

451.

INSULIN RESISTANCE

WITH CESSATION OF TRAINING

CHANGES IN INSULIN RESISTANCE

IN TRAINED ATHLETES

UPON CESSATION OF TRAINING

BY

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ABSTRACT

CHANGES IN INSULIN RESISTANCE IN TRAINED ATHLETES UPON CESSATION OF TRAINING

This study was designed to investigate possible changes in insulin sensitivity (IS) with cessation of training. Six endurance trained athletes were studied at 12, 60 h and 7 days following cessation of training. In-vivo IS was established by a glucose clamp technique (Greenfield et al. Diabetes 30, 1981) and expressed as the metabolic clearance rate of glucose (MCR) in ml. plasma cleared $\text{kg}^{-1} \cdot \text{min}^{-1}$. At 12 h after the last training session the mean MCR was 15.6 ± 1.8 compared with 7.8 ± 1.2 ($p < 0.01$) in age and weight matched sedentary controls. The MCR decreased to 10.1 ± 1.0 after 60 h and decreased significantly to 8.5 ± 0.5 ($p < 0.05$) after 7 days of detraining. In-vitro IS was measured by determining the insulin binding of fractionated young erythrocytes by the method of Polychronakos et al. (Clin. Inves. Med. 4, 14B, 1981). Insulin binding was $10.4 \pm 0.9\%$ at 12 h and decreased significantly to $8.1 \pm 0.7\% / 4 \times 10^9$ cells after 60 h of detraining ($p < 0.001$). In conclusion: 1) detraining of endurance athletes resulted in a rapid decrease in IS. After 7 days, glucose MCR reached values indistinguishable from sedentary controls. 2) changes in IS observed may be partially mediated by alterations in insulin binding to receptors. 3) since the high IS observed with endurance athletes on the initial test disappeared shortly after cessation of training, it is probably an acute effect of the last exercise bout rather than a chronic effect of training.

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CHAPTER I

INTRODUCTION

I.I. The Role of Insulin

The major biological function of insulin is to facilitate the transport of glucose from the plasma into the cells and to stimulate glycogen synthesis in muscle and liver. In order to properly accomplish these tasks, four sequential processes must occur:

- (a) The synthesis of insulin in the β cells of the pancreatic islets.
- (b) The controlled secretion of insulin into the circulation.
- (c) The interaction of insulin with receptors on the target cell membrane.
- (d) The influence of insulin on metabolism within the target cell.

The effectiveness of these processes may be altered by factors such as diseases, obesity, aging, diet and physical exercise.

As indicated by human and animal studies, acute exercise and a program of physical training affect tissue sensitivity to insulin. Investigations of endurance trained subjects show a lower basal insulin level and a lower insulin response to a glucose challenge when compared to sedentary controls (Owens et al. 1977, LeBlanc et al. 1979, Soman et al. 1979, Koivisto et al. 1979, Wirth et al. 1979). Similarly, glucose uptake at a given insulin concentration is greater in trained rats than in sedentary controls (Berger et al. 1979, Mondon et al. 1980).

A program of physical training has been shown to improve insulin sensitivity in diabetic and obese patients (Bjorntorp et al. 1977, Pedersen et al. 1980, Koivisto et al. 1980, Reaven & Chang 1981). However, these populations show smaller changes in tissue responsiveness compared to healthy non-obese subjects, suggesting some limitations induced by their initial state.

Changes in dietary patterns are also known to affect insulin activity (Bar et al. 1976, Wirth et al. 1980, Goodman & Ruderman 1980). Total caloric intake as well as diet composition appear to have a significant effect on tissue sensitivity to insulin (Olefsky et al. 1977, Olefsky et al. 1979, LeBlanc et al. 1981, Melvin et al. 1981, Fell et al. 1982). Insulin binding to receptors increases with weight loss (Rizza et al. 1981) and glucose uptake by skeletal muscle is enhanced with starvation in rats (Goodman & Ruderman 1980).

The change in glucose tolerance observed with physical training and with controlled diet seems to involve more than one site of the hormonal mechanism. Insulin concentration is lower and the percentage of insulin binding to receptors is higher in well trained compared to control subjects (Bar et al. 1976, Olefsky & Reaven 1977, Koivisto et al. 1979, LeBlanc et al. 1979) and in active compared to sedentary rats (Galbo et al. 1977, Galbo et al. 1981). The inverse relationship between insulin level and insulin receptor binding has been observed at rest and during exercise in healthy (Soman et al. 1978, Soman et al. 1979, Koivisto et al. 1979) and in diabetic patients (Pedersen et al. 1980). Recently, it has been suggested that post-receptor events respond to physical activity and

to food restriction and play an important role in determining insulin sensitivity (Amatruda et al. 1975, Kalant et al. 1978, Soman et al. 1979, Olefsky et al. 1979, Mondon et al. 1980, Olefsky & Kolterman 1981).

