glucose production (gluconeogenesis) from cultured hepatocytes derived from leptin- and vehicle-treated rats. Hepatocytes were incubated in the presence and absence of various concentrations of lactate. The cultured hepatocytes were isolated from fasted rats. Data expressed as means ± s.e.m. (control group n=7, leptin-treated group n=7). *$P < 0.05$ vs. leptin

References

1. Halaas, J.L., et al., *Weight-reducing effects of the plasma protein encoded by the obese gene.* Science, 1995. **269**(5223): p. 543-6.

2. Zhang, Y., et al., *Positional cloning of the mouse obese gene and its human homologue.* Nature, 1994. **372**(6505): p. 425-32.

3. Lin, C.Y., et al. *Central leptin increases insulin sensitivity in streptozotocin-induced diabetic rats.* Am J Physiol Endocrinol Metab 2002 May [cited 282 5]; E1084-91].

4. Lee, G.H., et al., *Abnormal splicing of the leptin receptor in diabetic mice.* Nature, 1996. **379**(6566): p. 632-5.

5. Tartaglia, L.A., et al., *Identification and expression cloning of a leptin receptor, OB-R.* Cell, 1995. **83**(7): p. 1263-71.

6. Torres, T.P., et al., *Restoration of hepatic glucokinase expression corrects hepatic glucose flux and normalizes plasma glucose in zucker diabetic fatty rats.* Diabetes, 2009. **58**(1): p. 78-86.

7. Hidaka, S., et al., *Chronic central leptin infusion restores hyperglycemia independent of food intake and insulin level in streptozotocin-induced diabetic rats.* Faseb J, 2002. **16**(6): p. 509-18.

8. da Silva, A.A., et al., *Chronic antidiabetic and cardiovascular actions of leptin: role of CNS and increased adrenergic activity.* Am J Physiol Regul Integr Comp Physiol, 2006. **291**(5): p. R1275-82.

9. Chinookoswong, N., J.L. Wang, and Z.Q. Shi, *Leptin restores euglycemia and normalizes glucose turnover in insulin-deficient diabetes in the rat.* Diabetes, 1999. **48**(7): p. 1487-92.

10. Rossetti, L., et al., *Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action.* J Biol Chem, 1997. **272**(44): p. 27758-63.

11. Nemecz, M., et al., *Acute effect of leptin on hepatic glycogenolysis and gluconeogenesis in perfused rat liver.* Hepatology, 1999. **29**(1): p. 166-72.

12. Ceddia, R.B., et al., *Acute effects of leptin on glucose metabolism of in situ rat perfused livers and isolated hepatocytes.* Int J Obes Relat Metab Disord, 1999. **23**(11): p. 1207-12.

13.   Cohen, B., D. Novick, and M. Rubinstein, *Modulation of insulin activities by leptin.* Science, 1996. **274**(5290): p. 1185-8.

14.   Wang, Y., et al., *Leptin receptor action in hepatic cells.* J Biol Chem, 1997. **272**(26): p. 16216-23.

15.   Wang, Y.J., et al., *Primary hepatocyte culture in collagen gel mixture and collagen sandwich.* World J Gastroenterol, 2004. **10**(5): p. 699-702.

16.   Thurman, R.G. and R. Scholz, *Interaction of mixed-function oxidation with biosynthetic processes. 2. Inhibition of lipogenesis by aminopyrine in perfused rat liver.* Eur J Biochem, 1973. **38**(1): p. 73-8.

17.   Miller, L.L., et al., *The dominant role of the liver in plasma protein synthesis; a direct study of the isolated perfused rat liver with the aid of lysine-epsilon-C14.* J Exp Med, 1951. **94**(5): p. 431-53.

18.   Adams, M.D., P. Raman, and R.L. Judd, *Comparative effects of englitazone and glyburide on gluconeogenesis and glycolysis in the isolated perfused rat liver.* Biochem Pharmacol, 1998. **55**(11): p. 1915-20.

19.   Peterson, J.I. and D.S. Young, *Evaluation of the hexokinase-glucose-6-phosphate dehydrogenase method of determination of glucose in urine.* Anal Biochem, 1968. **23**(2): p. 301-16.

20.   Rigoulet, M., et al., *Stimulation by glucose of gluconeogenesis in hepatocytes isolated from starved rats.* Biochem J, 1987. **245**(3): p. 661-8.

