Aus der Medizinischen Klinik und Poliklinik
der Albert Einstein College of Medicine of Yeshiva University
Vorstand: Dr. Allen M. Spiegel

Inhibiting Gluconeogenesis (GNG) Prevents the Effects of Free Fatty Acids (FFA) on Hepatic Glucose Effectiveness (GE)

Inaugural-Dissertation
zur Erlangung der Doktorwürde
der Medizinischen Fakultät
der
Julius-Maximilians-Universität zu Würzburg

Vorgelegt von
Sylvia Kehlenbrink
aus Frankfurt am Main

Würzburg, März 2010

Referent: Prof. Dr. B. Allolio

Koreferent: Prof. Dr. P.-G. Schlegel

Dekan: Prof. Dr. med. M. Frosch

Tag der mündlichen Prüfung: 11. Mai 2010

Die Promovendin ist Ärztin.

Table of Contents

1. Introduction

1.1. Type 2 Diabetes Mellitus

1.1.1. The Diabetes Epidemic- A Global Burden

Type 2 Diabetes Mellitus (T2DM) is increasingly becoming a major international health concern due to its rising incidence and its serious complications (1). According to the World Health Organization (WHO) an estimated 30 million people worldwide had diabetes in 1985. By 1995, just one decade later, this number increased to an estimated 135 million. The WHO currently estimates the number of people with diabetes worldwide at over 170 million. This number is expected to rise to 370 million people by the year 2030 (2). Each year about 3.2 million deaths are attributed to the serious complications of diabetes. The top ten countries with the most individuals suffering from diabetes are India, China, USA, Indonesia, Japan, Pakistan, Russia, Brazil, Italy, and Bangladesh. This causes great concern, given that much of the increase of diabetes will occur in developing countries, due to population growth, obesity, aging, sedentary lifestyles, and unhealthy diets. Whereas most diabetics in developed countries are above the age of retirement, the most frequently affected age group in developing countries is much younger- between 35 and 64 years- the most productive years of their lives. Additionally, type 2 diabetes mellitus is increasingly occurring at a younger age and in adolescents (3). Considering that type 2 diabetes accounts for about 90% of cases worldwide, many people will be affected.

In the United States an estimated 20.8 million people had diabetes in 2005 (approximately 7% of the population) (4). According to the Center for Disease Control and Prevention (CDC) the diagnosis was made in only about two thirds of those affected. In 2004 diabetes was the 6th most common cause of death in the United States and the 5th most common cause of death in New York City. It is currently the leading cause of adult blindness, end stage renal disease, and nontraumatic lower extremity amputation in the United States. Furthermore, T2DM is essentially the only major health problem in the U.S. that is rapidly getting worse, primarily due to rising obesity and reduced activity levels. Having recognized the gravity of the problem and the major burden T2DM imposes on the individual affected by it, as well as on the health care system, the New York City Board of Health recently took a step to better monitor this epidemic. Since January 2006 the glycosylated hemoglobin A1C (HbA1C) values are

required to be reported by laboratories to New York City's Department of Health and Mental Hygiene (4).

Through primary prevention, namely lifestyle modification, and secondary prevention, in particular control of blood glucose levels, a significant impact can be made on the incidence of diabetes and development of its complications (3). Hence, growing awareness and concern about diabetes and the possibility of preventing T2DM and its grave complications lead us to seek answers pertaining to its exact pathophysiology and most optimal treatment.

1.1.2. Diabetes Mellitus- A Brief Characterization

Diabetes mellitus (DM) is the term collectively used to describe a number of common metabolic disorders that share the phenotype of hyperglycemia. On the basis of a complex interaction between genetics, life-style choices, and environmental factors a number of diverse types of DM exist. DM is accompanied by numerous acute and chronic complications, which are caused by secondary pathophysiological changes in many organ systems due to the metabolic dysregulation (5).

A. Classification of Diabetes Mellitus

The classification of DM is based on the pathogenesis leading to hyperglycemia. The 2006 classification criteria issued by the American Diabetes Association (ADA) is as follows (6):

1. *Type 1 diabetes mellitus*

   Due to ß-cell destruction in the pancreas generally leading to absolute insulin deficiency.

2. *Type 2 diabetes mellitus*

   Due to variable degrees insulin deficiency, a progressive insulin secretory defect, and increased endogenous glucose production.

3. *Other specific types of diabetes due to other causes*

   Due to diseases of the exocrine pancreas (such as cystic fibrosis), genetic defects in ß-cell function, chemical or drug induced DM.

4. *Gestational diabetes mellitus (GDM)*

   Due to pregnancy.

T2DM, the most common and rapidly increasing type of diabetes, accounts for about 90% of all cases (3) and is the focus of this paper.

B. Diagnosis

Based on the current recommendations of the American Diabetes Association there are 3 different ways to diagnose diabetes mellitus in nonpregnant adults (6). Unless unequivocal symptoms of diabetes are present each test much be confirmed on a subsequent day.

1. Random blood glucose concentration of ≥ 11.1 mmol/l (200 mg/dl) *plus* symptoms of diabetes (polyuria, polydipsia, and weight loss)
2. Fasting plasma glucose (FPG) of ≥ 7.0 mmol/l (126 mg/dl)
3. 2-hour plasma glucose ≥ 11.1 mmol/l (200 mg/dl) following an oral glucose tolerance test (OGTT)

The FPG is the preferred diagnostic test for pregnant adults.

In addition to the above tests, a serum hemoglobin A1C (HbA1C) value of ≥ 6.5 % has most recently been recommended as a diagnostic marker for diabetes mellitus (7).

C. Pathophysiology of Type 2 Diabetes Mellitus

Glucose homeostasis is an intricate metabolic equilibrium between peripheral glucose uptake and utilization and hepatic glucose production. Various factors regulate this precise balance, of which insulin is the most important. Other hormones, such as glucagon, as well as neural input and metabolic signals are also a part of this regulation and together account for the integrated control of glucose utilization and supply. In nondiabetic individuals, low insulin levels in the fasting state increase glucose production by increasing glycogenolysis and gluconeogenesis. Similarly, glucagon stimulates glucose production by the liver and renal medulla (5).

Fasting hyperglycemia in T2DM is characterized by

1. Decreased peripheral glucose clearance,
2. Impaired insulin secretion, and
3. Increased hepatic endogenous glucose production (EGP) (8, 9).

1. Decreased peripheral glucose clearance

Postprandial hyperglycemia in T2DM is greatly due to decreased peripheral glucose clearance, or insulin resistance. The precise molecular mechanism of insulin resistance in T2DM has not been completely elucidated. However, insulin postreceptor defects are believed to play a key role. Current studies are focused on a phosphatidylinositol 3-kinase (PI3-kinase) signaling defect, which among other abnormalities, results in a decreased glucose transporter GLUT-4 translocation to the plasma membrane and thus decreased glucose uptake into the cell (5). Recent studies propose that elevated free fatty acids (FFA), which are commonly seen in obesity (71), may contribute in a number of ways to the pathogenesis of T2DM (10, 80). The impact of FFA on insulin resistance in particular could be linked to the ability of FFA to impair glucose utilization in skeletal muscle. FFA seem to directly influence the expression (118) and translocation (121) of skeletal muscle glucose transporters.

2. Impaired Insulin Secretion

In T2DM insulin secretion deteriorates gradually as the course of the disease progresses. To maintain normal glucose tolerance, insulin secretion is initially increased in response to insulin resistance. With time, however, a severe insulin secretory defect develops. The reason for this is still not well understood. However, the metabolic environment of diabetes may negatively affect pancreatic islet cell function. Paradoxically, chronic hyperglycemia impairs pancreatic islet cell function. This is termed 'glucose toxicity' and contributes to the worsening of hyperglycemia (11). Additionally, dietary fat and elevated FFA may worsen pancreatic islet cell function and induce ß-cell apoptosis ('lipotoxicity'), thereby decreasing insulin secretion (12, 13).

3. Increased hepatic endogenous glucose production (EGP)

Increased hepatic glucose production primarily accounts for fasting hyperglycemia and occurs early in the course of diabetes (8, 9, 14). As plasma glucose levels increase above ~140 mg/dl basal EGP progressively increases, significantly contributing to the worsening diabetic state in T2DM (8). In nondiabetic subjects (ND) EGP is suppressed by insulin (15, 16) and directly by hyperglycemia *per se* (17, 18, 19,

20, 21, 22), as both a doubling of plasma glucose (23) and a 4-5 fold increase in insulin levels (24) result in a 50% suppression of EGP. Since EGP is increased in T2DM, the ability of glucose to suppress EGP and increase its own utilization at basal insulin levels, termed *glucose effectiveness*, is clearly impaired. The phenomenon of glucose effectiveness is of great relevance to the regulation of glucose tolerance in T2DM (23). Therefore it shall be much the focus of this paper and further discussed in the following.

D. Complications of T2DM

Diabetes mellitus is associated with a number of serious complications, of which diabetic neuropathy is the most common (3). According to the WHO up to 50% of T2DM individuals are affected with neuropathy to some degree. In developed nations diabetes mellitus is the leading cause of peripheral neuropathy, the hallmark of which is distal symmetric sensorimotor polyneuropathy (25). Neuropathy is the major cause of impotence in diabetic men and can result in sensory loss and damage to the limbs. Another important and grave complication of diabetes is cardiovascular disease (CVD). Between 50% and 80% of deaths among people with diabetes result from heart disease (3). Primary culprits leading to CVD are poor glycemic control over the long term and other risk factors such as hypertension, smoking, elevated levels of blood lipids or cholesterol, and obesity. Diabetic retinopathy, one of the leading causes of visual disability and adult blindness, is an additional complication of diabetes. The WHO estimates that after 15 years of diabetes about 10% of people develop serious visual handicap and about 2% become blind (3). Furthermore, diabetic nephropathy develops in about one third of those affected with T2DM (2, 26) and is among the leading causes of kidney failure, which is related to the duration and severity of the disease and varies in frequency between populations (3). Finally, one of the major complications of diabetes is diabetic foot disease. It is the most common cause of nontraumatic lower limb amputation. About 15 percent of persons with diabetes develop foot ulcers (27). Ulceration is usually the result of advanced distal motor, sensory, and autonomic deficits as well as peripheral vascular disease (3, 25).

1.2. Glucose Effectiveness

Glucose effectiveness represents the ability of glucose *per se* to inhibit endogenous glucose production and increase glucose disposal in the nondiabetic

individual under basal insulin conditions. There are two components to glucose effectiveness that contribute importantly to glycemic homeostasis- a hepatic and a peripheral component. Each will be delineated in the following.

1.2.1. Hepatic Glucose Effectiveness

Hepatic glucose effectiveness is the ability of hyperglycemia *per se* to inhibit hepatic glucose output (EGP) in nondiabetic subjects independent of changes in insulin or other hormone levels. Both glucose production and peripheral glucose uptake are directly affected by acute elevations in plasma glucose levels. EGP is suppressed by elevations in plasma glucose levels independent of hormonal signals, which enables rapid modulation of plasma glucose levels. In nondiabetic individuals this contributes to the maintenance of euglycemia (28). More importantly, in T2DM subjects, 99% of glucose uptake following carbohydrate ingestion is due to glucose effectiveness, considering the minimal contribution of insulin to glucose uptake due to severe insulin resistance and depressed insulin secretion (23).

The two main pathways of EGP are glycogenolysis, the breakdown of glycogen stores, and gluconeogenesis (GNG), the *de novo* synthesis of glucose from 3-carbon precursors such as glycerol, lactate, and alanine. EGP occurs predominantly in the liver, with a renal contribution via GNG of only ~4-18% in overnight-fasted humans (29). Insulin exerts suppressive effects on EGP (15, 16). In addition, however, glucose *per se* can inhibit EGP in nondiabetic individuals (17, 18, 19, 20, 21, 22). Both a doubling of plasma glucose levels (23) and a 4-5 fold increase in insulin levels (24) result in a ~50% suppression of EGP.

A number of studies have demonstrated the specific effects of glucose *per se* on EGP in the absence of any hormonal effects using the 'pancreatic clamp' technique (17, 21, 40, 44). This technique uses infusions of somatostatin to inhibit all pancreatic hormone secretion, while replacing insulin and glucagon at fixed, basal infusion rates. Indeed, these studies verified the phenomenon referred to as 'glucose effectiveness', showing that hyperglycemia *per se* can inhibit EGP in nondiabetic individuals independent of changes in insulin or other hormonal signals.

Various groups have studied the mechanisms by which hyperglycemia *per se* inhibits hepatic glucose production. Rossetti et al. demonstrated that acute

hyperglycemia suppressed EGP in conscious rats by ~50% in the presence of basal plasma insulin and glucagon (30). This was due to a > 2 fold increase in the rate of 'glucose cycling', which is the net effect of glucose/glucose-6-phosphate cycling, and inhibition of glycogenolysis. These findings were confirmed in similar studies in the presence of higher insulin levels (~800 μU/ml) in normal mice (31). Furthermore, in overnight-fasted nondiabetic individuals the inhibitory effects of hyperglycemia *per se* on glycogenolysis also seem to be operative overnight (32). This primarily seems to be mediated through the inhibition of glycogen phosphorylase flux. Finally, it has been suggested that glucose-induced reductions in plasma FFA levels account for the majority of suppression of EGP by glucose in nondiabetic subjects (23).

1.2.2. Peripheral Glucose Effectiveness

The ability of hyperglycemia to stimulate glucose uptake under basal insulin conditions is termed peripheral glucose effectiveness. In nondiabetic individuals about 50% of glucose disposal following an oral glucose tolerance test (OGTT) is due to glucose effectiveness (23). Glucose enhances its own disposal via 3 different mechanisms: mass action, transporter recruitment, and enzyme activation. These 3 mechanisms are synergistic and together exert multiplicative effects to enhance glucose utilization. By law of mass action, hyperglycemia at basal insulin levels increases flux of glucose across capillary endothelium and into cells primarily through the insulin-independent GLUT-1 and GLUT-2 glucose transporters located on various cell membranes, and to a small degree though insulin-dependent GLUT-4 glucose transporters (33, 34). Glucose uptake by the brain is already saturated at the fasting glucose level, therefore the central nervous system contributes little or nothing to glucose effectiveness (35, 36). Muscle tissue, however, would be expected to greatly increase glucose uptake with hyperglycemia, considering it contains GLUT-1 transporters and constitutes about 50% of body mass (23).

