4206

DOES PRIOR EXERCISE AFFECT GLYCEMIC RESPONSE TO A GLUCOSE LOAD?

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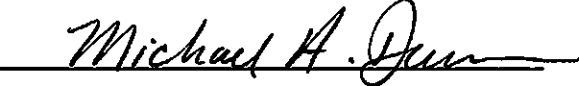
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ABSTRACT

When oral glucose tolerance tests (OGTT) are repeated in individuals, relatively large variations often occur in the magnitude of the blood glucose response from one occasion to another. Little is known about what causes this within-subject variability. One potential contributor to this variability may be the subject's prior extent of physical activity and/or the amount of stored glycogen present at the time of the OGTT. This research tested the effect of a bout of exercise (of the type known to significantly deplete muscle glycogen) performed within 24 hours prior to an OGTT on blood glucose and insulin responses. Ten male endurance athletes underwent an OGTT without prior exercise on one occasion and after a glycogen-depleting bout of exercise the day prior to testing on another occasion. Venous blood was sampled at standard intervals (0, 15, 30, 45, 60, 90, and 120 minutes) for 2 hours following consumption of an OGTT beverage containing 50 grams of glucose. Capillary blood samples also were taken at the same intervals by finger stick. Venous and capillary blood samples were analyzed for glucose. Venous samples also were analyzed for insulin concentration. Glucose and insulin areas under the curve (AUC) were calculated. There was no significant difference between the glucose AUC measured by capillary and venous blood samples and no significant difference between the exercise and non-exercise conditions. Insulin AUC values were significantly lower on the days following exercise (P=0.03). There was a significant difference between exercise and non-exercise glucose levels when insulin levels were held constant (P=0.03). Thus, when comparing people at the same insulin level, glucose AUC levels were significantly greater the day after exercise. There was no predictable difference in insulin sensitivity between exercise and non-exercise conditions based on

Insulin Sensitivity Index (ISI) values. Based on this evaluation of ten male endurance athletes, it does not appear that glycogen-depleting exercise has a predictable effect on the OGTT.

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GI	Glycemic Index
OGTT	Oral Glucose Tolerance Test
AUC	Area Under the Curve
ISI	Insulin Sensitivity Index
FAO	Food and Agriculture Organization
WHO	World Health Organization
SGLT1	Sodium-Glucose Transporter 1
GLUT	Glucose Transporter
UDP-glucose	Uridine diphosphate glucose
AMPK	AMP-activated protein kinase
FFA	Free Fatty Acid
DEXA	Dual Energy X-ray Absorptiometry
ACSM	American College of Sports Medicine
BMI	Body Mass Index

CHAPTER 1: LITERATURE REVIEW

Glucose Tolerance

Blood glucose levels are measured for both diagnostic and research purposes. As a diagnostic test, oral glucose tolerance tests are used to help diagnose diabetes as well as hypoglycemia. In research, glucose tolerance testing is used to determine the glycemic index (GI) of foods. However, glucose levels may have significant within-subject variability from day-to-day that may affect both the diagnosis of diabetes and the measurement of GI. Glucose tolerance may be affected by various factors such as the rate of gastric emptying when the glucose was ingested [1], the meal eaten the day prior [2], glycogen status [3,4], insulin release, insulin sensitivity [5], rate of disappearance of glucose [6], use of various medications, stress [7], and recent exercise [8,9].

When oral glucose tolerance tests (OGTT) are repeated in individuals, relatively large variations often occur in the magnitude of the blood glucose response from one occasion to another. Little is known about what causes this within-subject variability. One potential contributor may be the subject's prior extent of physical activity and/or the amount of stored glycogen present at the time of the OGTT.

Heath et al. [10] looked at glucose tolerance in well trained athletes after their normal workout regimen as well as after 10 days of inactivity. The trained subjects' glucose tolerance in the trained state was normal with an average peak value of only 104 ± 6 mg/ 100 mL, 30 minutes after ingestion of 100 g of glucose. Glucose tolerance was significantly reduced after 10 days of physical inactivity. A single bout of exercise after 11 days without exercise caused their glucose tolerance to return to almost the same level

as occurred in the trained state. This shows the pronounced effect exercise can have on the glycemic response to glucose ingestion.

Glycemic Index

The GI is a physiological method for classifying carbohydrate-containing foods according to their effect on blood glucose levels. It was developed to provide a numeric classification of carbohydrate foods that might be useful in the management of impaired glucose tolerance [11]. Individual foods are assigned a value based on the extent to which they raise blood glucose levels during the postprandial period. The GI is defined as the area under the blood glucose curve (AUC) after the consumption of 50 g carbohydrate from a test food divided by the AUC after eating a similar amount of a control food, usually glucose in the form of an OGTT beverage [12]. Related to this variability seen in standard OGTTs, the GI measurements of various foods often have substantial variance associated with published values [13,14]. Therefore, evaluation of the factors that may contribute to the within-subject variation seen in OGTT may lead to improved protocols for OGTT. One possible variable that may contribute to this variation is a glycogen-depleting bout of exercise performed the day prior to testing.

To generate a glycemic response curve when testing the GI of a food, blood samples are taken by a finger-stick method (to obtain capillary glucose levels) at fasting (zero), then at 15, 30, 45, 60, 90, and 120 minutes after a subject consumes the standard reference food (usually 50 g of glucose). Capillary blood is usually the standard type of blood used when determining the GI of foods because it has been found to be more sensitive to glycemic responses than venous blood or plasma [12]. However, due to the variability seen between glucose AUC when comparing capillary to venous blood,

previous research has suggested the need to compare capillary and venous blood samples when testing the glycemic index of various foods [14]. Venous blood glucose tends to have greater variability within subjects when compared to capillary blood glucose. Wolever et al. found that the GI values of foods are more precisely determined using capillary than venous blood sampling [14].

The Food and Agriculture Organization/World Health Organization (FAO/WHO) has adopted the GI concept and recommend developing national GI databases [15]. A GI database for food frequency questionnaires (FFQ) that are used in epidemiologic studies also is being developed. Despite the large number of publications on the topic of GI, there were no standard food composition databases with this information for use with standard dietary assessment instruments. Neuhouser et al. [16] recently developed a glycemic index database for use with the FFQ utilized in the Women's Health Initiative, which is a study of health among 165,000 postmenopausal women.

Glycemic index testing as a way of classifying carbohydrates is still controversial. One reason is that the methodology for obtaining GI of foods is not succinct. Identifying confounding variables in OGTT procedures, may help to establish a more accurate and usable system.

Glucose Uptake

After ingestion, glucose is absorbed into the mucosal cells of the small intestine by an active transport dependent mechanism. The glucose carrier involved in this absorption is called the sodium-glucose transporter 1 (SGLT1) and is dependent on the active transport of sodium out of the cell by a Na+/K+-ATPase pump. Most glucose that is absorbed into the mucosal cell is transported out of the cell into the blood by a carrier

in the basolateral membrane. The majority of glucose entering the liver is released to the systemic blood system and distributed to other tissues like muscle, kidney, and adipose tissue for energy use or storage.

In order for glucose to enter cells, it must cross the plasma membrane of the cell. Since glucose is highly polar, it cannot pass directly through the nonpolar lipid bilayer of cells. Instead it is passively admitted into most cells by a facilitated transport mechanism called glucose transporters (GLUT). The movement of glucose into cells is insulin dependent in adipocytes, heart, and skeletal muscle, and insulin independent in liver and brain [17]. Glucose uptake can be stimulated by at least two independent pathways in skeletal muscle: an insulin-signaling pathway that is activated when insulin binds to its receptor and a pathway that is activated by exercise/contractions and hypoxia [18,19].

Rate of appearance and disappearance

Plasma glucose concentration is a function of both the rate of appearance of glucose into the systemic circulation as well as the rate of disappearance of glucose, or glucose uptake from the blood by tissue. Most assume that if a food has a low GI that it is a result of a slower rate of digestion which slows the absorption of glucose into the circulation [6]. The rate of appearance is influenced by the intrinsic properties of carbohydrates (amylose-to-amylopectin ratio) and the fiber, protein and fat content of the food consumed [6]. The rate of disappearance, or glucose uptake from the blood by tissues, is dependent on insulin secretion and action on tissue [20].

Schenk et al. [6] looked at glucose kinetics that are responsible for the different glycemic responses of breakfast cereals with a low (bran cereal) or high (corn flakes) GI. They found that the high GI of corn flakes compared to bran cereal (132 vs. 55) was not

due to differences in rate of appearance of glucose. Bran cereal showed a lower GI due to an earlier increase in rate of disappearance of glucose which was associated with an earlier and higher insulin response during the initial 20 minute postprandial period. Therefore, even though bran cereal and corn flakes had a similar rate of glucose entry into the blood, the bran cereal had a more rapid insulin-mediated increase in tissue glucose uptake, lessening the overall increase in blood glucose concentration. Due to the fact that insulin mediates tissue glucose uptake, the blood insulin AUC, which reflects the rate of disappearance of glucose from the blood, may help to explain the variability in the blood glucose response for repeated OGTTs that may be observed in some individuals.

GLUT4 Transporters

There are 13 known members of the GLUT family [21]. One of these transporters, GLUT4, is insulin dependent. When present, insulin rapidly stimulates glucose uptake in skeletal muscle by stimulating the synthesis of GLUT4 transporters from ribosomes on the golgi apparatus. The transporters are then packaged and the vesicle-bound GLUT4 isoform is transported from the intracellular compartment to the cell membrane. The molecular mechanism that causes this translocation of GLUT4 remains unknown and is under investigation. Once the vesicles containing the GLUT4 transporters fuse with the plasma membrane, they release the transporters and allow them to position themselves in the membrane. Once insulin is removed from its receptor, membrane-bound transporters are retranslocated back into the intracellular compartment.

Insulin

Insulin is a polypeptide hormone that is produced in beta cells of the pancreas and controls carbohydrate metabolism. It is composed of 51 amino acids in two peptide chains (A and B) linked by two disulfide bonds. Besides controlling the uptake of glucose by cells, it also causes an increase in glycogen synthesis. When insulin levels decrease, glycogen is broken down into glucose which can then be used for energy by the body. As blood glucose concentration increases, insulin levels increase as well, and glucose is taken up by the cells to be used for energy or stored as glycogen for future energy needs.

Insulin Sensitivity Index (ISI)

Insulin's action on glucose transport is characterized in terms of insulin sensitivity and insulin responsiveness [22]. Insulin sensitivity is the capacity of cells to respond to insulin-stimulated glucose uptake following ingestion of carbohydrate and is defined in terms of the concentration of insulin required to cause 50% of its maximal effect on glucose transport [23]. In clinical investigation, the measurement of insulin sensitivity is important because of its key role in diabetes.

The hyperinsulinemic-euglycemic glucose clamp technique, described by Defronzo *et al.* [24], is considered the most definitive method to measure insulin sensitivity in humans but it is experimentally demanding and expensive. Therefore, the homeostasis model assessment (HOMA) introduced by Matthews *et al.* [25] was developed in 1985 and became the most simple index for evaluating insulin sensitivity. HOMA takes into account both glucose and insulin sensitivity using a structural model of the glucose-insulin feedback system in the fasted state and is solved by a computer

program using fasting glucose and insulin concentrations. Since its introduction, some limitations have been reported, including the need for a specialized computer program [26]. In an attempt to create a methodology that is more realistic for clinical use, researchers have since developed new insulin sensitivity indices that are calculated from plasma glucose and plasma insulin concentrations after an OGTT [27-29].

Matsuda and DeFronzo [28] developed a composite measurement of whole body insulin [ISI(composite) or COMP] sensitivity during an OGTT, which is shown in the following equation:

$$COMP = 10,000/\sqrt{[(FPG * FPI) * (meangluc * meanins)]}.$$

where 10,000 represents a constant that allows one to obtain numbers ranging from 0 to 12, FPG is the fasting plasma glucose value (mg/dL), FPI is fasting plasma insulin (μU/mL), meangluc is the mean glucose concentration during the OGTT, and *meanins* is the mean insulin concentration during the OGTT. The square-root conversion is used to correct the nonlinear distribution of values.