Exercise training is usually associated with an increase in aerobic capacity and a reduction in body fat and some authors have suggested that the enhanced insulin sensitivity following physical training may be linked to alterations in these variables. Since longitudinal training has been found to improve maximal oxygen consumption and to enhance insulin activity in sedentary subjects independently of changes in body composition (Bjorntorp et al. 1970, Bjorntorp et al. 1977, Soman et al. 1979), it is unlikely that the latter is predominant in relation to glucose metabolism and exercise. Alternatively, repeated exposure to catecholamines due to exercise may suppress the pancreatic β cells activity and result in low insulin concentration and high insulin efficiency observed with training (Galbo et al. 1977, Galbo et al. 1981).

In addition to chronic effects of training, acute exercise has been found to affect glucose uptake by peripheral tissues. One bout of exercise enhances insulin binding to receptors in healthy untrained, obese and diabetic subjects (Soman et al. 1978, Koivisto et al. 1979, Koivisto et al. 1980, Pedersen et al. 1980). Similarly, skeletal muscles of starved rats and carbohydrate (CHO) restricted rats following exhausting exercise take up more glucose compared to fed rats (Goodman & Ruderman 1980) and to animals on a high CHO diet (Fell et al. 1982). Since glycogen stores in skeletal muscle are

related to CHO intake, the level of muscle glycogen itself may affect the rate of which glucose is taken up.

In light of the facts that tissue sensitivity and tissue responsiveness to insulin is susceptible to short-term treatments, the degree of insulin resistance in healthy subjects may be considered as a transient rather than a stable state. This hypothesis raises the question whether enhanced sensitivity observed with training is a long lasting effect or whether it is an acute effect from the last training bout. LeBlanc and co-workers (1981) investigated insulin behaviour in highly trained athletes three days after cessation of training and found an increase in the hormonal response to a glucose challenge. When cessation of training was accompanied by caloric restrictions, the beneficial effects of training were retained. According to LeBlanc's findings no conclusion can be made concerning the kinetics of the change.

In order to give a further insight on kinetics of insulin resistance with detraining, subjects should be investigated during intensive physical activity and immediately after cessation of training until a steady state in insulin resistance is achieved. Investigation of different sites of the hormonal system may propose possible mechanism(s) responsible for the changes observed.

Aerobic capacity and adiposity seem to play a role in determining insulin sensitivity, yet, may be indirectly involved in the hormonal adaptations. Other factors such as muscle glycogen stores and plasma glucose levels undergo rapid changes when exposed to short-term treatments. Their contribution to an enhanced glucose

metabolism in relation to physical exercise should be further investigated.

I.2. Statement of the Problem

Human and animal studies indicate an enhanced tissue sensitivity and responsiveness to insulin with physical activity (Owens et al. 1977, LeBlance et al. 1979, Soman et al. 1979, Koivisto et al. 1979, Berger et al. 1979, Wirth et al. 1980, Mondon et al. 1980) and food restriction (Bar et al. 1976, Olefsky et al. 1977, Olefsky et al. 1979, Goodman & Ruderman 1980, Melvin et al. 1981, Fell et al. 1982).

More than one site of the hormonal system has been found to respond to a variety of treatments. Yet, no evidence exists for a major factor underlying the variations in the hormonal and metabolic efficiency. The rate of insulin secretion (Pruett 1970, LeBlanc et al. 1977, LeBlanc et al. 1979), insulin binding to receptors (Soman et al. 1978, Soman et al. 1979, Koivisto et al. 1979, Pedersen et al. 1980) and post-receptor events (Kalant et al. 1978, Soman et al. 1979, Olefsky et al. 1979) are all altered by physical training and diet manipulation. Since these alterations have been observed with longitudinal (Soman et al. 1979, LeBlanc et al. 1979, Pedersen 1980) as well as with short-term treatments (Soman et al. 1978, et al. 1980, Fell et al. 1982) the question arises whether the benefits induced by training are short lived and disappear with detraining.

Recent evidence (LeBlanc et al. 1981) suggests that highly trained athletes lose their ability to respond with low insulin levels to a given glucose load, shortly after cessation of training.

Whether the enhanced insulin sensitivity observed with these individuals is the acute effect of the last exercise session, or whether it is a chronic effect of longitudinal training is still unknown.

I.3 Purpose of the Study

The purpose of this study was to assess in-vivo insulin resistance in endurance trained athletes.

The study was designed:

(a) to confirm previous observations suggesting a low insulin resistance among a highly trained population.