Studies have shown that a second mechanism by which hyperglycemia may mediate peripheral glucose disposal is by mimicking insulin action, directly stimulating GLUT-4 glucose transporter translocation to the surface of skeletal muscle. GLUT-4 transporter abundance at the cell membrane was doubled by hyperglycemia without hyperinsulinemia in a study by Galante et al. (37). Moreover, a study by Nolte et al.

demonstrated that there are mechanistic differences between glucose-dependent and insulin-dependent activation of glucose transport (38). Insulin acts via a PI3-kinase mediated mechanism, as opposed to glucose which acts via a calcium-dependent mechanism.

The third pathway which contributes to peripheral glucose effectiveness is the activation of certain key enzymes by glucose. Glucokinase, for example, is a hepatic glucose-dependent enzyme which is rate limiting for hepatic glucose uptake and consequently glycogen synthesis (39). Independent of changes in insulin whole-body glucose disposal would be affected in response to fluxes in hepatic glycogen production and degradation (23).

1.2.3. Loss of glucose effectiveness in T2DM

The effect of hyperglycemia *per se* to inhibit EGP is clearly impaired in T2DM (17, 40, 41). Also, the ability of hyperglycemia to stimulate glucose uptake has been shown to be blunted in T2DM by a number of investigators using the 'pancreatic clamp' technique (42, 43, 44). This in vivo technique uses infusions of somatostatin to inhibit all pancreatic hormone secretion, replacing insulin and glucoregulatory hormones at fixed levels. Interestingly, this loss of glucose effectiveness seems to be directly proportional to the metabolic abnormalities in T2DM. Individuals with moderate-to-poorly controlled T2DM lack this regulation (17, 40). On the other hand, glucose effectiveness is retained in diabetic subjects with optimal glycemic control (40, 41, 44). In a study by Nagasaka et al. stimulation of glucose uptake by glucose was preserved in a group of subjects with T2DM in good control (mean HbA1c= 6.6%) (41). Additionally, 72 hours of intense insulinization has been shown to completely restore glucose effectiveness together with normalization of plasma FFA levels (40). It is important to note that there was no relationship between basal insulin requirements or body mass index (BMI) with this defect, thus confirming that this is an entirely independent phenomenon from insulin resistance. Consequently, it is possible that regulation of glucose fluxes by hyperglycemia may take place both in nondiabetic and T2DM individuals (44), but may be activated at a higher set point in moderate-to-poorly controlled T2DM.

One possible explanation for the loss of glucose effectiveness with chronic hyperglycemia may be the decreased availability of glucose transporters at the plasma membrane, making glucose less able to stimulate its own uptake by mass action (38). It has been proposed that glucose stimulates its own disposal in skeletal muscle cells via direct activation of the insulin receptor kinase, most likely due to cytosolic translocation of protein kinase C (PKC)- alpha (45). In the presence of chronic hyperglycemia this postulated direct effect of glucose may be downregulated.

1.3. Hepatic Autoregulation of Glucose Fluxes

As mentioned previously, the two main pathways of glucose production by the liver are gluconeogenesis (GNG) and glycogenolysis. Via these two pathways the liver responds directly to fluxes in plasma glucose levels to maintain normoglycemia. When sufficient glycogen stores are available, it appears that changes in the rate of glycogenolysis play a primary role in the hepatic autoregulatory response in nondiabetic individuals. However, the rate of GNG can also be altered in response to changes in plasma glucose levels (46).

In T2DM GNG is the pathway inappropriately increased, contributing to the high total rates of EGP (47, 48). However, the overall rates of EGP are not affected by isolated changes in GNG when insulin is kept at least at physiologic levels because of compensatory changes in glycogenolysis (101, 102, 103, 104, 105). A study by Trimmer et al. showed that increasing plasma glycerol levels in nondiabetic individuals both at rest and during exercise stimulated GNG (49). Importantly, however, neither overall EGP, nor an exercise-induced decrease in blood glucose levels, were affected by this increase in GNG. Similarly, in a study by Jenssen et al. there was no increase in overall EGP or fasting plasma glucose levels after infusing nondiabetic subjects with sodium lactate under fixed hormonal conditions (101). Consequently, in most physiological settings in nondiabetics or T2DM subjects, marked decreases or increases in GNG alone are not sufficient to alter EGP.

The phenomenon of hepatic ‘autoregulation’ points to the significance of the intracellular glucose-6-phosphate (Glc-6-P) pool, which is the relative flux through the two key hepatic enzymes glucokinase (GK) and glucose-6-phosphatase (G-6-Pase) which regulate the rate of hepatic glucose production. Glc-6-P seems to play a central

role in the reciprocal regulation of the pathways of glucose production, since it appears to regulate hepatic glycogenolysis through the inactivation of the phosphorylase enzyme, which catalyzes the breakdown of glycogen (50). Animal models with increased hepatic G-6-Pase expression (51, 52, 81) and decreased hepatic glucokinase activity (53, 84) demonstrate increased rates of EGP which are not appropriately regulated by hyperglycemia. T2DM is associated with increased activity of G-6-Pase and decreased activity of GK (69, 70), which would both decrease the intracellular Glc-6-P pool, thereby removing an important inhibitory signal to EGP. Therefore, it is probable that these impairments contribute to the inability of hyperglycemia *per se* to inhibit EGP appropriately in T2DM (54).

1.4. Circulating Free Fatty Acid Levels in T2DM and Glucose Effectiveness

The metabolic environment characteristic of T2DM is associated with elevated fasting plasma FFA levels (10, 71). Also, postprandial FFA levels have been found to be higher in subjects with insulin resistance (55) and T2DM (56, 71). The plasma FFA concentration results from the balance between uptake and release, through lipolysis of adipose tissue stores and intravascular lipolysis of triglyceride-rich lipoproteins (10). Adipose tissue release and storage of fatty acids, as well as lipolysis and triglyceride storage in insulin-sensitive tissues are severely abnormal and detectable in insulin resistance syndrome (IRS) even before the complete development of T2DM (10). Furthermore, as glycemic control in T2DM deteriorates, plasma FFA levels rise proportionately (58). These elevations in plasma FFA levels are due to adipocyte resistance to both glucose and insulin.

Although the negative impact of elevated plasma FFA levels on the effects of insulin have been recognized (10, 91), the effects of increased plasma FFA on glucose effectiveness have not been well established. Best et al. have suggested that the ability of glucose to suppress lipolysis, thereby reducing plasma FFA levels, may account for the majority of hepatic EGP suppression by glucose in the nondiabetic individual (23). The results of a study by Hawkins et al., in which T2DM subjects with optimal glycemic control displayed normal suppression of FFA levels by hyperglycemia and retained normal glucose effectiveness compared, favor this idea (40). Hence, increased plasma FFA levels appear to have stimulatory effects on EGP and be at least in part

accountable for the inability of moderate-to-poorly controlled T2DM individuals to adapt their hepatic glucose output to increasing glucose levels.

It has been demonstrated that increased plasma FFA stimulate GNG (72, 73, 105). A number of biochemical reactions distinguish the pathway of GNG from that of glycolysis, overcoming the energy barriers hindering a direct reversal of glycolysis. The enzymes responsible for catalyzing these reactions are pyruvic carboxylase, phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase, and glucose-6-phosphatase (72). There are several mechanisms which may account for the stimulatory effects of FFA on GNG:

1. Increased gene expression of PEPCK (73) and
2. Increased production of the following gluconeogenic regulators:
    a. Fructose-1,6-bisphosphatase (97)
    b. Fatty acyl Co-A and acetyl Co-A, which allosterically activate pyruvic carboxylase (72, 98)
    c. Citrate, which inhibits phosphofructokinase-1 (99, 100)
    d. Adenosine triphosphate (ATP), an energy source for GNG (63)
    e. Nicotinamide adenine dinucleotide (NADH), which enhances the conversion of 1,3-bisphosphoglycerate to glyceraldehyde-3-phosphate (63).

Moreover, increased plasma FFA have been shown to alter the activity and/or gene expression of the two main hepatic enzymes responsible for the regulation of EGP. Specifically, plasma FFA *decrease* the expression of hepatic glucokinase (GK) (66) and *increase* gene expression of glucose-6-phosphatase (G-6-Pase) (64, 65). Acute as well as chronic elevations in plasma FFA furthermore apply stimulatory allosteric effects on G-6-Pase and decrease the activity of GK (67, 68, 57). This would most likely affect EGP and glucose effectiveness, since increased G-6-Pase would stimulate EGP by both GNG and glycogenolysis, and the liver's ability to 'sense' rising glucose levels would be impaired with defective GK activity (58, 59).

In light of this, however, it is important to consider that basal EGP was not increased despite significant stimulation of GNG by elevated plasma FFA in overnight-fasted humans and dogs (105, 106, 107, 108). Furthermore, there was no effect on basal EGP in 5-hour-fasted rats after lowering plasma FFA levels with the anti-lipolytic

nicotinic acid analog acipimox (109). Hepatic 'autoregulation' ensures that EGP remains constant despite FFA-induced increases in GNG under conditions of stable hormone and plasma glucose levels. There are, however, a number of conditions under which this 'autoregulation' does not appear to be operative, such as hormonal or neuronal counterregulation (110) and prolonged fasting (111). This was confirmed in studies, such as by Song et al. (97) and Lam et al. (63), in which basal EGP was increased in overnight-fasted/ liver glycogen-depleted rats in the presence of elevated plasma FFA levels from a high-fat diet or prolonged infusion of lipid emulsion, respectively. With depleted hepatic glycogen stores from overnight fasting (97, 111) glycogenolysis is already limited. It may therefore not be possible to further reduce glycogenolysis in order to prevent increases in EGP. Conversely, both GNG and EGP were decreased in 24-hour fasted T2DM subjects when lowering their plasma FFA levels with nicotinic acid (60).

In addition, hepatic 'autoregulation' in response to increases in plasma FFA levels only seems to function in the presence of stable or decreasing plasma glucose levels. Studies show that during euglycemia changes in FFA levels do not change the rates of EGP (104, 105). On the other hand, Shah et al. demonstrated that GNG and EGP were increased in nondiabetic women upon elevating plasma FFA levels to ~ 1mmol/l for 8 hours, while plasma glucose levels were raised to ~ 8.3 mmol/l (150 mg/dl) within the first hour of the study (61). It is likely, therefore, that circulating plasma FFA levels inhibit the effect of hyperglycemia on EGP by influencing the action of the hepatic enzymes GK and G-6-Pase. A number of experimental models studying the impact of isolated changes in GK or G-6-Pase activity support this idea. Neither disrupting the GK allele in transgenic mice (84), nor acutely inhibiting GK in normal rats (53) impacted the rates of EGP during euglycemia. Both approaches, however, significantly blunted the suppression of EGP by hyperglycemia. Furthermore, Trinh et al. showed that G-6-Pase overexpression in the liver of normal rats had no effect on blood glucose levels under fasting conditions, but caused further increases in plasma glucose levels with oral glucose loading (81).

Studies by Kishore et al. recently demonstrated that effects of increased FFA on glucose effectiveness are time-dependent and reversible (62). In studies with poorly controlled T2DM these were rapid improvements in hepatic glucose effectiveness after

only 2 hours of FFA lowering by nicotinic acid infusion and a complete restoration of glucose effectiveness after 5 hours of FFA normalization. These findings were also consistent with rapid changes in the two key hepatic enzymes GK and G-6-Pase that are responsible for regulating EGP (50, 63, 64, 65, 66, 67, 68, 69, 70). Thus, there is increasing evidence that the loss of glucose effectiveness in T2DM is closely related to high plasma FFA levels.

1.5. Significance and Aim of the Current Study

In nondiabetic individuals glucose and insulin normally suppress endogenous glucose production and increase peripheral glucose uptake. In T2DM, however, this regulation seems to be lost, considering EGP is elevated despite hyperglycemia and hyperinsulinemia. In fact, increased EGP appears to be the main source of fasting hyperglycemia in T2DM (8, 9). This increase in EGP seems to be secondary to the chronic 'diabetic milieu' in T2DM, since by normalizing plasma glucose and FFA levels glucose effectiveness is completely restored. T2DM is generally accompanied by sustained elevations in FFA in addition to hyperglycemia (71). Past studies have revealed important effects of increased fatty acids (FFA) on glucose effectiveness and the up-regulation of GNG (72, 73). Of the two main pathways of EGP, GNG is the one inappropriately increased in T2DM. However, it is important to note that inhibiting GNG alone does not decrease EGP under normoglycemic conditions because of compensatory rises in glycogenolysis (hepatic 'autoregulation') (74).

At present long-term lowering of FFA seems to be a fairly elusive goal (75). Studies have shown rebound elevations in plasma FFA with long-term therapy with nicotinic acid (76). These are most likely responsible for the insulin resistance that is seen with chronic use of this agent (77). Furthermore, there has been some inconsistency in studies regarding peroxisome proliferator-activated receptor (PPAR)-alpha agonist gemfibrozil and its ability to decrease plasma FFA levels and improve various parameters of glucose metabolism (75, 78, 79). Therefore it would be of benefit to establish ways of decreasing EGP despite increased FFA. Since hyperglycemia inhibits glycogenolysis, these studies addressed the hypothesis that inhibiting GNG in the presence of hyperglycemia would decrease EGP and prevent the negative impact of FFA on glucose effectiveness.

In order to study this question we examined seven subjects with three independent studies each. To establish the effects of elevating glucose levels on EGP in the absence of hormonal effects, we used infusions of somatostatin to inhibit all pancreatic hormone secretion, subsequently replacing insulin and glucoregulatory hormones (glucagon and GH) at fixed, basal infusion rates ('pancreatic clamp'). Under these 'pancreatic clamp' conditions we first measured glucose uptake (GU) and EGP during an initial euglycemic phase followed by an acute hyperglycemic phase. During the second 'pancreatic clamp' study we infused the study subjects with a lipid emulsion to increase FFA to those levels seen in moderate-to-poorly controlled T2DM, determining the effects of FFA on glucose effectiveness. In the final study we infused both a lipid emulsion and ethanol, which inhibits GNG. These studies showed that concurrent inhibition of glycogenolysis (by hyperglycemia) and GNG (with ethanol) significantly reduced EGP despite increased plasma FFA.

2. RESEARCH DESIGN AND METHODS

2.1. Subject Characteristics

Nondiabetic subjects aged 23-60 years were recruited and voluntary. Informed written consent was obtained in accordance with the policies of the Institutional Committee on Clinical Investigations (Table 1). The nondiabetic (ND; n=7) healthy volunteers were taking no medications, had no family history of T2DM, and were not involved in any other research study. A 2 hour oral glucose tolerance test (OGTT) with 75g dextrose was performed to ensure normal glucose tolerance (Fisherbrand Sun-Dex, Fisher Health Care, Houston, TX). Blood samples were drawn at 10 min. prior to and 120 min. after glucose consumption. All subjects were in general good health.