Gutt et al. [27] developed the $ISI_{0,120}$ from the SI index (degree of peripheral insulin sensitivity), which was developed and published by Cederholm and Wibell [30]. The following formula consists of a few parts and was taken directly from Gutt et al.'s paper [27]. The first part of the formula is used to calculate the glucose uptake rate in peripheral tissues, designated as m (mg/min), using the 0 and 120 minute glucose values (mg/l) obtained from the OGTT, where the term 0.19 × BW denotes glucose space, and BW is body weight (kg):

$$m = (75{,}000 \text{ mg} + (0 \text{ min glucose} - 120 \text{ min glucose}) \times 0.19 \times BW)/120 \text{ min}$$

Mean plasma glucose (MPG), the mean of the 0 and 120 min glucose values (mg/l) from

the OGTT, is used to obtain the metabolic clearance rate (MCR), which corrects for the

potential influence of different blood glucose concentrations on the glucose uptake rate.

$$MCR = m/MPG$$

To correct for skewness of distribution, the mean serum insulin (MSI) calculated as the

mean of 0 and 120 minute insulin values (mU/l), is logarithmically transformed. The

$ISI_{0,120}$ insulin sensitivity index is then calculated:

$$ISI_{0,120} = MCR/\log MSI = m/MPG/\log MSI$$

The equation predicting insulin sensitivity proposed by Stumvoll et al. [29] also

uses insulin and glucose concentrations during an OGTT:

$$ISI_{Stumvoll} = 0.157 - 0.00004576 \times I_{120} - 0.00519 \times G_{90} - 0.000299 \times I_0.$$

I_{120} and I_0 are insulin concentrations (pmol/L) at 120 and 0 minutes of the OGTT,

respectively, and G_{90} is glucose concentration (mmol/L) in the 90th minute of the OGTT.

This index was proposed using stepwise linear regression.

Stumvoll also proposed an equation that utilized anthropometric parameters. The

following equation includes body mass index (BMI) of the subject because they found

that some of the changes seen in insulin sensitivity may be mediated through changes in

body composition [29].

$$ISI_{Stumvoll (BMI)} = 0.226 - 0.0032 \times BMI - 0.0000645 \times I_{120} - 0.00375 \times G_{90}$$

Kanauchi et al. [31] validated the ISI in a Japanese population by comparing

insulin sensitivity measured by the "gold standard" euglycemic-hyperinsulinemic glucose

clamp technique (*M*-value) to the HOMA index and to the three formulas proposed above

(Matsuda, Gutt, and Stumvoll). They found a weak inverse correlation between the

HOMA index and *M*-value. Matsuda, Gutt, and Stumvoll's formulas correlated

significantly with the *M*-value. Although the euglycemic-hyperinsulinemic clamp

method is thought to be the best way to measure insulin sensitivity, it is invasive and has

a high cost leaving it with limited use in clinical practice. Therefore, by using less

invasive formulas, that only require OGTT and insulin values obtained in the same time

interval, one can assess insulin sensitivity in subjects [31].

Glycogen

Upon ingestion, glucose is absorbed into the blood and transported to the body

tissues for use or stored in the liver and muscles as glycogen. Glucose must be brought

into the cell and then bound together to form glycogen, which is the major form of stored

carbohydrate in human tissue. Glycogen is a highly branched polyglucose molecule

composed of chains of individual glucose molecules that are linked in an alpha-1,4

configuration. Approximately every ten residues contains a branch with a glucose

molecule linked in the alpha-1,6 configuration [17].

Control of glycogen synthesis and breakdown is under hormonal control. When

there are adequate levels of blood glucose, insulin levels rise to stimulate cells to take up

this glucose. Insulin acts as a signaling molecule and stimulates the synthesis of

glycogen which stores glucose for future needs. Epinephrine and glucagon have the

opposite effect from that of insulin. When these hormones act on the cell, it leads to

glycogen breakdown and the use of glucose for energy metabolism in muscle cells and

the potential release of glucose from liver cells. Epinephrine acts more strongly on

muscle cells and their stores of glycogen, while glucagon acts more strongly on liver cells [17].

After entering the cell, glucose is phosphorylated to glucose 6-phosphate by the enzyme glucokinase in the liver and hexokinase in the muscle. After transferring the phosphate to the 1-carbon, uridine monophosphate is added to the glucose 1-phosphate to form uridine diphosphate glucose (UDP-glucose). Glucose is incorporated into glycogen as UDP-glucose and this reaction is catalyzed by glycogen synthase. Some preformed glycogen, in the form of a primer, is required for the glucose units to attach to. The primer involved is a protein called glycogenin which catalyzes the attachment of glucose units to form chains of up to eight units, at which time glycogen synthase finishes the elongation of the glycogen chain. Glycogen synthase exists in two forms, the active (dephosphorylated) form and the less active form (phosphorylated) form. The stimulating role of insulin on the synthesis of glycogen is due to its facilitation of the dephosphorylation of glycogen synthase into its active form. Therefore, the glycogen synthase reaction is the primary target and regulator of insulin's stimulation of glycogen synthesis.

Glycogen storage is localized primarily in liver and skeletal muscle. Human skeletal muscle normally contains about 14-18 g•kg^{-1} of glycogen, but the range can be much wider than this depending on muscle mass and glycogen status. The total amount of glycogen stored in the liver varies greatly. It has been reported that in the postabsorptive state, an average 70 kg male with 15% body fat has approximately 80 g of glycogen stored in his liver. This is much less than glycogen stored in muscles which is

about 300-400 g. A 12-hour fast can cause liver glycogen to decrease by more than half, where as without exercise, muscle glycogen has little depletion after a fast [32].

Glycogen Depletion and Repletion

Glycogen stores have been shown to be depleted after 2 hours of endurance exercise, including swimming, skiing, bicycling, and running [33]. The ability of muscle to synthesize glycogen is increased following a bout of strenuous exercise [34]. This increased ability to synthesize glycogen can last for days if the carbohydrate intake is restricted to keep muscle glycogen content low [3,35]. After depleting glycogen stores through exercise, glycogen repletion is not immediate and may take up to two days to complete [33]. Volek *et al.* also found that glycogen synthase activity was significantly increased immediately following exercise in overweight men [36].

Bogardus *et al.*[37] concluded that insulin sensitivity was improved in subjects 15 hours after a glycogen depleting bout of exercise. This was determined by the rate of glucose disposal during a euglycemic clamp procedure. They also found that the rate of glucose disposal was positively correlated with the rate of muscle glycogen synthesis and the activation of glycogen synthase. This would lead one to believe that glycogen depletion may increase glucose uptake and glucose tolerance, after the ingestion of glucose, through the activation of glycogen synthase and increased muscle glycogen synthesis.

Glycogen synthase, as mentioned above, is strongly stimulated after muscular exercise and remains more active when glycogen stores are depleted than when they are replenished resulting in a faster rate of glycogen resynthesis following exercise [38]. Therefore, the highest rates of muscle glycogen storage occurs during the first hour after

exercise [39]. Ivy et al. found that failure to consume carbohydrate immediately after exercise leads to a very low rate of glycogen repletion until feeding occurs. They also determined that even though early feeding may be important when there is only 4-8 hours between exercise sessions [39], it may have less of an impact over a longer recovery period. Overall, it appears that meeting total carbohydrate requirements for the day is more important than the pattern of intake, at least with long-term recovery of glycogen stores [40].

The most important dietary factor affecting muscle glycogen storage is the amount of carbohydrate consumed. Data from various studies that have monitored muscle glycogen storage after 24-hours of recovery from glycogen depleting exercise show a direct and positive relationship between the quantity of dietary carbohydrate and post-exercise glycogen storage. Two different studies looked at glycogen storage by feeding different amounts of carbohydrate to trained individuals over a 24-hour recovery period [41,42]. They both showed an increase in glycogen storage with increasing carbohydrate intake and a threshold for maximal glycogen storage at a daily carbohydrate intake of around 7-10 g•kg^{-1} body mass [41,42]. Thus, by keeping a carbohydrate intake below this amount after a glycogen depleting bout of exercise, it would suggest that glycogen stores will not be fully replenished after 24 hours.

Exercise and Glycemic Response

The effect of physical activity in improving glucose tolerance has been explained previously by a combination of several factors, including the enhanced insulin binding to receptors [43], an increased permeability of muscle cell membranes to glucose [19], an

insulin-independent activation of glucose transport [18,22,44] and a reduced glycogen concentration within the muscle cells [10].

The lasting effect of a strenuous bout of exercise that causes normal or improved glucose tolerance, despite lower insulin levels, may be explained by the above mechanisms. In 1979, Leblanc *et al.* determined that an increase in tissue sensitivity to insulin may be mediated by increased binding of insulin to its receptors on the cell membrane [43]. However, at the time of this study, no claim could be made as to whether this action was due to differences in the number of receptors or in their affinity for insulin [43].

The exercise-induced increase in muscle cell permeability to glucose can last for a long period after muscle contraction stops [3,19]. As the acute effect of muscle contractions on glucose transport reverses, it is replaced by a large increase in insulin sensitivity [45]. This does not appear to be mediated by an increase in the insulin signal, but rather the increase in insulin sensitivity is mediated by translocation of a larger number of GLUT4 transporters to the cell surface in response to a given insulin stimulus [46].

Insulin-independent mechanisms have also been found that activate glucose transport during and following exercise. Muscle contractions [44] and hypoxia [18] both induce increases in muscle glucose transport by stimulating movement of GLUT4 from intracellular storage sites to the cell surface [22]. The effects of contraction, hypoxia and insulin are additive but they stimulate glucose transport by separate pathways [47].

Finally, depletion of muscle and liver glycogen improves glucose tolerance which Heath *et al.* believe makes available "glucose storage space"[10]. In rats subjected to

exhausting exercise on the preceding day, the rate of glucose uptake by perfused skeletal muscle was significantly higher at the same insulin concentration in animals where muscle glycogen was kept low than in those in which glycogen was raised by carbohydrate feeding [3].

In humans, glucose tolerance is increased after an acute bout of exercise despite a normal or even diminished insulin response [10,37]. This increased insulin action appears to occur only after an exercise bout of sufficient intensity and duration to decrease muscle glycogen concentration [48]. A decrease in blood glucose response to a glucose load, or an increased glucose tolerance, is also frequently shown when comparing trained versus untrained individuals. A single bout of exercise can cause acute changes that cause this lower response but this is only temporary [10]. Over time, physical activity contributes to various mechanisms that decrease blood glucose response to an oral glucose load.

Similarly to the effects of insulin, a single bout of exercise increases the rate of glucose uptake into the contracting muscles [37], a process that is regulated by the translocation of GLUT4 glucose transporters to the plasma membrane. These effects can last for several hours after the exercise ends [49]. Translocation of GLUT4 and the increase in AMP-activated protein kinase (AMPK) activity are enhanced in low muscle glycogen states and these mechanisms could mediate the glycogen effects on glucose uptake [50].

GLUT4

GLUT4 transporters are the major insulin sensitive glucose transporters in skeletal muscle. Skeletal muscle GLUT4 levels increase rapidly in response to exercise training

[51] and decrease with detraining [52]. This response is seen in both previously sedentary people as well as trained individuals. Houmard *et al.* demonstrated an increase in skeletal muscle GLUT4 glucose transporter protein content after 12-14 weeks of endurance-exercise training in sedentary middle-aged men along with an increase in insulin sensitivity [53].

Similar to the effect of exercise, insulin also causes GLUT4 translocation in skeletal muscle [54]. It would seem logical that since blood flow is increased during exercise, the increase in GLUT4 translocation is due to an increased delivery of insulin to the muscle. However, when hindlimb skeletal muscles are contracted in situ without the presence of insulin, GLUT4 concentration is increased in the plasma membrane to a similar degree as when exercise is performed in vivo [55]. This shows that muscular contraction can recruit GLUT4 to the plasma membrane in rat skeletal muscle independent of insulin.

Exercise and Insulin Sensitivity

Insulin's action on glucose transport is characterized in terms of insulin sensitivity and insulin responsiveness [22]. Insulin sensitivity is defined in terms of the concentration of insulin required to cause 50% of its maximal effect on glucose transport. The fact that exercise increases the sensitivity of glucose transport to insulin in skeletal muscle was first discovered by Richter *et al.* [56]. Subsequent studies have found that muscle contractions stimulate glucose transport in the absence of insulin [18,22,44].

In a healthy person, it has been shown that a single bout of exercise can increase insulin stimulated whole body glucose uptake for at least 16 hours post-exercise [8,37]. In addition, as a result of a single bout of exercise a blunted insulin response may result

during an OGTT as well as an increased sensitivity to insulin [10,57]. LeBlanc *et al.* [43] reported that the insulin response to a glucose load was lessened while glucose tolerance remained unchanged in untrained subjects 18 hours after 60 minutes of exercise. They also found that plasma insulin levels after a glucose load are higher after three days of inactivity in trained individuals. From this information Heath *et al.* deduced that a single bout of exercise can reduce the insulin response to a glucose load whether the subjects are trained or not [10].

Ivy et al. [58] also discovered that following a mixed diet for one day after glycogen depleting exercise, the insulin response, represented by the area under the insulin curve, was 28.3% lower than that for the control treatment in which the subjects ate a mixed diet for three days following the same type of exercise bout. Even with a lower insulin response, the subjects had similar glucose AUC indicating an improvement in insulin sensitivity. Englert *et al.* [59] found that an acute bout of prior exercise produced a lower 2-hour insulin response to a CHO-rich energy bar compared to non-exercise immediately following the exercise bout. The authors explained this phenomenon by the possibility that acute exercise reduces insulin secretion through an increase in circulating catecholamines [59].

