EFFECT OF AEROBIC EXERCISE ON PERIPHERAL GLUCOSE UPTAKE AND ENDOGENOUS GLUCOSE PRODUCTION IN TYPE 2 DIABETES MELLITUS

DISSESSATION

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ABSTRACT

**Background:** Previous investigations have examined the effect of aerobic exercise training on peripheral glucose uptake (PGU) and endogenous glucose production (EGP) in humans with type 2 diabetes mellitus (DM). These studies often use lengthy training durations that improve both body fat levels and maximal oxygen consumption. Improvements to either of these measures can improve PGU and EGP, making it difficult to assess the independent effect of aerobic exercise on PGU and EGP. An aerobic exercise training duration of seven days can improve glucose tolerance and insulin action in humans with DM without altering body fat levels or maximal oxygen consumption. It is not known if the improved glucose tolerance resulting from exercise is due to an improved capacity of insulin to stimulate PGU, suppress EGP, or a combination of these events. The purpose of this study was to determine the effect of seven days of aerobic exercise on PGU and EGP during isoglycemic/ two-step hyperinsulinemic clamp conditions. It was hypothesized that seven days of aerobic exercise would result in increased insulin stimulation of PGU and increased insulin suppression of EGP during the post-absorptive state (i.e., no insulin infusion) and during each of low and high insulin infusion rates. It was further hypothesized that these changes to PGU and EGP would occur in a dose-response fashion relative to rate of insulin infusion. **Methods:** Eighteen obese, mildly diabetic humans were randomly assigned to one of two groups:
the diet only group consumed an isocaloric diet that was provided for them by the GCRC metabolic kitchen staff consisting of 50% carbohydrate, 30% fat and 20% protein for 15 days. The diet and exercise group consumed a similar diet over the 15 days and performed 50 minutes of treadmill walking at an intensity of 70% of each subject’s maximal oxygen consumption during the second seven days of the 15 day study period. Caloric expenditure from exercise was replaced with food energy. In addition to measuring maximal oxygen consumption and body fat levels before and after the intervention, each subject underwent an isoglycemic/ two-step hyperinsulinemic glucose clamp after the first and second week of participation in the study, where PGU and EGP were measured using 3-[^3]H labeled glucose. As anticipated by the study design, maximal oxygen consumption and body fat levels did not change in response to the intervention in either group. The exercise training intervention did not have an impact on PGU or EGP during post-absorptive insulin levels, or during the low-insulin infusion period of the isoglycemic clamp, and it did not alter EGP during the high-insulin infusion period. However, seven days of aerobic exercise training significantly increased PGU (p < 0.05) and muscle insulin sensitivity (p < 0.0001) during the high-insulin infusion period. **Conclusion:** Improvements to glucose tolerance and insulin action that have been observed by others in response to seven days of aerobic exercise training are likely due to changes in peripheral insulin sensitivity, not hepatic insulin sensitivity.
This work is dedicated to my family for their unwavering support during my educational pursuits
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CHAPTER 1

INTRODUCTION

In the United States today, there are millions of people who have been diagnosed with type 2 diabetes mellitus (DM), and millions who unknowingly have the disease. In addition to the tremendous financial burden DM places on our health care system, it can more importantly impact both the quality and length of life. Although years of research, have led to a better understanding of the pathology of this disease, there is no cure for DM.

Despite an apparently strong genetic influence on the development of DM, there is also strong evidence supporting a role of environmental factors in the development of the disease. Of these factors, obesity and inactivity have received much attention. Epidemiological research has clearly demonstrated that as an individual’s body fat percentage increases and/or participation in physical activity decreases, the risk of developing DM increases dramatically (Ohlson, 1985; Wei, 1999; Sesso, 2000; DPP Research Group, 2002). Indeed, the “correction” of each of these factors after DM has developed can result in a significant improvement of glucose metabolism and reduce elevated fasting levels of blood glucose, the hallmark characteristics of DM (Ivy, 1999).
In addition to a better understanding of factors which increase the likelihood of developing DM, there has also been a great deal of work done in an attempt to elucidate the mechanisms responsible for the development of DM. Although the development of DM is often multi-factorial, it is most often characterized by insulin resistance, relative pancreatic β-cell dysfunction and resultant hyperglycemia (Ivy, 1999). From this perspective, it is believed that DM is precipitated by insulin resistance in adipose, hepatic and skeletal muscle tissues. This state of insulin resistance can result in elevated rates of hepatic glucose output and reduced glucose uptake by peripheral tissues. The body’s response to this state is to release greater quantities of insulin, however, eventually pancreatic β-cells become unable to supply the body’s increasing demand for insulin to maintain blood glucose homeostasis, resulting in hyperglycemia.

Of the three tissues previously identified as those in which insulin resistance develops, it is likely that insulin resistance of skeletal muscle and liver contribute to the development of DM to the greatest extent. In DM, the rates of glucose uptake at a steady state insulin concentration are lower compared to those who are non-diabetic (Wright, 1988; Arciero, 1999; Nagasaka, 1999). The mechanisms by which this observed reduction in insulin sensitivity occur are numerous, but appear to include reduced expression of key 2nd messenger proteins involved in the insulin signaling cascade (Zierath, 1998), a reduction in Glut 4 movement to the muscle cell surface in response to insulin (Broznick, 1994), and a reduced capacity to metabolize glucose via oxidative and non-oxidative pathways (Golay, 1988; Wright, 1988; Shulman, 1990; Thorburn, 1990).
An elevated rate of hepatic glucose output has been suggested to be the primary contributor to elevated fasting plasma glucose levels in DM (DeFronzo, 1988). Although there are many different hormonal and substrate availability factors common to DM that affect hepatic glucose output, it has been suggested that the most important factor is insulin, as hepatic insulin resistance has been shown to persist in DM. While the mechanisms by which hepatic insulin resistance occur have received far less attention compared to skeletal muscle, it appears to include elevated rates of glycogenolysis and gluconeogenesis in combination with reduced glycogen synthesis (Magnusson, 1992; Rossetti, 1993; Gastaldelli, 2000; Paquot, 2002). At the cellular level, it has been revealed that chronic hyperglycemia, the hallmark characteristic of DM, contributes to an increase in the expression of the G6Pase gene and protein (Massillon, 1996; Clore, 2000) as well as a reduction in the expression of the GK protein (Clore, 2000). Altered expression of these rate-limiting enzymes for hepatic glucose output, and impaired insulin induced regulation of their activity are likely to play significant roles in the observed elevated rates of hepatic glucose output in DM.

Traditionally, the disease state of DM has been treated using pharmacological means. However, in recent years the employment of regular exercise training has gained widespread acceptance as an effective non-conventional modality of treatment for DM. Previous research has demonstrated that exercise can have an acute effect (i.e., lowering of blood glucose during exercise) on blood glucose by increasing the rate of uptake and disposal of blood glucose in peripheral tissues (Minuk, 1981), as well as a chronic effect (Ivy, 1999).
The mechanism by which long-term exercise training improves glucose metabolism in DM likely involves improvement of the insulin resistant state. Previous research has demonstrated that regular aerobic exercise training can result in greater rates of glucose uptake at given concentrations of insulin (Rodnick, 1987; Arciero, 1999). The mechanisms by which the exercise-induced gains in sensitivity of peripheral tissues to insulin occur are numerous. It is likely that the most important changes include upregulation of the expression of Glut 4 protein (Dela, 1994), upregulation and increased activation of proteins involved in the 2nd messenger cascade of insulin (Chibalini, 2000), greater translocation of Glut 4 to the cell surface in response to insulin (Goodyear, 1992; Dela, 1994), and a greater capacity of skeletal muscle to metabolize glucose via oxidative and non-oxidative pathways (Ren, 1994).

In addition to gains in skeletal muscle insulin sensitivity in DM, hepatic tissue can also experience favorable changes in insulin sensitivity in response to aerobic exercise training. Previous research has demonstrated that a single bout of exhaustive exercise can result in reduced rates of endogenous glucose production at basal and elevated insulin levels (Devlin, 1987), while other studies have demonstrated that long-term exercise training can also result in improved insulin-induced suppression of endogenous glucose production (Segal, 1995). In contrast to skeletal muscle, there are few studies which have attempted to characterize the mechanisms by which hepatic insulin sensitivity is increased. However, it is likely that these gains are caused in part by improved hormonal (i.e., insulin) regulation of the expression and activity of rate-limiting enzymes of endogenous glucose production and hepatic glucose output including GK, G6Pase and PEPCK.
A number of previous studies have examined the impact of seven days of aerobic exercise training on glucose metabolism in DM. While one may wonder the purpose of determining the impact of such a short training duration on glucose metabolism in DM, it is important because it limits certain whole body responses to exercise. Longer training duration may result in significant changes in body fat levels and/or VO$_2$ max. Because each of these factors can have a significant impact upon glucose metabolism in DM, their change can confound meaningful determination of the independent effect of exercise on endogenous glucose production and peripheral glucose uptake. However, there are limitations to each of these studies which can be improved upon in an attempt to better characterize the independent effect of exercise on glucose metabolism in DM. Kang et al. (1996) provided data suggesting that seven days of vigorous (70% VO$_2$ max) aerobic exercise did not result in improved insulin sensitivity assessed by OGTT in DM. However the authors acknowledged that this level of response was likely due to relative hypoinsulinemia of some of the diabetic subjects. Rogers et al. (1988) showed that seven days of aerobic exercise training resulted in improved insulin action and glucose tolerance, however, this was after the non-inclusion of data from subjects who were hypoinsulinemic. Moreover, neither of these studies employed methodologies that would allow the assessment of insulin sensitivity using the so-called gold standard technique of the hyperinsulinemic glucose clamp. Furthermore, the relative contribution of hepatic and peripheral tissues to improved insulin action is not known. Therefore, the purpose of this study was to determine the impact of seven days of aerobic exercise, using a protocol previously shown to improve glucose metabolism in DM without altering body composition or VO$_2$ max, on endogenous glucose production and peripheral glucose uptake.
uptake using the isoglycemic/hyperinsulinemic clamp technique, at post-absorptive insulin levels, and each of two insulin infusion rates. It was hypothesized that exercise training would reduce endogenous glucose production and increase peripheral glucose uptake in a dose-response manner at post-absorptive insulin levels, as well as at each of two pre-determined levels of insulin infusion.
CHAPTER 2

REVIEW OF LITERATURE

Type 2 diabetes mellitus (DM) is a disease that is characterized by elevated fasting blood glucose levels, and/or impaired glucose tolerance, and accounts for approximately 90-95% of all diabetes cases in the United States. Although our understanding of the mechanisms that precipitate the development of DM has increased dramatically in the past 30 years, there is still no cure for this disease state. In fact, according to National Health Interview Survey (NHIS) data presented by the CDC, from 1980 to 2002 the number of self reported cases of DM has increased from 5.8 million to 13.3 million. Moreover, these figures underestimate the number of cases of diabetes because approximately one-third of individuals who have DM remain undiagnosed. It has been suggested that as the prevalence of obesity and life expectancy continue to increase, and participation in physical activity declines, the incidence and prevalence of DM will increase above the current levels. This is unfortunate, because in addition to the soaring health care costs ascribed to DM, it can also have an impact on the quality and length of life of those who suffer from it. The purpose of this literature review is to describe the problem of DM, discuss mechanisms by which its development is likely precipitated and discuss the positive impact aerobic exercise can have on glucose metabolism in DM.
**Descriptive Data**

The CDC has reported that from 1980 to 2002, the incidence and prevalence of DM increases with advancing age, and have increased in all age groups. In 2002, the incidence rate of DM diagnosis in people aged 45-64 years (12.6 diagnoses per 1000 population) was 4.5 times that of people aged 18-44 years (2.8 diagnoses per 1000 population). Similarly, the prevalence rates of those between the ages of 65-74 (16.8%) are 14 times that of individuals less than 45 years of age (1.2%). Because of advances in medicine resulting in greater life expectancy, this age-related increase in DM incidence and prevalence is expected to contribute to the projected dramatic increase in the number of people suffering from DM.

In addition to advancing age being a significant risk factor for the development of DM, disparities across race also exist (Figure 2.1). The current prevalence rates of DM is greater in blacks than in whites, with the highest rates being observed in black females. Moreover, from 1980 to 2002, the age-adjusted prevalence rates have increased in all racial groups (except Hispanics, who did not have data reported in 1980). In 2002, the self-reported prevalence rates of DM in people of Hispanic origin were similar to those of African-Americans.
Figure 2.1: Reported prevalence rates of DM by race and gender from 1980 to 2002. Data are presented as reported by the Centers for Disease Control.

Regardless of race, gender or ethnicity, those individuals with DM experience a greater risk of experiencing negative health-related outcomes than those who do not have DM. In addition to having a greater risk of suffering from a stroke or coronary heart disease, individuals with DM have a greater risk of suffering from any cardiovascular disease condition, while the risk in this population increases with advancing age. The CDC reported that in 2000, 47% of those with DM who are between the ages of 65 and 74 years suffer from cardiovascular disease. Due primarily to this increase in cardiovascular disease risk for those with DM, health care costs to treat DM related complications have increased dramatically. In 1969, the total estimated amount of money
spent for health care of DM related complications was $2.6 billion ($12.44 billion after adjusting for inflation), while in 1997 direct costs were estimated to be $44.14 billion. Moreover, the ADA has estimated that the combination of direct and indirect costs range from $98.2 to $137.7 billion. Due to a combination of these factors, research is being performed to attempt to determine the pathology responsible for the disease state of DM.

Pathology of DM:

Experimental Data

As stated previously, the hallmark characteristics of DM are elevated fasting blood glucose levels and/or impaired glucose tolerance, accounting for dysregulation of glucose metabolism either in the fasted state or in response to a glucose challenge, respectively. Insulin resistance is believed to precipitate both of these factors, and one of the mechanisms by which insulin resistance is manifested, is the observed reduction in blood glucose disposal in DM. Previous research has indicated that insulin can have a significant impact on glucose disposal through both oxidative and non-oxidative pathways. Mandarino et al. (1987) used insulin infusions in healthy subjects to determine its effect on skeletal muscle enzymes involved in both of these pathways. Results of this study indicated that in addition to stimulating glucose disposal in a dose-response manner to the rate of insulin infusion, insulin also stimulates an increase in the activity of pyruvate dehydrogenase (PDH) and glycogen synthase (GS), but not phosphofructokinase (PFK). Moreover, the rate of oxidative glucose disposal was significantly correlated with PDH activity, and the rate of non-oxidative glucose disposal was
significantly correlated with GS activity. These data indicate the importance of insulin in the regulation of glucose metabolism, likely through, in part, the activation of these enzymes that are rate-limiting for the oxidative and non-oxidative disposal of glucose.

In DM, studies have consistently demonstrated that glucose uptake at basal insulin levels is normal (Vaag, 1992) it is reduced at elevated insulin levels (Wright, 1988; Arciero, 1999; Nagasaka, 1999), indicating the state of insulin resistance. One likely mechanism by which this observed dysregulation occurs, is an impaired capacity to metabolize glucose through both oxidative and non-oxidative pathways.

Boden et al. (1983) studied glucose metabolism in obese controls, and obese women with DM before, during and after the ingestion of a meal that was high (50% of total kcals) in carbohydrate using indirect calorimetry. Results of this study indicated that those subjects that did not have DM responded to the meal with higher insulin levels, greater C-peptide levels (an indicator of insulin secretion), and lower blood glucose. Despite the greater insulin secretory response in the control group, blood insulin levels were similar in the two groups, indicating fasting hyperinsulinemia in the DM group. Furthermore, the use of indirect calorimetry to measure oxidative and non-oxidative glucose disposal indicated that despite similar insulin levels, total oxidative and non-oxidative glucose disposal was lower in DM compared to controls over the four hour measurement period (Figure 2.2). The authors suggested that the likely mechanism to explain why both oxidative and non-oxidative glucose disposal is lower in DM compared to controls is reduced insulin sensitivity, as indicated by the lower biological response to insulin in DM despite similar circulating insulin levels. Of note, the ingestion of the meal also resulted in (presumably) insulin-induced suppression of free fatty acids (FFA) in the
control group, which might also have a significant impact on liver glucose production during these circumstances. This topic will be discussed in greater detail later in this literature review.

The results of Boden et al. (1983) are further supported by Bogardus et al. (1984), who observed that both oxidative and non-oxidative glucose disposal were lower in DM compared to controls at super-physiological insulin levels induced during the condition of a euglycemic clamp. These authors suggested that the mechanism responsible for reduced oxidative glucose disposal was the observed elevated FFA levels in DM in response to insulin infusion (similar results found by Boden et al. (1983)), that may precipitate

**Figure 2.2:** Oxidative and non-oxidative glucose disposal in obese controls and DM subjects in response to a meal. Data taken from Boden et al. (1983).
differences in the so-called glucose-fatty acid cycle between disease states. This idea would dictate that the greater availability of FFA would lead to an increase in their oxidation rate, as was observed in both of these studies (Boden, 1983; Bogardus, 1984). The mechanism by which the authors attributed the reduced non-oxidative glucose disposal observed in DM was a reduction in the activation of GS by elevated circulating insulin levels (Figure 2.3; Wright, 1988), a hypothesis that has been supported in subsequent studies. These differences in the capacity for oxidative and non-oxidative

![Graph](image)

**Figure 2.3**: Fractional Velocity of skeletal muscle glycogen synthase in response to a 7 kcal/ kg liquid meal containing 45% carbohydrate. Data taken from Wright et al. (1988).
glucose metabolism between those with and without DM have been shown to occur in non-obese DM populations as well (Thorburn, 1990; Golay, 1988; Shulman, 1990), indicating that these differences are not in response to obesity, but rather to the disease state of DM.