2.2. Experimental Design

All subjects were admitted to the General Clinical Research Center (GCRC) of Jack D. Weiler Hospital of the Albert Einstein College of Medicine the evening before the day of the study for observation. They were given a meal of standard composition at J.D. Weiler Hospital (~800 kcal, 50% carbohydrates, 20% protein, 30% fat) at ~1730 and a small snack (~325 kcal, 50% carbohydrates, 20% protein, 30% fat) at 2200, then fasted thereafter.

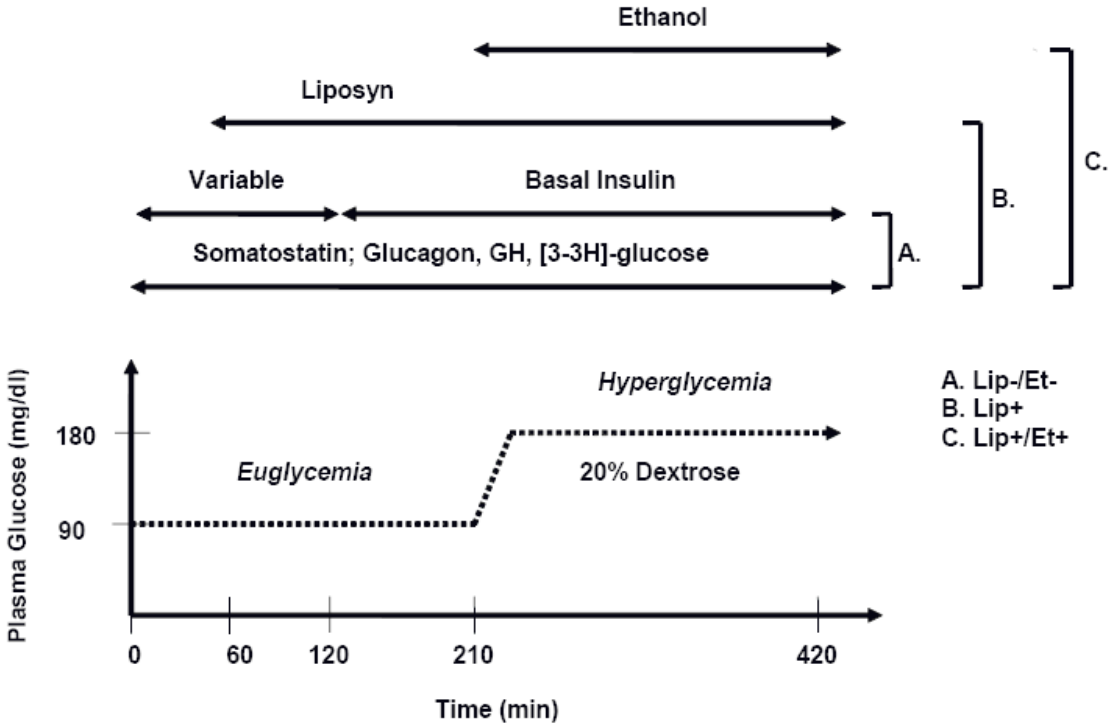
2.2.1. Euglycemic/Hyperglycemic Pancreatic Clamp Studies

At 0730 on the morning of the study, an 18-gauge intravenous catheter was inserted in an antecubital vein in the left arm for infusions and a contralateral hand vein was cannulated in a retrograde fashion for arterialized venous blood sampling. In order to obtain arterialized venous blood, this hand was kept in a warming blanket (Battlecreek® Thermophore® Muff, Battle Creek Equipment Co., Battle Creek, MI) maintained at 55°C. The experimental protocols lasted 7 hours and consisted of an initial 3.5-hour euglycemic period followed by a 3.5-hour hyperglycemic period (15,80). An overview of the 'pancreatic clamp' studies is given in Figure 1 (page 16).

At t=0 min, a primed-continuous infusion of high performance liquid chromatography (HPLC)-purified [3-^{3}H]-glucose (New England Nuclear, Boston, MA) was started (prime infusion 22 μCi), and then continued at 0.15 μCi/min for 7 hours.

Infusions containing somatostatin, growth hormone, glucagon and insulin were also initiated at t=0 min (15,72). Plasma glucose concentrations were measured from intravenous blood draws (0.5 ml) at 10-15 min intervals during the initial 210 min of the study and maintained at normal fasting concentrations (~5 mmol/l) by frequently adjusting the infusion rate of insulin during the first 120 min and maintaining these optimal insulin infusion rates for the duration of the studies. At 210 min, plasma glucose concentrations were acutely increased to 10 mmol/l and then clamped at this level with variable 20% glucose infusions. [3-^{3}H]-glucose (~0.1 μCi/ml) was added to the "cold" 20% glucose to maintain constant glucose specific activity (81). From zero to 420 min, blood samples were obtained to measure plasma glucose, insulin, glucagon, C-peptide, cortisol, growth hormone, free fatty acids, glycerol, lactate, and 3-^{3}H-glucose specific activity. All infusions were stopped at 420 min.

Figure 1. *Pancreatic Clamp Study Protocols*



2.2.2. General Clamp Study Protocol

All calculations were based on the individual study subject's weight, height, body surface area, and age. To keep the venous blood sampling line patent, normal saline solution (saline bag) was infused through the cannulated right hand vein. All other solutions were infused through the cannulated antecubital vein. The preparation of the infusion solutions was as follows:

1. Saline Bag:

3.5 ml heparin sodium (1000 USP Units/ml) (American Pharmaceutical Partners, Inc., Schaumberg, Illinois, USA) were added to a bag of 1000 ml normal saline solution (0.9% Sodium Chloride Injection USP, Baxter Healthcare Corporation, Deerfield, Ilinois).

2. Tracer ([3-^{3}H]-glucose):

0.15 mCi (5.55 MBq) of [3-^{3}H]-glucose (PerkinElmer® Life and Analytical Sciences, Boston, Massachusetts, USA) were added to 10 ml of normal saline solution. 6 ml thereof were added to the tracer infusate bag and 4 ml to a 20% dextrose solution (20% Dextrose Injection USP, Baxter Healthcare Corporation, Deerfield, Illinois, USA).

3. Insulin solution: 50 mU/ml

From a 1000ml normal saline solution bag 45 ml saline solution were removed and 5 ml albumin and 50 U of regular insulin (Novolin R®, Novo Nordisk® Pharmaceuticals, Princeton, New Jersey, USA) were added.

4. Hormone Bag:

2.5 ml albumin (ZLB Bioplasma AG, Bern, Switzerland), 36 ml normal saline solution, 2.5 ml somatostatin, growth hormone, and glucagon were added to a 100 ml normal saline solution bag. Rate of infusion: 0.3 ml/min.

4.1. Somatostatin Solution Preparation

Total dose: 250 μg/hr= 4.16 μg/min

One vial of somatostatin (Clinalfa®, Merck Biosciences AG, Laeufelfingen, Switzerland) contains 2.5 mg somatostatin/ml. 2ml of normal saline solution were added to the vial. 2.5 ml of this solution were drawn with a filter and added to the hormone bag.

4.2. Growth Hormone Solution Preparation:

The original growth hormone solution concentration was 1 mg/ml (Nutropin®, Genentech, Inc., South San Francisco, California, USA). For dilution 1 ml of growth hormone solution was added to 9 ml of normal saline solution for a growth hormone concentration of 0.1 mg/ml. To adjust the amount of growth hormone (GH) administered to the study subject following formula was employed:

GH = 3 ng/kg/min * Weight (kg) * 0.3 ml/min.

The calculated amount of growth hormone was taken with an insulin syringe and added to the hormone bag.

4.3. Glucagon Solution:

The original glucagon concentration was 1 mg/ml (GlucaGen®, Bedford Laboratories, Bedford, Ohio, USA). To dilute this concentration, 1 ml of glucagon was added to 9 ml of normal saline solution for a final glucagon concentration of 0.1 mg/ml. The amount of glucagon added to the hormone bag was calculated as follows:

Glucagon = 1 ng/kg/min * Weight (kg) * 0.3 ml/min.

5. Glucose Bolus:

The volume (in ml) of glucose 20% (20% Dextrose Injection USP, Baxter Healthcare Corporation, Deerfield, Illinois, USA) to be given as a bolus at the beginning of the hyperglycemic phase (t= 210 min) was calculated using the patient's weight (kg) and the anticipated distribution of glucose based on prior studies (40).

6. Insulin Infusion Rate:

The insulin infusion rate was calculated as follows:

Infusion rate (ml/min) = I * Weight (kg)/ C

I = Dose of insulin wanted in mU/kg/min

C = Concentration of the insulin solution in mU/ml

2.2.3. Study Conditions

To determine the impact of inhibiting GNG in the presence of elevated FFA on glucose effectiveness, rates of EGP were compared between euglycemia and hyperglycemia in nondiabetic subjects under the conditions outlined below. *All subjects were studied under the following three conditions, each study at least one month apart (Figure 1):*

a) **Lip–/Et-** : Baseline 7 hour saline control studies (normoglycemic/hyperglycemic pancreatic clamp studies; n=7)

b) **Lip+ (**n=7): Normoglycemic/hyperglycemic pancreatic clamp studies with infusion of lipid emulsion (*Liposyn* 20%, 0.42 ml/min) for the final 6 hours of the 7 hour clamp studies to reproduce the moderately elevated FFA levels observed in poorly controlled T2DM

c) **Lip+/Et+ (**n=7): Infusion of both lipid emulsion (*Liposyn*) *for the final 6 hours* of the studies and *ethanol* (to inhibit GNG) *during the hyperglycemic phase* (t=210-420) of the 7 hour normoglycemic/hyperglycemic pancreatic clamp studies.

<u>Liposyn infusions:</u>

To reproduce the moderately elevated FFA levels observed in poorly controlled T2DM, Liposyn 20% (Abbott Laboratories, North Chicago, IL) was infused at 0.42 ml/min for the final 6 hours of the Lip+ and Lip+/Et+ clamp studies. This duration was used since 6 hours of FFA elevation had maximal effects on glucose effectiveness (80).

<u>Ethanol infusions</u>**:**

Ethanol infusions titrated to reach plasma levels of 0.08 g/dl or 80% were started at t=210 min. To avoid venous irritation, 98% ethanol was diluted with 0.9% saline for

a final concentration of 6% (preparation by the central chemical laboratory of J.D. Weiler Hospital). The physiologically-based pharmacokinetik (PBPK) model, a three compartment model of alcohol mass flow rate, comprised of the liver, vasculature, and peripheral body water, was used to calculate the ethanol infusion rates (82). To characterize the kinetics of the individual subjects, four clinical parameters (height, weight, age, and gender) and an index of recent drinking history were taken into account. Those four parameters were entered into the PBPK model of alcohol distribution and elimination (MATLAB 6.5 Simulink 5, Mathworks, Boston, MA) and consequently transformed into a second set of physiologic parameter values for that particular individual: cardiac rate, total body water, vascular water volume, and limiting mass ethanol elimination rate. Based upon those values the PBPK model of alcohol distribution and elimination calculated the ethanol infusion rate for each individual. Target ethanol levels were established within the first 20 minutes after ethanol infusions began and monitored by measuring breath alcohol levels every 15 minutes using a hand held breath alcohol analyzer, Alcosensor IV meter (Intoximeters Inc., Saint Louis, Missouri, USA). Moreover, blood ethanol measurements were performed at t=300, 360, and 420 min. to confirm the accuracy of the breath ethanol measurements. Adjustments in the ethanol infusion rate were made as necessary to maintain the target ethanol levels (based on the observation that breath ethanol levels were ~ 80% of blood levels). Based on the National Institute on Alcohol Abuse and Alocholism (NIAAA) guidelines for the study of ethanol, participants who were pregnant or had a history of liver disease, depression, or alcohol problems were excluded from the study (83).

GNG Measurements

All GNG measurements were made during the euglycemic study phase. Accurate measurements of GNG could not be performed in the presence of changing glucose levels and exogenous glucose infusion. Maintaining glucose levels at ~ 180 ml/dl would require substantially greater rates of exogenous, unlabeled glucose infusion relative to rates of hepatic glucose production. The infused unlabeled glucose would consequently dilute the deuterated glucose generated by GNG, artificially lowering the measured rates of GNG (data not shown). During the Lip+/Et+ studies described above, ethanol infusions were only initiated during the hyperglycemic phase of the studies, in

order to avoid prolonged ethanol exposure. Therefore, to accurately determine rates of GNG in the Lip+/Et+ group, we performed additional short *euglycemic* pancreatic clamp studies in n=4 subjects infusing both Liposyn and ethanol (Lip+/Et+) for the duration of 3.5 hours.

Subjects drank deuterated water (total of 5 g D_2O/kg total body water) at 8pm, 11 pm and 3 am, the night before each study. Body water was estimated to be 50% of body weight in women and 60% in men. Deuterated water was ingested slowly over about 30 minutes per dose, to avoid dizziness. Any other ingested water was enriched to 0.5% with D_2O to maintain isotopic steady state. Blood was drawn to determine the C 5/2 ratio during the final 15 minutes of the euglycemic study phase (t=210).

2.3. Analytical procedures

Plasma glucose was measured with a Beckman glucose analyzer (Beckman Instruments, Fullerton, California, USA) by use of the glucose oxidase method, through conversion of glucose and oxygen to gluconate and H2O2. Plasma [3-^{3}H]-glucose radioactivity was measured by the Somogyi procedure, that is in duplicates in the supernatants of ZnSO4 and Ba(OH)2 precipitates of plasma samples (25 μl) after evaporation to dryness to eliminate tritiated water. Plasma tritiated water specific activities were measured before and after evaporation to dryness by liquid scintillation counting (Ultima Gold scintillation cocktail, PerkinElmer Life and Analytical Sciences, Boston, Massachusetts, USA) of the protein-free supernatant (Somogyi filtrate) (15, 84, 85). Plasma insulin was measured by radioimmunoassay using porcine and rat insulin standards (86). C-peptide (Human C-Peptide RIA Kit, Linco Research, Inc., Saint Charles, MO) and glucagon (Glucagon RIA Kit, Linco Research, Inc., Saint Charles, Missouri, USA) were also measured by radioimmunoassay. FFA (NEFA C, Wako Chemicals USA, Inc., Richmond, Virginia, USA) and glycerol (Free Glycerol Determination Kit FG0100, Sigma®, Saint Louis, Missouri, USA) were determined using colorimetric enzymatic methods (87, 88). An enzymatic spectrophotometric assay (Olympus System Lactate reagent, Olympus America Inc., Melville, New York, USA) was used to measure lactate (84). Blood ethanol measurements were performed using an enzymatic in vitro assay (Ethyl Alcohol Kit, Roche Diagnostics GmbH, Mannheim, Germany). Measurements of gluconeogenesis were performed at Case Western Reserve

University School of Medicine, using an established method that measures the deuterium enrichment at carbons 2 and 5 on plasma glucose (89, 90).