The increase in insulin-stimulated glucose uptake, or insulin sensitivity, in the post-exercise period has been correlated with the amount of glycogen utilization during exercise [4]. Muscle glycogen is also inversely related to both basal and insulin-stimulated glucose uptake [60]. Another factor that increases glucose uptake occurs due to insulin-stimulated GLUT4 translocation being greater under low glycogen conditions [61]. Derave *et al.* found that the glycogen depleting effect of insulin on glucose

transport and cell surface GLUT-4 content does not involve initial mechanisms but possibly more downstream signaling events [61]. Bogardus *et al.* [37] reported that the action of insulin, as determined by the rate of glucose disposal during an euglycemic clamp procedure, was improved in subjects 15 hours after a bout of exercise that significantly reduced their muscle glycogen concentration. Acute exercise has also been shown to decrease the insulin response to an OGTT [57], which may suggest that peripheral insulin sensitivity is increased.

The rate and duration of increased glucose uptake after exercise has been shown to be clearly influenced by the amount of glycogen depletion that occurs [56]. The reversal of the increased glucose uptake and insulin sensitivity can be manipulated by muscle glycogen concentration. Carbohydrate intake after exercise, that increases glycogen concentrations in muscle, will accelerate the return to basal rates of glucose uptake [62] and carbohydrate restriction that keeps glycogen levels low slows down this reversal process [45]. On the other hand, a low-carbohydrate diet lasting a few days decreases insulin sensitivity and causes apparent glucose intolerance [63].

As mentioned above, Heath *et al.* [10] looked at glucose tolerance in well trained athletes after their normal workout regimen as well as after 10 days of inactivity. The rise in plasma insulin concentration in response to a 100 g oral glucose load was much greater after the 10 days of inactivity than it was in the trained state. The extent of the increase in insulin response seemed to be out of proportion to the change in blood glucose response after 10 days of inactivity. They also found that while blood glucose concentration during the OGTT was only 10-25% higher following 10 days without exercise, plasma insulin concentration was from 55-120% higher. This decreased insulin

response to a glucose load following exercise has been a consistent finding in individuals who exercise regularly [43,64,65]. Additionally, the blunted insulin response and increased insulin sensitivity have been related to long-term adaptations to exercise rather than to an effect from the last bout of exercise [43,65].

John Holloszy [22] has undergone numerous studies looking at the exercise-induced increase in muscle insulin sensitivity. It is known that the stimulation of muscle glucose transport by insulin and by contractions/hypoxia is mediated by the movement of GLUT4 from intracellular storage sites to the cell surface. He has developed a hypothesis to explain why an increase in sensitivity to glucose transport remains even after the bout of exercise has terminated. When the stimulus that caused GLUT4 translocation is removed (muscle contraction, hypoxia, insulin), and its effect reverses, the GLUT4 leaves the cell surface and moves into an intracellular compartment. In this compartment, the transporters are highly susceptible to recruitment by either a weak insulin signal or contraction/hypoxia signal. The increase in sensitivity persists for as long as the GLUT4 remains in this "high-susceptibility compartment" [22]. According to Holloszy [22], all of the information that has been discovered in regards to the post-exercise increase in insulin sensitivity has been descriptive. Researchers have found various components that cause this increase, "but essentially no progress has been made in elucidating the mechanism responsible for mediating this phenomenon" [22].

INTRODUCTION

When oral glucose tolerance tests (OGTT) are repeated in individuals, relatively large variations often occur in the magnitude of the blood glucose response from one occasion to another. Little is known about what causes this within-subject variability. One potential contributor to this variability may be the subject's prior extent of physical activity and/or the amount of stored glycogen present at the time of the OGTT.

A previous study following a glycemic index assessment protocol reported significant within-subject variability on three repeated OGTTs [66]. Measurements of the area under the curve (AUC) for the blood glucose response to 50 g of glucose revealed within subject coefficients of variation (cv) ranging from 17 to 40% with a mean cv of 27%. Glycemic indexes for glucose solutions have also been reported as 96 or 114 with standard deviations of ± 22 and 28 respectively [13]. This degree of variability raises questions about the validity and utility of glycemic index testing and raises potential concerns regarding the proper protocols for OGTTs used for clinical diagnostic purposes. Prior physical activity and muscle glycogen levels may be variables that affect the within-subject variability in the glycemic response. Therefore, this study investigated the effect of extensive endurance exercise (of the type known to significantly decrease muscle glycogen) performed the day prior to testing the glycemic response.

Although many studies in the literature have shown significant between-subject and within-subject variation among OGTT [13,14,66] only a few have attempted to determine what causes this variation, although many researchers have speculated why it occurs [14,67,68]. Various conditions including the meal eaten the day prior [2], glycogen status [3,4], hormonal changes [69], and medications [70] have all been

considered but glycogen depletion acquired solely through exercise within 24 hours of testing has not been tested, to our knowledge.

Sparti and Decombaz [71] tested various diets to achieve glycogen depletion or repletion, along with exercise 36 hours prior to an OGTT to determine whether there was a glycogen depleting effect on glucose AUC. Due to their diet protocol (low-carbohydrate, high-fat) they did not see an effect from glycogen depleting exercise on glucose tolerance because the interrelationship of free fatty acids (FFA) and glucose may have overridden the exercise-induced changes in insulin-stimulated glucose uptake. This may be because high FFA concentrations can have an inhibitory effect on the rate of glucose utilization [72].

Sparti and Decombaz [71] had their subjects perform an exercise bout 36 hours prior to testing. It would seem fitting to have subjects perform an exercise bout, within 24 hours of testing, to maintain adequate glycogen depletion through exercise rather than diet. By standardizing the total number of carbohydrates in each subject's diet the day prior to both testing protocols, with a prior exercise bout as well as without an exercise bout, it will help to limit total glycogen repletion before the OGTT the following morning. Therefore, it will keep the subject's diets constant and test only the difference between the exercise and no-exercise treatments.

Identifying a factor that contributes to the variability seen in OGTTs could lead to better standardization of protocols for use in clinical OGTTs and in glycemic index studies. The specific aims of this study were to: 1) Determine if a glycogen-depleting type of exercise has a predictable effect on glycemic response. We hypothesized that compared to non-exercise, an acute bout of glycogen-depleting exercise within 24 hours

of an OGTT will result in a lower total increase in blood glucose concentration and therefore a lower area under the glucose curve. 2) Compare glycemic response as measured by capillary blood to that measured by venous blood samples. We hypothesized that glycemic response would be greater in venous glucose measurements compared to capillary glucose measurements. 3) Determine the body composition of participants by Dual Energy X-ray Absorptiometry (DEXA) to compare data from this study to the results of possible future studies using participants with different body composition and levels of habitual activity. Evaluate the relationship between body composition and the glycemic response. We hypothesized that having a greater body fat percentage may augment glycemic response to a glucose load. 4) Evaluate if the variability in the blood glucose response is due in part to variability in insulin response. We hypothesized that insulin levels would be lower after a glycogen depleting bout of exercise.

Previous research has shown that exercise training has an effect on glucose uptake via increased GLUT-4 translocation as well as increased insulin sensitivity [45,49]. However, to our knowledge, it is not known whether a glycogen depleting bout of exercise, within 24 hours prior to an OGTT has an effect on glucose AUC or insulin AUC. Determining whether an acute bout of exercise has an effect on the glycemic response of male endurance athletes may help to delineate one potential variable associated with variability in glycemic response. Due to the fact that glycemic index testing utilizes OGTTs to compare the blood glucose response to various foods, determining possible mechanisms involved in the within-subject variability seen in OGTTs may help to improve glycemic index testing protocols.

METHODS

Experimental Design

Subjects were recruited from local bicycle and triathlon shops, training clubs, and races, by flyers and presentations. Subjects were screened for eligibility criteria which included: 1) must have participated in an Olympic Distance Triathlon (1.5k swim, 40k bike, 10k run) or in running a marathon (26.2 miles) within the past 12 months; 2) be able to exercise at their usual intensity for at least 1 ½ hours; 3) be apparently healthy, as defined by American College of Sports Medicine (ACSM) guidelines [73].

Exclusion criteria included having: 1) diabetes; 2) heart disease; 3) taking any of the following medications: blood pressure medications, diuretics, corticosteroids, seizure medications; 4) refusal to discontinue vitamin and mineral supplements, multivitamins, herbal supplements, performance enhancing or recreational drugs including alcohol for 72 hours prior to testing, unless prescribed by a physician; 5) taking high doses of vitamin C (>1000mg day). With informed consent, eligible subjects were enrolled.

Subjects were required to attend three study visits at the Kapiolani Clinical Research Center. The first visit included enrollment and orientation procedures, including diet assessment and directions for subsequent visits. Subjects were given written and verbal instruction concerning diet, hydration, exercise, and fasting prior to each study visit. Preparation for the final two study visits included a glycogen depleting exercise protocol within 24 hours of study procedures and a non-exercise protocol requiring no exercise for at least 36 hours prior to study procedures. The order of study visits (exercise and non-exercise) was randomly assigned to each subject (Table 1).

Table 1. Randomly chosen subject visit profile

Subject	First Visit
1	Exercise
2	Non-Ex
3	Exercise
4	Non-Ex
5	Exercise
6	Exercise
7	Exercise
8	Exercise
9	Exercise
10	Non-Ex

Note. Subjects were randomly assigned to their first study visit
Non-Ex = non-exercise study visit

Subjects

Ten male endurance athletes were selected for the study. None of the subjects

were taking any medication or had a family history of diabetes mellitus. The percentage

of body fat was estimated using Dual Energy X-ray Absorptiometry (DEXA) which was

performed using the GE Lunar Prodigy DEXA scanner operated by a licensed operator.

The subjects were informed of the purpose and methods of the study and gave their

written consent before participating. The experimental protocol was approved by the

University of Hawaii's Committee on Human Studies and the Hawaii Pacific Health

Institutional Review Board.

Exercise Session

The day prior to testing in which a glycogen depleting type of exercise was

required, the subjects completed either 1 ½ - 2 hours of running, 2-4 hours of cycling, or

a combination of both running and cycling not to exceed 4 hours total at their usual

training intensity, in order to simulate glycogen depletion. The day prior to testing for the

non-exercise condition, the subjects were asked to refrain from exercise for at least 36 hours prior to testing in order to allow significant muscle glycogen repletion.

Diets

Each subject was required to keep a dietary record for the day prior to each study visit. At the initial study visit each subject consulted with the principle investigator (PI) to plan their food intake for each meal to be consumed the day prior to testing as well as during the exercise session, using their usual dietary pattern. Food Processor (Version 7.1.1, ESHA Research, Salem, OR) was used to determine the amount of carbohydrate in each food consumed. Carbohydrate intake was standardized for each individual subject to not exceed 5 g/kg body weight for the day prior to testing to ensure carbohydrate intake was unlikely adequate to significantly restore glycogen stores after the bout of exercise. To replicate their pretrial diet, the subjects received a copy of their dietary protocol and were asked to follow and record it again. Each subject fasted overnight and was told to drink 8-8oz. glasses of water the day prior to testing and drink 16 oz. of water on the morning of each test to maintain euhydration.

Analytical Procedures

Glucose tolerance test

The subjects arrived at the clinic between 0700 and 0800 hours after an overnight fast of at least 10 hours. Diet and exercise records were collected at the beginning of each visit. Just after arrival, subjects were given a choice of inserting a catheter into an antecubital vein or having multiple venous punctures to remove venous blood samples. If the subject preferred the catheter, it was kept patent with an isotonic saline infusion.

Subjects had 5 ml of venous blood drawn each for fasting glucose and insulin concentration measures. Glucose samples were collected in BD Vacutainer tubes (Sodium Fluoride 5mg/Potassium Oxalate 4mg) and the insulin draws were collected in BD Vacutainer SST (serum separator tubes).

A baseline capillary blood was also obtained by finger stick and analyzed for glucose with a MediSense Precision PCx glucometer (Abbott laboratories, Bedford, MA). Just after fasting blood samples were taken, subjects ingested 50 g of glucose in solution (EVER Scientific Glucose Drink 050, 50 grams dextrose, 10 fl. oz.). Venous glucose and insulin measurements were obtained at 15, 30, 45, 60, 90 and 120 minutes according to the standard GI measurement procedure [12].

Capillary glucose measurements by fingerstick were obtained at the same intervals from the opposite arm of the catheter or venous sticks. Venous glucose and insulin measurements were analyzed by Clin Labs Hawaii using Roche Hitachi 911 for glucose and chemiluminescent Immunoassay (Beckman DXI) for insulin. Fingerstick blood samples were obtained by trained Clinical Research Center staff and analyzed with the Precision PCx glucometer.