21.   Yu, X., et al., *Making insulin-deficient type 1 diabetic rodents thrive without insulin.* Proc Natl Acad Sci U S A, 2008. **105**(37): p. 14070-5.

22.   Zhao, A.Z., et al., *Leptin induces insulin-like signaling that antagonizes cAMP elevation by glucagon in hepatocytes.* J Biol Chem, 2000. **275**(15): p. 11348-54.

23.   Unger, R.H., *Glucagon physiology and pathophysiology.* N Engl J Med, 1971. **285**(8): p. 443-9.

24.   Muller, G., et al., *Leptin impairs metabolic actions of insulin in isolated rat adipocytes.* J Biol Chem, 1997. **272**(16): p. 10585-93.

25.   Scott, D.K., et al., *The repression of hormone-activated PEPCK gene expression by glucose is insulin-independent but requires glucose metabolism.* J Biol Chem, 1998. **273**(37): p. 24145-51.

26. Groen, A.K., et al., *Control of gluconeogenesis in rat liver cells. I. Kinetics of the individual enzymes and the effect of glucagon.* J Biol Chem, 1983. **258**(23): p. 14346-53.

27. She, P., et al., *Phosphoenolpyruvate carboxykinase is necessary for the integration of hepatic energy metabolism.* Mol Cell Biol, 2000. **20**(17): p. 6508-17.

28. Groen, A.K., et al., *Control of gluconeogenesis in rat liver cells. Flux control coefficients of the enzymes in the gluconeogenic pathway in the absence and presence of glucagon.* Biochem J, 1986. **237**(2): p. 379-89.

29. Exton, J.H. and C.R. Park, *Control of gluconeogenesis in liver. 3. Effects of L-lactate, pyruvate, fructose, glucagon, epinephrine, and adenosine 3',5'-monophosphate on gluconeogenic intermediates in the perfused rat liver.* J Biol Chem, 1969. **244**(6): p. 1424-33.

Chapter 5

Conclusions

Uncontrolled hepatic glucose production contributes significantly to hyperglycemia in type 2 diabetic patients. Leptin plays important roles in the regulation of blood glucose homeostasis. Chronic central leptin treatment normalizes circulating blood glucose concentrations in diabetic rats. Leptin-treated rats had nearly a 4-fold lower glucose response to lactate gavage than did vehicle-treated rats, which was consistent with the finding that hepatic glucose production from infusion of lactate was severely blunted in leptin-treated rats as compared to rats that were vehicle-treated in isolated perfused rat liver study. However, the results of hepatic glucose production from fructose were different from the results from lactate. When fructose was used as the gluconeogenic precursor, there was no difference in the hepatic glucose production between leptin- and vehicle-treated rats.

In the isolated hepatocytes studies, in the presence of various concentration of lactate, we measured glucose production from gluconeogenesis in both freshly prepared and cultured hepatocytes. A consistent finding was exhibited in cultured hepatocytes, in that the lactate-induced production of glucose in cells derived from leptin-treated rats was significantly reduced by 40-58%. However, there is no significant difference in lactate-induced glucose production in the freshly prepared hepatocytes derived from leptin-

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treated rats as compared with vehicle-treated rats. This may reflect differences in how the hepatocytes were prepared prior to the incubation with lactate.

Overweight and obesity are the most common nutritional disorders in the United States, affecting the majority of adults in the country. Obesity, particularly central obesity, is a well established risk factor for insulin resistance and type 2 diabetes mellitus, as well as other features of the metabolic syndrome, such as dyslipidemia, cardiovascular disease and hypertension. While insulin is important for maintaining glucose homeostasis, adipokines such as leptin may enable the body to use insulin more efficiency. During conditions of either leptin resistance or leptin deficiency, the central leptin signal is inadequate, resulting in not only increased body weight or even obesity, but also resistance to circulating insulin. Therefore, this could be one of the reasons why obese people are at risk for developing diabetes. Through these studies, we have found that chronic central leptin inhibits hepatic gluconeogenesis to help control whole body glucose levels preventing the overproduction of glucose in diabetic rats. These results indicate that the uncontrolled diabetes can be rescued without insulin by leptin to eliminate hepatic overproduction of glucose. Determining the mechanism by which this occurs may lead to new therapeutic agents that help control diabetes, independent of or in conjunction with insulin.