2.4. Calculations

Rates of glucose appearance (Ra) and glucose uptake (Rd) were calculated using Steele's steady state equation (89). In the steady state the rate of glucose appearance (Ra) is calculated by the total labeled glucose infusion rate (Total I) divided by the plasma specific activity (SAp) multiplied by weight:

$$Ra = \frac{\text{Total I (cpm/kg/min)}}{\text{Avg. SAp (cpm/mg)}}$$

Under stable and steady conditions the rate of glucose appearance and uptake should be equal, therefore:

Rd (mg/kg/min)= Ra (mg/kg/min).

Rates of endogenous glucose production were calculated by subtracting the glucose infusion rate (GIR) from the rate of glucose disappearance (Rd):

EGP= Rd (mg/kg/min) - GIR (cpm/kg/min) (91).

Data for glucose turnover, plasma hormones and substrate concentrations represent the mean values during the final 60 min of the euglycemic period (t=150-210 min) and the final 60 min of the hyperglycemic period (t=360-420 min).

2.5. Statistical analysis

Analysis of the data was performed using SPSS Version 11.5 (SPSS Inc., Chicago, IL). For averaged data, Student's t-tests were employed, using paired t-tests for comparisons of euglycemic and hyperglycemic intervals. ANOVA was used when comparisons were made among the Lip-/Et-, Lip+ and Lip+/Et+ studies. All data are presented as mean ± standard error of measurement (SEM) unless otherwise specified. A P value of < 0.05 was considered significant.

3. RESULTS

3.1. Baseline (fasting) patient characteristics (Table 1)

Following an overnight fast, plasma insulin concentrations averaged 16.01±3.2 μU/ml in all subjects (averaged for all studies). Fasting plasma glucose levels averaged 100.07±1.9 mg/dl (averaged for all studies). Basal (t=0) plasma FFA levels were 490.4±80.3 μmol/l, *P*= not significant (NS). There were no differences in these parameters on different study days.

3.2. General clamp study conditions

During the clamp studies, plasma glucose levels averaged 103.8±2.9 mg/dl in the euglycemic study period and 183.1±1.5 mg/dl during the hyperglycemic study period, respectively, and did not differ among the study types. Glucose specific activity was constant following tracer equilibration during both euglycemia and hyperglycemia in each group.

The average insulin infusion rates (IIR) required to maintain euglycemia were comparable in the three study types and averaged 0.14±0.02 μU/kg/min. Importantly, plasma insulin levels did not differ in either the euglycemic or hyperglycemic study periods in any of the study types. Plasma glucagon levels also remained stable in all three study types. Hence, basal insulin and glucagon levels were maintained in all studies (Table 1). C-peptide levels were suppressed by somatostatin infusion in all studies and did not differ among the three studies in either basal, euglycemic or hyperglycemic periods. Plasma lactate levels were stable in the saline control and Lip+ studies. During the hyperglycemic phase of the Lip+/Et+ studies there was a significant increase in lactate (Table 1). These findings are consistent with previous studies that report ethanol-induced lactate elevations (92, 93), presumably due to decreased consumption of lactate in GNG.

Table 1. *Plasma Hormone and Substrate Values*

	Insulin (µU/ml)	Glucagon (pg/ml)	CPeptide (nmol/ml)	Lactate (mmol/l)	Glycerol (µmol/l)	FFA (µmol/l)
Lip-/Et-						
Basal (t=0 min)	15.4±5.1	79.03±9.9	0.78±0.2	1.31±0.1	114.6±29.9	476.6±49.9
Euglycemia (t=150-210 min)	17.1±2.2	71.9±11.4	0.19±0.02	0.99±0.1	91.0±25.4	229.03±32.1
Hyperglycemia (t=210-420 min)	19.1±2.8	68.7±12.0	0.40±0.01	0.95±0.1	58.9±13.1	198.9±28.3
Lip+						
Basal (t=0 min)	14.5±2.5	70.7±10.2	0.88±0.2	1.57±0.2	111.7±38.6	518.8±110.3
Euglycemia (t=150-210 min)	19.6±3.8	73.3±6.8	0.19±0.02	1.00±0.2	252.8±52.4*	528.3±47.4*
Hyperglycemia (t= 210-420 min)	18.2±3.0	65.9±8.0	0.38±0.1	0.84±0.2	216.2±30.6*	602.4±59.1*
Lip+/Et+						
Basal (t= 0 min)	18.1±2.1	84.2±11.1	0.48±.12	1.64±0.1	100.3±21.6	475.7±80.7
Euglycemia (t= 150- 210 min)	17.4±2.9	80.0±12.1	0.17±.01	0.95±0.1	297.6±61.5*	549.0±115.0*
Hyperglycemia (t= 210- 420 min)	16.0±1.9	70.5±12.2	0.30±.06	1.53±0.1†	320.8±66.6*	510.9±97.1*

*P < 0.5 vs. Lip-/Et-

†P < 0.5 vs. Lip-/Et- and Lip+

3.3. Saline Control Studies

The rate of glucose infusion required to maintain the target hyperglycemic plateau during the last 60 minutes of the hyperglycemic period averaged 3.9±0.5 mg/kg/min. EGP was suppressed by 61.4±4.3% with hyperglycemia (Figure 2). Furthermore, the percent increase in GU between the euglycemic and hyperglycemic study periods was 110.4±18.5% (Table 2; Figure 3). Plasma FFA concentrations for the study subjects were 229.0±32.0 μmol/l during euglycemia and 198.9±28.3 μmol/l during hyperglycemia (Table 1) respectively, which are statistically not different.

3.4. LIP+ studies

The infusion of Liposyn raised FFA to levels comparable to those seen in poorly controlled T2DM (Table 1). Significant elevations in FFA levels were attained after ~1 hour of Liposyn infusion and maintained throughout the studies. Glycerol levels were also substantially elevated during Liposyn infusion in Lip+ studies (Lip+=216.2±30.6 vs. Lip-/Et-=58.9±13.1 μU/ml). The average rate of glucose infusion required to maintain the target hyperglycemic plateau during the last 60 minutes of the hyperglycemic period was significantly lower during the Lip+ studies as compared with the saline control studies in the same subjects (Lip+ =2.0±0.5 *vs*. Lip-/Et- =3.9±0.5 mg/kg/min, *P*=0.048) (Table 2). Plasma insulin levels in the Lip+ studies did not differ from those in the saline control studies.

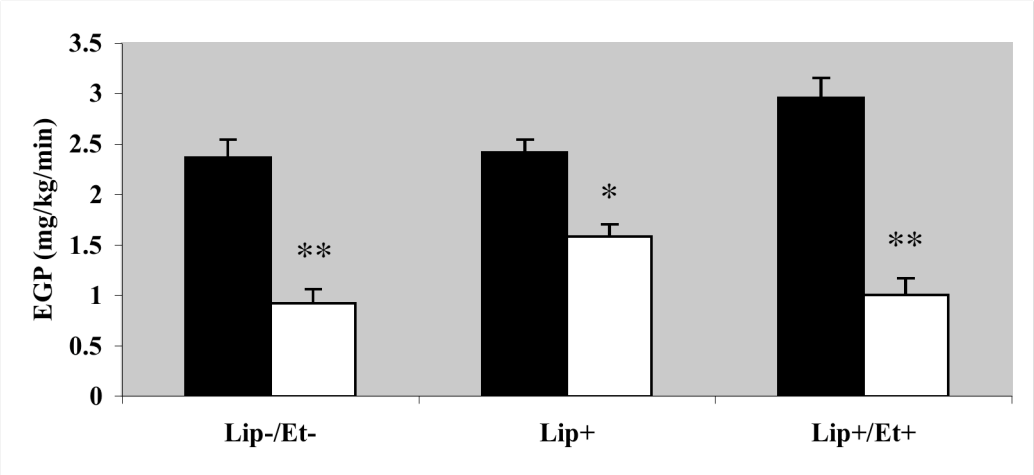
The elevated FFA levels resulted in a significant blunting of the % suppression of EGP with hyperglycemia in Lip+ studies (Lip+ =34.2±3.7% vs. Lip-/Et-=61.4±4.4%, *P*=0.0097) (Table 2; Figure 2). Although the % increase in GU during hyperglycemia in the Lip+ study type was lower relative to the saline control studies in the same subjects, the difference was not statistically significant (Lip+=53.3±21.0% vs. Lip-/Et-=110.4±18.5%, *P*=0.092) (Table 2; Figure 3).

Table 2. *GU, EGP, and GIR*

	GU		EGP		GIR
	mg/kg/min	**% change†**	**mg/kg/min**	**% change†**	**mg/kg/min**
Lip-/Et-					
Euglycemia (t=150-210 min)	2.37±0.2		2.37±0.2		
Hyperglycemia (t=210-420 min)	4.82±0.3	110.4±18.5	0.92±0.1	61.4±4.3	3.93±0.5
Lip+					
Euglycemia (t=150-210 min)	1.93±0.3		2.41±0.1		
Hyperglycemia (t= 210-420 min)	3.63±0.5	53.3±21.0	1.58±0.1	34.2±3.7‡	2.03±0.5
Lip+/Et+					
Euglycemia (t= 150- 210 min)	2.38±0.4		2.96±0.2		
Hyperglycemia (t= 210- 420 min)	3.24±0.3	10.2±8.9*	1.00±0.2	65.8±5.1	2.24±0.4

† % change between Eu- and Hyperglycemia
* $P < 0.5$ vs. Lip-/Et-
‡ $P < 0.5$ vs. Lip-/Et- and Lip+/Et+

A.



B.

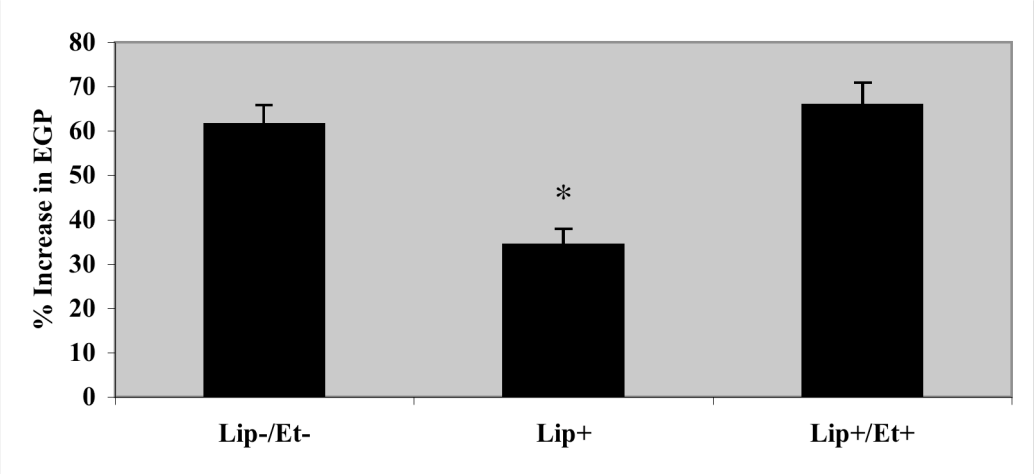
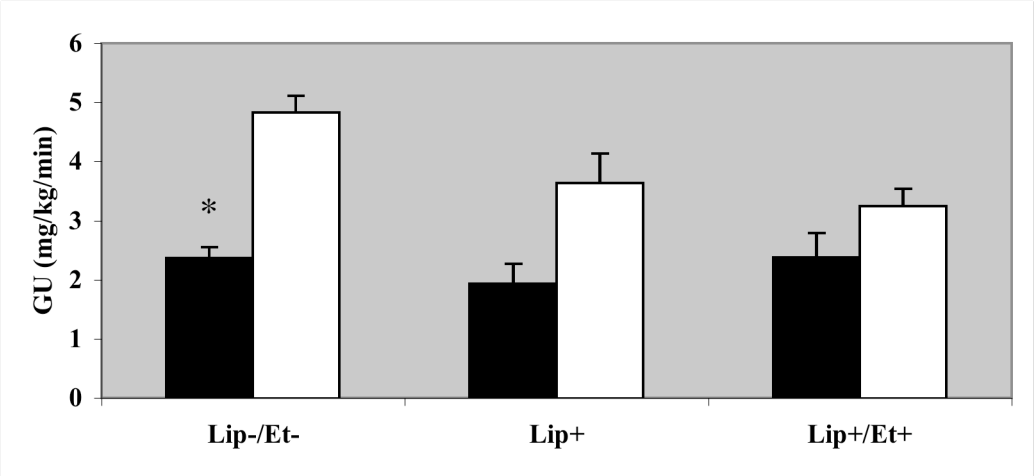


Figure 2. ***A.*** *Rates of endogenous glucose production* (EGP; mg/kg/min) during the euglycemic study period (filled bars) and the hyperglycemic study period (open bars) for the three study types: Lip-/Et-, Lip+, and Lip+/Et+. ***P*<0.0001 euglycemia vs. hyperglycemia in groups Lip-/Et- and Lip+/Et+. **P*<0.05 euglycemia vs. hyperglycemia in group Lip+. ***B.*** *Percent decrease in endogenous glucose production* (EGP) between the euglycemic and hyperglycemic phases. * *P*<0.05 in groups Lip+ vs. Lip-/Et- and Lip+ vs. Lip+/Et+.

A.



B.