As previously indicated, oxidative and non-oxidative glucose disposal in DM is lower in response to a glucose challenge compared to non-DM subjects. However, as it has been demonstrated that skeletal muscle glucose uptake rates are lower in DM at a given insulin level, it is certainly possible that the capacity of skeletal muscle to transport glucose from the blood into the cell in DM is compromised. One of the first areas of interest with respect to this hypothesis is analysis of the expression of the Glut 4 protein in DM. Having lower levels of the Glut 4 protein inside the muscle cell would likely explain a reduction in cellular glucose transport that would contribute to the observed differences in oxidative and non-oxidative glucose metabolism. Garvey et al. (1992) used lean controls, obese controls, and obese DM subjects to test this hypothesis, and observed that despite lower rates of glucose uptake in obese subjects (26% lower) and DM subjects (74% lower) during euglycemic clamp studies, there were no differences in intracellular Glut 4 protein or mRNA levels among these groups. This led the authors to conclude that the observed reduction in glucose uptake was not due to differences in Glut 4 levels in skeletal muscle, but rather it likely involves altered Glut 4 function or translocation. This hypothesis was supported by Broznick et al. (1994) who observed four-fold greater glucose uptake rates in lean rats compared to obese Zucker rats in response to insulin
infusion. The authors ascribed this increase to the observed significantly greater incorporation of the Glut 4 protein into the plasma membrane of lean rats in response to insulin despite no measured differences in total Glut 4 content.

The previously mentioned studies have indicated that glucose transport from the blood into the muscle cell are compromised in DM not because of differences in Glut 4 levels, but rather because Glut 4 is not translocated from within the cell to the sarcolemma where glucose transport occurs. It is therefore likely that the reduced capacity of skeletal muscle in DM to transport glucose from the blood into skeletal muscle comes from cellular events upstream of Glut 4 translocation, which, in other words, is the 2nd messenger cascade that occurs in skeletal muscle in response to insulin. To date, the entire insulin-induced cascade that leads to the translocation of Glut 4 to the plasma membrane has not been fully elucidated, however it likely involves a series of phosphorylation reactions of key 2nd messenger proteins, including the insulin receptor itself, insulin-receptor substrate (IRS) and PI-3 kinase (Figure 2.4). Downstream from PI-3 kinase are the proteins PDK and Akt, which likely play an active role in the insulin signaling cascade resulting in Glut 4 translocation, however intermediate proteins involved in this cascade have yet to be identified.
Figure 2.4: Insulin signaling cascade leading to the translocation of the Glut 4 protein from within intracellular vesicles, to the sarcolemmal membrane, where glucose transport can occur. Picture taken from Endotext.com; Chapter 25: Exercise and the regulation of blood glucose. Author: Jack Youngren.

More recent work has been done to identify the role these proteins play in the previously described reductions in Glut 4 movement to the cell membrane in response to insulin. In a study that examined the effect of obesity on post-insulin receptor activity, Goodyear et al. (1995) found that 2-deoxyglucose uptake in muscle strips of obese female subjects was 53% lower compared to non-obese females. From a mechanistic perspective, it was observed that in response to insulin infusion, insulin receptor phosphorylation, IRS-1 phosphorylation and PI-3 kinase activity were lower in obese subjects compared to non-obese subjects. The authors concluded that these important
differences in downstream events involved in insulin signaling likely contributed to the observed reduction of glucose uptake in the obese subjects. However, the results of this study do not apply to the disease state of DM, as subjects with DM were not studied.

Using the murine model of DM (ob/ob mice) Saad et al. (1992) examined the phosphorylation of the insulin receptor and IRS-1 protein in both skeletal muscle and liver tissues in response to insulin infusion. Results of this study indicated that IRS-1 phosphorylation in diabetic mice was reduced by 50% in both skeletal muscle and liver tissues in response to insulin infusion, and that IRS-1 phosphorylation is dependent primarily on insulin receptor kinase activity.

The findings of the previous two studies have also been tested in human cases of DM. Nolan et al (1994) subjected lean, obese non-DM and obese DM individuals to four different insulin infusion rates (0, 40, 120 and 1200 mU/ m²/ minute), taking a muscle biopsy before and after insulin infusion to determine insulin receptor kinase activity. During each clamp study, 3-3H glucose was used to determine glucose uptake rates. Results of this study indicated that glucose disposal rates were greatest in lean subjects, followed by obese subjects and finally obese DM subjects, and that these differences occurred in a dose-response fashion with insulin infusion rate. Results of the insulin receptor kinase activity portion of the study indicated that there were no differences in lean subjects when compared to obese and DM subjects. Curiously, however, the authors observed that insulin receptor kinase activity was greater in obese subjects compared to DM subjects. This was a surprising outcome, because given that glucose uptake rates were greatest in lean subjects compared to obese and DM subjects, one would expect that if insulin receptor kinase activity played a role in insulin resistance in DM, then the lean
subjects would be expected to have greater kinase activity compared to the other two groups. This led to a conclusion that the role of insulin receptor kinase activity in the manifestation of insulin resistance is likely secondary to other post-receptor defects in DM.

In a more comprehensive study of insulin signaling in DM, Bjornholm et al. (1997) studied obese DM subjects and obese controls by taking skeletal muscle biopsies before and after insulin infusion. The insulin infusion led to a ten-fold increase in circulating insulin levels (i.e., 60 pmol/ L to 650 pmol/ L). The primary outcome measures for this study was glucose transport using 3-O-methyl glucose methodology, IRS-1 phosphorylation and PI-3 kinase activity. Results of this study indicated that in response to insulin infusion, 3-O-methyl glucose transport was 40% higher in controls compared to DM. Furthermore, insulin infusion led to a six-fold increase in IRS-1 phosphorylation and a two-fold increase in PI-3 kinase activity controls (as measured by determining the protein level of IRS-1 bound to PI-3 kinase; Figure 2.5), while neither of these outcomes changed in DM in response to insulin infusion. This inability of insulin to stimulate changes in IRS-1 phosphorylation and PI-3 kinase activity might raise the question of whether levels of these proteins were different between groups. However, further analysis revealed that IRS-1 protein levels were not different, leading to the conclusion that the observed reduction in glucose transport observed in DM is likely due, in part, to reduced capacity of insulin to activate these proteins.

The results reported by Bjornholm et al. (1997) have been supported by subsequent studies by Krook et al. (2000). In this study, the authors subjected obese controls and individuals with DM to muscle biopsies prior to and after varying levels of
insulin infusion. Results of this study indicated that although insulin receptor phosphorylation was similar in DM compared to controls in response to all levels of insulin infusion, glucose transport, IRS-1 phosphorylation, and PI-3 kinase activity, despite being similar at basal insulin levels, was significantly lower in DM during insulin infusion. Moreover, the authors noted that protein levels of the insulin receptor, IRS-1 and PI-3 kinase were similar between groups. The results of this study further demonstrate the likely significant impact that reduced activation of these key 2nd messenger proteins in response to insulin can have on glucose transport in DM.
In conclusion, the rate of glucose uptake by peripheral tissues in DM appears to be “normal” at basal insulin levels. However, the insulin resistant state observed in DM that can be precipitated by obesity and/or physical inactivity, results in significantly reduced glucose disposal during insulin infusion that mimics the post-prandial state. The mechanisms responsible for this reduction likely include (but are not limited to) decreased activity of proteins downstream of insulin receptor binding.

As noted previously, DM is characterized by elevated fasting blood glucose and/or impaired glucose tolerance. Although insulin resistance characteristic of skeletal muscle likely contributes to both elevated fasting blood glucose measurements and impaired glucose tolerance, this is not the only contributing mechanism. Rather insulin resistance of the liver, the major source of glucose production in humans, is also thought to play a significant, if not primary role in the dysregulation of glucose metabolism in DM (DeFronzo, 1988; Consoli, 1992).

Although insulin resistance, as would be observed in response to elevated insulin levels, is believed to occur in liver, it is first necessary to review the pathology of hepatic tissue in DM during the fasting state in order to gain a perspective on the underlying mechanisms responsible for the observed insulin resistance in liver tissue. One likely mechanism responsible for the observed elevated rates of glucose production by hepatic tissue in DM is elevated gluconeogenesis. Magnusson et al. (1992) observed that rates of gluconeogenesis were elevated in women with and without DM after a 23 hour fast. This study indicated that women with severe DM (mean FPG = 262 mg% and mean HbA1C = 12) experience increased total glucose output (defined as the total rate of appearance of glucose), increased gluconeogenesis and surprisingly, reduced glycogenolysis compared
to non-DM women. Furthermore, gluconeogenesis accounted for a greater percent of total glucose production in DM compared to non-DM. Results of this study indicate that there is a dysregulation of hepatic glucose production (defined as the cumulative glucose production from gluconeogenesis, glycogenolysis and G6Pase:GK activity) in DM, and that this dysregulation occurs mainly in response to elevated rates of gluconeogenesis. However, it is important to note that the observed increase in total glucose output is not solely caused by elevated gluconeogenesis. Rather it is due, in part, to a reduced capacity for glycogen synthesis (which is responsible for the observed reduced glycogenolysis) caused by reductions in the G6P pool in response to elevated activity of G6Pase relative to GK in DM during the post-absorptive state (Lam, 2003).

In another study, Gastaldelli et al. (2000) observed that after a 15 hour fast, total glucose output was increased in DM compared to both obese and non-obese control subjects without DM. Using tracer methodology, the authors noted that increased rates of gluconeogenesis were primarily responsible for this increase in total glucose output, and observed that while gluconeogenesis accounted for 47% of total glucose output in non-DM subjects and 68% of the already elevated total glucose output in DM. Moreover, it was observed that glucagon concentrations were elevated in DM during this 15 hour fast, which correlated significantly with the increased observed rates of gluconeogenesis.

Hormonal mechanisms responsible for elevated fasting glucose production in DM have also been evaluated. In DM, plasma levels of glucagon are typically elevated. Baron et al. (1987) observed that under basal conditions, subjects with DM had significantly higher blood glucose, insulin and glucagon levels. Furthermore, they noted that suppression of glucagon using somatostatin resulted in a significant reduction in glucose
output compared to conditions of basal glucagon levels, indicating the effect of this hormone to stimulate glucose output of the liver. In support of these results, Shah et al. (2000) noticed that during the post-prandial period (after 50 grams of glucose was ingested), the suppression of glucagon resulted in significantly lower glucose AUC in DM, indicating improved glucose tolerance, and the authors ascribed this improved glucose tolerance to reduced hepatic glycogenolysis, while glycogen synthesis did not change. The contribution of gluconeogenesis to improved glucose tolerance was not measured in this study. The authors argued that the role of glucagon in the stimulation of glycogenolysis occurs in a more acute time frame compared to its effect on gluconeogenesis, which takes longer to occur.

Another factor that can have a significant impact on hepatic glucose production is the presence of ambient hyperglycemia. After a healthy individual ingests carbohydrate, blood glucose concentrations rise. This rise in blood glucose has two primary independent outcomes (independent from the release of insulin), which include the stimulation of glucose uptake in peripheral tissues and the suppression of glucose production by the liver. In a study using rats as subjects, Rossetti et al. (1993) observed that glucose infusion-induced hyperglycemia, in the presence of somatostatin-induced basal pancreatic hormone levels, resulted in a significant reduction of hepatic glucose production and total glucose output due to suppression of hepatic glycogenolysis and stimulation of glycogen synthesis and GK activity. When compared under similar experimental conditions to a group with pancreatectomy-induced DM, it was observed that the DM group had increased glycogenolysis and gluconeogenesis, and reduced GK
activity in response to hyperglycemia, each of which likely contributed to the observed increase in total glucose output during hyperglycemia and basal levels of pancreatic hormones.

In a study with human subjects, Mevorach et al. (1998) also observed that the capacity of hyperglycemia per se to suppress total glucose output is compromised. For this study, the researchers used the euglycemic-hyperglycemic pancreatic clamp during continuous infusion of insulin, glucagon and growth hormone at basal levels to investigate the effects of hyperglycemia on glucose metabolism. The first important point of this paper is that during conditions of euglycemia (90 mg%), rates of peripheral glucose uptake were not different between those with and without DM. Furthermore, this study also indicated that during the condition of hyperglycemia (180 mg%) and basal levels of insulin and counterregulatory hormones, peripheral glucose uptake was increased by 69% in control subjects but only 49% in subjects with DM when compared with euglycemic blood glucose levels (90 mg%), a difference of 30% between the two disease conditions (Figure 2.6). The authors noted that this difference during hyperglycemia was likely due, in part, to “a tendency” for glucose oxidation rates to be higher in control subjects compared to those with DM, as there was no difference in glycogen synthesis rates between groups during hyperglycemia. Together, these data provide evidence that hyperglycemia characteristic of DM likely has a significant effect on whole body glucose uptake in DM during the post-absorptive state.
Figure 2.6: Rates of peripheral glucose uptake during conditions of euglycemia and hyperglycemia in obese DM subjects and obese controls. Data taken from Mevorach et al. (1998).

Another important aspect of the Mevorach et al. (1998) study was the examination of hepatic glucose metabolism in response to hyperglycemia in DM. Results from this portion of the study indicated that endogenous glucose production was higher in control subjects compared to those with DM during euglycemia (Figure 2.7). However, hyperglycemia resulted in a significant reduction in endogenous glucose production in control subjects, while those with DM showed no change. Additionally, total glucose output was reduced by 35% in controls during hyperglycemia, while total glucose output did not change in the DM group. The authors also noted that this inability of
Figure 2.7: Endogenous glucose production (EGP) and total glucose output (TGO) in response to euglycemia and hyperglycemia in obese non-DM controls (upper panel) and obese subjects with DM (lower panel). Data taken from Mevorach et al. (1998).

Hyperglycemia per se to suppress endogenous glucose production and total glucose output in DM are likely due to impaired regulation of G6Pase and/or GK activity in DM during conditions similar to those observed during the post-absorptive state.
Work has also been performed in an attempt to determine the mechanisms responsible for the observed dysregulation of hepatic glucose metabolism in DM. Brichard et al. (1993) performed an experiment examining the effect of “glucose toxicity” on the expression of hepatic genes involved in glucose metabolism. For this study, the authors used control rats, then induced DM by streptozotocin in two separate groups of rats, where one group was left untreated, and the other was treated with phlorizin, a drug that inhibits renal glucose reabsorption. Results of this study indicated that phlorizin treatment (resulting in normoglycemia) in STZ-induced DM, increased GK activity and decreased PEPCK activity compared to animals experiencing chronic hyperglycemia (no phlorizin treatment). These data provide evidence for a role of hyperglycemia per se to alter the expression and/or regulation of these key rate-limiting enzymes involved in total glucose output and endogenous glucose production. Furthermore, the phlorizin treated animals showed reduced activity of pyruvate kinase and glucokinase while at the same time demonstrating increased PEPCK activity compared to control animals. These data indicate the likely important role of insulin in the expression and/or regulation of these proteins involved in hepatic glucose metabolism, and may be a contributing mechanism responsible for the observed dysregulation of hepatic glucose metabolism in response to insulin in DM.

These data are supported by work done by Massillon et al. (1996). These investigators observed that hyperglycemia in mice, induced by pancreatectomy, resulted in increased expression of G6Pase mRNA and protein. Furthermore, under conditions of insulin restoration, and during conditions of hyperglycemia correction with phlorizin (with no insulin infusion), the levels of G6Pase mRNA and protein were restored to
normal levels. Unfortunately, other rate-limiting enzymes involved in hepatic glucose metabolism were not examined. Finally, the question of whether dysregulation of hepatic glucose metabolism in DM is due, in part, to altered expression and/or activity of G6Pase and GK has been investigated in humans. Clore et al. (2000) took liver biopsies from morbidly obese controls and DM subjects undergoing gastric bypass surgery. Results indicated that in the disease state of DM, total glucose output was elevated in DM compared to controls. Liver biopsy data indicated that G6Pase activity was elevated and GK activity was lower in DM compared to non-DM subjects. Although the results of this study do not indicate a cause-effect relationship between the activity of these enzymes and endogenous glucose production, it is likely, based on previously described animal data, that the activity of these enzymes likely plays an important role in the observed dysregulation of hepatic glucose metabolism in DM. Moreover, it has been demonstrated in both animals (Rossetti, 1997; Ferre, 1996) and humans (Hawkins, 2002) that increasing the activity of GK relative to G6Pase in DM can act to improve, if not normalize hepatic glucose metabolism in DM.