Body Composition

Body mass was measured in the fasted state on a SECA 767 electronic scale. Height was measured at the same time using a Heightronic digital stadiometer. These values were used to determine body mass index (BMI). Body composition was estimated using the GE Lunar Prodigy DEXA scanner which was operated by a licensed DEXA study coordinator.

Calculations

The glycemic response was estimated by using the incremental area under the glucose curve. The AUC values for glucose and insulin, were calculated with a formula provided by Thomas Wolever [12].

Insulin Sensitivity Index (ISI)

Blood glucose and insulin values were used to calculate three different ISI values for comparison of insulin sensitivity within-subjects between exercise and non-exercise testing conditions. The formulas were obtained from the original papers by Matsuda, Gutt, and Stumvoll [27-29].

The ISI by Matsuda and DeFronzo (COMP) [27] was calculated as follows:

1. $COMP = 10,000/\sqrt{[(FPG * FPI) * (meangluc * meanins)]}$.

where 10,000 represents a constant that allows one to obtain numbers ranging from 0 to 12, FPG is the fasting plasma glucose value (mg/dL), FPI is fasting plasma insulin ($\mu U/mL$), *meangluc* is the mean glucose concentration during the OGTT, and *meanins* is the mean insulin concentration during the OGTT. The square-root conversion was used to correct the nonlinear distribution of values.

Gutt *et al.* ($ISI_{0,120}$) [27]:

2. $ISI_{0,120} = MCR/\log MSI = m/MPG/\log MSI$

$m = (75,000 + (0 \text{ min glucose} - 120 \text{ min glucose}) \times 0.19 \times BW)/120 \text{ min}$

$MPG = $ mean of 0 and 120 min glucose values (mg/l)

$$\text{MSI} = \text{mean of 0 and 120 minute insulin values (mU/l)}$$

The first part of the formula is used to calculate the glucose uptake rate in peripheral tissues, designated as m (mg/min), using the 0 and 120 minute glucose values (mg/l) obtained from the OGTT, where the term $0.19 \times \text{BW}$ denotes glucose space, and BW is body weight (kg):

Mean plasma glucose (MPG), the mean of the 0 and 120 min glucose values from the OGTT, is used to obtain the metabolic clearance rate (MCR), which corrects for the potential influence of different blood glucose concentrations on the glucose uptake rate. To correct for skewness of distribution, the mean serum insulin (MSI) calculated as the mean of 0 and 120 minute insulin values (mU/l), is logarithmically transformed. The $\text{ISI}_{0,120}$ insulin sensitivity index is then calculated:

Stumvoll *et al.* ($\text{ISI}_{\text{Stumvoll}}$) [29]:

3a. $\text{ISI}_{\text{Stumvoll}} = 0.157 - 0.00004576 \times I_{120} - 0.00519 \times G_{90} - 0.000299 \times I_0$.

3b. $\text{ISI}_{\text{Stumvoll (BMI)}} = 0.226 - 0.0032 \times \text{BMI} - 0.0000645 \times I_{120} - 0.00375$

I_{120} and I_0 are insulin concentrations (pmol/L) at 120 and 0 minutes of the OGTT, respectively, and G_{90} is glucose concentration (mmol/L) in the 90th minute of the OGTT. Equation 3b includes body mass index (BMI) which is calculated as body weight in kilograms divided by height in meters squared.

Statistical Analysis

Two-tailed paired t-tests were used to compare AUC values and individual time points during exercise and non-exercise OGTTs. Separate two-tailed paired t-tests as well as a multiple regression analysis were performed to compare venous and capillary

blood glucose levels. A one-tailed paired *t*-test was utilized, based on the directional hypothesis, to compare insulin AUC values for exercise and non-exercise testing conditions. ISI values also were analyzed using two-tailed paired *t*-tests to compare insulin sensitivity between exercise and non-exercise testing conditions. A separate multiple regression model using glucose AUC values as the outcome, which included capillary and venous blood samples as well as insulin AUC for exercise and non-exercise conditions, also was performed. The repeated glucose measurements in both regression models were treated as clusters within study participants to identify that the two values (exercise and non-exercise glucose) belonged to the same person. A separate model looking at percent body fat and glucose AUC for venous and capillary blood also was analyzed. Regression models were fit using generalized estimating equations.

RESULTS

Participants and Protocol Compliance

Physical characteristics of the subjects are shown in Table 2. Subjects were

young, non-obese, physically active male triathletes, with the exception of one who was

overweight (BMI=27.3; percent body fat=25.5%). Two other subjects were considered

overweight by BMI (BMI>25) but had body fat percentages of 9.0 and 7.9% which is

considered "athlete" according to the ACSM [73].

Table 2. Subject summary of anthropometric data

Subject	Age (yr)	Weight (kg)	Height (cm)	BMI (kg/m2)	Body Fat %
1	27.6	75.9	185.2	22.1	7.3
2	25.9	76.3	178.4	24.0	5.3
3	29.2	91.1	185.8	26.4	9.0
4	33.2	80.9	184.8	23.7	12.6
5	31.7	75.9	174.4	25.0	7.9
6	35.4	77.6	185.1	22.6	17.5
7	20.9	65.4	176.0	21.1	11.9
8	31.1	71.7	179.8	22.2	5.2
9	25.8	70.7	182.2	21.3	11.5
10	31.9	84.8	176.2	27.3	25.5
Mean	29.3	77.0	180.8	23.6	11.4
STD	4.29	7.31	4.38	2.12	6.22
CI	26.6, 32.0	72.5, 81.5	178.1, 183.5	22.3, 24.9	7.5, 15.2
Range	20.9 - 35.4	65.4 - 91.1	176.0 - 185.8	21.1 - 27.3	5.2 - 25.5

Note. BMI = Body Mass Index; STD = standard deviation; CI = confidence interval

All participants were able to complete the protocol. Questionnaires revealed that

all subjects abstained from alcohol and supplement use for 72 hours, and no one currently

smoked cigarettes or consumed performance enhancing drugs. Each subject fasted for at

least 10 hours prior to testing and consumed at least 16 ounces of water the morning of

the test. Carbohydrate intake was standardized for each individual subject to not exceed

5 g/kg body weight for the day prior to testing. Three subjects consumed over the

recommended amount (Table 3). All other subjects appeared to have followed their diet

protocol according to the food records they kept the day prior to both OGTTs.

Table 3. Total carbohydrate (CHO) intake and body
weight (BW) adjusted intake during the days prior to testing

Subject	Total CHO (g)	CHO g/kg BW
1	421	5.55
2	354	4.64
3	375	4.12
4	515	6.37
5	470	6.19
6	245	3.16
7	295	4.51
8	315	4.39
9	350	4.95
10	305	3.60
Mean	364.5	4.7
STD	83.34	1.05
CI	312.9, 416.2	4.1, 5.3

Note. STD = standard deviation; CI = confidence interval

Glucose and Insulin Responses

Incremental glucose areas under the curve (AUC) and insulin AUCs were

determined for capillary and venous samples after exercise and non-exercise conditions

for each subject (Table 4). Paired t-tests indicated no significant difference between

exercise and non-exercise glucose AUC means for venous ($P=0.24$) and capillary

($P=0.11$) blood independently (Table 5). Although, the mean insulin AUC value was

significantly lower on the days following exercise ($P=0.026$, one-tailed t-test) (Table 5),

there were no statistically significant differences between insulin concentrations at any of

the time points of the OGTT between the conditions of exercise and no exercise the day

prior to testing (Figure 1, Table 6).

Table 4. Area under the curve (AUC) data for exercise (Ex) and non-exercise (Non-Ex) conditions

Subject	Venous Glucose		Capillary Glucose		Insulin	
	Ex	Non-Ex	Ex	Non-Ex	Ex	Non-Ex
	(mg∙min/dL)	(mg∙min/dL)	(mg∙min/dL)	(mg∙min/dL)	(uIU∙min/L)	(uIU∙min/L)
1	1881	4024	1348	2453	579	950
2	5441	1102	4628	845	1657	1208
3	1385	1382	1959	1108	578	1080
4	5708	3535	6046	4304	793	1245
5	4628	2071	6480	3754	434	416
6	3420	3489	5685	4575	2522	2759
7	6008	5123	5055	4035	1938	2856
8	3511	485	3772	2445	1015	887
9	4005	4195	4845	5108	949	1200
10	1195	3226	3356	5010	1825	2558
Mean	3718	2863	4318	3364	1229	1516
STD	1776	1519	1701	1557	708	870
P-value*	0.24		0.10		*0.026*	

*Comparison of exercise vs non-exercise means; Italicized value indicates a significant *P*-value using a one-tailed test where P<0.05. Note. Ex = exercise; Non-Ex = non-exercise; Ven Glu = venous glucose; Cap Glu = capillary glucose; STD = standard deviation.

Table 5. Mean differences from paired *t*-tests for exercise and non-exercise conditions using venous and capillary blood glucose AUC values and insulin AUC values

Blood Type	Venous Glucose	Capillary Glucose	Insulin
Mean Diff	855.12 (-680, 2390)	953.88 (-225.4, 2133.2)	-287 (-577.9, 4.0)
P-Value	0.24	0.10	*0.026*

Mean Diff = mean difference between testing conditions; ( ) indicate confidence intervals; Ex = exercise; Non-Ex = non-exercise. Italicized value indicates a significant *P*-value using a one-tailed test and P<0.05.

Table 6. Paired *t*-test *P*-values comparing exercise and non-exercise conditions using insulin AUC values and venous and capillary blood glucose AUC vales

Blood Type	Fasting	15 min	30 min	45 min	60 min	90 min	120 min
Insulin	0.20	0.38	0.44	0.90	0.08	0.47	0.07
Venous	0.37	0.27	0.84	0.36	0.86	*0.037*	0.17
Capillary	*0.022*	0.08	0.60	0.66	0.98	0.50	0.65

Italicized value for 90 min (venous) and fasting (capillary) indicates a significant *P*-value comparing exercise and non-exercise conditions for venous blood and capillary blood respectively *P*<0.05.

However, at 90 minutes venous glucose levels were significantly higher after exercise than no exercise (*P*=0.037) (Figure 2, Table 6). Capillary glucose at baseline was also significantly lower after the glycogen-depleting bout of exercise (*P*=0.022) (Figure 3, Table 6). There was no significant difference between capillary and venous glucose AUC (*P*=0.106) (Table 7). When comparing all capillary glucose values to all venous glucose values using a regression model, no significant differences were found (*P*=0.106).

Figure 1. Venous blood insulin levels during two hours following ingestion of 50 g of glucose after exercise and non-exercise days (means ± SEM)
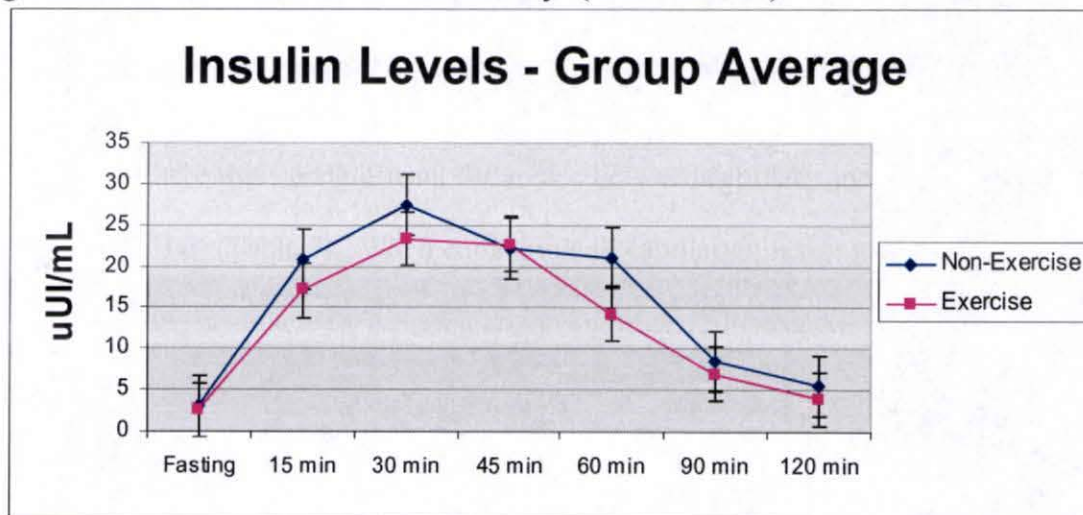
Figure 2. Venous blood glucose levels during two hours following ingestion of 50 g of glucose after exercise and non-exercise days (means ± SEM)