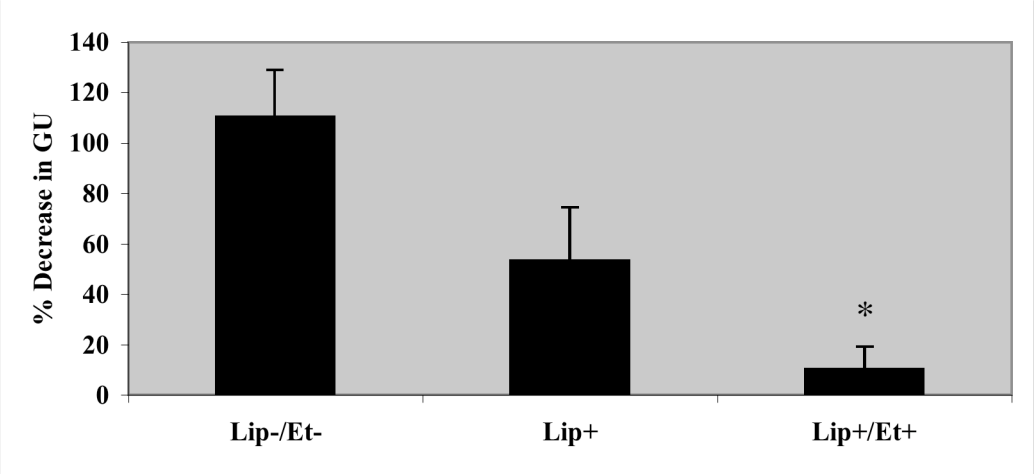


Figure 3: ***A.*** *Rates of glucose uptake* (GU; mg/kg/min) during the euglycemic study period (filled bars) and the hyperglycemic study period (open bars) for the three study groups: Lip-/Et-, Lip+, and Lip+/Et+. **P*<0.05 compared to the Lip-/Et- hyperglycemic phase. ***B.*** *Percent increase in glucose uptake* (GU) between the euglycemic and hyperglycemic phases. **P*<0.05 in group Lip+/Et+ vs. Lip-/Et-.

3.5. Lip+/Et+ Studies

Plasma ethanol levels were 90.4±5.2 mg/dl at t=300 min, 85.6±4.9 mg/dl at t=360 min, and 86.3±3.3 mg/dl at t=420. The average was 87.4±4.4 mg/dl and remained stable throughout the studies (*P*=NS by ANOVA). The FFA values attained during the final hour of the Liposyn and ethanol co-infusion studies did not differ from the Lip+ studies (Lip+/Et+=510.8±97.1 vs. Lip+=602.4±59.1 μM/l, *P*=0.17). Similarly, there was no statistical difference in the glycerol levels between the Lip+ and co-infusion studies. There was, however, an upward trend in glycerol levels with the onset of the ethanol infusion, which is likely to be caused by a decrease in GNG (Table 1).

Importantly, there was significantly greater suppression in EGP with hyperglycemia in the Liposyn and ethanol co-infusion studies when compared to the Lip+ studies (Lip+/Et+=65.8±5.1% vs. Lip+=34.2±3.7%, *P*=0.004) (Table 2; Figure 2). The % decrease in EGP in the co-infusion studies was comparable to that in the saline control studies (Lip+/Et+=65.8±5.1% decrease in EGP vs. Lip-/Et-=61.4±4.4%, *P*=0.6), suggesting the restoration of glucose effectiveness. The % increase in glucose uptake during the hyperglycemic phase of the Lip+/Et+ studies was significantly lower than that of the Lip-/Et- studies (Lip+/Et+=10.2±8.8% vs. Lip-/Et-=110.4±18.5%, *P*=0.001) (Table 2; Figure 3). GU trended downwards in the co-infusion studies when compared to the Lip+ studies, but did not reach statistical significance (Lip+/Et+=10.2±8.8% vs. Lip+=53.3±21.0%, *P*=0.1). This is consistent with previous studies confirming that insulin-mediated GU is significantly reduced in the presence of systemic ethanol, particularly in ND individuals (94).

3.6. GNG Measurements

Under euglycemic, pancreatic clamp (Lip-/Et-) conditions, rates of GNG averaged 0.9±0.1 mg/kg/min (accounting for ~ 39% of EGP) (Figure 4). When FFA levels were elevated by Liposyn infusion throughout the 3.5-hour studies, rates of GNG increased to 1.4±0.1 mg/kg/min (*P*=0.006). However, ethanol completely prevented the Liposyn-induced rise in GNG, with GNG decreasing significantly to baseline rates of 0.9±0.1 mg/kg/min (*P*=0.008 between Lip+ and Lip+/Et+ studies; *P*= 0.999 for Lip-/Et- vs. Lip+/Et+).

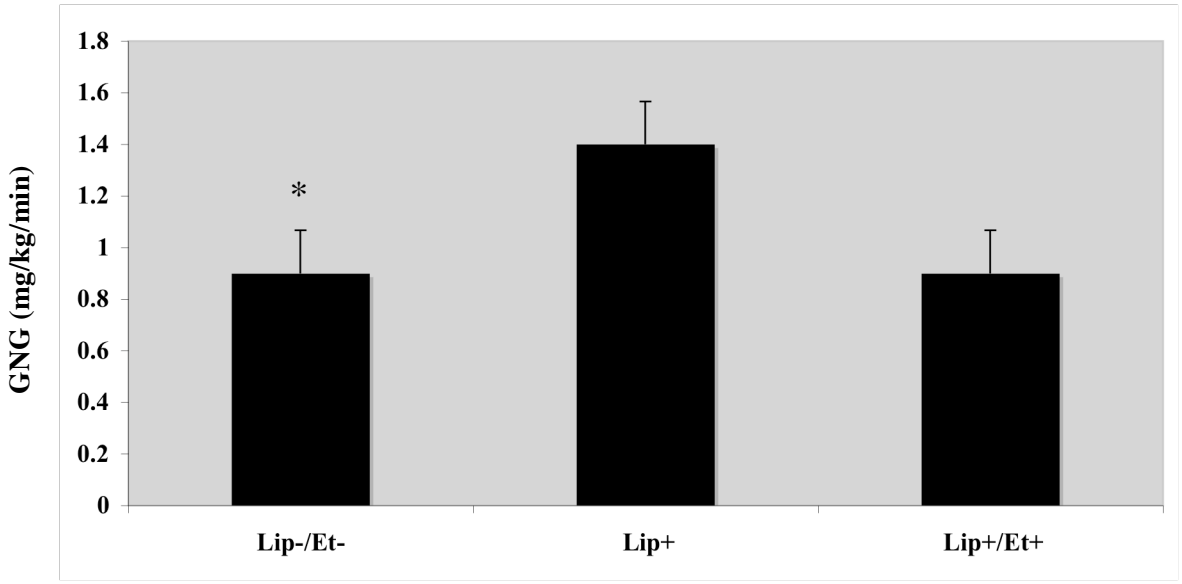


Figure 4. *Rates of gluconeogenesis (GNG)* for the three study types Lip-/Et-, Lip+, and Lip+/Et+. Blood sample was taken at t= 210 min. * $P < 0.05$ comparing Lip+ to Lip-/Et- and Lip+ to Lip+/Et+.

4. Discussion

Loss of the ability of glucose to suppress hepatic glucose production ('glucose effectiveness') significantly contributes to worsening hyperglycemia in T2DM. An increasing number of studies have recently suggested that elevated plasma FFA levels not only inhibit the effects of insulin on hepatic glucose metabolism (15, 16) but also impair glucose effectiveness in T2DM (18, 19, 20, 21, 22), leading to increased EGP despite the presence of hyperinsulinemia and hyperglycemia. Since long-term lowering of plasma FFA levels is not currently feasible in T2DM, it would be of benefit to find ways to decrease EGP in the face of increased plasma FFA levels. Considering that a rise in GNG is an important process responsible for increased EGP in T2DM and increased FFA levels potently stimulate GNG (72, 73), we hypothesized that inhibiting GNG with ethanol would prevent the negative impact of FFA on glucose effectiveness. Indeed, these studies show significant increases in GNG after only 2 hours of Liposyn infusion. Furthermore, FFA-induced loss of glucose effectiveness was completely restored by ethanol infusion, likely due to inhibition of GNG, in the face of increased plasma FFA levels.

4.1. Increased plasma FFA inhibit hepatic glucose effectiveness

The current studies confirmed past findings that increased circulating FFA inhibit the reduction of EGP that normally occurs with hyperglycemia. During the saline control studies EGP was suppressed by 61% with the onset of hyperglycemia. However, EGP only decreased 34% in response to hyperglycemia when Liposyn was infused.

The major cause of fasting hyperglycemia in T2DM is believed to be increased EGP (8, 9). Ader and Bergman (95) reported that insulin only has a minor direct effect on EGP suppression. They suggested that the site at which insulin primarily regulates hepatic glucose output is at the adipocyte. An investigation by Rebrin et al. demonstrated the inhibitory effects of insulin on lipolysis, which in turn lowered EGP (96). In addition, glucose suppresses lipolysis which lowers the FFA signal to the liver, decreasing hepatic glucose output. Best and colleagues hypothesized that this suppression of lipolysis and concurrent lowering of plasma FFA could establish the value of the hepatic suppression component of glucose effectiveness (23). Therefore, in

ND individuals the majority of suppression of EGP by glucose is thought to be due to glucose-induced reductions in FFA levels (23).

The important effects of plasma FFA on glucose metabolism are becoming increasingly clear. Lewis et al. illustrated the deleterious effects of increased plasma FFA on insulin's regulation of glucose metabolism (10). Shah and colleagues observed that elevated FFA impaired glucose metabolism in women in the presence of combined hyperinsulinemia and hyperglycemia by inhibiting the suppression of splanchnic glucose production, whole- body glucose disposal, and muscle glucose uptake (61). Furthermore, the emerging theory that plasma FFA levels play a critical role in glucose effectiveness and hepatic EGP are supported by studies that show a rise of plasma FFA in proportion to worsening glycemic control. Hawkins et al. have reported that in individuals with optimal glycemic control, EGP shows normal suppression of FFA by hyperglycemia and therefore normal glucose effectiveness (40). Furthermore, 72 hours of intensive insulinization in poorly controlled T2DM restored glucose effectiveness together with the normalization of plasma FFA levels (40). More recently, Kishore et al. observed a rapid improvement in hepatic glucose effectiveness after ~ 2 hours of FFA lowering and a complete restoration of glucose effectiveness after ~ 5 hours of FFA lowering (62). The current studies similarly demonstrate this phenomenon. During the Lip+ euglycemic/hyperglycemic pancreatic clamp studies there was a significant blunting of the percent decrease in EGP with onset of hyperglycemia. Since insulin signaling and other parameters can be affected by supra-physiologic rises in plasma FFA levels, the effects of moderate elevations in FFA levels typical of T2DM were examined here.

Of the two main pathways of hepatic glucose production, GNG and glycogenolysis, GNG is inappropriately increased in T2DM and accounts for the high overall rates of EGP. As previously described, increased levels of plasma FFA have been shown to potently stimulate hepatic GNG through various mechanisms that include the enhanced gene expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) (73), and increased generation of fructose-1,6-bisphosphatase (97). Furthermore, increased plasma FFA levels promote the generation of the energy sources ATP and NADH (63), as well as acetyl-CoA and fatty acyl-CoA (72, 98). Both acetyl-CoA and fatty acyl-CoA allosterically activate

pyruvate carboxylase and generate citrate (99, 100), which inhibits the enzyme phosphofructokinase.

Of note, all GNG measurements in the current studies were taken during the euglycemic study phase. Maintaining glucose levels at ~180 mg/dl would require substantially greater rates of exogenous, unlabelled glucose infusion relative to rates of hepatic glucose production. The infused unlabelled glucose would consequently dilute the deuterated glucose generated by GNG, artificially lowering the measured rates of GNG (data not shown). Therefore, to accurately quantify GNG, it was necessary to measure GNG during euglycemia. To minimize ethanol exposure, ethanol was only infused during the hyperglycemic phase of the studies. Therefore, we performed a small number of additional studies in which the combined effects of FFA and ethanol on GNG were quantified under stable, euglycemic conditions. Indeed, the elevation of plasma FFA for only 2 hours stimulated GNG significantly.

Importantly, marked increases or decreases in GNG alone are not enough to change EGP in most physiological settings (101, 102, 103, 104, 105). In overnight-fasted humans and dogs basal EGP was not increased upon elevating plasma FFA levels, despite considerable stimulation of GNG (105, 106, 107, 108). In accordance with this, Lee et al. showed that basal EGP was also not affected by lowering plasma FFA levels with nicotinic acid in 5-hour-fasted rats (109). Boden et al. demonstrated both stimulation and inhibition of GNG in both nondiabetic and T2DM subjects with increases and decreases in plasma FFA levels, respectively (60). However, total EGP was not altered despite these changes in GNG. A hepatic 'autoregulatory' mechanism maintains constant EGP and plasma glucose in the presence of stable hormone and plasma glucose levels. This is due to compensatory changes in glycogenolysis. During euglycemic conditions this mechanism remains intact, even in the face of FFA-induced increases in GNG (60). Our studies confirm this finding, given that Liposyn infusion increased rates of GNG but failed to alter rates of EGP. As autoregulation would be intact under euglycemic conditions, ethanol should likewise not affect basal EGP during euglycemia, since its inhibitory effects on GNG should be compensated by increases in glycogenolysis (110). This observation was likewise confirmed in our studies. Increases in plasma glucose levels, however, rapidly inhibit glycogenolysis in nondiabetic individuals and would impair this hepatic autoregulation. Although we did

not measure glycogenolysis directly in these studies, we would predict based on the findings of Chu et al. (108) that increased FFA in the presence of hyperglycemia would suppress glycogenolysis. Indeed, the current studies demonstrate the loss of hepatic autoregulation, evidenced by a rise in EGP in response to Liposyn infusion during hyperglycemia.

4.2. Inhibiting GNG in the presence of hyperglycemia impacts EGP

Since hyperglycemia inhibits glycogenolysis, we predicted that changes in GNG would significantly impact EGP in the presence of hyperglycemia. Rossetti et al. demonstrated that hyperglycemia causes a marked inhibition of EGP mainly through the increase in glucokinase flux and the suppression of glycogenolysis, with no evident changes in the fluxes through glucose-6-phosphatase and gluconeogenesis (30). Similarly, Peterson and colleagues described the inhibition of hepatic glycogenolysis by hyperglycemia primarily through inhibition of glycogen phosphorylase flux in a pancreatic clamp study in humans (32).

Ethanol has been shown to inhibit gluconeogenesis (111). Krebs and colleagues were among the first to demonstrate this phenomenon (112). They concluded that GNG is inhibited primarily through the interaction of ethanol with the enzyme alcohol dehydrogenase, which causes a decrease of the [NAD+]/[NADH] ratio. This decrease lowers the concentration of pyruvate, which is the immediate cause of the inhibition of gluconeogenesis from alanine, serine, and lactate. Thus, the rate of the pyruvate carboxylase reaction, one of the rate-limiting reactions of gluconeogenesis, is decreased. Others studied the incorporation of gluconeogenic substrates such as alanine (113) and lactate (114) into glucose before and during ethanol administration in humans. In both studies this incorporation, and therefore gluconeogenesis, was clearly impaired by ethanol. Of note, there was a significant rise in lactate was measured during the hyperglycemic phase of the Lip+/Et+ studies. This is most likely due to the inhibitory effects of ethanol on gluconeogenesis and the resulting decreased consumption of lactate. A number of other studies likewise reported this effect, such as Avogaro et al. (78), Sarkola et al. (93), and Lieber et al. (115), where significant elevations in blood lactate concentrations accompanied ethanol metabolism in humans.