In one particularly interesting study, Hawkins et al. (2002) used fructose infusions to activate GK activity in humans with DM. The authors noted in their introduction that during the post-absorptive state, GK is compartmentalized in the nucleus of the liver cell. Then, when fructose (or insulin) is introduced to the system, the GK is released from the nucleus where it can resume its metabolic activity. During the euglycemic-hyperglycemic pancreatic clamp procedures, it was noted that the infusion of fructose at two different rates resulted in a reduction of glucose output in a dose-response fashion. Furthermore, while fructose infusion did not alter glucose production in healthy controls, the high dose
of fructose infusion in DM almost completely normalized glucose production during hyperglycemia relative to controls. These data provide further evidence of the important role of GK in the regulation of glucose production in DM.

Although it has been suggested that elevated rates of glucose production by hepatic tissues during the fasted state are primarily responsible for the chronic hyperglycemia in DM, the previous studies were performed during hyperglycemia and basal replacement infusions of pancreatic hormones. However, hepatic insulin resistance has also been observed in DM, which would indicate that hepatic glucose metabolism is altered during the post-prandial state. In a particularly interesting study by Pacquot et al (2002) significant insulin resistance of hepatic tissues was observed in DM. During a standardized infusion of glucose, it was observed that obese subjects without DM and subjects with DM required increased insulin infusion rates of 1.5 and 4 times that of lean non-DM controls, respectively, to maintain isoglycemia. It was also observed that despite this lower insulin infusion rate in the lean healthy controls, endogenous glucose production (defined as the production of glucose through a combination of gluconeogenesis, glucose cycling and glycogenolysis) was 67% lower, glucose cycling (defined as glucose production from the flux of substrate through GK and G6Pase) was 63% lower, and glucose uptake of peripheral tissues was 46% higher (Figure 2.9).

On a different day, the healthy lean controls were infused with insulin at a rate similar to that of the DM group trial. Despite the fact that insulin infusion rates were similar, plasma insulin levels in the healthy lean controls was still 2.5 times less than that of the DM group (Figure 2.8), indicating a reduced capacity to clear insulin from the blood, which might contribute to the observed fasting hyperinsulinemia in DM.
Figure 2.8: Insulin and substrate levels in response to insulin infusion in lean, non-DM control subjects at low and high (similar to the insulin infusion rate from the DM trial) insulin infusion rates, and obese non-DM and DM subjects during insulin infusion to maintain isoglycemia. Data taken from Pacquot et al. (2002).

This condition of similar insulin infusion resulted in 146% lower total glucose output (total glucose rate of appearance), 93% lower endogenous glucose production, 214% less glucose cycling and 55% more glucose uptake (Figure 2.9). However, it is noteworthy that as can be seen in Figure 2.10, plasma levels of FFA in control subjects was consistently lower than those observed in DM, which might confound the observed differences in hepatic glucose metabolism. Increased flux of FFA from the adipose tissue to the liver during the post-absorptive state (Gastaldelli, 2002) or in response to a high fat meal (Oakes, 1997), can result in greater rates of endogenous glucose production and total glucose output due to greater oxidation of these FFA resulting in elevated levels of
acetyl CoA and other TCA cycle intermediates. This can result in elevated levels of substrate that can be converted into glucose through the process of gluconeogenesis (Lam, 2003). Furthermore, although FFA are also elevated in obesity without DM (Figure 2.8), this is most likely counteracted by a preserved capacity to store the newly produced glucose as glycogen, a process that is compromised in DM due, in part, to insulin resistance and reduced glucokinase activity (Seoane, 1996). Regardless of the mechanisms involved, however, results of this experiment indicate a number of important differences in glucose and insulin metabolism between lean healthy controls

![Figure 2.9: Total glucose output (TGO), endogenous glucose production (EGP) and glucose cycling (GC) in response to insulin infusion in lean, non-DM control subjects at low and high (similar to the insulin infusion rate from the DM trial) insulin infusion rates, and obese non-DM and DM subjects during insulin infusion. Data taken from Pacquot et al. (2002).](image-url)
and those with DM during conditions that mimic the post-prandial state, including an enhanced capacity to clear insulin from the blood in the absence of DM, and resistance to the effects of insulin on glucose metabolism in key tissues including skeletal muscle and the liver.

In conclusion, it has been clearly demonstrated that dysregulation of hepatic glucose metabolism in DM contributes to the observed elevated fasting glucose levels and reduced glucose tolerance. During the fasted state, it is likely that a combination of insulin and chronically elevated counterregulatory hormones such as glucagon, and even the presence of elevated blood glucose levels contribute to this observed dysregulation. From a mechanistic perspective, these conditions result in altered expression and/or activity of key rate-limiting enzymes of hepatic glucose metabolism including PEPCK, G6Pase and GK. During experimental conditions designed to mimic the post-prandial state, the dysregulation of hepatic glucose metabolism between those with and without DM become more apparent.

**Impact of Exercise on DM**

**Epidemiological Data**

While prescription medication is often used to treat the symptoms of DM, exercise is a non-conventional modality of treatment that can be effective for the management of DM and its complications. Much of the initial motivation for using exercise training as a therapeutic modality for the treatment of DM has come from epidemiological studies that show the significant impact exercise can have on the development and progression of DM.
One of the earlier studies exploring the impact of exercise on health-related outcomes is the Harvard Alumni Study, developed by Paffenbarger and colleagues. Although several publications have come from the collection of data from this study, I will focus on one of the more recent publications by Sesso et al. (2000). In this study, 12,516 Harvard Alumni were administered questionnaires designed to measure health-related outcomes and exercise behavior. Results of this study indicated an inverse relationship between total weekly physical activity and incidence of coronary heart disease, where no additional protective effect of weekly caloric expenditure against CHD incidence for physical activity volumes greater than 1000 kcals per week was observed (Table 2.1).

<table>
<thead>
<tr>
<th>Physical Activity (kcal/ week)</th>
<th>&lt;500</th>
<th>500-1000</th>
<th>1000-2000</th>
<th>2000-3000</th>
<th>&gt; 3000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-adjusted RR</td>
<td>1.0</td>
<td>0.85</td>
<td>0.75</td>
<td>0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>95% Confidence Interval</td>
<td>***</td>
<td>0.74-0.97</td>
<td>0.66-0.85</td>
<td>0.63-0.84</td>
<td>0.64-0.84</td>
</tr>
</tbody>
</table>

Table 2.1: Relative Risk of CHD as a function of weekly caloric expenditure. Data taken from Sesso et al. (2000).

Although these data indicate that caloric expenditure from physical activity can have a protective effect against the incidence of CHD, it does not take into consideration other factors that can increase the likelihood of developing CHD such as BMI, alcohol intake, hypertension, DM, smoking status and a family history of CHD. However, the authors of this study noted that even after adjusting for all of these variables, a protective effect of physical activity was observed, and resembled an L-shaped curve (Figure 2.10), further indicating an independent protective effect of exercise against the development of
CHD in males. Further analysis of collected data indicated that the type of physical activity engaged in can also have a significant impact on the incidence of developing CHD. This analysis indicated that participation in vigorous physical activity (> 6 METs), compared to participation in only light and moderate physical activity, can also reduce the risk of developing CHD.

![Figure 2.10: Relative Risk of CHD as a function of weekly caloric expenditure, after adjusting for BMI, alcohol intake, hypertension, DM, smoking status, and family history of CHD. Data taken from Sesso et al. (2000).](image)

As indicated previously, DM can significantly increase the likelihood of developing CHD, and thus intuition would dictate that because exercise can have a significant impact on the development of CHD, then it might also have a significant
impact on the development of DM. This hypothesis was tested by Wei et al. (1999) in a study that involved the recruitment of 8,633 men, of which 97% were white. Subjects were stratified into one of three groups based on their cardiorespiratory (CR) fitness, as measured by an objective VO2 max treadmill test, to include low, moderate and high fitness levels. DM in this study was diagnosed in individuals whose fasting plasma glucose were 126 mg% or greater. Results of this study indicated that after an average follow-up of 6 years, those individuals stratified into the low CR fitness group had a 3.7 times greater incidence of DM compared to the high CR fitness group. Furthermore, after adjusting for age, BMI, hypertension, triglyceride levels and family history of DM, those subjects stratified into the low CR fitness group had 2.6 times greater odds of developing DM compared to the high CR fitness group. These data suggest that exercise behavior patterns of higher volume and high CR fitness levels can have an independent protective effect against the development of DM in men. Furthermore, while the use of self-reported measures of physical activity and health related outcomes (i.e., DM) in the study by Sesso et al. (2000) is a significant limitation of that study, the use of objective measures for CR fitness and DM in the study by Wei et al. (1999) strengthen the validity of the interpretation that exercise can have a protective effect against the development of DM. However, both of these studies are limited in that they only included male subjects.

The disease state of DM is most often precipitated by insulin resistance, that leads to a reduced capacity to remove glucose from the blood and resultant chronic hyperglycemia. It is thus widely recognized that the improvement of the insulin resistant state can have a significant impact on the body’s ability to maintain “normal” glucose homeostasis and thus prevent negative health-related outcomes associated with chronic
hyperglycemia. A study performed by Mayer-Davis et al. (1998), known as the Insulin Resistance Atherosclerosis Study (IRAS), examined the relationship of physical activity behavior and insulin sensitivity across a multi-ethnic population of males and females.

In this study, 1467 men and women of all racial backgrounds (e.g., African-American, Hispanic, and non-Hispanic whites) were studied. The range of ages for this study was 40-69 years, and insulin sensitivity, as determined by IVGTT, characterized the population as having normal to mild DM. The results of this study indicated that those individuals participating in 5 or more days of physical activity per week had the highest measures of insulin sensitivity. Also observed, was a direct relationship between total physical activity and the quantity of vigorous physical activity, and insulin sensitivity. These results were consistent across gender and ethnicity, indicating the positive impact physical activity can have on insulin action in all of these groups of people. However, one of the limitations of this study is that it was a cross-sectional study, and thus it is not possible to determine the effect of exercise per se on insulin sensitivity.

More recent research has been conducted to determine the impact of lifestyle intervention, in the form of altering dietary patterns and physical activity, on glucose metabolism. One such study was performed by McAuley et al. (2002) that aimed to determine the effect of modest and intensive lifestyle modification on insulin sensitivity and various anthropometric and clinical measures in obese, insulin-resistant non-DM subjects. Results of this study indicated that while modest lifestyle modification had no effect on measured outcomes, intense lifestyle modification, in the form of reduced dietary fat intake and intense (80-90% of max HR) exercise five times per week, resulted in improved insulin sensitivity as determined using the euglycemic clamp, significant
weight loss, reduced body fat levels, lower resting blood pressure and improved cardiovascular fitness. These data indicate that intense lifestyle changes can result in improved insulin action and an improved cardiovascular disease risk profile in obese, insulin resistant subjects.

Another study (DPP, 2002) examined the impact of lifestyle intervention or use of the drug Metformin on the incidence of DM in 3,234 obese, multi-racial subjects of both genders that did not have DM at the outset of the study, but did have impaired glucose tolerance as diagnosed by OGTT. Results of this study indicate that after an average 2.8 years follow-up, lifestyle intervention, in the form of 7% weight loss goal and 150 minutes of physical activity per week, lowered the incidence of DM by 58% compared to the placebo group (Figure 2.11). Lifestyle intervention also proved more protective against the development of DM than the Metformin group, which showed a 31% lower incidence of DM at follow-up compared to the placebo group. Lifestyle intervention also resulted in reduced BMI and improved glucose tolerance in response to OGTT, indicating the significant impact of this intervention on these outcomes as well.
The previously outlined studies are useful because they use large sample sizes to determine the relationship between physical activity and health-related outcomes such as CHD and DM. However, it is also important to study the metabolic and cellular mechanisms that explain this observed therapeutic effect of exercise. Knowledge of these mechanisms can lead to the creation of treatment programs that can be used to manage the dysregulation of glucose metabolism observed in DM and reduce its contribution to the occurrence of negative health-related outcomes.
Impact of Exercise on DM

Experimental Data

In addition to epidemiological data indicating a significant preventive and therapeutic effect of exercise on glucose metabolism in DM, considerable experimental evidence regarding these effects also exists. Conventional wisdom regarding exercise as a therapeutic intervention for glucose metabolism in DM indicates that regularly performed exercise training results in a reduction of blood glucose levels acutely (i.e., in response to a single exercise training session) and after longer duration (i.e., several weeks) of exercise training (Ivy, 2000).

In a study done by Minuk et al. (1981) it was observed that 45 minutes of cycling exercise in obese DM subjects resulted in a significant reduction to plasma glucose levels (pre-exercise value of 226 mg% and post-exercise value of 190 mg%), while obese non-DM subjects did not experience any change in glycemic levels (86 mg% pre-exercise and post-exercise). The authors indicated that the mechanism responsible for the changes to blood glucose levels in DM was the observed reduction in counter-regulatory responses compared to controls. More specifically, it was observed that while peripheral uptake of glucose was similar in DM and control subjects, glucose production during exercise was lower in DM, which accounted for the reduction in blood glucose during exercise. Furthermore, it was noted that while plasma insulin levels were similar at baseline between these two groups, there was a significant decline for the control group, while plasma insulin levels did not change in the DM group. These data indicate that reduced insulin levels that are commonly observed during exercise, did occur in the control group,
although suppression of insulin release by the sympathetic nervous system and/ or reduced hepatic clearance of insulin during exercise may also be impaired in DM. Although plasma insulin levels in the DM group were higher during exercise, it did not result in elevated rates of glucose uptake and hepatic glucose suppression in DM, a clear manifestation of insulin resistance associated with this disease state.

In a similar study, Giacca et al. (1998) also observed that 45 minutes of aerobic exercise training at 50% of VO$_2$ max results in reduced plasma glucose levels in DM compared to lean and obese non-DM controls. The investigators showed that plasma glucose levels remained similar in the lean and obese non-DM groups. During exercise and after 195 minutes of recovery, plasma glucose levels in DM fell from approximately 7.6 mmol/ L at rest, to approximately 7.0 mmol/ L after 30 minutes of exercise, and finally to a low of approximately 6.5 mmol/ L after 40 minutes of exercise. Furthermore, plasma glucose remained significantly reduced for the entire 195 minute recovery period after exercise in the DM group (Figure 2.12).
Figure 2.12: Blood glucose levels before, during and after 50 minutes of aerobic exercise in lean controls, obese non-DM subjects and obese subjects with DM. Data taken from Giacca et al. (1998).

In contrast to the previously noted study by Minuk et al. (1981), it was observed that this reduction in plasma glucose was not in response to a blunted counter-regulatory response. Rather, it was observed that in response to exercise, glucose production increased approximately two-fold in all three groups during exercise, and declined similarly during recovery. However, glucose utilization in the DM group was greater during exercise compared to the lean group ($p < 0.05$) and tended to be greater compared to the obese non-DM subjects ($p = 0.066$). Because the metabolic clearance rate (i.e., rate of glucose uptake relative to circulating insulin levels) was not different between the three groups, it was proposed by the authors that the reduced plasma glucose levels in DM compared to the other two groups was due to the effect of the “mass action” of
glucose on rates of glucose disposal. Despite no observable differences in glucose production during rest in DM compared to lean and obese non-DM groups, glucose cycling (i.e., the flux of glucose through G6Pase) was elevated at rest in DM, indicating a dysregulation of hepatic enzyme function (most notably the lower ratio of glucokinase:G6Pase activity) despite elevated basal insulin levels. However, glucose cycling was significantly reduced in only the DM group both during and after exercise, which may, in part, contribute to the observed reduction in plasma glucose levels that were observed during these periods. Of note, one thing that was noticed when reading this paper (Giacca et al. 1998) is that both glucose production and glucose uptake rates were expressed as μmol/m^2/minute. This is a curious expression of the data, for which the authors gave no explanation. Because the previously noted paper by Minuk et al. (1981) expressed data as mg/minute, it is questionable whether the results of each paper are comparable, and it seems plausible that normalization of these values based on either body weight or preferably lean body mass would have resulted in different observations.

Evidence also exists that a single bout of variable intensity aerobic exercise training can improve whole-body glucose metabolism in DM for at least 24 hours. Devlin et al. (1987) observed that 24 hours after glycogen-depleting exercise in subjects with DM, insulin-induced glucose disposal was increased after exercise training. The authors attributed this to the observed increase in non-oxidative glucose disposal in DM after exercise (i.e., glycogen synthesis), as no changes were observed for oxidative glucose disposal after exercise training. This conclusion was further supported by the observation that glycogen synthase activity was increased after the exercise training bout in response to insulin infusion. From this study, it is also noteworthy that blood glucose levels were
also significantly lower in the DM group after the single bout of exercise. The baseline and one-day post-exercise values, respectively, were 197 and 164 mg%. This important difference may have had a significant impact on the rates of glucose disposal via changes in oxidative or non-oxidative pathways at basal insulin levels and each of two insulin infusion rates (40 and 400 mU/ m²/ minute for the low and high insulin infusion periods, respectively) by reducing the impact of blood glucose mass action on glucose disposal. Regardless of how glucose metabolism was improved in this study, the observed reduction in blood glucose levels demonstrates the efficacy of aerobic exercise to improve blood glucose metabolism in DM.