(b) to investigate changes in insulin resistance upon cessation of training.

(c) to investigate possible sites of change in the hormonal mechanism affecting insulin resistance.

LIST OF DEFINITIONS

For the purpose of the present study the following terms were used:

Diabetes Mellitus-A chronic disorder of carbohydrate metabolism due to disturbance of the normal insulin mechanism. Characterized by hyperglycemia, glycosuria and alterations of protein and fat metabolism, producing polyuria, thirst, weight loss, ketosis, acidosis and coma. A hereditary predisposition is present in most cases.

Glycolysis-The breaking down of sugars into simpler compounds chiefly pyruvate and lactate.

Glycogenesis-The conversion of glucose to glycogen for storage in the liver.

Gluconeogenesis-The synthesis of glucose by the liver and kidney from noncarbohydrate sources such as amino-acids.

Glucose Tolerance Test-Evaluation of the ability of the body to metabolize glucose by administration of a standard dose of glucose to a fasting individual and measurement of blood and urine glucose at regular intervals.

Insulin Resistance-A given amount of insulin that produces less than a normal biologic effect due to a decrease in tissue sensitivity or responsiveness to insulin, or a combination of both.

Juvenile Onset Diabetes-Diabetes mellitus which develops early in life, requires insulin replacement and is ketoacidosis prone.

Ketoacidosis-Acidosis due to accumulation of ketone bodies.

Maturity Onset Diabetes-Diabetes mellitus which develops later in life, characterized by more gradual development and less severe symptoms than juvenile onset diabetes.

Sensitivity-The degree of change or responsiveness of an organism with respect to some specific factor or stimulus.

LIST OF DEFINITIONS

Sites of the hormonal mechanism-Refers to the prereceptor, receptor and post-receptor steps in insulin action.

μU Insulin unit-The specific activity contained in 0.0455 mg of pure dried insulin hydrochloride.

CHAPTER II

Literature Review

II.1 Mechanisms for Glucose Homeostasis

II.1(1) Regulation of Blood Glucose Concentration

Glucose homeostasis in man is finely regulated by an integrated mechanism involving several endocrine glands. The pancreas plays an important role in CHO homeostasis and its islets affect metabolism mainly through insulin and glucagon activities. Normal blood glucose level (80-120mg/100ml) is maintained through interconversion of glycogen, glucose and lactate and is controlled by at least four hormones:

a) Epinephrine which is released from the adrenal medulla and stimulates the release of glucose from the liver.

b) Norepinephrine which is released from sympathetic nerve-ends and stimulates glycogenolysis in the liver.

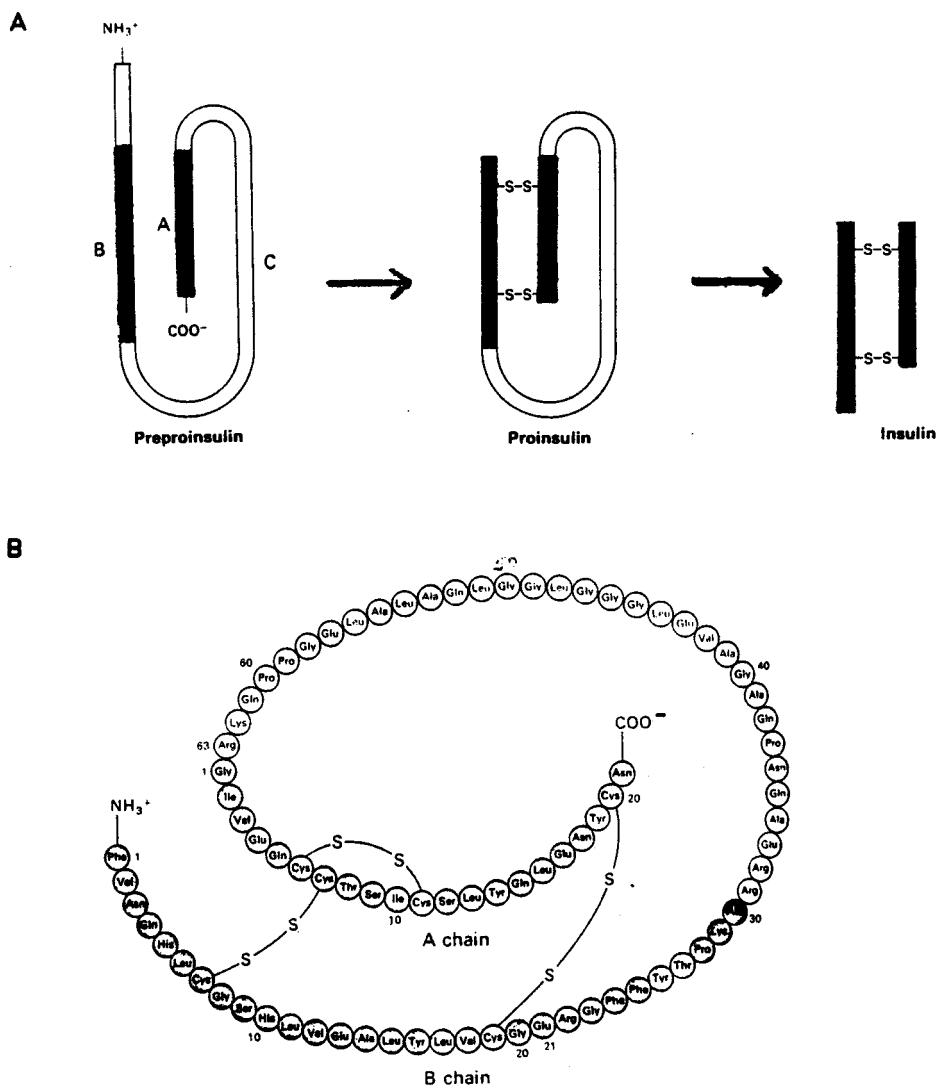
c) Glucagon which is secreted by the α cells of the pancreas and stimulates glycogenolysis in the liver.