As discussed above, glucose effectiveness was dramatically and rapidly blunted in the face of elevated FFA. Here we show that ethanol infusion in the presence of increased FFA completely restores glucose effectiveness. The current ethanol and Liposyn co-infusion studies demonstrated a significant decrease in EGP of 65.8% in the presence of hyperglycemia. Under euglycemic conditions we have demonstrated the inhibitory effects of ethanol on GNG. Although we were not able to directly measure GNG during hyperglycemia it is likely that the inhibition of GNG also accounted for the restoration of glucose effectiveness in the presence of elevated FFA. It cannot be excluded, however, that ethanol independently suppresses GNG and EGP under hyperglycemic conditions.

These studies imply that FFA-induced stimulation of GNG exerts substantial effects on glucose effectiveness and could be a beneficial target for intervention. Of note, moderate alcohol consumption has been shown to lower the risk of developing T2DM (116). Since ethanol is associated with other concerns, new approaches of inhibiting GNG are currently being developed. Recently, a fructose-1,6-bisphosphatase inhibitor has been shown to lower blood glucose in monkeys and diabetic rodents (117).

4.3. Increased FFA inhibit peripheral glucose effectiveness

Increased plasma FFA levels not only impaired hepatic glucose effectiveness, but also inhibited peripheral glucose effectiveness. The ability of hyperglycemia per se to stimulate whole-body glucose uptake was affected in both the Lip+ and the Lip+/Et+ co-infusion studies. This is consistent with previous studies in which elevated plasma FFA impaired peripheral glucose effectiveness. Hawkins et al. (80) established the ability of increased plasma FFA to inhibit glucose-stimulated glucose uptake. In addition, Long et al. demonstrated a reduction in glucose transporter GLUT-4 mRNA content in muscle and adipose tissue with increased plasma FFA, which would impair peripheral glucose effectiveness (118). The ability of glucose to stimulate its own uptake is considerably enhanced by insulin (23). It is important to note that the current studies were performed in the presence of low physiologic insulin levels. In past studies, increased FFA levels inhibited peripheral glucose effectiveness in the presence of physiologic insulin levels, but had no effect on glucose-mediated glucose uptake in the absence of insulin (119, 120). In T2DM the diminished ability of glucose to stimulate its

own uptake may also be due to a decreased number of glucose transporters at the plasma membrane (38), as FFA seem to directly influence the expression (118) and translocation (121) of skeletal muscle glucose transporters.

4.4. Effects of both ethanol and increased FFA on peripheral glucose uptake

In these studies there was a nearly complete loss of peripheral glucose effectiveness in the presence of both Liposyn and ethanol infusions. Glucose uptake increased by 110% during hyperglycemia in the saline control studies. However, during the ethanol and Liposyn co-infusion studies, although hepatic glucose effectiveness was completely restored, peripheral glucose uptake increased by only 10% with onset of hyperglycemia. It seems likely that this observation is due to the combined effects of increased plasma FFA and ethanol levels on peripheral glucose uptake. In support of this, Avogaro et al. (94) reported significantly decreased whole-body insulin-mediated glucose uptake with systemic ethanol infusion in both ND and T2DM individuals. This effect was particularly evident in the ND subjects. A study by Xu and colleagues confirmed that ethanol infusion in rats causes acute insulin resistance with marked decreases of glucose uptake in most skeletal muscles (122). Yki Jarvinen and Nikkila (123) reported that acute intake of alcohol in moderate doses by normal men induces resistance to insulin-stimulated glucose uptake. Similarly, Shelmet et al. described the inhibitory effects of ethanol on glucose disappearance and oxidation in healthy men (124). Given the inhibitory effects of ethanol as well as the negative impact of increased plasma FFA on peripheral glucose uptake, it is possible that their combined effects would account for the near complete loss of peripheral glucose effectiveness seen in the Lip+/Et+ studies.

4.5. Future implications

With ever increasing numbers of people affected by diabetes mellitus worldwide and the serious medical consequences of hyperglycemia, finding mechanisms to regulate glucose homeostasis is of vital importance. The important role of glucose effectiveness in normal glucose metabolism is becoming increasingly clear. The importance of glucose effectiveness, however, seems to be even greater in individuals with insulin-resistance and T2DM. In such states, not only is the time to achieve

maximal insulin action increased, but the overall effect of insulin is decreased due to cellular insulin resistance. Therefore, in individuals with T2DM, restored glucose effectiveness would improve glucose disposal and glycemic regulation even when insulin action is compromised. This would very likely influence the morbidity and mortality associated with poor glycemic control in T2DM (23). The results of the current study demonstrate that the loss of glucose effectiveness in T2DM may well be restored. This would lead us one step further in our pursuit of understanding the pathogenesis of T2DM and finding new treatment options.

Nonetheless, it is imperative to emphasize that lifestyle modification, in particular maintaining a healthy weight, consuming a well-balanced diet, and participating in regular physical activity, is the first choice in preventing and delaying the onset of T2DM. This has been highlighted by numerous well-designed randomized controlled trials (125, 126, 127). The Diabetes Prevention Program (DPP) demonstrated the superior efficacy of weight loss and moderate physical activity over use of medication in the prevention of diabetes (125). Therefore, the challenge in both the developed and the developing worlds remains to encourage and advocate a healthy lifestyle.

To conclude, these studies confirm the significant impact of increased plasma free fatty acid levels on glucose effectiveness. In particular, the substantial effects of increased FFA levels on the ability of glucose to properly suppress endogenous glucose production appear to be due in great part to FFA-induced stimulation of gluconeogenesis. In type 2 diabetes mellitus the loss of glucose effectiveness, likely secondary to the chronic diabetic ‘milieu’, contributes significantly to the worsening of hyperglycemia. Although glucose effectiveness is completely restored in such individuals by normalizing plasma glucose and FFA levels, this is a rather elusive goal and is therefore currently not a primary therapeutic objective. In light of this, inhibiting gluconeogenesis in the presence of hyperglycemia and increased FFA levels shows promise as a potential target for intervention.

5. Summary

Free fatty acids (FFA) modulate the effectiveness of glucose to suppress endogenous glucose production (EGP), and increased FFA levels contribute importantly to the loss of glucose effectiveness in type 2 diabetes mellitus (T2DM). Elevating FFA levels in nondiabetic (ND) subjects for at least 6h both increases gluconeogenesis (GNG) and impairs glucose effectiveness. Therefore, we wished to define the extent to which an increase in GNG is responsible for the loss of glucose effectiveness and whether EGP can be inhibited in the presence of elevated plasma FFA by inhibiting GNG with ethanol.

To determine the effect of inhibiting GNG on glucose effectiveness, EGP ([3-3H]-glucose) was measured during three separate 7h normoglycemic/hyperglycemic pancreatic clamp studies (somatostatin; basal glucagon/GH/insulin replacement) in n=7 ND subjects (1F/6M; age=45±5 yr; BMI=27.6±3.0 kg/m2). Following an initial 210 min interval of euglycemia (5 mmol/l), blood glucose levels were raised to hyperglycemic levels (10 mmol/l) from t=210-420 min. The first pancreatic clamp study was a baseline study with saline infusions (Lip-/Et-). Lipid emulsion (Liposyn 20%) was infused throughout the second and third study types (Lip+ and Lip+/Et+) to increase FFA to T2DM levels (~ 500 mmol/l). In addition to Liposyn, ethanol (Et) was infused during hyperglycemia in the third study type (Lip+/Et+), using a pharmacokinetic algorithm to attain GNG-inhibiting ethanol levels of 80 mg/dl within 20 min.

Under baseline conditions, hyperglycemia suppressed EGP by 61%. After raising plasma FFA to T2DM levels, suppression of EGP by hyperglycemia was impaired in Lip+ (34% decrease). During the Lip+/Et+ co-infusion studies the infusion of ethanol enhanced suppression of EGP by hyperglycemia (65.8% decrease, P=0.004 vs. Lip+) and thus restored glucose effectiveness (P=0.6 vs. Lip-/Et-). Thus, our results confirm the striking effects of elevated plasma FFA to impair glucose effectiveness and suggest that increased GNG contributes importantly to this loss of regulation. Inhibiting GNG could be an effective means of lowering EGP and improving glucose effectiveness in T2DM.

5.1. Zusammenfassung

Freie Fettsäuren (FFA) modulieren die Fähigkeit von Glukose die endogene Glukoseproduktion (EGP) zu unterdrücken und spielen eine wichtige Rolle bei dem Verlust der Glukoseeffektivität bei Typ-2-Diabetes mellitus (T2DM). Die Erhöhung freier Fettsäuren in Nicht-Diabetikern (ND) für mindestens 6 Stunden steigert die Glukoneogenese (GNG) und beeinträchtigt die Glukoseeffektivität. Ziel dieser Studien war es daher zu erkennen inwiefern die GNG für den Verlust der Glukoseeffektivität verantwortlich ist und ob die EGP in der Gegenwart von erhöhten FFA, durch die Inhibierung der GNG mit Ethanol, gehemmt werden kann.

Um die Auswirkung der Hemmung der GNG auf die Glukoseeffektivität zu bestimmen haben wir die EGP ([3-3H]-glucose) während drei verschiedener normoglykämischen/ hyperglykämischen 'Pancreatic Clamp' Studien (Infusion von Somatostatin; Ersetzung basaler Konzentrationen von Glukagon, GH, und Insulin) von jeweils 7 Stunden Dauer in n=7 ND Probanden (1W/6M; Alter=45±5 Jahre; BMI=27.6±3.0 kg/m^2) gemessen. Nach einer initialen Phase der Euglykämie (Blutglukosekonzentration bei 5 mmol/l; t=0-210 Minuten) wurde für den Zeitintervall t=210-420 Minuten die Blutglukosekonzentration auf 10 mmol/l erhöht. Die erste 'Pancreatic Clamp' Studie war eine Kontrollstudie mit Infusion einer NaCl-Lösung (Lip-/Et-). Eine Lipidemulsion (Liposyn 20%) wurde während der zweiten und dritten Studie (Lip+ und Lip+/Et-) infundiert, um die FFA Plasmaspiegel auf Konzentrationen zu erhöhen, die charakteristisch für den T2DM sind (~ 500 mmol/l). In Ergänzung zu Liposyn wurde Ethanol (Et) während der hyperglykämischen Phase der dritten Studie (Lip+/Et+) zugeführt. Mittels eines pharmakokinetischen Algorithmus wurden innerhalb von 20 Minuten Ethanolwerte erreicht die die GNG hemmen (~80 mg/dl).

In den Kontrollstudien verminderte sich die EGP um 61% mit Einsetzen der Hyperglykämie. Nach Infusion von Liposyn in den Lip+ Studien verminderte sich die EGP in Folge der Hyperglykämie jedoch nur um 34%. Die GNG wurde rasch durch die Infusion von Ethanol in den Lip+/Et+ Studien gehemmt und verbesserte signifikant die hyperglykämie-induzierte Suppression der EGP (65% Verminderung der EGP, P=0.004 vs. Lip+). Dadurch wurde die normale Glukoseeffektivität wiederhergestellt (P=0.6 vs. Lip-/Et-). Diese Ergebnisse bestätigen die markante Rolle erhöhter Plasma FFA-Spiegel

für die Beeinträchtigung der Glukoseeffektivität und deuten auf die Zentrale Rolle der GNG für den Verlust dieser Regulierung hin. Die Inhibierung der GNG könnte eine effektive Maßnahme sein, die EGP bei T2DM zu vermindern und die Glukoseeffektivität wiederherzustellen.

6. References

1. National Diabetes Data Group: Diabetes in America. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, NIH Publication No. 95-1468, 1995

2. Ruggenenti P, Fassi A, Ilieva AP, Bruno S, Iliev IP, Brusegan V, Rubis N, Gherardi G, Arnoldi F, Ganeva M, Ene-Iordache B, Gaspari F, Perna A, Bossi A, Trevisan R, Dodesini AR, Remuzzi G, the Bergamo Nephrologic Diabetes Complications Trial (BENEDICT) Investigators: Microalbuminuria in type 2 diabetes. *NEJM* 351 (16): 1941-51, 2004

3. World Health Organization. Diabetes 2006. (Accessed March 8, 2006, at http://www.who.int/dietphysicalactivity/publications/facts/diabetes/en/.)