Venous Glucose - Group Average
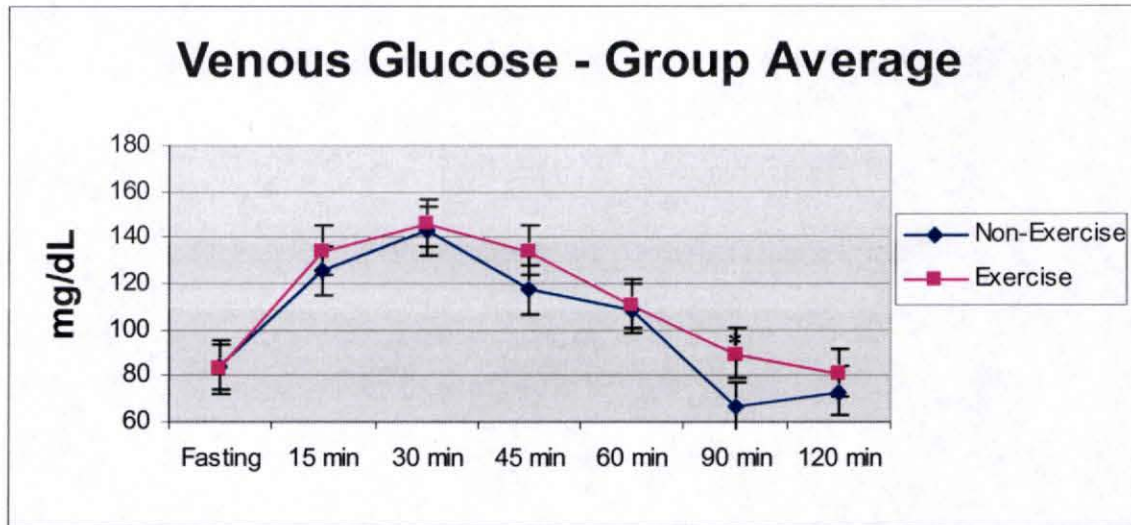
Note. *Glucose was significantly greater after the exercise day at 90 min; $P = 0.04$

Figure 3. Capillary blood glucose levels during two hours following ingestion of 50 g of glucose after exercise and non-exercise days (means ± SEM)

Capillary Glucose - Group Average
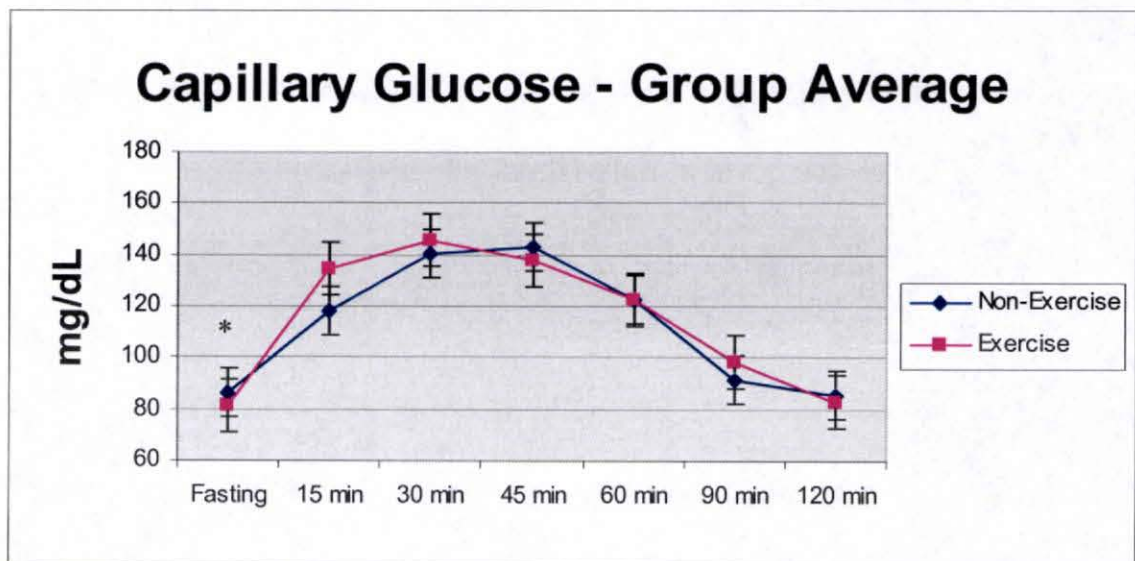
Note. *Glucose was significantly lower after the exercise day for the baseline measurement; $P = 0.02$

Glucose-Insulin Relationship

Multiple regression analysis was used to compare venous glucose levels with

exercise to venous glucose levels with non-exercise as well as capillary glucose levels

with exercise to capillary glucose levels with non-exercise (Table 7). Insulin AUC values

were significantly lower on the days following exercise (P=0.026) but there were no

significant differences seen in glucose AUC for the two separate visits (Table 4). The

lack of a significant difference between exercise and non-exercise blood glucose levels

may be due to a greater insulin sensitivity following exercise. Therefore, exercise and

non-exercise glucose levels were compared with insulin levels held constant, which

revealed that glucose AUC was significantly greater after exercise days when controlling

for insulin (P=0.029) (Table 7).

Table 7. Estimating differences between exercise and non-exercise blood glucose values
and capillary and venous blood glucose values with insulin levels held constant, by
multiple regression analysis

Variable	Estimate	Standard Error	95% CI	P-value
Exercise	1114.8	510.58	(114.07, 2115.5)	*0.029*
Non-Exercise	XX	XX	XX	XX
Capillary	550.05	340.02	(-116.39,1216.5)	0.106
Venous	XX	XX	XX	XX

Italicized values indicate a statistically significant difference P<0.05 for each condition; exercise
vs. non-exercise, and capillary vs. venous blood glucose
CI = confidence interval; XX = lines included for comparison purposes only

Thus, when comparing blood samples with the same insulin level, glucose AUC

levels were significantly greater the day after exercise. After comparing venous glucose

levels with exercise to venous glucose levels with non-exercise and capillary glucose

levels with exercise to capillary glucose levels with non-exercise, those with the same

insulin level tended to have higher capillary glucose levels compared to venous glucose

levels (P=0.106) and higher glucose after exercise compared to non-exercise (P=0.029)

regardless of type of blood (Table 7). With exercise and insulin levels held constant,

capillary samples had higher glucose levels than venous samples but were not

significantly different (P=0.106) (Table 7).

Insulin Sensitivity Index (ISI)

Based on ISI calculations from Matsuda, Gutt, and Stumvoll [27-29] there is no

predictable difference in insulin sensitivity between exercise and non-exercise conditions.

Insulin sensitivity calculated utilizing Gutt's equation and Stumvoll's equations with and

without BMI tended to increase after exercise although not significantly (P=0.20, 0.09,

0.13) (Table 8).

Table 8. Insulin Sensitivity Index (ISI) values calculated utilizing formulas by Matsuda [28], Gutt [27], and Stumvoll [29]

	Matsuda		Gutt		Stumvoll		Stumvoll w/BMI	
	Exer	Non-Ex	Exer	Non-Ex	Exer	Non-Ex	Exer	Non-Ex
Subject								
1	43.69	27.47	3.44	3.34	0.136	0.136	0.142	0.142
2	21.63	24.55	4.09	2.44	0.133	0.140	0.134	0.140
3	36.07	17.42	5.11	1.36	0.135	0.129	0.128	0.127
4	26.18	17.18	1.76	1.64	0.122	0.128	0.127	0.133
5	19.73	32.36	1.22	2.87	0.115	0.134	0.120	0.132
6	21.32	14.68	1.08	1.00	0.124	0.125	0.130	0.131
7	12.42	11.42	0.64	0.58	0.106	0.123	0.123	0.135
8	26.46	30.06	3.08	3.16	0.130	0.139	0.138	0.145
9	31.10	29.65	2.18	1.13	0.126	0.127	0.138	0.136
10	18.24	12.45	2.22	1.15	0.131	0.123	0.123	0.118
Mean	25.69	21.72	2.48	1.87	0.126	0.130	0.130	0.134
P-value*	0.216		0.203		0.133		0.094	

*Based on t-test values comparing exercise to non-exercise test conditions.
Exer = exercise; Non-Ex = non-exercise

Body Fat

When comparing exercise vs. non-exercise conditions, the differences seen in glucose AUC are not explained by body fat percentage; although, for each body fat percentage point increase, glucose AUC increases by 53.9 mg•min/dl. Body fat was not significantly associated with differences seen in glucose AUC when subjects had performed a bout of exercise or had not.

DISCUSSION

Glucose and insulin AUC values were calculated for male endurance athletes after a bout of exercise and after no exercise. Previous research has shown that a glycogen depleting bout of exercise causes an increase in insulin sensitivity, GLUT4 translocation, and increased glycogen synthase activity [37,38,44]; therefore it would appear that an increase in glucose uptake and subsequently a decreased glucose AUC would occur following a bout of exercise. On the contrary, our study found a non-significant increase in glucose AUC following exercise associated with a significant decrease in insulin AUC. Previous research demonstrated differences between glucose AUC when using capillary versus venous blood samples [14], however, we found no significant difference between capillary and venous blood samples for either testing condition.

Our results show a significant difference between OGTTs performed with exercise the day prior compared to no exercise when insulin levels were held constant. However, we did not find a significant difference between glucose AUC values when looked at independently, although they tended to be higher after the bout of exercise. The fact that there was a lower insulin AUC following exercise but glucose stayed the same demonstrates a slight increase in insulin sensitivity following exercise.

The result that glucose AUC was not significantly different after exercise is in agreement with previous work by Ben-Ezra *et al.* [74] and Englert *et al. [59]*. They found no difference in glucose AUC post-exercise compared to a non-exercise control condition but did find a lower insulin AUC following exercise. It is possible that glucose AUC was not lower after the exercise bout compared to non-exercise because of a decreased energy balance. Because the subjects consumed the same amount of calories

on both days but expended more energy on the exercise day, they were likely in a more negative energy balance on the mornings following exercise. Many subjects commented on being hungry the morning after their exercise session. Hepatic glucose production may have increased following the exercise bout to stabilize blood glucose levels and may have helped to maintain the glucose AUC on the post-exercise day. Without glucose rate of appearance and disappearance data, this remains a theoretical speculation.

Insulin levels decreased as expected following an exercise bout [59,74]. A study by Lohmann et al. concluded that under physiologic conditions the extent of insulin secretion is not dependent only upon the blood glucose levels. They found that a lack of insulin response can occur as a consequence of adaptation to physical training [65]. Our subjects were well trained endurance athletes, having competed in at least one Olympic distance triathlon (1.5 k swim, 40 k bike, 10 k run) during the past year. This adaptation to training may reflect the lowered insulin concentrations following exercise observed in the present study.

Another potential explanation for the blunted insulin response after exercise is the increased circulation of catecholamines. Catecholamines that are released as a result of the exercise bout [75] may have reduced insulin secretion [59]. The blunting of insulin release from the pancreas may carry over into the recovery period causing a diminished insulin response. Additionally, the decreased insulin response following exercise may be attributed to an increased insulin clearance. Tuominen et al. looked at insulin clearance following a marathon or 2-hour treadmill test and found the insulin clearance rate was significantly greater 12 and 44-hours after the exercise conditions than non-exercise [76]. Therefore, the actual amount of insulin released may have been similar to the non-

exercise condition, but the rate of clearance was may have been augmented resulting in a lower overall concentration. Again, without rate of appearance and disappearance data for insulin, this can only be speculated.

Insulin secretion and glycogen synthase activity are positively correlated. As the insulin concentration increases in the blood, glycogen synthase concentration also increases in order to synthesize glycogen from excess glucose [22]. Our results indicated that insulin AUC was lower after a bout of exercise and glucose concentrations were slightly, but not significantly, greater. The slightly augmented glucose concentration may also have been in part due to a decrease in glycogen synthase activity as a result of lower insulin levels, resulting in less glucose used for glycogen synthesis and therefore a subtle increase in blood glucose concentration.

As a result of a single bout of exercise, a blunted insulin response may result during an OGTT as well as an increased sensitivity to insulin [10,57]. Similarly, our subjects tended to have a blunted insulin response following exercise with no significant change in blood glucose, indicating an increase in insulin sensitivity. However, ISI values were not found to be significantly different, indicating that there was no predictable difference in insulin sensitivity between exercise and non-exercise conditions.

When controlling for insulin levels, glucose AUC values were significantly higher after exercise when compared to non-exercise conditions. This finding suggests that something other than insulin concentrations has an effect on glucose uptake after a glycogen depleting bout of exercise. Prior to testing we hypothesized that the amount of body fat may have an effect on the subjects' glucose tolerance. After testing, we found that percent body fat did not significantly affect glucose AUC. Although it was not

significant, glucose AUC values tended to be 53.9 mg•min/dL higher with each percentage point increase in body fat. Overall, body fat was not a significant predictor of glucose AUC after exercise.

Another possible explanation for the variability seen in the OGTTs was the participants' hydration status. Subjects were instructed to drink at least 64 oz of water the day prior to testing as well as 16 oz of water the morning of testing. This amount of water may not have been enough to maintain euhydration after water loss from their exercise session. Dehydration may cause a falsely high glucose concentration which would explain part of the increase in glucose AUC found after the glycogen depleting bout of exercise. On the contrary, this would not explain why insulin concentrations were significantly lower after exercise. Although his may be explained by the blunted insulin response seen previously after exercise [65].