d) Insulin which is secreted by the β cells of the pancreas, promotes glucose transport and uptake by peripheral cells and enhances the rate of glycogen synthesis in both muscle and liver. Gluconeogenesis, the conversion of lactate into glucose, is inhibited by insulin which acts antagonistically to glucagon and epinephrine (Cohen et al. 1979). The minute-to-minute modulation of blood glucose concentration widely depends on the balance between catecholamines - glucagon activity and insulin activity.

II.1(2) Insulin Structure

Insulin is a polypeptide hormone composed of 51 amino-acid (a.a.) residues that form two a.a. chains, the A-chain consists of 21 and the B-chain consists of 30 residues. The chains are covalently linked by 2 disulfide bonds. Insulin is derived from a single polypeptide chain (107 a.a.) known as pre-proinsulin (Chan et al. 1976-cited by Cohen et al. 1979). The 23 a.a. of the N-terminal are cleared at some stage during its transfer to the Golgi apparatus to yield the direct precursor, proinsulin.

Proinsulin contains more than 30 a.a. residues that are absent from the final insulin molecule. The sequence of these residues joins the carboxyl end of the B-chain and the amino terminus of the A-chain and is known as the connecting peptide (C-peptide). The conversion of proinsulin to the final and active form of the hormone involves a proteolytic reaction which releases the C-peptide by hydrolyzing the fragment at its C-terminus (Arginine-Lysine) and N-terminus (Arginine-Arginine). The conversion starts in the Golgi apparatus and continues within the secretory granules until the final structure of insulin is formed. The insulin molecule has a compact three-dimensional structure which is stabilized by several salt and hydrogen links in addition to its two disulfide interchain bonds. (Stryer 1981).



II.1(3) Insulin Storage and Secretion

Insulin and C-peptide are stored in the secretory granules in an equimolar ratio where the former is complexed with zinc.

When a stimulus for insulin secretion is applied, the content of the secretory granules is released into the circulation by exocytosis, the process in which the granules' membrane fuses with the plasma membrane of the β cells (Tager & Steiner 1974).

II.1(4) Kinetics of Insulin Release

Insulin secretion takes two forms: (a) insulin release in the basal state and (b) insulin release in response to external stimulus. The rate of insulin secretion in the absence of exogenous glucose stimulation, reflects the basal state of the pancreatic islets.

Insulin secretion in response to glucose overload has a biphasic pattern. The initial phase occurs within 30-60 seconds from the rise in glucose and reaches a peak which is followed by a fall in secretion rate. The magnitude of the first phase depends on glucose concentration and on the basic state of the islets. During the second phase, insulin release increases gradually until a steady level is reached. With cessation of glucose infusion, insulin level returns to baseline (Williams 1974). A two-compartment model of insulin storage has been proposed to explain the biphasic pattern of the islets response (Grodsky 1972,-cited by Williams, 1974).

II.1(5) Regulation of Insulin Secretion

Insulin release from the pancreas is regulated by a simple closed-loop system (Stryer 1981). The β cells of Langerhans islets

respond to high glucose concentration by the release of insulin which is circulated to its target cell. The hormone interacts with tissue receptors (e.g. fat cells, muscle cells) and facilitates glucose uptake and glycogen synthesis by a mechanism still unknown. It is mainly the fall in blood glucose induced by insulin that signals the endocrine gland to halt further hormonal secretion. In addition to the simple endocrine - control system, several substrates and hormones carefully regulate insulin secretion and counterbalance its activity (Stryer 1981).