Devlin et al. (1987) also observed that hepatic glucose metabolism was improved in response to the single bout of exercise training. The authors observed that 24 hours after the single exercise bout, endogenous glucose production during the basal state (i.e., no insulin infusions) was significantly reduced, and that this change was associated with the changes in fasting plasma glucose. This improved hepatic glucose metabolism also occurred during insulin infusion, as it was observed that endogenous glucose production was significantly lower during insulin infusion after exercise compared to baseline values.

It is apparent that a single bout of exercise can improve glucose metabolism in individuals with DM, which contributes to the notion that the benefit of exercise on glucose metabolism in DM is a “chronic-acute” affect. However, there are a number of studies that have investigated the effect of longer term exercise on glucose metabolism in DM. In a cross-sectional study that involved a comparison of various glucose metabolism measures between trained and sedentary individuals, Rodnick et al. (1987) observed that
glucose metabolism was better in the trained group compared to the untrained group. In this study, it was observed that in response to an oral glucose tolerance test, although blood glucose values were similar over the 3-hour post-prandial period, insulin levels were significantly lower after 30, 60 and 120 minutes in the trained group. These data indicate that although glucose tolerance was similar between the two groups (as demonstrated by similar blood glucose values during OGTT), insulin action was better in the trained group. As another marker of glucose metabolism, the researchers used the glycemic-pancreatic clamp at two different insulin infusion rates. During this test, it was observed that the trained subjects had significantly greater rates of glucose disposal and greater suppression of endogenous glucose production at each of two insulin infusion rates. Also observed, was a significant positive relationship between VO$_2$ max and rates of glucose uptake, indicating that greater rates of glucose uptake are observed in those with higher aerobic fitness. However, an obvious limitation of this study is that it was cross-sectional, and thus the differences detected may have been due to inter-individual differences instead of exercise training per se.

In a true-experimental design, Bogardus et al. (1984) examined the effect of 12 weeks of weight loss, in either the form of caloric restriction or exercise training, on glucose metabolism in subjects with glucose intolerance and DM. In this study, it was observed that both groups experienced a similar, significant change in body fat mass (a loss of approximately 8 kg of fat). Also in response to the intervention, both groups experienced significant reductions in basal endogenous glucose production, and improved hepatic insulin sensitivity of approximately 25% during insulin infusion and euglycemic clamp conditions (Figure 2.13).
Figure 2.13: Endogenous glucose production rates of glucose intolerant individuals during insulin infusion of 40 mU/ m²/ min, in response to 12 weeks of either diet therapy alone, or 12 weeks of diet and exercise training. Data taken from Bogardus et al. (1984).

Despite no change in rates of peripheral glucose uptake in the diet only group, the diet plus exercise training group experienced a significant increase in glucose uptake rates during the clamp (Figure 2.14). This occurrence was ascribed to significantly greater non-oxidative glucose disposal during insulin infusion in the diet plus exercise group, despite greater rates of oxidative glucose disposal for the diet only group. It is also noteworthy, that fasting plasma glucose concentrations were significantly reduced in both groups, although the diet plus exercise group’s fasting plasma glucose levels were reduced to a level that was significantly lower than the diet only group.
Figure 2.14: Glucose disposal rates of glucose intolerant individuals in response to 12 weeks of either diet therapy alone, or diet plus exercise training. Data taken from Bogardus et al. (1984).

In a similar study, Arciero et al. (1999) studied the effect of short-term diet and exercise on insulin action in individuals with abnormal glucose tolerance (some with DM were included in the study). In this study, the investigators determined the effect of 10 days of a low calorie diet or exercise training (where caloric restriction was achieved by the calories expended during each exercise session) on glucose metabolism in individuals with abnormal glucose tolerance. Exercise training consisted of 10 consecutive days of aerobic exercise at an intensity of 60-65% of VO$_2$ max. In response to hyperglycemic clamp conditions, the diet plus exercise group had significantly greater rates of glucose disposal compared to the diet only group, despite having lower plasma insulin
concentrations during the first 45 minutes of the 120 minute procedure. Of note, the investigators did not use concomitant tracer infusions to determine the rates of glucose uptake and glucose production. Instead, the investigators used the amount of glucose required to maintain hyperglycemia (~250 mg%) as their marker of glucose disposal rates. While this does allow the determination of blood glucose homeostasis control during intravenous glucose infusion, it does not allow the measurement of the relative contribution of the liver and peripheral tissues to maintain blood glucose homeostasis. Thus the increase in glucose disposal rates as defined by the authors after exercise training may have been due to factors other than simply glucose disposal (i.e., the suppression of liver glucose production).

There is abundant evidence that exercise training can significantly improve glucose metabolism in DM. However, there is a general misconception among those with DM (anecdotal report) that “feeling better” and having significantly reduced blood sugar levels from exercise training has led to a cure of their glucose metabolism pathology. However this is not the case, and as indicated earlier, exercise training’s benefit to glucose metabolism in DM is more likely a “chronic-acute” effect rather than a cure. This is supported by a study that was done by Segal et al. (1991). In this study, the investigators trained lean, obese, and obese-DM men for a 12 week period and investigated glucose metabolism during euglycemic clamp conditions before training and 4-5 days after the cessation of training. Results of this study indicated that 70 minutes of cycling exercise four times per week at an intensity equivalent to the ventilatory threshold did not result in any changes to peripheral glucose uptake rates. Similarly, fasting blood glucose levels and OGTT responses did not change in response to the
exercise training intervention in any of the three groups. However, hepatic glucose production in response to insulin infusion was 22% lower in DM after exercise training, while the other two groups did not experience any change. These data indicate that while the washout effect of exercise on peripheral changes is at most 4 days, the benefit of exercise training on hepatic glucose metabolism follows a different time course, and that exercise-induced factors that lead to improved glucose metabolism in both peripheral and hepatic tissues likely differ.

The previously described papers provide evidence that aerobic exercise training is an efficacious means by which blood glucose metabolism can be improved in those with DM. However, it is also important to understand, from a mechanistic perspective, how aerobic exercise training improves glucose metabolism in DM. The understanding of these mechanisms can lead to the development of medications and other therapeutic modalities that can be used to treat this disease.

As noted earlier, DM is characterized by insulin resistance, which progresses eventually to a point where the pancreas is unable to release quantities of insulin that are great enough to maintain “normal” glucose levels, resulting in chronic hyperglycemia. The insulin resistant condition is most often a result of a genetic predisposition to the development of DM, as well as obesity and physical inactivity. Thus it is intuitive that although a genetic predisposition for the development of DM cannot be treated at this time, exercise training can be used to treat obesity and the insulin resistant state.

Previous research has indicated that insulin secretion and effectiveness can be improved in response to exercise training. Krotkiewski et al. (1985) exercised subjects
three days per week for three months using intermittent high-intensity cycling training.

Results of this study indicated that in response to an oral glucose challenge of 100 grams, insulin secretion was improved in subjects with DM. Specifically, in response to the OGTT, those DM subjects who had initial values that were below normal for insulin secretion (as measured by C-peptide values), indicating hypoinsulinemia, increased their insulin secretion after exercise, while those who had elevated C-peptide values in response to the OGTT experienced a reduction in C-peptide values after exercise training. Both groups of DM subjects experienced improved glucose tolerance during OGTT after exercise training despite no change in blood glucose values. These results indicate that the regulation of insulin secretion is improved in response to exercise training, and that those individuals with different clinical entities (i.e., hypo- and hyper-insulinemia) respond differently to exercise training, and that these differential responses contribute to improved glucose tolerance.

Another tissue that is believed to contribute to improved glucose metabolism in response to exercise training is skeletal muscle. Because skeletal muscle is the largest series of tissues that contribute to glucose uptake, it has been studied extensively because of its obviously large contribution to glucose homeostasis in DM. The protein that facilitates the uptake of glucose from the blood into the muscle is Glut 4. In response to insulin signaling, a series of 2\textsuperscript{nd} messenger cascade reactions occur that eventually lead to the incorporation of Glut 4 to the skeletal muscle cell’s surface where glucose transport can occur (Figure 2.4). Because the incorporation of Glut 4 into the cell’s surface is reduced in DM, the effect of exercise on this process is one of the areas that has been most heavily studied regarding the benefit of exercise on glucose uptake in DM.
In one of the earlier studies examining the effect of exercise training on Glut 4 protein expression, Goodyear et al. (1992), observed that exercise training increased the rate of glucose uptake in the skeletal muscle of rats that were non-DM. In this study, it was observed that despite increased insulin-stimulated rates of glucose uptake and a greater quantity of glucose transporters located in the membrane after exercise, the total quantity of glucose transporters in the muscle homogenate did not change. These data indicate that exercise training does not increase the number of glucose transporters in the muscle, but rather it leads to an increase in the incorporation of glucose transporters to the cell’s surface in response to insulin. However, there are other studies that refute this evidence that Glut 4 protein expression is unaltered in response to exercise training.

Neufer et al. (1993) exercise trained rats for a period of one day and one week, and examined changes to Glut 4 gene transcription in response to the training intervention. Results of this study indicated that Glut 4 gene transcription was increased 3 hours after the cessation of exercise similarly for the one day exercise group (~1.4 fold increase in gene transcription) and the one week exercise trained group (~1.8 fold increase). Levels of Glut 4 protein were greater after one week of exercise training compared to non-exercised controls, although muscle from the one day exercise trained group was not examined. Results of this study indicate that Glut 4 gene transcription is increased in response to exercise training, and because of similar values in the one day and one week exercise trained groups, it was indicated that changes in Glut 4 protein levels were in response to a “chronic-acute” effect of exercise training, meaning that changes in Glut 4 gene transcription are in response to the last exercise bout instead of a cumulative effect.
The effect of exercise training on Glut 4 protein and mRNA has also been examined in humans with DM. Dela et al. (1994) observed that in response to exercise training that consisted of 30 minutes per day, 6-days per week for a total of 9 weeks, Glut 4 mRNA and protein increased in obese controls, and obese DM subjects (Figure 2.15). Although Glut 4 protein increased similarly in both groups, mRNA levels were consistently lower in DM. Results of this study indicate that aerobic exercise training can lead to increases in Glut 4 gene and protein expression in non-DM and DM subjects. However, the usefulness of this study is somewhat limited. The only purpose of this study was to determine the responses of the Glut 4 protein, while the analysis of blood glucose homeostasis was not determined. Although it is likely that the changes in Glut 4 protein expression in response to exercise training contributed to improved glucose metabolism, there was no measurement of whether glucose metabolism improved or not.
Figure 2.15: Glut 4 mRNA (upper panel) and protein (lower panel) responses to 9 weeks of aerobic exercise training in obese controls, and obese DM subjects. Data taken from Dela et al. (1994).
There are studies, however, that have shown the value of aerobic exercise training to improve glucose transport in DM. In particular, it has been shown that exercise training results in increased Glut 4 expression and insulin stimulated Glut 4 movement to the cell’s surface (Broznick et al. 1993; Etgen et al. 1997). Although all of the pieces of this puzzle have yet to be determined, the effect of exercise to increase insulin stimulated glucose uptake likely involves increased incorporation of Glut 4 to the muscle cell surface.

Another likely mechanism by which exercise training improves glucose transport is through greater amplification of the insulin signal. As was previously noted, key 2nd messenger proteins for insulin such as IRS and PI-3 are activated (i.e., phosphorylated) to a lesser extent by insulin in DM. Therefore, it is believed that this occurrence contributes to insulin resistance characteristic of DM. Chibalin et al. (2000) observed that in a non-DM rat model, exercise training resulted in improved glucose transport, increased Glut 4 movement to the muscle cell’s surface, and increased intramuscular glycogen levels in response to insulin infusion compared to control animals. Also performed was a comprehensive analysis of insulin signaling 2nd messenger protein activation. Results of this portion of the study revealed that after exercise training, there was increased insulin receptor, IRS, PI-3 and Akt phosphorylation in response to insulin infusion. Furthermore, all of these changes occurred despite the absence of change in IRS-1 or IRS-2 protein concentration. This indicates that exercise training improved the efficiency by which these post-translational modifications occur, and that these changes in the post-translational efficiency contributed to the increased glucose transport observed after exercise training.
Exercise training can also lead to increased non-oxidative disposal of glucose. Perseghin et al. (1996) observed that in response to exercise training for six weeks, first degree relatives of individuals with DM experienced improved insulin sensitivity, and that this improved insulin sensitivity was due, in part, to increased insulin-stimulated glucose transport, phosphorylation and glycogen synthesis. Furthermore, the observed increase in glycogen synthesis after six weeks of exercise training was greater than that observed after a single day of exercise training. This indicates that the benefit of exercise training on non-oxidative glucose disposal is likely more than an acute effect of exercise.

Because of the significant contribution of peripheral tissues (i.e., skeletal muscle) to clear glucose from the blood, changes in its “quality” after exercise training in DM has received a bulk of the attention from the exercise physiology community. However, this is unfortunate, because the contribution of the liver to maintain blood glucose homeostasis is not insignificant, and may be the primary site of blood glucose homeostasis dysregulation in DM (DeFronzo, 1988; Consoli, 1992). As noted previously, several studies (Devlin, 1987; Rodnick, 1987; Segal, 1991; Giacca, 1998) have indicated the efficacy of exercise to improve hepatic glucose metabolism. However, little is known to date about the impact of exercise on hepatic function in DM other than its capacity to suppress glucose production during basal and elevated insulin levels. To my knowledge, there has only been one paper that has been published that has attempted to investigate the effect of exercise on hepatic glucose metabolism in a DM model from a mechanistic perspective.
Heled et al. (2004) investigated the effect of 4 weeks of exercise training on hepatic glucose metabolism in a *Psammomys obesus* (aka, gerbil) model of DM. In this study, the investigators exposed animals to three different treatments, including a high-calorie diet, a high calorie diet plus exercise, and a low calorie diet. During the course of the intervention, it was expected that all of the animals would develop characteristics of DM. However, it was reported that while all animals in the high calorie diet developed these conditions (determined by fasting glucose and insulin concentrations), none of the low calorie diet or the exercise trained animals did. During the post-prandial state, PEPCK activity was significantly higher in the high calorie group compared to both the low calorie and exercise trained groups (Figure 2.16). Also observed, was higher G6Pase activity in both the exercise trained (with high calorie diet) and the high calorie control group compared to the low calorie diet group (Figure 2.16). Glucose production was not measured.

This portion of this study is the first (to my knowledge) to examine the effect of exercise on hepatic glucose metabolism in DM from a mechanistic perspective. The results of this study indicate that exercise can contribute to the protection from the development of DM, and that this phenomenon might occur due in part to improved regulation of key rate-limiting enzymes involved in hepatic glucose production. Additionally, although the reported data are informative, the study could have been strengthened by the inclusion of data regarding opposing rate limiting enzymes. For example, although G6Pase activity in the exercise trained group was similar to the DM group, this measurement alone is not necessarily meaningful. Because the rate limiting enzymes for total hepatic glucose output are G6Pase and glucokinase, the inclusion of
glucokinase activity data in response to exercise training would have been most beneficial for further insights regarding glucose homeostasis.

Figure 2.16: PEPCK (upper panel) and G6Pase (lower panel) activity in response to high-calorie diet, high-calorie diet and exercise, and low calorie diet in a DM model of Psammomys obesus. Data taken from Heled et al. (2004).
In another arm of the study, similar groups of animals were studied in the fasted state, 30 minutes after an interperitoneal injection of insulin to determine the activity of 2nd messenger proteins involved in the insulin signaling cascade. Results of this study showed that tyrosine phosphorylation of the insulin receptor, and the association of IRS-2 with PI3-kinase was greater in the low calorie group, and the exercise trained group, compared to the high calorie diet group. These data indicate that the improvements to hepatic glucose output in response to exercise training are likely, in part, due to improved efficiency of the 2nd messenger cascade of insulin.

Finally, although this study was a good first attempt to determine the role of liver in exercise-mediated prevention of DM, much more work needs to be done. Future studies should focus on issues such as the effect of exercise on processes such as gluconeogenesis, glucose cycling and glycogenolysis, the influence of insulin and counter-regulatory hormones on the regulation of these processes, and the molecular mechanisms by which these factors are mediated in those individuals who already have DM. Such studies are expected to contribute to the development of medications used to treat DM, and help unravel the mystery of DM.
 CHAPTER 3

METHODS

Subjects: Subjects who participated in this study were randomly assigned to one of two treatment groups: dietary control only, or dietary control plus seven days of aerobic exercise training. The methods of this study were approved by The Ohio State University's Institutional Review Board for human subject research. Each subject was required to meet the following criteria to be eligible to participate in the study:

- Previously sedentary for 6 months
- Obese (28 < BMI < 40)
- Ages 30-60
- Taking 2 diabetic medications or less
- Have DM as determined from OGTT responses
- Hemoglobin A1C value of 7.5 or less

Previously sedentary for 6 months. Because the primary intervention of this study was aerobic exercise training, all subjects were required to have similar baseline physical activity patterns. Only those subjects who reported having been sedentary for the previous six months were eligible to participate. Physical activity was measured using a
questionnaire developed by Godin et al. (1985). Those subjects reporting greater than one day of moderate or strenuous physical activity per week over the previous six months were not eligible to participate.