4. Steinbrook R: Facing the Diabetes Epidemic- Mandatory Reporting of Glycosylated Hemoglobin Values in New York City. *New England Journal of Medicine* 354: 545-548, 2006

5. Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL: Diabetes mellitus. In *Harrison's Principles of Internal Medicine* 16th edition (pp. 2152-80). New York: McGraw-Hill, 2005

6. American Diabetes Association: Standards of medical care in diabetes- 2006. *Diabetes Care* 29: S4-S42, 2006

7. International Expert Committee: International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care* 32: 1327- 34, 2009

8. DeFronzo R: The triumvirate: beta cell, muscle and liver; a collusion responsible for T2DM. *Diabetes* 37: 667-87, 1988

9. Bogardus C, Lillioja S, Howard B, Reaven G, Mott D: Relationship between insulin secretion, insulin action and fasting plasma glucose conc. in nondiabetic & T2DM subjects. *J Clin Invest* 74: 1238-46, 1984

10. Lewis GF, Carpentier A, Adeli K, Giacca A: Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* 23: 201-29, 2002

11. Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi: Glucose Toxicity in ß-cells: Type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes* 52: 581-87, 2003

12. Cnop M, Hannaert JC, Hoorens A, Eizirik DL, Pipeleers DG: Inverse relationship between cytotoxicity of free fatty acids in pancreatic islet cells and cellular triglyceride accumulation. *Diabetes* 50: 1771-77, 2001

13. Shimabukuro M, Zhou Y, Levi M, Unger RH: Fatty acid-induced ß cell apoptosis: a link between obesity and diabetes. *Proc Natl Acad Sci US* A95: 2498-2502, 1998

14. Mitrakou A, Kelley D, Veneman T, Jenssen T, Pangburn T, Reilly J, Gerich J: Contribution of abnormal muscle and liver glucose metabolism to postprandial hyperglycemia in NIDDM. *Diabetes* 39: 1381-90, 1990

15. Mittelman SD, Fu YY, Rebrin K, Steil G, Bergman RN: Indirect effect of insulin to suppress endogenous glucose production is dominant, even with hyperglucagonemia. *J Clin Invest* 100(12):3121-30, 1997

16. Fisher SJ, Kahn CR: Insulin signaling is required for insulin's direct and indirect action on hepatic glucose production. *J Clin Invest* 111(4):463-8, 2003

17. Mevorach M, Giacca A, Aharon Y, Hawkins M, Shamoon H, and Rossetti L: Regulation of Endogenous Glucose Production by Glucose per se is Impaired in Type 2 Diabetes Mellitus. *J Clin Invest* 102:744-753, 1998

18. Bergman R: Interaction of insulin and glucose in the control of hepatic glucose balance. *Am J Physiol* 227: 1314-22, 1974

19. Liljenquist JE, Mueller GL, Cherrington AD, Perry JM, Rabinowitz D: Hyperglycemia per se (insulin and glucagon withdrawn) can inhibit hepatic glucose production in man. *J Clin Endocrinol Metab* 48: 171-75, 1979

20. Sacca L, Hendler R, Sherwin RS: Hyperglycemia inhibits glucose production in man independent of changes in glucoregulatory hormone. *J Clin Endocrinol Metab* 47: 1160-63, 1978

21. DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wahren J: Regulation of splanchic and peripheral glucose uptake by insulin and hyperglycemia in man. *Diabetes* 32: 35-45, 1983

22. Bell PM, Firth RG, Rizza RA: Effect of hyperglycemia on glucose production and utilization in humans. *Diabetes* 35:642-48, 1986

23. Best JD, Kahn SE, Ader M, Watanabe RM, Ni TC, Bergman RN: Role of glucose effectiveness in the determination of glucose tolerance. *Diabetes Care* 19:1018-30, 1996

24. Roden M, Perseghin G, Petersen KF, Hwang JH, Cline GW, Gerow K, Rothman DL, Shulman GI: The roles of insulin and glucagon in the regulation of hepatic glycogen synthesis and turnover in humans. *J Clin Invest* 97(3):642-8, 1996

25. The Diabetes Control and Complications Trial Research Group: The effect of intensive diabetes therapy on the development and progression of neuropathy. *Ann Intern Med* 122:561-568, 1995

26. Remuzzi G, Schieppati A, Ruggeneti P: Nephropathy in patients with type 2 diabetes. *N Engl J Med* 346: 1145-51, 2002

27. Boulton A, Kirsner R, Vileikyte L: Neuropathic diabetic foot ulcers. *N Engl J Med* 351: 48-55, 2004

28. Campbell P, Mandarino L, Gerich J: Quantification of the relative impairment in actions of insulin on hepatic glucose production and peripheral glucose uptake in non-insulin-dependent diabetes mellitus. *Metabolism* 37: 15-21, 1988

29. Moller N, Rizza RA, Ford GC, Nair KS: Assessment of postabsorptive renal glucose metabolism in humans with multiple glucose tracers. *Diabetes* 50(4): 747-51, 2001

30. Rossetti L, Giaccari A, Barzilai N, Howard K, Sebel G, Hu M: Mechanism by which hyperglycemia inhibits glucose production in conscious rats. *J Clin Invest* 92: 1126-34, 1993

31. Rognstad R: Glucose-6-phosphatase flux and the hepatic glucose balance model. *Am J Physiol* 271 (6 Pt 1): E1125-7, 1996

32. Petersen KF, Laurent D, Rothman DL, Cline GW, Shulman GI: Mechanism by which glucose and insulin inhibit net hepatic glycogenolysis in humans. *J Clin Invest* 101 (6): 1203-9, 1998

33. Mueckler M: Family of glucose-transporter genes: implications for glucose homeostasis and diabetes. *Diabetes* 39: 6-11, 1990

34. Bell GI, Kayano T, Buse JB, Burant CF, Takeda J, Lin D, Fukumoto H, Seino S: Molecular biology of mammalian glucose transporters. *Diabetes Care* 13: 198-208, 1990

35. Lang CH: Rates and tissue sites of non-insulin and insulin-mediated glucose uptake in diabetic rats. *Proc Soc Exp Biol Med* 199: 81-89, 1992

36. Crone C: Facilitated diffusion of glucose from blood to brain tissue. *J Physiol* 181:103-113, 1965

37. Galante P, Mosthaf L, Kellerer M, Berti L, Tippmer S, Bossenmaier B, Fujiwara T, Okuno A, Horikoshi H, Haring HU: Acute hyperglycemia provides an insulin-independent inducer for GLUT4 translocation in C2C12 myotubes and rat skeletal muscle. *Diabetes* 44: 646-651, 1995

38. Nolte LA, Rincon J, Wahlstrom EO, Craig BW, Zierath JR, Wallberg-Henriksson H: Hyperglycemia activates glucose transport in rat skeletal muscle via a Calcium-dependent mechanism. *Diabetes* 44: 1345-48, 1995

39. Printz RL, Magnuson MA, Granner DK: Mammalian glucokinase. *Ann Rev Nutr* 13: 463-96, 1992

40. Hawkins M, Gabriely I, Wozniak R, Reddy K, Rossetti L, Shamoon H: Glycemic control determines hepatic and peripheral glucose effectiveness in type 2 diabetic subjects. *Diabetes* 51: 2179-89, 2002

41. Nagasaka S, Tokuyama K, Kusaka I, Hayashi H, Rokkaku K, Nakamura T, Kawakami A, Higashiyama M, Ishikawa S, Saito T: Endogenous Glucose Production and Glucose Effectiveness in Type 2 Diabetic Subjects Derived From Stable-Labeled Minimal Model Approach. *Diabetes* 48: 1054-1060, 1999

42. Del Prato SD, Matsuda M, Simonson DC, Groop LC, Sheehan P, Leonetti F, Bonadonna RC, De Fronzo RA: Studies on the mass action effect of glucose in NIDDM and IDDM: evidence for glucose resistance. *Diabetologia* 40: 687-97, 1997

43. Forbes A, Elliott T, Tildesley H, Finegood D, Meneilly GS: Alterations in non-insulin-mediated glucose uptake in the elderly patient with diabetes. *Diabetes* 47: 1915-19, 1998

44. Nielsen MF, Basu R, Wise S, Caumo A, Cobelli C, Rizza RA: Normal Glucose-Induced Suppression Of Glucose Production But Impaired Stimulation Of Glucose Disposal in Type 2 Diabetes. *Diabetes* 47: 1735-1747, 1998

45. Caruso M, Miele C, Oriente F, Maitan A, Bifulco G, Andreozzi F, Condorelli G, Formisano P, Beguinot F: In L6 skeletal muscle cells, glucose induces cytosolic translocation of protein kinase C-alpha and trans-activates the insulin receptor kinase. *J Biol Chem* 274: 28637-44, 1999

46. Moore MC, Connolly CC, Cherrington AD: Autoregulation of hepatic glucose production. *Eur J Endocrinol* 138 (3): 240-48, 1998

47. Magnusson I, Rothman DL, Katz LD, Shulman RG, Schulman GI: Increased rate of gluconeogenesis in type 2 diabetes mellitus. *J Clin Invest* 90: 1323-27, 1992

48. Boden G, Chen X, Stein TP: Gluconeogensis in moderately and severly hyperglycemic patients with type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab* 280: E23-E30, 2001

49. Trimmer JK, Casazza GA, Horning MA, Brooks GA: Autoregulation of glucose production in men with a glycerol load during rest and exercise. *Am J Physiol Endocrinol Metab* 280 (4): E657-68, 2001

50. Aiston S, Anderson B, Agius L: Glucose-6-phosphate regulates hepatic glycogenolysis through inactivation of phosphorylase. *Diabetes* 52(6): 1333-9, 2003

51. Massillon D, Chen W, Barzilai N, Prus-Wertheimer D, Hawkins M, Liu R, Taub R, Rossetti L: Abnormal regulation of HEGP by hyperglycemia in mice with a disrupted glucokinase allele. *J Biol Chem* 273: 228-34, 1998

52. Aiston S, Trinh KY, Lange AJ, Newgard CB, Agius L: Glucose-6-phosphatase overexpression lowers glucose-6-phosphate and inhibits glycogen synthesis and glycolysis in hepatocytes without affecting glucokinase translocation. Evidence against feedback inhibition of glucokinase. *J Biol Chem* 274 (35): 24559-66, 1999

53. Barzilai N, Hawkins M, Angelov I, Hu M, Rossetti L: Glucosamine-induced inhibition of liver glucokinase impairs the ability of hyperglycemia to suppress endogenous glucose production. *Diabetes* 45: 1329-35, 1996

54. Basu A, Basu R, Shah P, Vella A, Johnson CM, Nair KS, Jensen MD, Schwenk WF, Rizza RA: Effects of type 2 diabetes on the ability of insulin and glucose to regulate

splanchnic and muscle glucose metabolism: evidence for a defect in hepatic glucokinase activity. *Diabetes* 49(2): 272-83, 2000

55. Coppack SW, Evans RD, Fisher RM, Frayn KN, Gibbons GF, Humphreys SM, Kirk ML, Potts JL, Hockaday TD: Adipose tissue metabolism in obesity: lipase action *in vivo* before and after a mixed meal. *Metabolism* 41: 264-272, 1992

56. Lewis GF, O'Meara NM, Soltys PA, Blackman JD, Iverius PH, Pugh WL, Getz GS, Polonsky KS: Fasting hypertriglyceridemia in noninsulin-dependent diabets mellitus is an important predictor of postprandial lipid and lipoprotein abnormalities. *J Endocrinol Metab* 72: 934-944, 1991

57. Gustafson LA, Neeft M, Reijngoud DJ, Kuipers F, Sauerwein HP, Romijn JA, Herling AW, Burger HJ, Meijer AJ: Fatty acid and amino acid modulation of glucose cycling in isolated rat hepatocytes. *Biochem J* 358(Pt 3): 665-71, 2001

58. Hawkins M, Gabriely I, Wozniak R, Vilcu C, Shamoon H, Rossetti L: Fructose improves the ability of hyperglycemia per se to regulate glucose production in type 2 diabetes. *Diabetes* 51(3):606-14, 2002

59. Basu A, Basu R, Shah P, Vella A, Johnson CM, Jensen M, Nair KS, Schwenk WF, Rizza RA: Type 2 diabetes impairs splanchnic uptake of glucose but does not alter intestinal glucose absorption during enteral glucose feeding: additional evidence for a defect in hepatic glucokinase activity. *Diabetes* 50(6):1351-62, 2001

60. Boden G, Chen X, Capulong E, Mozzoli M: Effects of free fatty acids on gluconeogenesis and autoregulation of glucose production in type 2 diabetes. *Diabetes* 50: 810-16, 2001

61. Shah P, Vella A, Basu A, Basu R, Adkins A, Schwenk WF, Johnson CM, Nair KS, Jensen MD, Rizza RA: Effects of free fatty acids and glycerol on splanchnic glucose metabolism and insulin extraction in nondiabetic humans. *Diabetes* 51(2): 301-10, 2002

62. Kishore P, Tonelli J, Koppaka S, Fratila C, Bose A, Lee D, Reddy K, Hawkins M: Time-dependent effects of free fatty acids on glucose effectiveness in type 2 diabetes. *Diabetes* (in press).

63. Lam TK, Carpentier A, Lewis GF, van de Werve G, Fantus IG, Giacca A: Mechanisms of the free fatty acid-induced increase in hepatic glucose production. *Am J Physiol Endocrinol Metab* 284(5):E863-73, 2003

64. Massillon D, Barzilai N, Hawkins M, Prus-Wertheimer D, Rossetti L: Induction of hepatic glucose-6-phosphatase gene expression by lipid infusion. *Diabetes* 46:153-7, 1997

65. van de Werve G, Lange A, Newgard C, Mechin MC, Li Y, Berteloot A: New lessons in the regulation of glucose metabolism taught by the glucose 6-phosphatase system. *Eur J Biochem* 267(6): 1533-49, 2000

66. Jump DB, Clarke SD, Thelen A, Liimatta M: Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. *J Lipid Res* 35:1076-84, 1994

67. Lam TK, van de Werve G, Giacca A: Free fatty acids increase basal hepatic glucose production and induce hepatic insulin resistance at different sites. *Am J Physiol Endocrinol Metab* 284:E281-90, 2003

68. Oakes N, Cooney G, Camilleri S, Chisholm D, and Kraegen E: Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. *Diabetes* 46: 1768-74, 1997

69. Clore J, Stillman J, Sugarman H: Glucose-6-Phosphatase flux in vitro is increased in type 2 diabetes. *Diabetes* 49:969-74, 2000

70. Caro J, Triester S, Patel V, Tapscott E, Frazier N, and Dohm G: Liver glucokinase: decreased activity in patients with type II diabetes. *Horm Met Res* 27:19-22, 1995

71. Reaven GM, Hollenbeck C, Jeng CY, Wu MS, Chen YD: Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM. *Diabetes*. 37(8):1020-4, 1988

72. Williamson JR, Kreisberg RA, Felts PW: Mechanism for the stimulation of gluconeogenesis by fatty acids in perfused rat liver. *Proc Natl Acad Sci USA* 56: 247-254, 1966

73. Antras-Ferry J, Le Bigot G, Robin P, Robin D, Forest C: Stimulation of phosphoenolpyruvate carboxykinase gene expression by fatty acids. *Biochem Biophys Res Commun* 203: 385-391, 1994

74. Clore JN, Glickman PS, Nestler JE, Blackard WG: In vivo evidence for hepatic autoregulation during FFA-stimulated gluconeogenesis in normal humans. *Am J Physiol* 261 (4 Pt. 1): E425-9, 1991

75. Whitelaw DC, Smith JM, Nattrass M: Effects of gemfibrozil on insulin resistance to fat metabolism in subjects with type 2 diabetes and hypertriglyceridaemia. *Diabetes, Obesity and Metabolism* May (4) 3: 187-94, 2002

76. Vaag AA, Beck-Nielsen H: Effects of prolonged acipimox treatment on glucose and lipid metabolism and on in vivo insulin sensitivity in patients with non-insulin dependent diabetes mellitus. *Acta Endocrinol (Copenh)* 127(4): 344-50, 1992

77. Saloranta C, Taskinen MR, Widen E, Harkonen M, Melander A, Groop L: Metabolic consequences of sustained suppression of free fatty acids by acipimox in patients with NIDDM. *Diabetes* 42(11): 1559-66, 1993

78. Avogaro A, Miola M, Favaro A, Gottardo L, Pacini G, Manzato E, Zambon S, Sacerdoti D, de Kreuzenberg S, Piliego T, Tiengo A, Del Prato S: Gemfibrozil improves insulin-sensitivity and flow-mediated vasodilation in type 2 diabetic patients. *Eur J Clin Invest* 31(7): 603-9, 2001