Glucose is the main, yet not the only substrate that affects insulin secretion. Gastrointestinal (GI) hormones are released as response to food ingestion and are known to enhance insulin secretion. Amino-acids (mainly Arginine) stimulate the release of insulin and glucagon which have an antagonistic effect on fuel metabolism. In the presence of insulin, a.a. are taken up by the muscle and are stored as proteins. Meanwhile, glucagon stimulates glycogen breakdown by the liver. The synchronized activity of both insulin and glucagon protects the brain from hypoglycemia which would occur if a.a. stimulated insulin release only (Williams 1974).

Stimulation of the α and the β adrenergic receptors induces a negative and positive effect on insulin secretion, respectively. The net effect of epinephrine and norepinephrine stimulating the adrenergic receptors, results in inhibition of insulin release (Williams 1974).

II.1(6) Mechanism of Insulin Action

A. Insulin Binding to Receptors

The first step in insulin action is binding to a specific receptor on the plasma membrane of target cells. (Cuatrecasas et al. 1972-cited by Cohen 1979). The receptors are proteins bound to the outer surface of the membrane, which serve to recognize the active hormone. The binding of hormone to receptor is highly specific and reversible. The hormone-receptor complex formed by this interaction initiates intracellular metabolic events. The nature of activating these reactions varies with different hormones and for some of them this mechanism is not yet entirely clear (Bajaj, 1977). The magnitude of the hormonal stimulation depends upon (a) concentration of circulating hormone (b) receptor affinity for a specific hormone and (c) receptor concentration on the cell membrane. The responsiveness of a target tissue to a given hormonal concentration is determined by the number and affinity of its receptors (Stryer 1981). Receptor Concentration is largely affected by the hormone itself by a process known as "down regulation of insulin receptors" (Olefsky et al. 1980). This mechanism has important implications in vivo. Hyperinsulinemia is often accompanied by reduction in receptor number. Conversely, physical training is usually associated with low insulin levels and an increase in receptor concentration. (LeBlanc et al. 1979, Soman et al. 1979). Receptor Affinity is altered as a function of the hormonal concentration and the receptor occupancy. Low insulin levels and high percentages of unoccupied receptor sites, enhance receptor affinity. Circulating antibodies suppress affinity by occupying either the binding site or an adjacent region on the specific receptor. A fall in blood pH (e.g. ketoacidosis), results

in a significant reduction in receptor affinity for insulin (Williams 1974).

The extent of receptor occupancy which is required for stimulating a certain biological event by insulin, depends upon the nature of the event and the target tissue (Freychet 1978). Glucose uptake in soleus muscle is fully stimulated at less than 20% of full receptor occupancy, whereas glycogen synthesis requires a higher level of receptor saturation. Amino-acid uptake by liver cells is directly proportional to receptor binding.

B. Insulin Action

The mechanisms of insulin action distal to its binding to specific receptors are largely unknown. The "second messenger" model is regarded as the mechanism by which epinephrine affects intracellular metabolism. It appears that insulin actions are not mediated by changes in cyclic Adenosine monophosphate (cAMP), however the concept of a "second messenger" seems to apply to insulin (Cohen et al. 1979).

The mechanism by which epinephrine stimulates glycogenolysis in muscle does not require the entry of the hormone into its target cell. Like insulin, epinephrine binds to specific receptor sites on the outer membrane of the cell. Receptors can be of two types α and β and the hormonal interaction with each of them is mediated by different factors. The interaction of epinephrine with β receptors activates adenylate cyclase, an intracellular enzyme which raises cAMP concentrations and consequently activates the enzyme cAMP-dependent protein kinase. The latter catalyzes phosphorylation of phosphorylase-kinase which activates glycogen phosphorylase. The

sequence of these reactions results in an enhanced rate of glycogenolysis (Cohen et al. 1979). Stimulation of glycogenolysis in the liver may proceed through interaction of catecholamines with α receptors, which unlike the β receptor interaction, does not activate adenylate cyclase (Huston et al. 1976, Cherrington et al. 1976 - cited by Cohen 1979).

The "second messenger" model is based on the hypothesis that it is not the hormone itself, but an intracellular messenger that directly affects metabolic events. The cascade effect of enzymatic reactions provides amplification and regulation of the hormonal activity. Larner et al. (1979) reported that interaction of insulin with target receptors, generated a mediator substance which inhibited CAMP-dependent protein kinase and resulted in a decreased binding of CAMP to protein kinase. In addition, it activated phosphatase which by dephosphorylation converted glycogen synthase into its active form.