Sparti and Decombaz [71] found that a bout of glycogen depleting exercise performed 36 hours prior to an OGTT, along with diet modification to maintain glycogen depletion (i.e., high-fat, low-carbohydrate) caused a significant increase in glucose AUC. The researchers proposed that due to the interrelationship between FFA concentration and glucose uptake, an elevated FFA concentration may have contributed to the increase in glucose AUC observed following exercise. Our subjects were assumed to have followed the same dietary intake the day prior to both testing scenarios but individuals were not observed during their meal time. It is possible that subjects may have strayed from their recommended food intake. The dietary analysis that was performed on each subject only included total carbohydrate; therefore, an undetected increase in fat intake

could have contributed to the reduced insulin and augmented glucose response following exercise.

A study performed by Wilkerson *et al.* [63] determined that a low-carbohydrate diet lasting for a few days decreases insulin sensitivity and causes diminished glucose tolerance. Our subjects were following a diet that consisted of fewer carbohydrates than they normally consume, which may have had an effect on their insulin and glucose responses. However, if this were the case, insulin sensitivity should have decreased in the relatively more carbohydrate restricted condition following the glycogen-depleting exercise day and it did not.

CONCLUSION

Based on this evaluation of ten male endurance athletes, it does not appear that glycogen-depleting exercise has a highly predictable effect on glucose tolerance. Glycogen-depleting exercise had no significant effect on the blood glucose AUC induced by an OGTT. However, the insulin AUC during OGTTs was significantly lower on test days that followed exercise days. When glucose AUC values were compared with insulin levels held constant, the blood glucose AUC was significantly greater after exercise days. Although these results indicate a significant blunting of insulin release and slight (but insignificant) increase in glucose AUC following exercise, these responses were not observed consistently in all subjects. Capillary glucose AUC was not significantly different than venous glucose AUC for either testing condition, nor did body composition have a significant effect on glycemic and insulinemic responses. These results indicate that prior exercise may contribute somewhat to the significant within-subject variability commonly observed in the response to repeated OGTTs. However, a more complete understanding of this relationship requires additional research with more stringently controlled conditions to delineate the causes of this variability.

APPENDIX

TABLE 1A. Area under the curve (AUC) data for exercise (Ex) and non-exercise (Non-Ex) conditions

	Venous Glucose		Capillary Glucose		Insulin	
	Ex	Non-Ex	Ex	Non-Ex	Ex	Non-Ex
Subject	(mmol•min/L)	(mmol•min/L)	(mmol•min/L)	(mmol•min/L)	(uIU•min/L)	(uIU•min/L)
1	104	224	75	136	32	53
2	302	61	257	47	92	67
3	77	77	109	62	32	60
4	317	196	336	239	44	69
5	257	115	360	209	24	23
6	190	194	316	254	140	153
7	334	285	281	224	108	159
8	195	27	210	136	56	49
9	223	233	269	284	53	67
10	66	179	186	278	101	142
Mean	207	159	240	187	68	84
STD	99	84	95	86	39	48
P*-value	0.24		0.10		*0.026*	

*Comparison of exercise vs non-exercise means; Italicized value indicates a significant *P*-value using a one-tailed test where P<0.05. Note. Ex = exercise; Non-Ex = non-exercise; Ven Glu = venous glucose; Cap Glu = capillary glucose; STD = standard deviation.

FIGURE 1.A. Venous blood glucose levels for all subjects during two hours following ingestion of 50 g of glucose after exercise and non-exercise days.
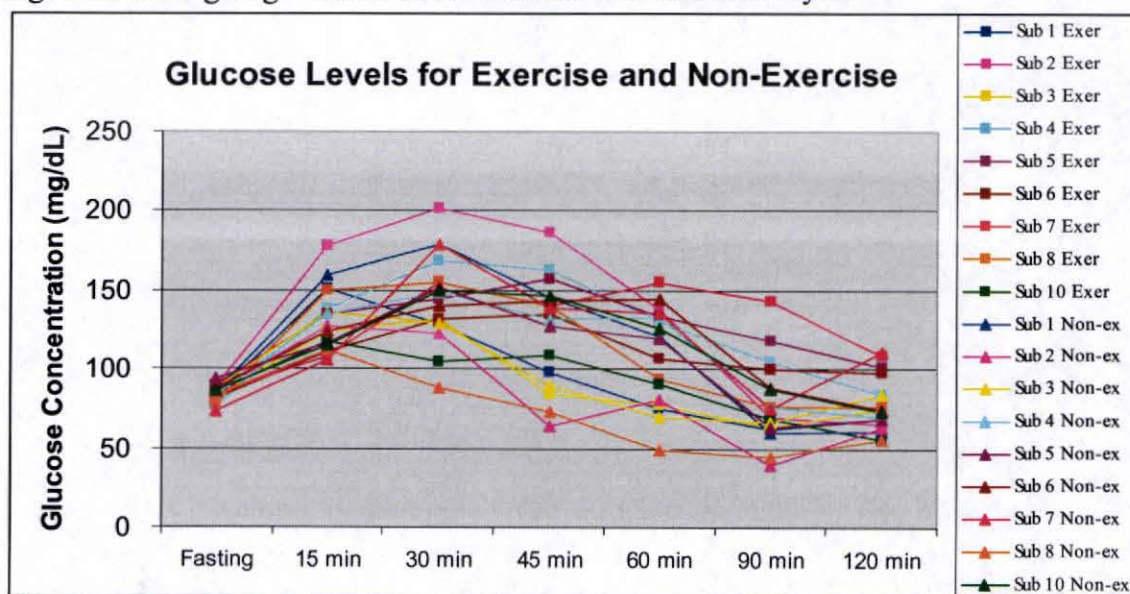
Note. Sub = subject #; Exer = exercise; Non-ex = non-exercise

FIGURE 2.A. Glucose area under the curve (AUC) for each subject

	1	2	3	4	5	6	7	8	9	10
■ Venous Exercise	104	302	77	317	257	190	334	195	223	66
■ Capillary Exercise	75	257	109	336	360	316	281	210	269	186
▨ Venous Non-Ex	224	61	77	196	115	194	285	27	233	179
▤ Capillary Non-Ex	136	47	62	239	209	254	224	136	284	278
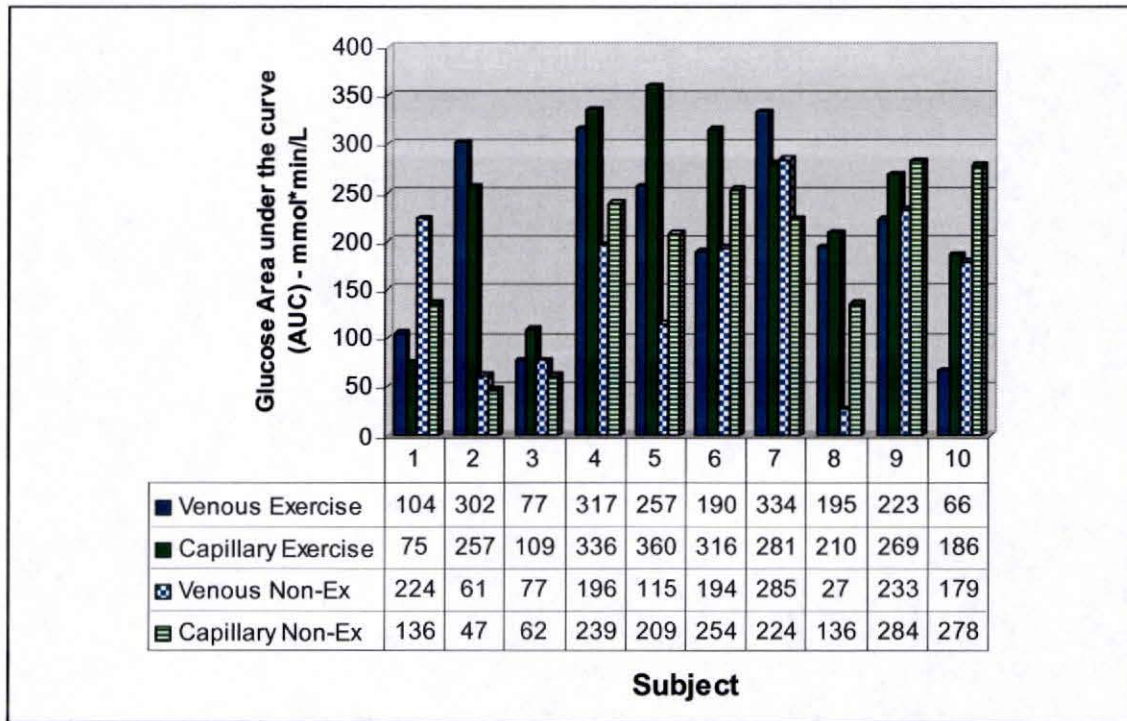
Subject

FIGURE 3.A. Insulin areas under the curve (AUC) for each subject

	1	2	3	4	5	6	7	8	9	10
■ Insulin Exercise	32	92	32	44	24	140	108	56	53	101
■ Insulin Non-Exercise	53	67	60	69	23	153	159	49	67	142