28 < BMI < 40. Approximately 90% of patients with DM are obese. Therefore, the use of obese subjects allowed the application of the results of this study to the largest possible subgroup of diabetics. However, because of the significant volume of exercise performed, the subjects’ body fat levels should not be excessive, as this might make it more difficult to complete the training regimen. Moreover, a BMI of 40 was the high end of this criteria, as body fat levels greater than this invites the possibility of an interaction between excessive body fat levels (i.e., visceral adiposity) and the expected benefit of exercise on glucose metabolism. Body composition was assessed using bioelectric impedance analysis to further define the subjects’ clinical characteristics as accurately as possible.

Males and Females. Both men and non-pregnant women were eligible to participate in the study. Females were not permitted to participate if pregnant because of the potential for radiation exposure during the clamp procedure to damage the fetus. Women of childbearing age took a urinary pregnancy test during their screening visit and prior to the performance of each isoglycemic clamp procedure to ensure that they were not pregnant.

Age 30-60. This age range was used because of the intensity of exercise the subjects were required to perform. The exercise training program required the subjects to exercise at 70% of their individual VO\textsubscript{2} maximum. Because individuals with type 2 diabetes are more likely to develop micro- and macro-vascular complications (i.e., coronary artery disease, peripheral vascular disease, etc.) it is more likely that individuals greater than 60
years of age will have developed these complications. Therefore, their participation in the proposed exercise protocol may lead to a greater risk of experiencing exercise-induced complications than those individuals aged 60 years or less.

**Taking two diabetic treatments or less.** Because the intention of this study was to examine the independent effect of exercise training on endogenous glucose production and peripheral glucose uptake in type 2 diabetes mellitus, it was necessary for subjects to discontinue their usual medication regimens at the outset of the dietary control portion of the study to eliminate the possibility of an exercise-medication interaction on the measured outcomes. Discontinuance of prescribed medication is more easily justified if the subjects are at a stage of their treatment that requires them to take two medications or less, indicating a better level of diabetic control. All subjects were taking medications such as secretagogues, glucosidase inhibitors or insulin sensitizers that have short half-lives (i.e., one week or less). Individuals who take medications which have longer “wash out” periods (i.e., TZD’s), and those individuals taking insulin were not eligible to participate. Justification for discontinuance of usual medication regimens was the adoption during the study of a well-controlled diet and exercise as the primary means of treating the diabetic disease state.

**Type 2 diabetes mellitus (DM).** The presence of DM was determined based upon responses to a 75 gram OGTT. Those subjects with blood glucose levels >199 mg% two hours after the ingestion of a 75 gram oral glucose load were considered to have DM, and thus be eligible to participate in the study. Additionally, a hemoglobin A1C level less
than 7.5 was necessary for consideration to participate in the study, because it was most likely that these individuals would not experience a “dangerous” change in blood glucose homeostasis after discontinuing their medication regimen.

All of the subjects were in good overall health (with the exception of DM), and able to perform a strenuous aerobic exercise training program. This was determined by a physical examination and clearance to participate in the study by a physician specializing in the treatment of DM.

*Exclusion Criteria:* Subjects were not considered eligible to participate in the study if they reported uncontrolled hypertension, experienced an adverse cardiac or blood pressure response to exercise during the stress test (i.e., ischemia, arrhythmias, claudication, etc.), or reported a history of neuropathy, nephropathy, or retinopathy, or musculoskeletal conditions which would make participation in regular exercise difficult or dangerous.

*Estimation of Sample Size:* Based upon previous research by Devlin et al. (1987), it was estimated that using an effect size of 1.0, nine subjects per group would be required to detect significant F-ratios between groups (pre-exercise vs. post-exercise) for the outcome variables with adequately high power (0.8).

*Study Overview:* The purpose of this study was to determine the effect of seven days of aerobic exercise training on endogenous glucose production and peripheral glucose uptake in individuals with DM. Prior to the determination of the impact of aerobic exercise training on endogenous glucose production and peripheral glucose uptake, each subject was required to undergo a battery of tests in the OSU General Clinical Research Center (GCRC) that characterized their DM as well as their overall health and ability to
participate in the study (Figure 3.1). These series of tests were followed by seven days of dietary control, after which each subject underwent their first isoglycemic/hyperinsulinemic clamp to determine endogenous glucose production and peripheral glucose uptake in the post-absorptive state (i.e., no insulin infusion) and at each of two insulin infusion rates. This clamp was followed by a minimum of seven more days of dietary control (depending on when their second clamp procedure was scheduled) for every subject, and seven days of aerobic exercise in the exercise training group only. Approximately eight days after the first clamp procedure, subjects underwent a second isoglycemic/hyperinsulinemic clamp procedure to determine the effect of the intervention on endogenous glucose production and peripheral glucose uptake. A final visit was made to the GCRC to determine body fat levels and VO2 max to determine if either of these variables changed in response to the intervention.
Resting Metabolic Rate Measurement: Resting metabolic rate (RMR), was used to prescribe caloric intake for the dietary control aspect of this study. RMR was determined via indirect calorimetry and respiratory exchange ratios for 20-30 minutes while the subject rested comfortably in a hospital bed. Attainment of RMR was defined as oxygen consumption rates that differed by < 2% over a 5 minute period. Final estimation of daily energy expenditure was increased by 30% to account for the thermic effect of a meal and daily activity above resting levels. During exercise training (day 0 through day 7), daily caloric intake was adjusted, for the exercise group only, on a per subject basis to account for the energy expenditure from exercise (determined based upon O₂ consumption and
RER during the stage of the VO\textsubscript{2} max test corresponding to the exercise intensity prescribed during training). Each exercise session generated a caloric expenditure of approximately 400 kcals.

**Oral Glucose Tolerance Test (OGTT):** Not less than one week before the baseline clamp procedure, each subject underwent an OGTT as a determination of whether they were diabetic. This test was performed after an overnight fast (12 hours). After the collection of baseline blood samples, each subject consumed a 75 g beverage of dextrose within a 10-minute period. Thereafter, blood was collected 15, 30 and 120 minutes after consumption of the beverage for determination of plasma glucose and insulin. A plasma glucose concentration $>$199 mg\% at the 120 minute time point classified the subjects as diabetic. Baseline blood samples were also used to measure hemoglobin A1C. A value of 7.5 or less was necessary to participate in the study.

**Body Fat Measurement:** Body fat percentage was measured using bioelectric impedance analysis (BIA) at baseline (Visit #1) and post-intervention (Visit #5). Body fat percentage was estimated for BIA based upon the equation of Segal et al. (1988).

**VO\textsubscript{2} max/ EKG Testing:** Prior to the VO\textsubscript{2} max test, each subject received a physical examination from a physician to ensure that they were physically able to participate in the study. Each VO\textsubscript{2} max test was performed in the presence of a physician with 12 lead EKG monitoring for two purposes: the first purpose was to detect the presence of significant negative side effects the individual may experience during exercise (i.e., ventricular arrhythmias, ischemia, claudication, etc.) which might make their participation in the study a significant health risk. The second was to measure VO\textsubscript{2} max, while at the same time monitoring \(O_2\) consumption at different workloads. This provided
information so an appropriate treadmill speed and grade would be used during aerobic training, requiring each subject to consume 70% of their measured VO₂ max during the exercise training period. This test was performed not less than one week before the baseline clamp procedure (day 0) to allow adequate time for the “washing out” of the effects of this exercise bout on the variables measured during the first clamp. A modification to the Balke 3.0 protocol was used for the stress test. The Balke 3.0 starts the subjects walking at 2.0 mph at 0% grade for 2 minutes followed by 3.3 mph at 2.5% grade for two minutes, followed by 2.5% grade increments every two minutes without altering the treadmill speed. This is an appropriate test to solicit a VO₂ max, however this does not allow for the attainment of a steady-state of oxygen consumption at submaximal workloads. Consequently, the duration of each stage was increased to three minutes to allow the attainment of a steady state in oxygen consumption during each stage, and provide greater confidence that the work load prescribed for exercise (70% VO₂ max) is accurate (Reeves, 2004). The measurement of VO₂ max was considered valid if two of the following four criteria were met: 1) a maximum RER measurement of 1.10 or more; 2) Attainment of a maximum measured heart rate within 10 beats of the age predicted maximum (220 – age); 3) no change in measured VO₂ despite an increase in workload; and, 4) Perceived exertion level of 9 or more on a 10 point Borg scale. A second VO₂ max test was administered during Visit #5 to determine whether exercise training brought about a change in VO₂ max.

*Dietary Control:* Nutrient intake was controlled throughout the duration of this study, beginning one week prior to the first clamp. It has been observed in healthy subjects that the consumption of a high carbohydrate diet can alter the expression of glucose
metabolism-related proteins. The primary example of this is the Glut 4 protein, which is expressed in greater quantities in response to high carbohydrate feedings post-exercise (Kuo, 1999). Altering expression of this protein could affect glucose uptake in peripheral tissues independent of the effect of exercise training. Although this effect of a high carbohydrate diet on the expression of Glut 4 has yet to be validated in humans with DM, diet was controlled in an attempt to exclude this factor as a primary source of variability in the outcome measures. Beginning one week before the first clamp, subjects began consuming an isocaloric diet consisting of a target of 50% carbohydrate, 30% fat and 20% protein, a diet recommended for hyperinsulinemic DM individuals. The meal plan was a three-day cycling diet, meaning food was prepared by the GCRC metabolic kitchen and picked up by the subject every fourth day or the meals were delivered to them. Specific directions were provided for the subjects by a registered dietician to ensure that they understood the quantity of food they are allowed to consume throughout the course of the day. Strict management of the subjects’ diet was required as this can be considered an “alternative treatment” for DM after discontinuance of their usual diabetic medications on day -7. Control of diet began one week prior to the first clamp procedure, allowing adequate wash out time of the medications previously taken by the subjects and stabilization of blood glucose in response to the newly prescribed diet. The use of all nutritional supplements was discontinued at the outset of dietary control.

**Isoglycemic/ Hyperinsulinemic Clamp Studies:** The Isoglycemic/ hyperinsulinemic clamp technique (Figure 3.2) was used because it allows the concomitant measurement of endogenous glucose production and peripheral glucose uptake in the basal state, and at each of two insulin infusion rates.
On the morning of each clamp procedure, subjects were admitted to the GCRC at 7:30 am after a 12 hour fast. Subjects were also instructed to consume a minimum of 8 glasses of water during the day prior, as well as before sleep and upon awakening. Upon arrival to the GCRC, a 20 gauge needle was inserted into the antecubital vein for infusions, while a 20 gauge needle was also inserted into the contralateral vein for periodic blood draws. A heating pad was kept over the drawing vein for assistance with the collection of arterialized blood samples.

After baseline blood samples were drawn (Figure 3.2), subjects were infused with a priming dose of $3^3\text{H}$ glucose tracer over a 10 minute period as prescribed by Hother-Nielsen et al. (1990) and modified by Harper et al. (1995), based on fasting plasma glucose levels. The priming dose was followed by a continuous infusion of the tracer at a rate of 0.25 $\mu$Ci/ minute for the remainder of the first two hour phase of the clamp procedure. During this period when only the tracer was being infused, the IV infusion line was kept patent by the infusion of 0.9% normal saline solution at a rate of 15 mL/ hr. During the final 30 minutes of this basal phase of the clamp, arterialized blood samples were collected and analyzed for the determination of endogenous glucose production and peripheral glucose uptake. Also during the final 30 minutes of this first phase of the clamp, blood glucose values, taken every five minutes, were averaged to give the blood glucose level at which the subject would be “clamped.”
Figure 3.2: Schematic Representation of the Isoglycemic/hyperinsulinemic clamp. Note that although not included, 3-\(^3\)H labeled glucose was also infused as described in the text.

The second and third-two hour phases of the clamp procedure were characterized by the primed (DeFronzo, 1979), continuous infusion of regular human insulin (Humilin, Eli Lilly and Company; Indianapolis, IN) at 20 and 40 mU/ m\(^2\)/ minute, respectively. The infusion rate of the tracer was reduced to 50\% of the basal level after 20 minutes of insulin infusion, and 25\% of basal after 40 minutes of insulin infusion, and this level was maintained for the remainder of the procedure. Blood glucose was monitored during insulin infusion using a bedside plasma glucose analyzer (Yellow Springs Instruments; Yellow Springs, OH), and maintained at the desired isoglycemic level by the variable infusion of a 20\% (w/v) dextrose solution that was labeled with tracer according to the
work of Neely et al. (1990) to maintain specific activity. Similar to the basal phase of the clamp procedure, blood samples were collected and analyzed during the final 30 minutes of each insulin infusion phase of the clamp for the determination of endogenous glucose production and peripheral glucose uptake.

**Calculations:** Plasma enrichment of 3-\(^3\text{H}\) was measured by scintillation counting (LS 6500, Beckman Instruments), and Steele’s non-steady state equation was used to quantify the rates of appearance and disappearance of glucose (Steele, 1959). Endogenous glucose production was calculated by subtracting the rate of exogenous glucose infusion from total rate of appearance. Insulin sensitivity was determined by expressing rates of glucose uptake and endogenous glucose production during the final 30 minutes of each clamp phase per average insulin level during this period.

**Additional Blood Variables Measured:** The schematic representation of the timing of blood collection for analysis is displayed in **Figure 3.2.** Insulin, glucagon, cortisol (Diagnostic Products Corporation; Los Angeles, CA), and catecholamines (Rocky Mountain Diagnostics; Colorado Springs, CO) were analyzed by commercially available radioimmunoassay kits. Plasma glucose (Yellow Springs Instruments; Yellow Springs, OH), lactate (SUNY Buffalo; Buffalo, NY), glycerol (Sigma Diagnostics; St. Louis, MO), and free fatty acids (Wako Diagnostics; Wako, TX) were measured using enzymatic assays. Serum radioactivity was determined as described previously (Angus, 2002), after consultation via email with one of the paper’s authors (Dr. Mark Hargreaves; Deakin University, Australia).
Exercise Training: The exercise training program took place in a supervised setting (The Center for Wellness and Prevention at the Camera Center), where an exercise physiologist was in attendance to ensure that the subjects performed the exercise as prescribed. One subject performed only six of the seven exercise sessions (day seven was missed), and thus compliance with the intervention was greater than 98%. The exercise training protocol mimicked that used by Rogers et al. (1988), which improved insulin action in seven days without altering VO\(_2\) max or body fat percentage. Exercise on days 1-3 and 5-7 required subjects to begin with a five minute warm-up consisting of light stretching and slow walking, followed by 2 x 25 minute bouts of aerobic exercise (treadmill walking) with a 10 minute break between bouts. The intensity of the supervised exercise sessions was 70% VO\(_2\) max. Following this aerobic exercise session, subjects performed a 5-minute “cool down” period of slow walking. Day 4 of exercise was also supervised and required subjects to exercise for 60 minutes at 60% of their age-predicted maximum heart rate (220-age), a lower intensity than the other six days of exercise. Subjects were provided with heart rate monitors on day 4, allowing easy measurement of heart rate during this exercise bout.

Statistical Analysis: ANOVA (exercise x time x insulin infusion rate) with repeated measures was used for analysis of endogenous glucose production, peripheral glucose uptake, and insulin sensitivity. In the event of significant F-ratios for main effects or an interaction between the independent variables, post-hoc comparisons were made as appropriate. Statistical significance was set at p < 0.05. Body composition and VO\(_2\) max data was analyzed using ANOVA (exercise x time) with repeated measures. Resting metabolic rate and hemoglobin A1C data were analyzed using unpaired Student’s t-test.
CHAPTER 4

RESULTS

Subject characteristics: Baseline demographics and basic physiological measurements are presented in Table 4.1. A total of 18 obese men and women with type 2 diabetes mellitus completed the study. Of the variables presented in Table 4.1, no differences were detected between the diet only group and the diet plus exercise group.

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<th>Variable</th>
<th>Diet Only Group</th>
<th>Diet &amp; Exercise Group</th>
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<td>99.5 ± 15.0</td>
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<td>34.9 ± 3.1</td>
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<tr>
<td>Body Fat (%)</td>
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<td>43.0 ± 6.1</td>
</tr>
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<td>Hemoglobin A1C (%)</td>
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<td>6.5 ± 0.4</td>
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<td>VO2 max (mL*kg^{-1}*minute^{-1})</td>
<td>20.4 ± 4.5</td>
<td>22.6 ± 3.4</td>
</tr>
</tbody>
</table>

Table 4.1: Baseline characteristics for 18 subjects that completed the study. Values are presented as mean ± sd. No differences were detected between groups for any of the outcomes listed (p > 0.05).