II.1(7) Stimulation of Glycogen Biosynthesis by Insulin

Glycogen biosynthesis and degradation proceed in distinct pathways. The reactions are catalyzed by different enzymes and are regulated by an antagonistic hormonal activity.

A. Glycogen Synthesis

Glycogen is synthesized in an elongation reaction with Uridine diphosphate glucose (UDPG) serving as glucose donor. UDPG is an activated form of glucose, synthesized from Glucose-1 phosphate (G,1-P) and Uridine triphosphate (UTP) in a reaction catalyzed by UDP-glucose pyrophosphorylase. Hydrolysis of pyrophosphate is essential

to assure the irreversibility of the reaction and the formation of UDPG:

1. $\text{Gl,1-P} + \text{UTP} \longrightarrow \text{UDPG} + \text{PPi}$
2. $\text{PPi} + \text{H}_2\text{O} \longrightarrow 2\text{Pi}$ (Stryer 1981)

Glucose transfer from UDPG to a growing glycogen chain is catalyzed by the enzyme glycogen-synthase. The activated glucosyl unit of UDPG is transferred to the hydroxyl group and is linked to the C-4 terminus of glycogen to form an α -1,4-glycosidic linkage. In this reaction UDP is displaced by the terminal hydroxyl group of the growing glycogen chain. Since glycogen synthase can add glucosyl residues only to a chain containing more than 4 residues, an additional primer enzyme is needed for a complete biosynthesis of glycogen. A distinct enzyme catalyzes the formation of α -1-6-linkages and creates the branching of the glycogen molecule by the cleavage of an α -1,4-link and the transfer of a 7 residues block to an interior site of the molecule. This sequence of residues includes the nonreducing terminus derived from a chain of 11 residues or more. The new branch point will be at least 4 residues away from an existing branch point (Stryer 1981).

B. Activation of Glycogen Synthase

Regulation of glycogen synthesis and degradation is coordinated at the hormonal level. Insulin enhances glycogen build up whereas epinephrine and glucagon stimulate the breakdown of the molecule in muscle and liver, respectively (Stryer 1981).

Initiation of glycogenolysis by epinephrine has been previously described (see Mechanism of Insulin Action II.1(6) B.). In brief,

epinephrine initiates an enzymatic cascade reaction mediated by cAMP, which results in phosphorylation and activation of glycogen-phosphorylase and in an enhanced rate of glycogenolysis.

Phosphorylase in skeletal muscle exists in two interconvertible forms. Phosphorylation of a single serine residue (Ser-14) converts the inactive (phosphorylase b) to the active form (phosphorylase a) of the enzyme. This reaction is reversible by hydrolysis of the phosphoryl group. High cAMP concentrations in cell have a positive allosteric effect, whereas high levels of ATP and glucose 6 phosphate (G,6-P) have a negative allosteric effect on phosphorylase b (Cohen et al. 1979). Under resting conditions, phosphorylase b is predominant in muscle.

Analogous to phosphorylase in the glycogenolysis pathway, glycogen synthase exists in two forms in skeletal muscle. Incubation of glycogen synthase a with MgATP results in conversion of the a into the b form of the enzyme, suggesting that glycogen synthase b is the phosphorylated form of a (Friedman & Larner 1963-cited by Cohen et al. 1979). Glycogen synthase a is almost fully activated in the absence of G,6-P whereas the b form depends on the presence of glucose for its activity. Both enzymatic forms are inhibited by ATP, but inhibition of the a form is easily reversed by low concentrations of G,6-P (Piras et al. 1968-cited by Cohen et al. 1979). The dephosphorylated a form of glycogen synthase appears to be the most active enzymatic form in vivo (Cohen et al. 1979).

Insulin increases the "activity ratio" (the enzymatic activity in the absence of G,6-P, relative to the activity in the presence of G,6-P) of glycogen synthase. Conversely, epinephrine decreases the