Subject
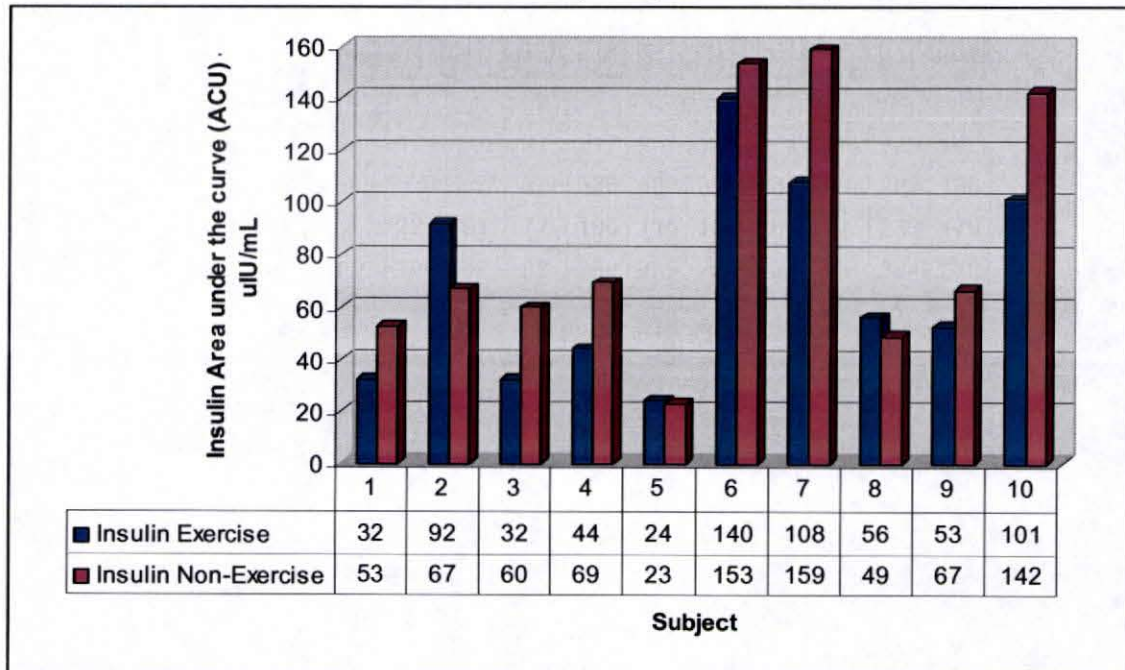
FIGURE 4.A. Insulin sensitivity index (ISI) for exercise and non-exercise testing conditions using a formula by Matsuda and DeFronzo
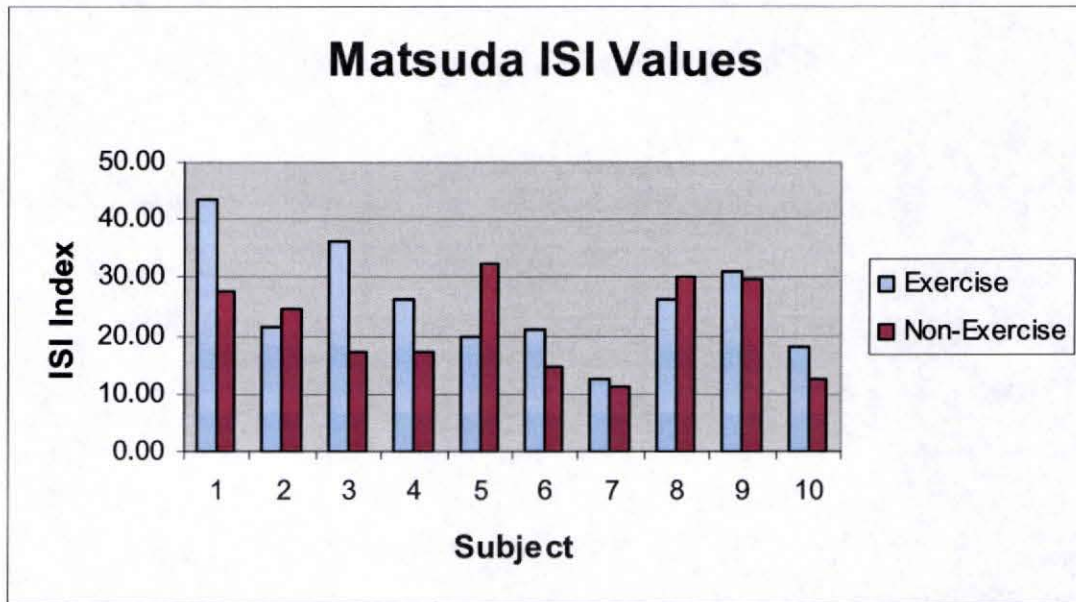
FIGURE 5.A. Insulin sensitivity index (ISI) for exercise and non-exercise testing conditions using a formula by Gutt *et al.*
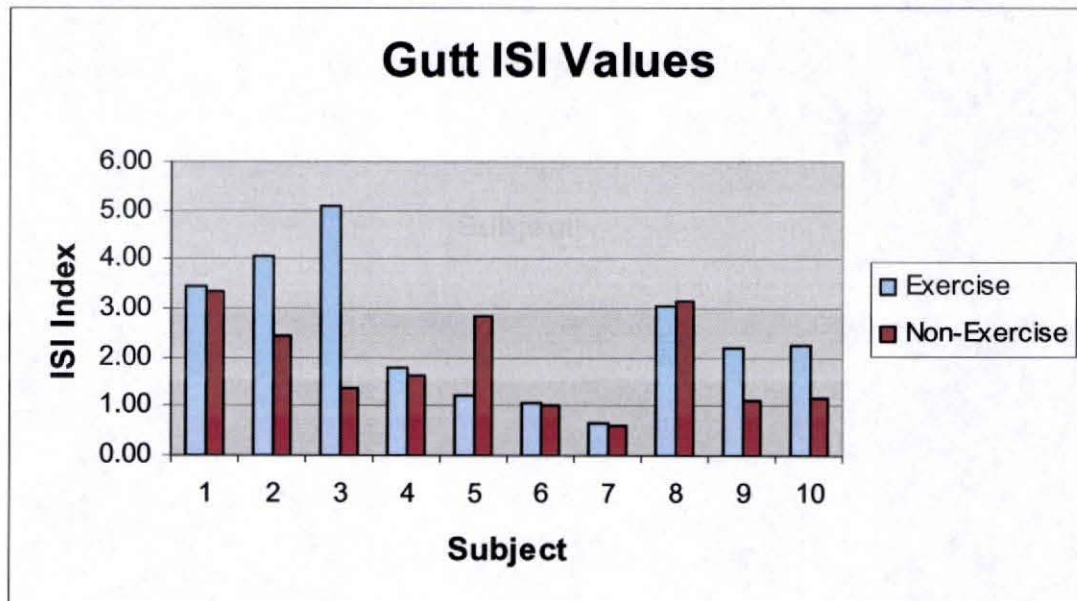
FIGURE 6.A. Insulin sensitivity index (ISI) for exercise and non-exercise testing conditions using a formula by Stumvoll *et al.*
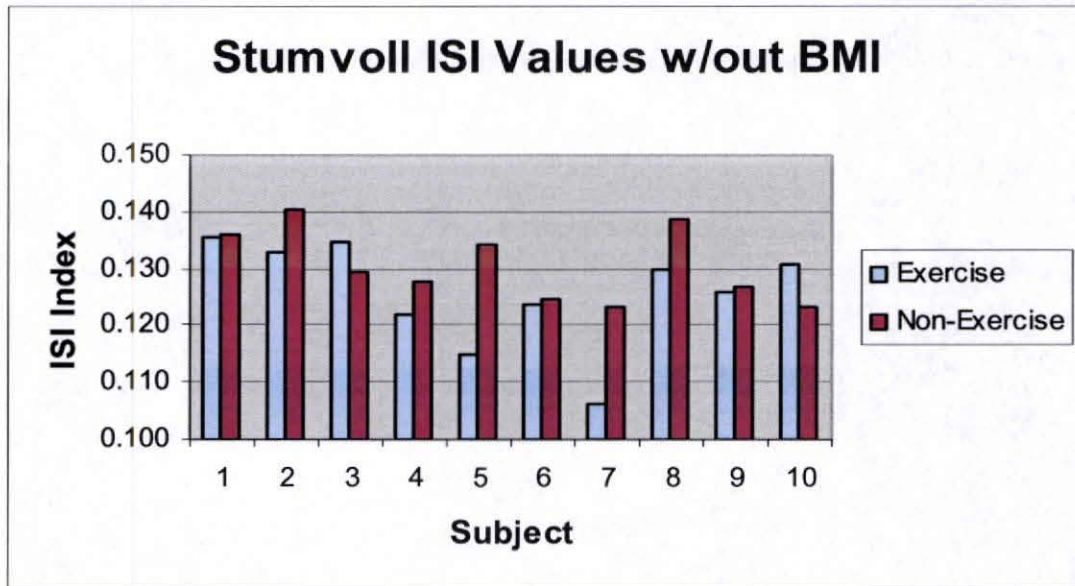
FIGURE 7.A. Insulin sensitivity index (ISI) for exercise and non-exercise testing conditions using a formula Stumvoll *et al.* including body mass index (BMI)
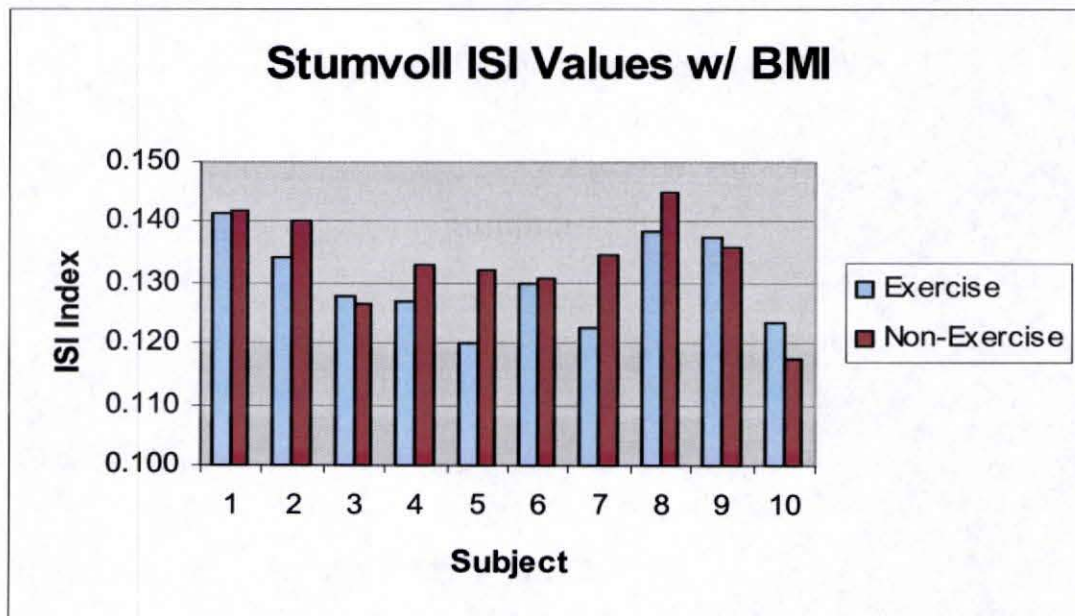
TABLE 2.A. Raw data for blood glucose and insulin values during exercise and non-exercise testing conditions – mg/dL (venous and capillary glucose); uIU/L (insulin)

Subject	Date	Visit	Blood	0"	15"	30"	45"	60"	90"	120"	AUC
1	15-Dec	Exer	Venous	83	151	128	98	75	62	69	1880.9
1	15-Dec	Exer	Capillary	84	116	124	95	93	59	67	1348.2
1	22-Dec	Non-Ex	Venous	85	160	179	145	121	60	61	4023.7
1	22-Dec	Non-Ex	Capillary	93	77	172	144	128	74	62	2453.0
1	15-Dec	Exer	Insulin	1.74	17.66	12.23	9.98	4.02	1.98	1.78	578.9
1	22-Dec	Non-Ex	Insulin	1.72	2.16	13.74	12.05	16.8	10.53	1.99	949.5
2	26-Dec	Exer	Capillary	85	152	183	170	129	76	75	4627.9
2	26-Dec	Exer	Venous	88	178	201	186	138	70	66	5441.5
2	20-Dec	Non-Ex	Capillary	89	89	127	109	85	72	83	845.0
2	20-Dec	Non-Ex	Venous	85	127	123	65	81	40	64	1101.7
2	26-Dec	Exer	Insulin	1.92	24.98	33.28	34.29	17.3	2.37	1.3	1657.5
2	20-Dec	Non-Ex	Insulin	2.57	27.32	21.44	13.77	19.72	2.58	1.99	1208.3
3	13-Dec	Exer	Capillary	82	142	129	109	73	71	72	1959.4
3	13-Dec	Exer	Venous	82	124	131	84	78	64	75	1385.0
3	20-Jan	Non-Ex	Capillary	85	133	99	102	59	69	79	1107.9
3	20-Jan	Non-Ex	Venous	87	136	129	89	70	67	84	1381.6
3	13-Dec	Exer	Insulin	2.14	16.2	18.76	9.48	2.74	1.08	0.74	578.1
3	20-Jan	Non-Ex	Insulin	4.26	35.32	38.8	10.94	3.69	1.44	2.68	1080.3
4	6-Jan	Exer	Capillary	87	148	170	170	158	124	82	6046.4
4	6-Jan	Exer	Venous	77	138	168	163	130	105	84	5707.5
4	23-Dec	Non-Ex	Capillary	85	135	143	158	152	89	78	4304.3
4	23-Dec	Non-Ex	Venous	83	135	149	131	133	77	70	3534.6
4	6-Jan	Exer	Insulin	2.28	11.15	15.89	13.36	9.22	6.29	3.16	793.1
4	23-Dec	Non-Ex	Insulin	3.53	21.98	24.43	18.14	18.21	7.29	2.98	1245.3
5	8-Jan	Exer	Capillary	83	140	153	158	155	130	111	6480.0
5	8-Jan	Exer	Venous	89	135	144	157	134	119	101	4627.5
5	4-Jan	Non-Ex	Capillary	89	106	140	142	158	106	73	3753.9
5	4-Jan	Non-Ex	Venous	94	116	152	127	120	64	69	2071.1
5	8-Jan	Exer	Insulin	3.88	10.19	10.03	9.36	9.07	5.81	2.88	433.9
5	4-Jan	Non-Ex	Insulin	2.13	6.52	7.2	7.48	10.44	2.45	1.68	415.9
6	30-Dec	Exer	Capillary	76	118	144	138	134	124	100	5685.0
6	30-Dec	Exer	Venous	81	111	131	135	107	100	98	3420.0
6	19-Jan	Non-Ex	Capillary	94	123	132	166	162	124	98	4575.0
6	19-Jan	Non-Ex	Venous	87	124	140	143	144	88	74	3488.6
6	30-Dec	Exer	Insulin	1.52	15.98	45.83	56.74	20.42	11.74	6.87	2522.0
6	19-Jan	Non-Ex	Insulin	2.34	18.36	24.13	33.98	51.61	18.76	10.1	2759.3
7	3-Jan	Exer	Capillary	72	103	127	120	132	119	91	5055.0
7	3-Jan	Exer	Venous	84	108	155	140	155	143	109	6007.5
7	13-Jan	Non-Ex	Capillary	82	111	171	150	122	82	105	4035.0
7	13-Jan	Non-Ex	Venous	73	106	179	139	136	75	111	5122.5
7	3-Jan	Exer	Insulin	3.15	10.25	31.09	16.91	22.5	22.81	15.22	1938.2