Hemoglobin A1C: Hemoglobin A1C (Table 4.1), measured only at the outset of the study, was not different between the diet only group (6.7 ± 0.19; %, mean ± sem) and the diet plus exercise group (6.5 ± 0.14).
**Resting Metabolic Rate:** Resting metabolic rate (RMR) was measured at the outset of the study, and used to estimate the daily caloric needs of each subject while they participated in the dietary control portion of the study. RMR was not different between the diet only group (1628.0 ± 111.8; kcals/ 24 hours) and the diet plus exercise group (1707.3 ± 77.3).

**Oral Glucose Tolerance Test (OGTT) glucose responses:** A 75 gram OGTT was performed on each subject during visit #1 to determine whether the individual had DM. As was expected in individuals with DM, there was a steady rise in plasma glucose values from minute zero (i.e., immediately after the 75 gram glucose drink was completely ingested) to minute 120. Plasma glucose values (Figure 4.1) averaged 119.9 ± 10.2 (mg/ dL), 118.4 ± 10.9, 156.2 ± 13.1, 198.8 ± 17.0, and 248.3 ± 16.0 for the diet only group at time points –15, 0, 15, 30, and 120 minutes, respectively. Plasma glucose values for the diet plus exercise group over the same time points in response to the OGTT averaged 125.3 ± 8.2, 128.6 ± 8.9, 167.5 ± 11.1, 203.3 ± 13.4, and 236.9 ± 19. Although plasma glucose increased at each time point from minute zero through minute 120 of the OGTT, there were no differences in plasma glucose values between groups at any time point, indicating similar glucose tolerance between groups at the outset of the study. Intra-assay coefficient of variation averaged < 1.0%.

**OGTT Insulin Responses:** In response to the 75 gram OGTT, insulin increased over time as expected. Plasma insulin values (Figure 4.1) averaged 21.9 ± 5.6 (µIU/ mL), 21.6 ± 5.3, 42.4 ± 4.3, 51.2 ± 9.2, and 92.8 ± 17.9 for the diet only group at time points –15, 0, 15, 30, and 120 minutes, respectively. Plasma insulin values for the diet plus exercise group over the same respective time points in response to the OGTT averaged 23.7 ± 3.8,
24.2 ± 3.9, 36.2 ± 5.3, 46.0 ± 6.6, and 78.6 ± 15.9 μIU/ mL. Plasma insulin levels (of both groups combined) were significantly greater at minute 30 and 120 compared to the baseline measurement (p < 0.05). There were no differences in the plasma insulin levels between the diet only and diet plus exercise groups during any of the time periods. Intra-assay coefficient of variation averaged 2.5% and inter-assay coefficient of variation averaged 9.2%.

**Figure 4.1:** Plasma insulin and glucose responses over a two hour period to a 75 gram oral glucose challenge. No significant differences were detected between groups for either glucose or insulin at any of the indicated time points (p > 0.05).

**Caloric Intake:** One of the aims of the methods of this study was to eliminate the contribution of diet to cause insulin resistance in our subjects. To do this, all subjects were given all of their food over the two week study period. The aim of the dietary
portion of the study was two fold, and included the control of blood sugar, and prevention of the loss of body fat. Caloric intake of diet only group during the first week of the study (i.e., the week leading up to the first isoglycemic clamp), averaged 2267.3 ± 164.0 kcals, while during the first week of the study the diet plus exercise group consumed 2411.4 ± 124.6 kcals. During the second week of the study (i.e., the period between isoglycemic clamps), the estimated caloric expenditure of exercise was replaced, while the diet only group continued to consume a similar nutrient intake as the first week. During week 2 of the study, diet only subjects consumed 2293.0 ± 178.8 kcals, while the diet plus exercise group consumed 2834.3 ± 166.7 kcals. Caloric intake was similar between the diet only group and diet plus exercise group during the first week of feeding. However, during the second week of the study, the diet plus exercise group consumed significantly more kcals than they did during the first week, and more kcals than the diet only group did during the second week of the study (p < 0.04 for each). Average percent of calories consumed by the diet only group over the entire study period was 49.4 ± 0.70 % carbohydrate, 30.2 ± 0.55 % fat and 20.3 ± 0.40 % protein. The balance of caloric intake percentage (i.e., 0.1%) in the diet only group was due to alcohol consumption. On three separate occasions a subject reported consuming one alcoholic beverage). Average percent of calories consumed by the diet plus exercise group over the entire study period was 49.6 ± 0.45 % carbohydrate, 30.1 ± 0.35 % fat and 20.3 ± 0.25 % protein. No differences in the percent of calories from carbohydrate, fat and protein were observed between the diet only group and the diet plus exercise group during either of the two weeks of the study.
Body Mass Index (BMI): ANOVA revealed that there were no significant differences in BMI at baseline between the diet only group (32.0 ± 1.8; kg * m$^{-2}$) and the diet plus exercise group (34.9 ± 1.8), and that there were no differences in post-intervention BMI values between the diet only group (31.9 ± 1.8) and diet plus exercise group (34.8 ± 1.0). There was also not a significant difference in BMI in either group from the baseline measurement to the post-intervention measurement (p > 0.05).

Body Fat Levels: Body fat levels (Figure 4.2) were measured using bioelectric impedance analysis before and after the intervention. ANOVA revealed that there were no differences in body fat percentage at baseline between the diet only group (39.5 ± 2.3; % body fat) and the diet plus exercise group (43.0 ± 2.0), and body fat percentage was not different after the intervention between the diet only group (38.9 ± 2.4) and the diet plus exercise group (42.6 ± 2.0). As well, there was not a significant difference in body fat percentage in either group from the baseline measurement to the post-intervention measurement (p > 0.05).
Figure 4.2: Body fat percentage of the diet only and diet & exercise groups before and after the intervention. No significant differences were detected between groups or over time (p > 0.05).

**VO₂ Maximum**: Maximal oxygen consumption (VO₂ max) was measured at baseline, and post-intervention in most subjects (Figure 4.3). In total eight subjects from the exercise group and six subjects from the control group had complete pre-post VO₂ max data. One subject from the diet plus exercise group did not have a valid follow-up test because the test had to be stopped prematurely due to the complaint of chest pain. In the control group, one subject showed signs of significant right AV-blockage during the pre-intervention stress test and therefore a follow-up test may have been a significant health risk (as determined by the physician’s expert opinion), one subject had what was considered an unsafe drop in blood pressure in response to the exercise stimulus during the post-intervention test and thus this test had to be stopped prematurely, and finally, one
subject did not comply, and simply did not come in for the post-intervention stress test within a week of completing the other portions of the study. Of the data that was collected, ANOVA revealed that VO$_2$ max was not different at pre-intervention between the diet only group (20.4 ± 1.7; mL * kg$^{-1}$ * min$^{-1}$) and the diet plus exercise group (22.6 ± 1.2) and that there were no differences in VO$_2$ max between the diet only group (21.5 ± 1.9) and the diet plus exercise group (22.1 ± 1.2) after the intervention. There was also not a significant difference in VO$_2$ max in either group from the pre-intervention measurement to the post-intervention measurement (p > 0.05).

**Figure 4.3:** VO$_2$ maximum of the diet only and diet & exercise groups before and after the intervention. No significant differences were detected between groups or over time (p > 0.05).
**Fasting Plasma Glucose Values:** Fasting plasma glucose was measured on the morning of each visit for the isoglycemic clamp. Average fasting plasma glucose level for the diet only group prior to the pre-intervention isoglycemic clamp was 131.0 ± 11.5, and the fasting plasma glucose level prior to the pre-intervention clamp for the diet and exercise group averaged 131.4 ± 12.3 mg/ dL. Prior to the post-intervention isoglycemic clamp, fasting plasma glucose in the diet only group averaged 123.3 ± 10.5 mg/ dL, while the average fasting plasma glucose levels in the diet and exercise group was 127.7 ± 17.3 mg/ dL. No differences were observed between the diet only and diet plus exercise groups during the pre-intervention or post-intervention clamps. Also, there were no changes in fasting plasma glucose levels from the pre-intervention to the post-intervention clamp in either the diet only or the diet plus exercise groups. Intra-assay coefficient of variation averaged < 1.0%.

**Hormonal Responses during the Isoglycemic Clamp:**

**Insulin:** Plasma insulin levels *(Figures 4.4 – 4.6)* were determined throughout the isoglycemic clamp procedure to assess insulin sensitivity. Blood samples were taken for this purpose every five minutes during the final 30 minutes of each phase of the isoglycemic clamp procedure, and averaged to provide steady state insulin values. The steady state insulin values for the diet only group during the pre-intervention clamp averaged 15.6 ± 3.6, 33.9 ± 4.0, and 69.0 ± 4.8 μIU/ mL during the post-absorptive, low and high insulin infusion rates, respectively. In the diet plus exercise group, the steady state insulin values averaged 15.4 ± 2.4, 37.8 ± 1.7, and 64.9 ± 3.6 μIU/ mL during the same respective time periods. During the post-intervention isoglycemic clamp procedure, the average insulin level for the diet only group was 15.0 ± 4.2, 31.7 ± 3.0, and 64.1 ± 3.4
μIU/ mL during the post-absorptive, low and high insulin infusion rates, respectively. The average insulin level in the diet plus exercise group was 13.0 ± 2.2, 29.8 ± 2.2, and 58.6 ± 2.4 μIU/ mL during the same respective time periods. There were no differences in insulin levels between the diet only group and the diet plus exercise group during any of the indicated time periods. As reported by the GCRC corelab, both intra- and inter-assay coefficient of variation averages are < 5.0%.

**Figure 4.4:** Steady state plasma insulin levels during the post-absorptive period (i.e., during the first phase of the clamp) of the isoglycemic clamp. No significant differences were detected between groups or over time (p > 0.05).
Figure 4.5: Steady state plasma insulin levels during the low insulin infusion period (i.e., during the second phase of the clamp) of the isoglycemic clamp. No significant differences were detected between groups or over time (p > 0.05).
Figure 4.6: Steady state plasma insulin levels during the high insulin infusion period (i.e., during the third phase of the clamp) of the isoglycemic clamp. No significant differences were detected between groups or over time (p > 0.05).

Cortisol: Plasma cortisol levels (Table 4.2) were determined at time 0 (i.e., prior to the beginning of the clamp), and at the 90, 210 and 330 minute time points of the clamp procedure. The latter three values correspond with the time points at which PGU and EGP were measured during the post-absorptive insulin, low, and high insulin infusion rates, respectively. Average plasma cortisol level for the diet only group during the pre-intervention clamp was 20.6 ± 3.5, 10.9 ± 1.4, 11.7 ± 2.5, and 10.8 ± 2.0 μg/ dL during the 0, 90, 210 and 330 minute time points, respectively. Similarly, the average plasma cortisol level for the diet plus exercise group during the pre-intervention clamp procedure were 16.3 ± 2.4, 9.8 ± 2.0, 12.9 ± 2.3, and 12.5 ± 2.3 μg/ dL during the 0, 90, 210, and
330 time points, respectively. During the post-intervention clamp procedure, plasma cortisol levels for the diet only group averaged 16.1 ± 2.1, 9.9 ± 1.4, 10.2 ± 2.9, and 8.7 ± 1.8 μg/ dL during the 0, 90, 210, and 330 minute time points, respectively. The average plasma cortisol levels for the diet plus exercise group during the post-intervention clamp were 17.0 ± 2.4, 9.4 ± 2.3, 11.0 ± 2.3, and 12.1 ± 2.3 μg/ dL during the 0, 90, 210 and 330 minute time periods, respectively. No differences in plasma cortisol levels were observed during any of the time periods between the diet only and diet plus exercise groups during the pre-intervention clamp or the post-intervention clamp. During each of the two clamps, plasma cortisol levels were significantly lower during the 90, 210 and 330 minute time points compared to the 0 minute cortisol level (p < 0.0001 for each) in both the diet only group and the diet plus exercise group. Intra-assay coefficient of variation averaged 2.8% and inter-assay coefficient of variation averaged 7.3%.

**Glucagon:** Plasma glucagon levels (Table 4.2) were determined at minute 0 (i.e., prior to the beginning of the clamp), and at the 90, 210 and 330 minute time points of the clamp procedure. The latter three values correspond with the time points at which PGU and EGP were measured during the post-absorptive insulin, low, and high insulin infusion rates, respectively. Average plasma glucagon level for the diet only group during the pre-intervention clamp was 58.2 ± 9.2, 42.7 ± 6.3, 37.0 ± 5.5, and 31.5 ± 5.1 pg/ mL during the 0, 90, 210 and 330 minute time points, respectively. The average plasma glucagon level for the diet plus exercise group during the pre-intervention clamp procedure was 56.2 ± 6.8, 50.0 ± 4.9, 42.9 ± 3.4, and 40.7 ± 3.8 pg/ mL during the 0, 90, 210, and 330 minute time points, respectively. During the post-intervention clamp procedure, plasma glucagon levels for the diet only group averaged 52.4 ± 7.8, 38.6 ± 4.3, 41.8 ± 7.0, and 35.1 ± 6.5
pg/mL during the 0, 90, 210, and 330 minute time points, respectively. The average plasma glucagon levels for the diet plus exercise group during the post-intervention clamp were 58.3 ± 3.3, 48.5 ± 3.8, 47.5 ± 3.5, and 42.3 ± 4.9 pg/mL during the 0, 90, 210 and 330 minute time periods, respectively. There were no differences in glucagon levels between the diet only group and diet plus exercise group during any of the indicated time periods. However, glucagon levels at the 210 and 330 minute time periods were significantly lower than 0 minute glucagon levels (\(p < 0.0001\) for each), and glucagon levels at the 330 minute time period were significantly lower than those observed during the 210 minute time period (\(p < 0.03\)). Intra-assay coefficient of variation averaged 2.6% and inter-assay coefficient of variation averaged 2.4%.
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<th>210</th>
<th>330</th>
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<td>0.55 ± 0.04</td>
<td>0.22 ± 0.02</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>Plasma Lactate (μM/L)</td>
<td>Diet Only</td>
<td>Pre</td>
<td>947.2 ± 225.7</td>
<td>580.1 ± 54.5</td>
<td>561.5 ± 51.6</td>
<td>570.1 ± 93.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>556.9 ± 55.1</td>
<td>513.3 ± 34.1</td>
<td>480.0 ± 36.2</td>
<td>514.9 ± 64.6</td>
</tr>
<tr>
<td></td>
<td>Diet and Exercise</td>
<td>Pre</td>
<td>620.2 ± 50.6</td>
<td>439.6 ± 35.7</td>
<td>474.0 ± 54.7</td>
<td>473.3 ± 62.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>603.7 ± 89.1</td>
<td>610.6 ± 79.4</td>
<td>542.4 ± 72.2</td>
<td>460.1 ± 45.5</td>
</tr>
</tbody>
</table>

Table 4.2: Measured values of selected hormones and substrates during the isoglycemic clamp. No differences were observed between groups at any time point (p > 0.05).

**Epinephrine and Norepinephrine:** During each isoglycemic clamp, plasma samples were collected for the future determination of the catecholamines epinephrine and norepinephrine. Samples were collected in tubes containing EDTA as prescribed by the manufacturer of the assay kit, and stored in a -80 degree Celsius freezer for future analysis. Over the 4th of July holiday (2006), the freezer malfunctioned and all samples thawed. This freezer was equipped with temperature sensors that should have been activated in response to the temperature change, however this system failed. This caused concern of the integrity of samples. These concerns were supported when the samples...
were being assayed by RIA procedures, and unusually low yields of both epinephrine and norepinephrine were recovered, despite having appropriate $R^2$ values (i.e., $R^2 > 0.99$) on the standard curve that was generated. Yields for control samples provided by the manufacturer were also similar to expected values. Because of these events, there were a number of steps taken to determine whether it was likely that the samples had been compromised by the thawing. These steps included the following: 1) four subject samples were tested by themselves; 2) each of these four subject samples were then spiked with either standard B or standard C; 3) Fresh plasma samples were collected from each of three volunteers and analyzed; and 4) control samples provided by the manufacturer were also analyzed. Results for epinephrine and norepinephrine are shown in Figures 4.7 and 4.8, respectively.
**Figure 4.7**: Epinephrine concentrations during the trouble shooting trial.
Figure 4.8: Norepinephrine concentrations during the trouble shooting trial.