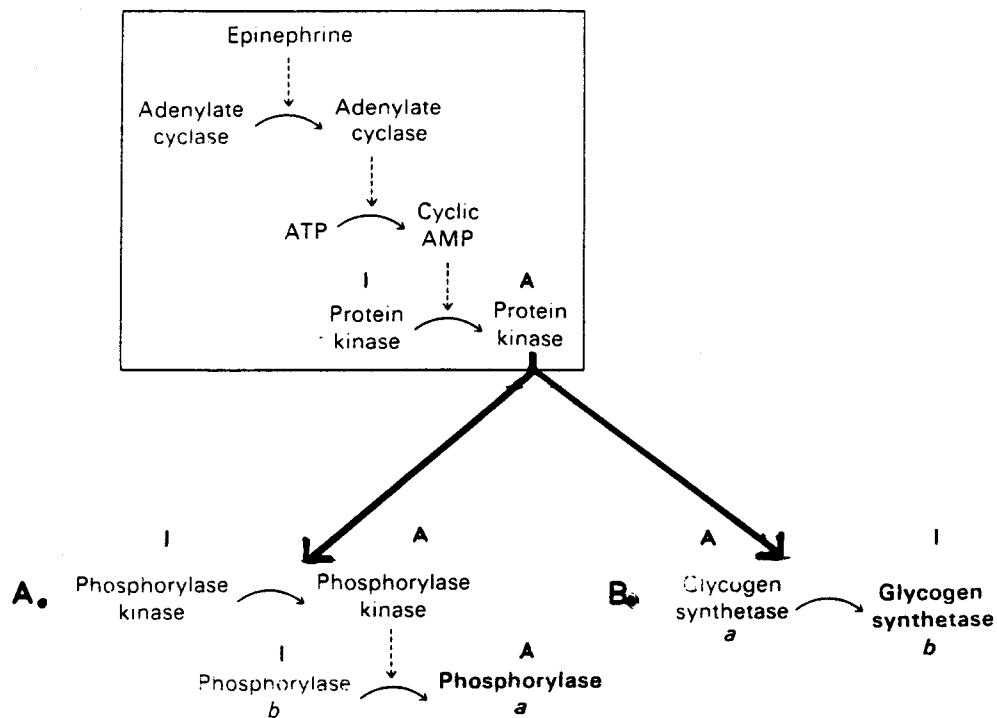
"activity ratio" in vivo. (Danforth 1965-cited by Cohen et al. 1979). The rate of glycogen synthesis has been found to correlate with the enzymatic activity in the absence of G,6-P. Thus, enhanced glycogen synthesis in the presence of insulin, appears to be a consequence of the hormonal activity and not of the increased rate of glucose entry into the cell (Danforth 1965-cited by Cohen et al. 1979). Phosphorylation activates glycogenolysis and inhibits biosynthesis. Insulin and epinephrine alter the enzymatic "activity ratio" by decreasing and increasing the degree of phosphorylation of glycogen synthase, respectively.

Since the conversion of glycogen synthase a into b by MgATP was stimulated by cAMP, it is accepted that the phosphorylation reaction is catalyzed by cAMP-dependent protein kinase. The effect of insulin on the degree of phosphorylation is either by decreasing the activity of cAMP-dependent protein kinase, or by increasing the activity of glycogen synthase phosphatase which reverses phosphorylation (Cohen et al. 1979).

Insulin has been found to lower cAMP concentrations that were previously elevated by epinephrine in adipose tissue and in the liver. The change in cAMP levels was accompanied by inhibition of lipolysis and glucose output in adipose tissues and in liver, respectively (Butcher et al. 1968, Robinson et al. 1971-cited by Cohen et al. 1979). Conversely, Fain (1974-cited by Cohen et al. 1979), demonstrated a lack of correlation between the effect of insulin on cAMP and its effect on metabolism in adipose tissues and in liver. In skeletal muscle, insulin did not alter cAMP

concentrations. It has been previously mentioned (see Mechanism of Insulin Action - II.1(6) B.) that epinephrine through interacting with α adrenergic receptors may affect glucose output by the liver independently of changes in cAMP. It is likely that the antagonistic effect of insulin on this process is unrelated to cAMP changes either (Cohen et al. 1979).

In summary, the competing pathways of glycogenolysis and glycogen synthesis are controlled in a synchronized manner. Phosphorylation activates glycogen degradation and inhibits glycogen biosynthesis to assure a coordinated activity of the two pathways and regulation of glycogen metabolism.



I indicates inactive form

A indicates active form

Figure 2: Control of glycogen metabolism

A. Glycogen degradation

B. Glycogen synthesis

(From: Stryer, Biochemistry, 1981)

II.1(8) Insulin Activity - a Feedback Mechanism with Blood Glucose

A. Glucose Homeostasis in the Fed State

Glucose is the major stimulus for insulin secretion, although some amino-acids are also known to act synergistically and to enhance insulin release by the islets. CHO ingestion stimulates some GI hormones that enhance insulin secretion from the β cells. The response of the GI hormones facilitates the insulinogenic effect of glucose and a.a. and may explain the higher insulin levels observed when glucose is given orally, compared to intravenously. (Fajans, 1972-cited by Williams 1974).