79. Mussoni L, Mannucci L, Sirtori C, Pazzucconi F, Bonfardeci G, Cimminiello C, Notarbartolo A, Scafidi V, Bittolo Bon G, Alessandrini P, Nenci G, Parise P, Colombo L, Piliego T, Tremoli E: Effects of gemfibrozil on insulin sensitivity and on haemostatic variables in hypertriglyceridemic patients. *Atherosclerosis* 148(2): 397-406, 2000

80. Hawkins M, Tonelli J, Kishore P, Stein D, Ragucci E, Gitig A, Reddy K: Contribution of elevated free fatty acid levels to the lack of glucose effectiveness in type 2 diabetes. *Diabetes* 52(11):2748-58, 2003

81. Trinh KY, O'Doherty RM, Anderson P, Lange AJ, Newgard CB: Perturbation of fuel homeostasis caused by overexpression of the glucose-6-phosphatase catalytic subunit in liver of normal rats. *J Biol Chem* 273(47):31615-20, 1998

82. Ramchandani VA, Bolane J, Li TK, O'Connor S: A physiologically-based pharmacokinetic (PBPK) model for alcohol facilitates rapid BrAC clamping. *Alcohol Clin Exp Res* 23(4): 617-23, 1999

83. NIAAA: Recommended Council Guidelines on Ethyl Alcohol Administration in Human Experimentation. Rockville, MD, 1989

84. Rossetti L, Chen W, Hu M, Hawkins M, Barzilai N, and Efrat S: Abnormal regulation of HEGP by hyperglycemia in mice with a disrupted glucokinase allele. *Am J Physiol* 273: E743-750, 1997

85. Shamoon H, Friedman S, Canton C, Zacharowicz L, Hu M, Rossetti L: Increased epinephrine and skeletal muscle responses to hypoglycemia in noninsulin-dependent diabetes mellitus. *J Clin Invest* 93: 2562-2571, 1994

86. Sotsky MJ, Shilo S, Shamoon H: Regulation of counterregulatory hormone secretion in man during exercise and hypoglycemia. *J Clin Endocrinol Metab* 68:9–17, 1989

87. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE: Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 108:1167–1174, 2001

88. Mannaerts, G.P., Debeer, L.J., Thomas, J., and DeSchepper, P.J.: Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats. *J Biol Chem* **254**:4585-4595, 1979

89. Landau BR, Wahren J, Chandramouli V, Schumann WC, Ekberg K, Kalhan SC: Contributions of gluconeogenesis to glucose production in the fasted state. *J Clin Invest* 98: 378-385, 1996.

90. Staehr P, Hother-Nielsen O, Landau BR, Chandramouli V, Holst JJ, Beck-Nielsen H: Effects of free fatty acids per se on glucose production, gluconeogenesis, and glycogenolysis. *Diabetes* 52: 260-267, 2003

91. Shah P, Basu A, Rizza R: Fat-induced liver insulin resistance. *Curr Diab Rep* 3(3): 214-8, 2003

92. Greenway CV, Lautt WW: Acute and chronic ethanol on heptatic oxygen ethanol and lactate metabolism in cats. *Am J Physiol* 258 (3 Pt 1): G411-8, 1990

93. Sarkola T, Iles MR, Kohlenberg-Mueller K, Eriksson CJ: Ethanol, acetaldehyde, acetate, and lactate levels after alcohol intake in white men and women: effect of 4-methylpyrazole. *Alcohol Clin Exp Res.* 26(2):239-45, 2002

94 . Avogaro A, Valerio A, Miola M, Crepaldi C, Pavan P, Tiengo A, del Prato S: Ethanol impairs insulin-mediated glucose uptake by an indirect mechanism. *J Clin Endocrinol Metab* 81 (6): 2285-90, 1996

95. Ader M, Bergman RN: Peripheral effects of insulin dominate suppression of fasting hepatic glucose production. *Am J Physiol* 258: E1020-32, 1990

96. Rebrin K, Steil GM, Getty L, Bergman RN: Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. *Diabetes* 44: 1038-45, 1995

97. Song S, Andrikopoulos S, Filippis C, Thorburn AW, Khan D, Proietto J: Mechanism of fat-induced hepatic gluconeogenesis: effect of metformin. *Am J Physiol Endocrinol Metab* 281(2): E275-82, 2001

98. Ruderman NB, Toews CJ, Shafrir E: Role of free fatty acids in glucose homeostasis. *Arch Intern Med* 123:299-313, 1969

99. Lam TK, Yoshii H, Haber CA, Bogdanovic E, Lam L, Fantus IG, and Giacca A: Free fatty acid-induced hepatic insulin resistance: a potential role for protein kinase C-. *Am J Physiol Endocrinol Metab* 283: E682-E691, 2002

100. Williamson JR, Browning ET, and Scholz R: Control mechanisms of gluconeogenesis and ketogenesis. I. Effects of oleate on gluconeogenesis in perfused rat liver. *J Biol Chem* 244(17):4607-16, 1969

101. Jenssen T, Nurjhan N, Consoli A, Gerich J: Failure of substrate- induced glucogenesis to increase overall glucose appearance in normal humans; Demonstration of hepatic autoregulation without a changing plasma glucose concentration. *J Clin Invest* 86: 489-97, 1990

102. Puhakainen I, Koivisto VA, Yki-Jarvinen H: No reduction in total hepatic glucose output by inhibition of gluconeogenesis with ethanol in T2DM patients. *Diabetes* 40:1319-27, 1991

103. Puhakainen I and Yki-Jarvinen H: Inhibition of lipolysis decreases lipid oxidation and gluconeogenesis from lactate but not fasting hyperglycemia or total hepatic glucose production in NIDDM. *Diabetes* 42:1694-1699, 1993

104. Stingl H, Krssak M, Krebs M, Bischof MG, Nowotny P, Furnsinn C, Shulman GI, Waldhausl W, Roden M: Lipid-dependent control of hepatic glycogen stores in healthy humans. *Diabetologia* 44(1):48-54, 2001

105. Chen X, Iqbal N, Boden G: The effects of free fatty acids on gluconeogenesis and glycogenolysis in normal subjects. *J Clin Invest* 103(3):365-72, 1999

106. Rebrin K, Steil GM, Mittelman SD, and Bergman RN: Causal linkage between insulin suppression of lipolysis and suppression of liver glucose output in dogs. *J Clin Invest* 98: 741-749, 1996

107. Roden, M, Stingl H, Chandramouli V, Schumann WC, Hofer A, Landau BR, Nowotny P, Waldhausl W, and Shulman GI: Effects of free fatty acid elevation on postabsorptive endogenous glucose production and gluconeogenesis in humans. *Diabetes* 49: 701-707, 2000

108. Chu CA, Sherck SM, Igawa K, Sindelar DK, Neal DW, Emshwiller M, and Cherrington AD: Effects of free fatty acids on hepatic glycogenolysis and gluconeogenesis in conscious dogs. *Am J Physiol Endocrinol Metab* 282: E402-E411, 2002

109. Lee KU, Park JY, Kim CH, Hong SK, Suh KI, Park KS, and Park SW: Effect of decreasing plasma free fatty acids by acipimox on hepatic glucose metabolism in normal rats. *Metabolism* 45: 1408-1414, 1996

110. Mokuda O, Tanaka H, Hayashi T, Ooka H, Okazaki R, Sakamoto Y. Ethanol stimulates glycogenolysis and inhibits both glycogenesis via gluconeogenesis and from exogenous glucose in perfused rat liver. Ann Nutr Metab 48: 276, 2004

111. Siler SQ, Neese RA, Christiansen MP, Hellerstein MK: The inhibition of gluconeogenesis following alcohol in humans. *Am J Physiol Endocrinol Metab* 275(5): E897 – 907, 1998

112. Krebs HA, Freedland RA, Hems R, Stubbs M: Inhibition of hepatic gluconeogenesis by ethanol. *Biochem J* 112: 117-124, 1969

113. Clore JN, Blackard WG: Suppression of gluconeogenesis after a three day fast does not deplete liver glycogen in patients with NIDDM. *Diabetes* 43: 256-262, 1994

114. Kreisberg RA, Siegal AM, Owen WC: Glucose-lactate interrelationships: effect of ethanol. *J. Clin. Invest.* 50: 175-185, 1971

115. Lieber CS, Leevy CM, Stein SW, George WS, Cherrick GR, Abelmann WH, Davidson CS: Effect of ethanol on plasma free fatty acids in man. *J Lab Clin Med* 59: 826-32, 1962

116. Polsky S, Howard AA, Bray GA, Brown-Friday J, Perreault L, Whittington T, Barrett-Connor E, Foo S, Ma Y, Edelstein S, Crandall J: Moderate alcohol consumption is associated with lower diabetes risk in the Diabetes Prevention Program. *Diabetes* 57 (Suppl. 1): 102-OR, 2008

117. Van Poelje PD, Potter SC, Fujitaki JM, Dang Q, Linemeyer DL, Erion MD: MB07803, a prodrug of a second generation fructose-1,6-bisphosphatase inhibitor, lowers blood glucose in diabetic rodents and in cynomolgus monkeys. *Diabetes* 57 (Suppl. 1): 350-OR, 2008

118. Long SD, Pekala PH: Regulation of GLUT4 gene expression by arachidonic acid. Evidence for multiple pathways, one of which requires oxidation to prostaglandin E2. *J Biol Chem* 271:1138-44, 1996

119. Baron AD, Brechtel G, Edelman SV: Effects of free fatty acids and ketone bodies on in vivo non-insulin-mediated glucose utilization and production in humans. *Metabolism* 38: 1056-1061, 1989

120. Piatti PM, Monti LD, Pacchioni M, Pontiroli AE, Pozza G: Forearm insulin- and non-insulin mediated glucose uptake and muscle metabolism in man: role of free fatty acids and blood glucose levels. *Metabolism* 40:926-933, 1991

121. Zierath JR, Housenecht KL, Gnudi L, Kahn BB: High-fat feeding impairs insulin-stimulated GLUT4 recruitment via an early insulin-signaling defect. *Diabetes* 46:215-223, 1997

122. Xu D, Dhillon AS, Davey CG, Fournier PA, Palmer TN: Alcohol and glucose metabolism in skeletal muscles in the rat. *Addict Biol* 1(1):71-83, 1996

123. Yki-Jarvinen H, Nikkila EA: Ethanol decreases glucose utilization in healthy men. *J Clin Endocrinol Metab* 61: 941-45, 1985

124. Shelmet JJ, Reichard GA, Skutchen CL, Hoeldtke RD, Owen OE, Boden G: Ethanol causes acute inhibition of carbohydrate, fat, and protein oxidation and insulin resistance. *J Clin Invest* 81: 1137-1145, 1988

125. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346: 393-403, 2002

126. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laasko M, Louheranta A, Rastas M, Salminen V, Uusitupa M: Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 344: 1343-50, 2001

127. Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, Lin J, Xiao JZ, Cao HB, Liu PA, Jiang XG, Jiang YY, Wang JP, Zheng H, Zhang H, Bennett PH, Howard BV:

Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance: the Da Qing IGT and Diabetes Study. *Diabetes Care* 20: 537-44, 1997

7. Appendix

7.1. Glossary

ADA	American Diabetes Association
ATP	adenosine triphosphate
BMI	body mass index
CDC	Center for Disease Control and Prevention
CVD	cardiovascular disease
DM	diabetes mellitus
DPP	Diabetes Prevention Program
EGP	endogenous glucose production
Et	ethanol
F	female
FFA	free fatty acids
FPG	fasting plasma glucose
G-6-Pase	glucose-6-phosphatase
GCRC	General Clinical Research Center
GDM	gestational diabetes mellitus
GH	growth hormone
GIR	glucose infusion rate
GK	glucokinase
Glc-6-P	glucose-6-phosphate
GLUT	glucose transporter
GNG	gluconeogenesis
GU	glucose uptake
HPLC	high performance liquid chromatography
HbA1C	hemoglobin A1C
IIR	insulin infusion rate
IRS	insulin resistance syndrome
Lip	Liposyn
M	male
ND	nondiabetic

NADH	nicotinamide adenine dinucleotide
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NS	not significant
OGTT	oral glucose tolerance test
PBPK	physiologically-based pharmacokinetic
PEPCK	phosphoenolpyruvate carboxykinase
PI-3-kinase	phosphatidylinositol 3-kinase
PKC	protein kinase C
PPAR	peroxisome proliferator-activated receptor
Ra	rate of appearance
Rd	rate of disappearance
SAp	plasma specific activity
SEM	standard error of measurement
T2DM	type 2 diabetes mellitus
W	weiblich
WHO	World Health Organization

6.2. Table Index

6.3. Figure Index

Acknowledgements

First and foremost, I would like to extend my deepest and sincere thanks to Dr. Meredith Hawkins for giving me the wonderful opportunity to work with her. Her constant encouragement, open door, and guidance over the past few years have made writing this dissertation an extremely enjoyable and exciting journey. I would also like to extend my greatest thanks to Prof. Dr. B. Allolio of the Department of Endocrinology at the Julius-Maximilians-University in Würzburg for supporting and overseeing this project in Germany. His thoughtful, helpful advice and encouragement have been invaluable to me. I also owe my deepest gratitude to Prof. Dr. Schlegel of the Department of Pediatrics at the Julius-Maximilians-University Würzburg for co-overseeing the paper.

Many thanks to Dr. Hawkins' lab team at Einstein: Dr. Preeti Kishore, Dr. Do-Eun Lee, Dr. Sudha Koppaka, Dr. Weijie Li, and Dr. Kehao Zhang. My warmest thanks particularly to Dr. Kishore for her great help, assistance, and patience putting the paper together. I would also very much like to thank Angela Stangarone for coordinating the studies and helping me with many computer technicalities.

Dr. Silvia Stefanescu, Dr. Septimiu Vele, and Robert Cashin deserve my deepest thanks for their indispensable help carrying out the clinical studies. I am furthermore greatly indebted to Robin Sgueglia and Greg Cruikshank for their great help with the hormone and lactose measurements, respectively. I would also like to extend my sincere gratitude to Dr. Sean O'Connor at Indiana University School of Medicine for the PBPK model of alcohol distribution and elimination and his kind help with it.

I am particularly grateful to Dr. Norman Fleisher for the privilege of working in the Department of Endocrinology at Albert Einstein College of Medicine. The opportunity to work with this brilliant group of scientists and teachers has been an extraordinary experience. I have learned more than I can express.