As can be seen in Figures 4.7 and 4.8, there is apparently low variability in samples 1-4 (patient samples), and the yields are very low. Furthermore, they yielded epinephrine and norepinephrine levels lower than what was determined for standard B, which had a concentration of 30 and 150 pg/mL, for epinephrine and norepinephrine, respectively. Expected values are ~100 and ~700 pg/mL for epinephrine and norepinephrine, respectively. Each graph also shows that spiked samples yielded similar catecholamine concentrations as standards used for spiking, further indicating the lack of assayable epinephrine and norepinephrine in the patient samples. Moreover, each of the volunteer samples that were taken the day prior to the test resulted in catecholamine concentrations that were within normal physiological ranges. Finally, each of the standards assayed (that were provided by the manufacturer) yielded values that were
within one standard deviation of the value provided by the manufacturer. Control sample number two data is not provided in the graph because it had very high epinephrine and norepinephrine concentrations that would skew what is being shown in each graph. The average intra-assay coefficient of variation for all samples tested was 1.6% for epinephrine and 2.1% for norepinephrine. These data led to the conclusion that the integrity of the catecholamine samples was compromised by unanticipated thaw.
Substrate Responses during the Isoglycemic Clamp:

**Glucose Specific Activity:** When using tracers to measure glucose kinetics, it is important to maintain steady state levels of specific activity to ensure that the measurement of the tracee’s rates of appearance and disappearance are as accurate as possible. Steady state specific activity during the final 30 minutes of each clamp phase for the diet only group averaged 2254.4 ± 134.3, 2273.3 ± 117.9, and 2296.7 ± 149.9 dpm/ mg during the post-absorptive, and low and high periods of insulin infusion, respectively during the pre-intervention clamp. For the diet and exercise group, specific activity averaged 2236.7 ± 85.9, 2197.9 ± 110.7, and 2208.9 ± 113.2 dpm/ mg over the same respective periods of insulin infusion during the pre-intervention isoglycemic clamp. During the post-intervention clamp, specific activity in the diet only group during the final 30 minutes of each clamp phase averaged 2355.8 ± 131.4, 2333.6 ± 150.2, and 2381.6 ± 196.5 dpm/ mg during the post-absorptive, low and high insulin infusion periods, respectively. For the diet and exercise group, specific activity averaged 2272.5 ± 202.4, 2106.7 ± 126.6, and 2053.4 ± 66.8 dpm/ mg during the same respective periods of insulin infusion. No differences in specific activity were detected between the diet only group and the diet plus exercise group during any of the insulin infusion periods of either the pre- or post-intervention clamp.

**Plasma Lactate:** Plasma lactate levels (Table 4.2) were determined at minute 0 (i.e., prior to the beginning of the clamp), and at the 90, 210 and 330 minute time points of the clamp procedure. The latter three values correspond with the time points at which PGU and EGP were measured during the post-absorptive insulin, low, and high insulin infusion rates, respectively. Average plasma lactate level for the diet only group during
the pre-intervention clamp was 947.2 ± 225.7, 580.1 ± 54.5, 561.5 ± 51.6, and 570.1 ± 93.5 μM/L during the 0, 90, 210 and 330 minute time points, respectively. The average plasma lactate level for the diet plus exercise group during the pre-intervention clamp procedure were 620.2 ± 50.6, 439.6 ± 35.7, 474.0 ± 54.7, and 473.3 ± 62.4 μM/L during the 0, 90, 210, and 330 minute time points, respectively. During the post-intervention clamp, plasma lactate levels for the diet only group averaged 550.9 ± 55.1, 513.3 ± 34.1, 480.0 ± 36.2, and 514.9 ± 64.6 μM/L during the 0, 90, 210, and 330 minute time points, respectively. The average plasma lactate levels for the diet plus exercise group during the post-intervention clamp were 603.7 ± 89.1, 610.6 ± 79.4, 542.4 ± 72.2, and 460.1 ± 45.5 μM/L during the 0, 90, 210 and 330 minute time periods, respectively. No differences were observed between the diet only group and the diet plus exercise group during any of the time periods. However, plasma lactate levels during the 90, 210 and 330 minute time periods were significantly lower than minute 0 plasma lactate levels (p < 0.01 for each).

Intra-assay coefficient of variation averaged 8.6% and inter-assay coefficient of variation averaged 10.1%.

**Plasma Glycerol:** Plasma glycerol levels (Table 4.2) were determined at minute 0 (i.e., prior to the beginning of the clamp), and at the 90, 210 and 330 minute time points of the clamp. The latter three values correspond with the time points at which PGU and EGP were measured during the post-absorptive, low, and high insulin infusion rates, respectively. Average plasma glycerol level for the diet only group during the pre-intervention clamp was 307.5 ± 63.4, 170.1 ± 19.2, 143.3 ± 18.6, and 120.3 ± 25.7 μM/L during the 0, 90, 210 and 330 minute time points, respectively. The average plasma
glycerol level for the diet plus exercise group during the pre-intervention clamp were
213.9 ± 39.6, 177.9 ± 28.4, 121.9 ± 8.8, and 128.7 ± 18.1 μM/ L during the 0, 90, 210, and
330 minute time points, respectively. During the post-intervention clamp, plasma
glycerol levels for the diet only group averaged 224.0 ± 43.8, 220.0 ± 42.0, 144.4 ± 35.8, and 129.3 ± 37.5 μM/ L during the 0, 90, 210, and 330 minute time points, respectively. The average plasma glycerol levels for the diet plus exercise group during the post-intervention clamp were 187.4 ± 24.5, 148.3 ± 12.0, 127.5 ± 11.4, and 70.9 ± 12.2 μM/ L during the 0, 90, 210 and 330 minute time periods, respectively. No differences were observed between the diet only group and diet plus exercise group at any of the indicated time periods. However, plasma glycerol levels were significantly lower during the 210 and 330 minute time periods compared to both the 0 and 90 minute time periods (p < 0.04 for each). Intra-assay coefficient of variation averaged 2.6% and inter-assay coefficient of variation averaged 9.6%.

Plasma Non-Esterified Fatty Acids (FFA): Plasma FFA levels (Table 4.2) were determined at minute 0 (i.e., prior to the beginning of the clamp), and at the 90, 210 and 330 minute time points of the clamp. The latter three values correspond with the time points at which PGU and EGP were measured during the post-absorptive, low, and high insulin infusion rates, respectively. Average plasma FFA level for the diet only group during the pre-intervention clamp was 0.53 ± 0.04, 0.53 ± 0.05, 0.19 ± 0.05, and 0.11 ± 0.03 mmol/ L during the 0, 90, 210 and 330 minute time points, respectively. The average plasma FFA level for the diet plus exercise group during the pre-intervention clamp were 0.60 ± 0.05, 0.56 ± 0.05, 0.18 ± 0.03, and 0.08 ± 0.01 mmol/ L during the 0, 90, 210, and 330 minute time points, respectively. During the post-intervention clamp procedure,
plasma FFA levels for the diet only group averaged 0.56 ± 0.08, 0.50 ± 0.05, 0.23 ± 0.05, and 0.10 ± 0.03 mmol/ L during the 0, 90, 210, and 330 minute time points, respectively. The average plasma FFA levels for the diet plus exercise group during the post-intervention clamp were 0.60 ± 0.05, 0.55 ± 0.04, 0.22 ± 0.02, and 0.08 ± 0.01 mmol/ L during the 0, 90, 210 and 330 minute time periods, respectively. No differences were observed between the diet only and diet plus exercise groups during any of the indicated time periods. However, plasma FFA were significantly lower during the 210 and 330 minute time periods compared to minute 0 (p < 0.0001 for each), and plasma FFA were significantly lower during the 330 minute time point of the clamp compared to the 210 minute time period of the clamp (p < 0.0001). Intra-assay coefficient of variation averaged 7.1% and inter-assay coefficient of variation averaged 6.3%.

**Measurement of Peripheral Glucose Uptake and Endogenous Glucose Production:**

**Isoglycemic Clamp Glucose Levels:** Plasma glucose levels were measured using a bedside glucose monitor (Yellow Springs Instruments, Yellow Springs, Ohio), and used for the calculation of PGU and EGP. This was done by averaging the plasma glucose levels during the final 30 minutes of each two-hour phase of the isoglycemic clamp to provide steady state glucose values for each subject. Steady state glucose level for the diet only group averaged 128.4 ± 0.8, 137.2 ± 0.4, and 135.4 ± 0.6 mg/ dL during the post-absorptive, and low and high periods of insulin infusion, respectively during the pre-intervention clamp. For the diet and exercise group, plasma glucose levels averaged 119.2 ± 0.7, 131.3 ± 0.3, and 123.0 ± 0.9 mg/ dL over the same respective periods of insulin infusion during the pre-intervention isoglycemic clamp. During the post-intervention clamp, plasma glucose levels in the diet only group averaged 121.3 ± 0.3,
130.9 ± 0.3, and 119.3 ± 1.4 mg/ dL during the post-absorptive, low and high insulin infusion periods, respectively. For the diet and exercise group, plasma glucose values averaged 116.1 ± 0.5, 129.2 ± 0.6, and 124.0 ± 0.6 mg/ dL during the same respective periods of insulin infusion. During the post-intervention clamp, plasma glucose levels during the post-absorptive period were significantly greater in the diet only group compared to the diet and exercise group (p < 0.05). No differences in plasma glucose levels were detected between the diet only group and the diet plus exercise group during the low or high insulin infusion periods of either the pre- or post-intervention clamp.

Peripheral Glucose Uptake: Peripheral glucose uptake (Figures 4.9 – 4.11) was measured using 3-3H labeled glucose for the determination of the rate of appearance and disappearance of glucose from the blood. Blood samples were taken for this purpose every five minutes during the final 30 minutes of each phase of the isoglycemic clamp procedure. The rates of disappearance, determined over five minute periods during the final 30 minutes of each phase of the clamp were then averaged to provide steady state rates of disappearance. The values for rate of disappearance were then expressed per unit of lean body mass as determined by BIA. The average rates of glucose disappearance for the diet only group during the pre-intervention isoglycemic clamp were 3.00 ± 0.18, 4.24 ± 0.84, and 6.43 ± 1.11 mg/ kgLBM/ minute during post-absorptive insulin levels, and low and high insulin infusion rates, respectively. During the pre-intervention clamp for the diet plus exercise group, the average rates of disappearance over the same respective time periods were 3.04 ± 0.24, 4.07 ± 0.38, and 6.31 ± 0.57 mg/ kgLBM/ minute. During the post-intervention isoglycemic clamp, the average rates of disappearance for the diet only group were 3.04 ± 0.20, 4.24 ± 0.56, and 6.80 ± 1.11 mg/ kgLBM/ minute during
post-absorptive insulin levels, and low and high insulin infusion rates, respectively, while rates of disappearance in the diet plus exercise group during the post-intervention clamp averaged $3.44 \pm 0.43$, $3.99 \pm 0.36$, and $10.01 \pm 1.22$ mg/kgLBM/minute during the same respective insulin infusion periods. Although there were no differences detected between the diet only and diet plus exercise groups during the post-intervention clamp at post-absorptive insulin levels and during the low insulin infusion period, the rate of disappearance (Rd) was significantly greater in the diet plus exercise group compared to the diet only group during the post-intervention clamp during the high insulin infusion rate ($p < 0.05$).

**Figure 4.9:** Peripheral glucose uptake during the post-absorptive period (i.e., the first phase of the clamp) of the isoglycemic clamp. No significant differences were detected between groups or over time ($p > 0.05$).
Figure 4.10: Peripheral glucose uptake during the low insulin infusion period (i.e., the second phase of the clamp) of the isoglycemic clamp. No significant differences were detected between groups or over time (p > 0.05).
Figure 4.11: Peripheral glucose uptake during the high insulin infusion period (i.e., the third phase of the clamp) of the isoglycemic clamp. * P < 0.05 for diet & exercise post-intervention measurement compared to the diet only post-intervention measurement.
**Endogenous Glucose Production:** Endogenous glucose production (Figures 4.12 – 4.14) was measured using $3^{-3}H$ labeled glucose. Blood samples were taken for this purpose every five minutes during the final 30 minutes of each phase of the isoglycemic clamp. The rates of appearance, determined over five minute periods during the final 30 minutes of each phase of the clamp were then averaged to provide steady state rates of appearance. The values for rate of appearance were then expressed per unit of lean body mass as determined by BIA. The average rates of glucose appearance for the diet only group during the pre-intervention isoglycemic clamp were $3.02 \pm 0.23$, $0.77 \pm 0.31$, and $0.51 \pm 0.23 \text{ mg/kgLBM/minute}$ during post-absorptive insulin levels, and low and high insulin infusion rates, respectively. During the pre-intervention clamp for the diet plus exercise group, the average rates of appearance over the same respective time periods were $3.04 \pm 0.24$, $0.58 \pm 0.16$, and $1.04 \pm 0.42 \text{ mg/kgLBM/minute}$. During the post-intervention isoglycemic clamp, the average rates of appearance for the diet only group were $3.05 \pm 0.25$, $0.79 \pm 0.25$, and $1.34 \pm 0.39 \text{ mg/kgLBM/minute}$ during post-absorptive insulin levels, and low and high insulin infusion rates, respectively, while rates of appearance in the diet plus exercise group during the post-intervention clamp averaged $3.44 \pm 0.43$, $0.59 \pm 0.16$, and $1.85 \pm 0.51 \text{ mg/kgLBM/minute}$ during the same respective insulin infusion periods. No differences in EGP between the diet only and diet plus exercise groups were observed during any of the three insulin infusion periods. However, EGP was greater during the post-intervention clamp compared to the pre-intervention clamp (i.e., the diet only and diet plus exercise groups combined) during the high insulin infusion rate ($p < 0.01$).
Figure 4.12: Endogenous glucose production during the post-absorptive period (i.e., the first phase of the clamp) of the isoglycemic clamp. No significant differences were detected between groups or over time (p > 0.05).
Figure 4.13: Endogenous glucose production during the low insulin infusion period (i.e., the second phase of the clamp) of the isoglycemic clamp. No significant differences were detected between groups or over time (p > 0.05).
Figure 4.14: Endogenous glucose production during the high insulin infusion period (i.e., the third phase of the clamp) of the isoglycemic clamp. No significant differences were observed between groups during the pre-intervention or post-intervention time period (p > 0.05).
**Whole Body Insulin Sensitivity:** Whole body insulin sensitivity was determined by expressing the average glucose infusion rate (e.g., during the insulin infusion periods of the clamp) in mg of glucose infused/ kgLBM/ minute per unit of plasma insulin during the final 30 minutes of both the low and high insulin infusion periods of the clamp. Average whole body insulin sensitivity for the diet only group during the pre-intervention isoglycemic clamp was 0.12 ± 0.02, and 0.09 ± 0.03 during the low and high insulin infusion rate periods, respectively. During the pre-intervention isoglycemic clamp in the diet plus exercise group, insulin sensitivity averaged 0.10 ± 0.01, and 0.10 ± 0.02 during the same respective insulin infusion periods. During the post-intervention clamp, whole body insulin sensitivity averaged 0.12 ± 0.02, and 0.09 ± 0.02 in the diet only group during the low and high insulin infusion rate periods, respectively. Whole body insulin sensitivity in the diet plus exercise group during the same respective periods averaged 0.12 ± 0.01, and 0.16 ± 0.03 during the post-intervention clamp. No differences were observed between the diet only and diet plus exercise groups during either of the insulin infusion periods, despite an apparent trend for whole body insulin sensitivity to be greater in the diet plus exercise group compared to the diet only group in response to high insulin infusion during the post-intervention clamp (p = 0.09).

**Peripheral Insulin Sensitivity:** Peripheral insulin sensitivity (Figures 4.15 – 4.17) was determined by expressing PGU per unit of ambient insulin. Peripheral insulin sensitivity values for the diet only group during the pre-intervention isoglycemic clamp were 0.25 ± 0.04, 0.13 ± 0.02, and 0.10 ± 0.02 mg/ kgLBM/ minute/ μU of insulin during post-absorptive insulin levels, and low and high insulin infusion rates, respectively. During the pre-intervention clamp for the diet plus exercise group, the average peripheral insulin
sensitivity over the same respective time periods were 0.23 ± 0.03, 0.11 ± 0.01, and 0.10 ± 0.01 mg/ kgLBM/ minute/ µU of insulin. During the post-intervention isoglycemic clamp, the average peripheral insulin sensitivity for the diet only group was 0.30 ± 0.06, 0.14 ± 0.02, and 0.11 ± 0.01 mg/ kgLBM/ minute/ µU of insulin during post-absorptive insulin levels, and low and high insulin infusion rates, respectively, while peripheral insulin sensitivity in the diet plus exercise group during the post-intervention clamp averaged 0.36 ± 0.10, 0.13 ± 0.01, and 0.18 ± 0.03 mg/ kgLBM/ minute/ µU of insulin during the same respective insulin infusion periods. No differences in peripheral insulin sensitivity were detected between the diet only and exercise groups, during post-absorptive insulin levels or during the low insulin infusion rate. However, during the post-intervention clamp, peripheral insulin sensitivity was higher during the high insulin infusion rate in the diet plus exercise group compared to the diet only group (p < 0.0001).
Figure 4.15: Peripheral insulin sensitivity, measured by expressing PGU per unit of insulin, during the post-absorptive period. No differences were detected between groups or over time (p > 0.05).
Figure 4.16: Peripheral insulin sensitivity, measured by expressing PGU per unit of insulin, during the low insulin infusion period. No differences were detected between groups or over time (p > 0.05).
Figure 4.17: Peripheral insulin sensitivity, measured by expressing PGU per unit of insulin, during the high insulin infusion period. * P < 0.0001 for diet & exercise post-intervention compared to diet only post-intervention measurement.