Subject	Date	Visit	Blood	0"	15"	30"	45"	60"	90"	120"	AUC
7	13-Jan	Non-Ex	Insulin	3.63	32.83	58.09	33.36	28.78	14.66	20.88	2856.4
8	15-Jan	Exer	Capillary	86	156	174	152	107	81	77	3771.9
8	15-Jan	Exer	Venous	78	150	155	140	94	77	76	3510.9
8	23-Jan	Non-Ex	Capillary	81	132	139	135	81	74	79	2445.0
8	23-Jan	Non-Ex	Venous	83	112	88	73	50	45	56	485.0
8	15-Jan	Exer	Insulin	2.57	24.37	22.00	19.7	8.02	3.42	1.11	1015.5
8	23-Jan	Non-Ex	Insulin	2.81	18.58	25.57	20.11	5.52	1.78	1.17	887.2
9	16-Jan	Exer	Capillary	72	139	125	115	122	111	79	4845.0
9	16-Jan	Exer	Venous	67	132	122	104	105	91	72	4005.0
9	22-Jan	Non-Ex	Capillary	82	148	146	142	133	107	106	5107.5
9	22-Jan	Non-Ex	Venous	67	XXX	153	125	128	88	88	4195.0
9	16-Jan	Exer	Insulin	2.2	20.19	14.88	9.61	9.14	9.21	2.93	948.6
9	22-Jan	Non-Ex	Insulin	1.45	XXX	14.86	14.29	19.82	11.46	8.28	1200.0
10	26-Jan	Exer	Capillary	85	131	130	152	121	93	76	3356.5
10	26-Jan	Exer	Venous	85	117	105	109	90	69	56	1195.4
10	18-Jan	Non-Ex	Capillary	89	127	138	182	147	119	96	5010.0
10	18-Jan	Non-Ex	Venous	86	115	150	147	126	87	73	3226.1
10	26-Jan	Exer	Insulin	3.25	23.04	20.75	34.88	34.37	6.72	2.07	1824.9
10	18-Jan	Non-Ex	Insulin	3.78	24.18	33.49	49.52	35.4	16.13	6.32	2557.8

* Exer=exercise, Non-Ex=non-exercise; XXX indicate no values were reported

TABLE 3.A. Raw data for blood glucose values – mmol/L

Subject	Date	Visit	Blood	0"	15"	30"	45"	60"	90"	120"	AUC
1	15-Dec	Exer	Capillary	4.6	8.4	7.1	5.4	4.2	3.4	3.8	104.5
1	15-Dec	Exer	Venous	4.7	6.4	6.9	5.3	5.2	3.3	3.7	74.9
1	22-Dec	Non-Ex	Capillary	4.7	8.9	9.9	8.1	6.7	3.3	3.4	223.5
1	22-Dec	Non-Ex	Venous	5.2	4.3	9.6	8.0	7.1	4.1	3.4	136.3
2	26-Dec	Exer	Capillary	4.7	8.4	10.2	9.4	7.2	4.2	4.2	257.1
2	26-Dec	Exer	Venous	4.9	9.9	11.2	10.3	7.7	3.9	3.7	302.3
2	20-Dec	Non-Ex	Capillary	4.9	4.9	7.1	6.1	4.7	4.0	4.6	46.9
2	20-Dec	Non-Ex	Venous	4.7	7.1	6.8	3.6	4.5	2.2	3.6	61.2
3	13-Dec	Exer	Capillary	4.6	7.9	7.2	6.1	4.1	3.9	4.0	108.9
3	13-Dec	Exer	Venous	4.6	6.9	7.3	4.7	4.3	3.6	4.2	76.9
3	20-Jan	Non-Ex	Capillary	4.7	7.4	5.5	5.7	3.3	3.8	4.4	61.6
3	20-Jan	Non-Ex	Venous	4.8	7.6	7.2	4.9	3.9	3.7	4.7	76.8
4	6-Jan	Exer	Capillary	4.8	8.2	9.4	9.4	8.8	6.9	4.6	335.9
4	6-Jan	Exer	Venous	4.3	7.7	9.3	9.1	7.2	5.8	4.7	317.1
4	23-Dec	Non-Ex	Capillary	4.7	7.5	7.9	8.8	8.4	4.9	4.3	239.1
4	23-Dec	Non-Ex	Venous	4.6	7.5	8.3	7.3	7.4	4.3	3.9	196.4
5	8-Jan	Exer	Capillary	4.6	7.8	8.5	8.8	8.6	7.2	6.2	360.0
5	8-Jan	Exer	Venous	4.9	7.5	8.0	8.7	7.4	6.6	5.6	257.1
5	4-Jan	Non-Ex	Capillary	4.9	5.9	7.8	7.9	8.8	5.9	4.1	208.5
5	4-Jan	Non-Ex	Venous	5.2	6.4	8.4	7.1	6.7	3.6	3.8	115.1
6	30-Dec	Exer	Capillary	4.2	6.6	8.0	7.7	7.4	6.9	5.6	315.8
6	30-Dec	Exer	Venous	4.5	6.2	7.3	7.5	5.9	5.6	5.4	190.0
6	19-Jan	Non-Ex	Capillary	5.2	6.8	7.3	9.2	9.0	6.9	5.4	254.2
6	19-Jan	Non-Ex	Venous	4.8	6.9	7.8	7.9	8.0	4.9	4.1	193.8
7	3-Jan	Exer	Capillary	4.0	5.7	7.1	6.7	7.3	6.6	5.1	280.8
7	3-Jan	Exer	Venous	4.7	6.0	8.6	7,8	8.6	7.9	6.1	333.8
7	13-Jan	Non-Ex	Capillary	4.6	6.2	9.5	8.3	6.8	4.6	5.8	224.2
7	13-Jan	Non-Ex	Venous	4.1	5.9	9.9	7.7	7.6	4.2	6.2	284.6
8	15-Jan	Exer	Capillary	4.8	8.7	9.7	8.4	5.9	4.5	4.3	209.6
8	15-Jan	Exer	Venous	4.3	8.3	8.6	7.8	5.2	4.3	4.2	195.0
8	23-Jan	Non-Ex	Capillary	4.5	7.3	7.7	7.5	4.5	4.1	4.4	135.8
8	23-Jan	Non-Ex	Venous	4.6	6.2	4.9	4.1	2.8	2.5	3.1	26.9
9	16-Jan	Exer	Capillary	4.0	7.7	6.9	6.4	6.8	6.2	4.4	269.2
9	16-Jan	Exer	Venous	3.7	7.3	6.8	5.8	5.8	5.1	4.0	222.5
9	22-Jan	Non-Ex	Capillary	4.6	8.2	8.1	7.9	7.4	5.9	5.9	283.8
9	22-Jan	Non-Ex	Venous	3.7	XXX	8.5	6.9	7.1	4.9	4.9	233.1
10	26-Jan	Exer	Capillary	4.7	7.3	7.2	8.4	6.7	5.2	4.2	186.5
10	26-Jan	Exer	Venous	4.7	6.5	5.8	6.1	5.0	3.8	3.1	66.4
10	18-Jan	Non-Ex	Capillary	4.9	7.1	7.7	10.1	8.2	6.6	5.3	278.3
10	18-Jan	Non-Ex	Venous	4.8	6.4	8.3	8.2	7.0	4.8	4.1	179.2

* Exer=exercise, Non-Ex=non-exercise; XXX indicate no values were reported

TABLE 4.A. Venous glucose values for exercise study visit – mg/dL

Subject	Fasting	15 min	30 min	45 min	60 min	90 min	120 min	AUC t	AUC Ŧ
1	83	151	128	98	75	62	69	1881	104
2	88	178	201	186	138	70	66	5441	302
3	82	124	131	84	78	64	75	1385	77
4	77	138	168	163	130	105	84	5708	317
5	89	135	144	157	134	119	101	4628	257
6	81	111	131	135	107	100	98	3420	190
7	84	108	155	140	155	143	109	6008	334
8	78	150	155	140	94	77	76	3511	195
10	85	117	105	109	90	69	56	1195	66
Mean	83	135	146	135	111	90	82	3718	207
STD	4	23	28	33	29	28	18	1776	99

t = mg•min/dL; Ŧ = mmol•min/L (STD = standard deviation); Data for subject 9 was incomplete

TABLE 5.A. Venous glucose values for non-exercise study visit – mg/dL

Subject	Fasting	15 min	30 min	45 min	60 min	90 min	120 min	AUC t	AUC Ŧ
1	85	160	179	145	121	60	61	4024	224
2	85	127	123	65	81	40	64	1102	61
3	87	136	129	89	70	67	84	1382	77
4	83	135	149	131	133	77	70	3535	196
5	94	116	152	127	120	64	69	2071	115
6	87	124	140	143	144	88	74	3489	194
7	73	106	179	139	136	75	111	5123	285
8	83	112	88	73	50	45	56	485	27
10	86	115	150	147	126	87	73	3226	179
Mean	85	126	143	118	109	67	74	2863	159
STD	5	16	28	33	33	17	16	1519	84

t = mg•min/dL; Ŧ = mmol•min/L (STD = standard deviation); Data for subject 9 was incomplete

TABLE 6.A. Capillary glucose values for exercise study visit – mg/dL

Subject	Fasting	15 min	30 min	45 min	60 min	90 min	120 min	AUC t	AUC Ŧ
1	84	116	124	95	93	59	67	1348	75
2	85	152	183	170	129	76	75	4628	257
3	82	142	129	109	73	71	72	1959	109
4	87	148	170	170	158	124	82	6046	336
5	83	140	153	158	155	130	111	6480	360
6	76	118	144	138	134	124	100	5685	316
7	72	103	127	120	132	119	91	5055	281
8	86	156	174	152	107	81	77	3772	210
10	85	131	130	152	121	93	76	3356	186
Mean	82	134	148	140	122	97	83	4259	237
STD	5	18	23	27	28	27	14	1794	100

t = mg•min/dL; Ŧ = mmol•min/L (STD = standard deviation); Data for subject 9 was incomplete

TABLE 7.A. Capillary glucose values for non-exercise study visit – mg/dL

Subject	Fasting	15 min	30 min	45 min	60 min	90 min	120 min	AUC t	AUC ꝉ
1	93	77	172	144	128	74	62	2453	136
2	89	89	127	109	85	72	83	845	47
3	85	133	99	102	59	69	79	1108	62
4	85	135	143	158	152	89	78	4304	239
5	89	106	140	142	158	106	73	3754	209
6	94	123	132	166	162	124	98	4575	254
7	82	111	171	150	122	82	105	4035	224
8	81	132	139	135	81	74	79	2445	136
10	89	127	138	182	147	119	96	5010	278
Mean	87	115	140	143	122	90	84	3170	176
STD	5	21	22	26	38	21	14	1518	84

t = mg•min/dL; ꝉ = mmol•min/L (STD = standard deviation); Data for subject 9 was incomplete

TABLE 8.A. Insulin values for exercise study visit – uIU/mL

Subject	Fasting	15 min	30 min	45 min	60 min	90 min	120 min	AUC
1	1.7	17.7	12.2	10.0	4.0	2.0	1.8	579
2	1.9	25.0	33.3	34.3	17.3	2.4	1.3	1657
3	2.1	16.2	18.8	9.5	2.7	1.1	0.7	578
4	2.3	11.2	15.9	13.4	9.2	6.3	3.2	793
5	3.9	10.2	10.0	9.4	9.1	5.8	2.9	434
6	1.5	16.0	45.8	56.7	20.4	11.7	6.9	2522
7	3.2	10.3	31.1	16.9	22.5	22.8	15.2	1938
8	2.6	24.4	22.0	19.7	8.0	3.4	1.1	1015
10	3.3	23.0	20.8	34.9	34.4	6.7	2.1	1825
Mean	2.5	17.1	23.3	22.7	14.2	6.9	3.9	1229
STD	0.8	5.9	11.5	16.1	10.3	6.8	4.6	708

(STD = standard deviation); Data for subject 9 was incomplete

TABLE 9.A. Insulin values for non-exercise study visit – uIU/mL

Subject	Fasting	15 min	30 min	45 min	60 min	90 min	120 min	AUC
1	1.7	2.2	13.7	12.1	16.8	10.5	2.0	950
2	2.6	27.3	21.4	13.8	19.7	2.6	2.0	1208
3	4.3	35.3	38.8	10.9	3.7	1.4	2.7	1080
4	3.5	22.0	24.4	18.1	18.2	7.3	3.0	1245
5	2.1	6.5	7.2	7.5	10.4	2.5	1.7	416
6	2.3	18.4	24.1	34.0	51.6	18.8	10.1	2759
7	3.6	32.8	58.1	33.4	28.8	14.7	20.9	2856
8	2.8	18.6	25.6	20.1	5.5	1.8	1.2	887
10	3.8	24.2	33.5	49.5	35.4	16.1	6.3	2558
Mean	3.0	20.8	27.4	22.2	21.1	8.4	5.5	1516
STD	0.9	11.0	14.8	13.9	15.3	6.8	6.4	870

(STD = standard deviation); Data for subject 9 was incomplete

TABLE 10A. Bone Mineral Density (BMD) for Subjects

	BMD (g/cm^2)	T-Score	Z-Score
1	1.243	0.3	0.4
2	1.252	0.4	0.5
3	1.342	1.5	0.9
4	1.232	0.1	0
5	1.327	1.3	1.4
6	1.256	0.4	0.5
7	1.171	-0.6	-0.1
8	1.43	2.6	2.9
9	1.237	0.2	0.5
10	1.339	1.5	1.2
Mean	1.2829	0.77	0.82
STD	0.075	0.933	0.870

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