Hepatic Insulin Resistance: Hepatic insulin resistance (Figures 4.18 – 4.20) was determined by expressing EGP per unit of ambient insulin. Hepatic insulin resistance values for the diet only group during the pre-intervention isoglycemic clamp were 0.258 ± 0.043, 0.021 ± 0.008, and 0.007 ± 0.003 mg/ kgLBM/ minute/ µU of insulin during post-absorptive insulin levels, and low and high insulin infusion rates, respectively. During the pre-intervention clamp for the diet plus exercise group, the average hepatic insulin resistance over the same respective time periods were 0.227 ± 0.027, 0.016 ± 0.004, and 0.016 ± 0.007 mg/ kgLBM/ minute/ µU of insulin. During the post-intervention isoglycemic clamp, the average hepatic insulin resistance for the control
group were $0.307 \pm 0.064$, $0.026 \pm 0.008$, and $0.020 \pm 0.006$ mg/kgLBM/minute/µU of insulin during post-absorptive insulin levels, and low and high insulin infusion rates, respectively, while hepatic insulin resistance in the diet plus exercise group during the post-intervention clamp averaged $0.357 \pm 0.104$, $0.020 \pm 0.006$, and $0.035 \pm 0.012$ mg/kgLBM/minute/µU of insulin during the same respective insulin infusion periods. No differences in hepatic insulin resistance were observed between the diet only and diet plus exercise groups at any time point, although during the post-intervention clamp, hepatic insulin resistance of both the diet only and diet plus exercise groups combined were greater during the high insulin infusion period compared with the pre-intervention clamp procedure.

**Figure 4.18:** Hepatic insulin resistance, measured by expressing EGP per unit of insulin, during the post-absorptive period. No differences were detected between groups at any time point or over time ($p > 0.05$).
Figure 4.19: Hepatic insulin resistance, measured by expressing EGP per unit of insulin, during the low insulin infusion period. No differences were detected between groups at any time point or over time (p > 0.05).
Figure 4.20: Hepatic insulin resistance, measured by expressing EGP per unit of insulin, during the high insulin infusion period. No differences were detected between groups at any time point or over time (p > 0.05).
CHAPTER 5

DISCUSSION

This study assessed the effects of seven days of aerobic exercise on endogenous glucose production (EGP) and peripheral glucose uptake (PGU) in obese humans with mild type 2 diabetes mellitus (DM), during post-absorptive insulin levels, and at a low and a high rate of insulin infusion. Another important component of this study was to gain control over certain aspects of the subjects’ lifestyle that could confound the measurement of EGP and PGU, namely the adoption of strict dietary control, the cessation of diabetic medications, and the prevention of a change in body fat levels and aerobic power that are not expected to occur in response to seven days of aerobic exercise training. It was hypothesized that seven days of aerobic exercise training during these conditions would result in reduced EGP and increased PGU, in a dose-response manner, at post-absorptive insulin levels, as well as during both low and high insulin infusion rates. The results indicate the following: first, the diet only and diet plus exercise groups demonstrated similar glucose tolerance (as determined by the OGTT) and similar levels of EGP and PGU during the pre-intervention isoglycemic clamp, indicating that each group had similar levels of insulin resistance at the outset of the study. Second, EGP and PGU did not change in the diet only group from the pre-intervention isoglycemic clamp to the post-intervention clamp, indicating stability of these measurements over the
intervention period. Third, while seven days of diet and exercise training had no apparent effect on EGP or hepatic insulin sensitivity at any insulin level, the diet plus exercise group showed greater rates of PGU and peripheral insulin sensitivity compared to the diet only group during the post-intervention isoglycemic clamp during the high insulin infusion rate. The higher rates of PGU during insulin infusion, along with no changes to EGP in response to exercise training that were observed in the current study indicate that improved glucose tolerance and insulin action, after seven days of aerobic exercise that has been reported previously (Rogers, 1988; Kang, 1996), are due primarily to changes in insulin mediated PGU, and not insulin mediated suppression of EGP.

A number of previous studies have investigated the effect of aerobic exercise training on PGU and EGP in obese humans with DM. These studies have observed that chronic aerobic exercise training results in improved glucose tolerance, and during glucose clamp conditions, improved insulin sensitivity. While these studies demonstrate the efficacy of aerobic exercise to improve whole body insulin sensitivity in DM, the experimental design often produces a loss of body fat or gains in aerobic power (i.e., VO$_2$ max). Each of these factors, independent of the effect of exercise training, can improve insulin sensitivity. Consequently, it is difficult to determine the independent effect of exercise training on insulin sensitivity from these studies. Therefore, a number of studies have employed exercise training interventions that are from 1-10 days in duration. With this short period of training, there are minimal, if any, changes to either body fat levels or aerobic power. Such designs permit the determination of the effects of exercise on glucose tolerance and insulin sensitivity independent of other factors. Such an exercise
intervention was used in the current study to determine the relative contribution of hepatic and peripheral tissues to improve whole body insulin sensitivity in obese humans with DM, an area that has yet to be investigated.

**Peripheral Glucose Uptake**

Previous research by Devlin et al. (1987) showed that a single bout of exhaustive aerobic exercise in humans with DM improved peripheral insulin sensitivity during euglycemic clamp conditions in the form of increased tracer measured glucose disposal per unit of insulin compared to a non-exercise control condition. Other studies have employed longer exercise training durations to try and determine the independent effect of aerobic exercise training on glucose tolerance and insulin sensitivity. Rogers et al. (1988) and Kang et al. (1996) both reported that seven days of aerobic exercise training, using a training protocol identical to the one used in the current study, resulted in improved glucose tolerance, manifested by reduced plasma glucose levels during OGTT after exercise training, as well as improved insulin action, as interpreted by the lower insulin levels in response to the OGTT after exercise training. Similarly, Arciero et al. (1999) showed that 10 days of aerobic exercise training in humans with impaired glucose tolerance or “mild” DM resulted in increased whole-body glucose disposal during hyperglycemic (i.e., 250 mg% clamp values) clamp conditions, as determined by the amount of intravenously infused dextrose required to maintain hyperglycemic conditions. While each of these three studies (Rogers, 1988; Kang, 1996; Arciero, 1999) have been critical in the development of our understanding of the independent effect of aerobic exercise training on whole body glucose tolerance and insulin sensitivity in humans with
DM, they do not provide information on the relative contributions of changes to PGU and EGP that are responsible for improved whole body glucose metabolism in response to exercise training.

The results of the present study indicate that seven days of aerobic exercise training, identical to that which was used by Rogers et al. (1988), in obese humans with DM, resulted in greater rates of PGU during physiologically relevant hyperinsulinemia (i.e., insulin levels of ~60 μU/mL). The fact that there was a dose-response increase in PGU relative to post-absorptive PGU during low and high insulin infusion rates indicates that the methods used during the isoglycemic clamp were valid.

In contrast with the lack of an extensive literature base examining the effect of aerobic exercise training on factors that regulate EGP, there has been significant work that has improved our understanding of how aerobic exercise training affects factors that regulate PGU. Peripheral glucose uptake by skeletal muscle is determined by the interaction of three factors: the delivery of glucose to the skeletal muscle (i.e., blood flow), the transport of glucose into the skeletal muscle cell, and the rate of glucose phosphorylation after it has entered the skeletal muscle cell. Although the current investigation clearly does not attempt to determine the mechanisms responsible for changes in PGU that were observed, it appears that any of these factors, either singly, or interactively contributed to the observed increase in PGU. Of the factors that limit the uptake of glucose into skeletal muscle, the delivery of glucose to the muscle has received the least amount of attention. Previous research has indicated that endothelial dysfunction (i.e., impaired NO-mediated blood flow) is present in DM (Williams, 1996). There are few investigations that have determined the effect of exercise training on blood flow...
during resting conditions in DM. Sakamoto et al. (1998) used a rat model of DM and demonstrated that exercise training resulted in improved nitric oxide-mediated vasodilation, which is the mechanism by which insulin exerts its actions. Furthermore, other investigations (Chakraphan, 2005; Amatyakul, 2006) have indicated that exercise training durations of 12-24 weeks can prevent endothelial dysfunction from developing in STZ-induced DM. Based on these studies, it appears that the role of exercise training to improve PGU during hyperinsulinemia in the current study may be mediated in part by improved endothelial function, although it is not clear whether these changes could have occurred in response to our relatively short training duration. Further investigation will be required to address this issue.

More thoroughly characterized are the adaptations of short term exercise training on glucose transport into the muscle and subsequent phosphorylation. In a comprehensive evaluation of these areas, Ren et al. (1994) exercised two groups of healthy rats for one or two days, and measured Glut 4 mRNA levels, Glut 4 protein levels, muscle hexokinase activity and glycogen synthesis during the rested state (i.e., not during exercise). Results of this study showed that only two days of exercise training resulted in a two-fold increase in Glut 4 mRNA, Glut 4 protein and hexokinase activity. Also observed was a doubling of Glut 4 translocation to the sarcolemma and glycogen synthesis in response to incubation of muscle strips with insulin and glucose. These data show the significant impact that short term aerobic exercise training can have to improve processes that limit PGU in skeletal muscle during insulin infusion, although a healthy rat model was used in this study. However, increases in Glut 4 protein levels have been shown to rise nearly four fold and glycogen concentrations can increase by 40% in healthy humans after seven
days of aerobic exercise training (Kraniou, 2004). Exercise training also has been shown to increase Glut 4 protein levels (Dela, 1994) and non-oxidative glucose metabolism in humans with DM. Thus it appears likely that a combination of these factors could also have contributed to the increase in insulin-mediated glucose uptake observed in the current study.

One of the objectives of the current study was to prevent changes to aerobic power (i.e., VO2 max), and because of this, it is unlikely that changes in oxidative disposal of glucose contributed to the observed increase in PGU. This is corroborated by the work of Black et al. (2005) who reported no change in oxidative glucose disposal during intravenous glucose infusion after a single week of aerobic exercise training in “at risk” obese humans that did not have DM. However, Black et al. (2005) did show that non-oxidative glucose disposal during the IVGTT was increased in response to the exercise training intervention in subjects with relative energy deficit, further supporting the likelihood that increased rates of non-oxidative, rather than oxidative glucose disposal, contributed to the increase in PGU that was noted in the current study.

**Endogenous Glucose Production**

It is generally accepted that aerobic exercise training can improve hepatic insulin sensitivity as indicated by lower rates of EGP both during the post-absorptive state, as well as in response to insulin infusion. Using a cross sectional design, Rodnick et al. (1987) observed that highly trained runners had two and three-fold lower rates of EGP during infusion-induced steady state insulin levels of 10 and 50 \( \mu \text{U}/ \text{mL} \), respectively compared to otherwise healthy non-runners. Bogardus et al. (1984) observed that 12 weeks of caloric restriction plus exercise in humans with glucose intolerance and DM
resulted in reduced EGP during post-absorptive insulin levels, as well as greater insulin-induced suppression of EGP. Devlin et al. (1987) observed that a single bout of exhaustive exercise in humans with DM resulted in reduced EGP during post-absorptive insulin levels as well as during insulin infusion similar to the high infusion dose used in our study. Segal et al. (1991) also noted that post-absorptive EGP was lower 4-5 days after the cessation of a 12 week training regimen, even after peripheral effects of the exercise had been lost (e.g., there was no longer improved PGU). Together, these studies demonstrate that aerobic exercise can have an acute (i.e., after a single bout), as well as a chronic (i.e., after 12 weeks of training) positive response on EGP in DM during the post-absorptive state and during hyperinsulinemia.

The results of the present study suggest that seven days of aerobic exercise training did not have a significant impact on EGP during post-absorptive insulin levels, and it did not affect EGP during the low insulin infusion rate. It was also observed that the high insulin infusion rate did not additionally suppress EGP beyond the level that was observed during the low insulin infusion rate. Insulin infusion did result in the suppression of EGP, indicating that the measurement of EGP with the methodology we used was valid.

The fact that the aerobic exercise training protocol used in the current study did not affect EGP is somewhat surprising, considering the number of studies using a similar intensity of exercise that have reported improved EGP during both post-absorptive insulin levels and during varying levels of physiologically relevant insulin infusion. However, there are a number of factors that might explain why these protocols observed improved hepatic glucose metabolism and the present study did not. Devlin et al. (1987)
reported that a single bout of exhaustive exercise resulted in lower rates of EGP during both post-absorptive insulin levels and during insulin infusion identical to the high insulin infusion rate used in the present study. However, it is important to recognize that exhaustive exercise has very different metabolic consequences on hepatic tissue compared to steady state endurance exercise similar to the type of exercise the subjects performed in the present study. Recently, Camacho et al. (2006) performed a study using a mouse model to compare the effects of exhaustive versus steady state endurance exercise on selected parameters that pertain to hepatic glucose physiology. The results revealed that steady state endurance exercise (i.e., exercise not to exhaustion), resulted in a significant rise in hepatic AMP levels compared to non-exercised control animals, which in turn resulted in a two-fold increase in the phosphorylation (i.e., activation) of AMPK in hepatocytes. However, there was a further increase in hepatic AMP levels compared to the non-exhaustive exercise group, and a robust seven-fold increase in AMPK phosphorylation in the group of animals that were exercised to exhaustion. It has been shown previously by Iglesias et al. (2002) that the administration of AICAR, an AMPK agonist, can ameliorate hepatic insulin resistance induced by a high fat diet, thus indicating the role of AMPK activation to regulate EGP. Thus when considering that the present study did not detect a change in EGP after seven days of steady state endurance training, it appears plausible that previous studies reporting improved insulin mediated EGP over longer training durations may be in part caused by a chronic low-level activation of AMPK that did not occur in the current study to the extent required for changes in EGP to occur.
Another relevant consideration when interpreting the results of the current study is the mode of delivery of insulin into the body. In the current study, insulin was infused intravenously, which limits its action on hepatic glucose metabolism. In fact, insulin has both direct (i.e., hepatic sinusoidal activation) and indirect (i.e., regulation of substrate and hormonal fluxes) effects on hepatic glucose metabolism. Cherrington (1997) noted that direct effects of insulin on hepatic glucose metabolism are mediated by the binding of insulin in the hepatic sinusoids, and include the suppression of hepatic glucose production by inhibiting the metabolic processes of glycogenolysis and gluconeogenesis, and a recent study by Edgerton et al. (2006) has indicated that the direct effect of insulin plays a dominant role in the regulation of in vivo hepatic glucose metabolism. Indirect effects of insulin on hepatic glucose physiology most relevant to the results of the current study include the following: 1) the inhibition of glucagon release from pancreatic α-cells; 2) the inhibition of lipolysis, which reduces the levels of circulating glycerol and FFA (gluconeogenic substrates); 3) the inhibition of muscle catabolism which reduces the levels of alanine and other gluconeogenic substrates; and 4) activation of hypothalamic insulin receptors which may contribute to the suppression of endogenous glucose production.

Because insulin was infused peripherally in the current study, it is likely that the noted peripheral effects of insulin played a dominant role in the suppression of EGP during both low and high insulin infusion rates. A series of studies performed by Sindelar et al. (1996, 1997) on the conscious dog indicated that during peripheral infusion of insulin, as occurs during a typical clamp study in humans, 25% of the observed reduction in EGP was due to increased levels of sinusoidal insulin (i.e., the direct effect), 25% was
due to reductions in the presence of FFA, glycerol and amino acids as gluconeogenic substrates, and 50% was due to the redirection of glycogenolytically derived G6P to lactate in the hepatocyte. Thus, the reduction in EGP in response to insulin infusion that was observed in the current study was likely due to a combination of hepatic sinusoid activation, as well as a reduction in circulating FFA and glycerol, and to a minimal, if any, extent the redirection of glycogenolytically derived G6P to lactate, as evidenced by the lack of an effect of insulin infusion on plasma lactate values. Moreover, the lack of an effect of the exercise training protocol to change any of the circulating levels of these gluconeogenic substrates in the current study likely contributes to the absence of an exercise effect on EGP. Thus, while reduced EGP may ensue after seven days of aerobic exercise training in response to an oral or intravenous glucose challenge due to endogenous insulin release, its contribution to improved glucose tolerance during these conditions is likely minimal. Considering the magnitude of the change in muscle insulin sensitivity observed in the current study, and the absence of a measured change in hepatic insulin sensitivity, improved whole body insulin sensitivity or glucose tolerance that has been reported after seven days of aerobic exercise training (Rogers, 1988; Kang, 1996) are likely caused primarily by improved peripheral insulin sensitivity.

In conclusion, this investigation demonstrates that seven days of aerobic exercise training is of sufficient length to produce changes to insulin sensitivity in obese humans with DM. Furthermore, these changes are caused by improved insulin sensitivity of peripheral tissues. However, because there were no changes in hepatic insulin sensitivity, it is likely that a training duration of a length of time greater than seven days is required to produce that effect in obese humans with DM.
BIBLIOGRAPHY


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