The delivery of glucose from the GI tract which involves several GI factors, elevates plasma glucose concentration and evokes insulin secretion. Plasma insulin level rises considerably within 20 minutes of CHO ingestion and induces a fall in hepatic glucose output by inhibition of glycogenolysis and gluconeogenesis. Glucose taken up by the liver is phosphorylated by glucokinase and is converted into glycogen. The reaction is catalyzed by glycogen synthase and regulated by insulin. Glucose that is not stored, is circulated and accounts for the elevated plasma glucose concentration. Hyperglycemia stimulates a further release of insulin which results in glucose translocation into muscle and adipose tissue. Glucose concentration declines within one hour due to the anabolic effect of insulin on liver and peripheral tissues. Insulin activity is counterregulated by other hormones (e.g. glucagon, catecholamines), that affect hepatic glucose output and glucose uptake by peripheral tissues to prevent a further fall in plasma glucose leading to hypoglycemia (Williams 1974).

B. Glucose Homeostasis in the Fasted State

In the postabsorptive state glucose concentration is maintained at the expenditure of other fuel sources (Cahill 1970-cited by Williams 1974). During a 24 hours fast, approximately 75 grams (g) of proteins and 160 g of triacylglycerols (TAG) are used to generate 180 g glucose by glycogenolysis and gluconeogenesis. The brain consumes 70% of the glucose produced. At this stage, the glucose pool ranges between 10-20 g with plasma glucose concentrations of 50-70 mg/100ml. When the glucose pool is depleted, hepatic glycogen stores are converted into free glucose via glycogenolysis, stimulated mainly by glucagon and to some extent by catecholamines (Cahill 1970-cited by Williams 1974).

By 24-48 hours of fasting, glycogen stores are depleted and glucose is derived from other sources. Pyruvate and lactate which originated in the glycolytic process and most a.a. (with the exception of Leucine) derived from proteolysis in muscle, are gluconeogenic precursors (Williams 1974). At this state, gluconeogenesis takes part mainly in the liver with minor contribution by the kidney. A decline in liver glucokinase and in insulin concentration together with a rise in glucagon, promote hepatic glycogenolysis and gluconeogenesis. A diminished glucose uptake by muscle and adipose tissue as well as enhanced lipolysis and proteolysis due to insulin suppression, result in a flow of gluconeogenic precursors to prevent acute hypoglycemia (Williams 1974).

With prolonged starvation, glucose output falls gradually to about 90 g per day with a simultaneous decline in protein catabolism. The kidney's contribution to gluconeogenesis increases considerably

and accounts for about 50% of the glucose regenerated. Lipolysis is enhanced due to low insulin concentration and high levels of the counter-regulatory hormones (Williams 1974).

Free fatty acids (FFA) taken up by the liver are oxidized via acetyl CoA to form ketone bodies which are metabolized to carbon dioxide and water or re-esterified to TAG's. When starvation is extended, ketone bodies partly substitute glucose as a fuel used by the brain. Prolonged starvation is characterized by suppression of insulin activity as the main anabolic hormone and activation of catabolic hormones. The adaptations of various organs to consume fat as the main energy source during food deprivation, may be regarded as a compensatory mechanism to minimize protein degradation and to protect the brain from hypoglycemia (Stryer 1981).

II.2 Insulin Related Abnormalities in CHO Homeostasis

II.2(1) Sites of Glucose Intolerance

Glucose intolerance associated with diabetes-mellitus and often with obesity results from abnormalities at different sites in the mechanism of insulin action. For insulin to complete its biologic role, it must be synthesized, secreted and transported to its target tissues. Insulin interaction with specific receptors on the target cell signals intracellular events which affect glucose metabolism. A defect in any of these steps results in an impaired CHO homeostasis.

Abnormalities in insulin action are related to one or more of the following conditions:

- (a) an abnormal β cell secretory product
- (b) circulating insulin antagonists
- (c) target tissue defects (Olefsky & Kolterman 1981, Rizza et al. 1981).

Abnormal β cell product: Synthesis and secretion of a molecule with an abnormal chemical structure is associated with defective biological function of the hormone. Similarly, inability to convert proinsulin to insulin and C-peptide, results in high levels of insulin precursor which features only 5% of the biological activity of insulin. In both cases tissue responsiveness to exogenous insulin may be perfectly normal (Olefsky & Kolterman 1981).

Circulating insulin antagonists: Insulin resistance may also be caused by hormonal or non-hormonal insulin antagonists. Excess of counterregulatory hormones such as glucagon, epinephrine, cortisol

and growth hormone, anti-insulin antibodies and anti-insulin receptor antibodies, all lead to a state of insulin resistance (Olefsky & Kolterman 1981).

Target tissue defects: A defect in the hormone-receptor interaction site, or at any point distal to receptor binding could also be expected to result in varying degrees of insulin resistance. Prereceptor defects are classified as insulin deficiency whereas receptor and postreceptor abnormalities are classified as tissue resistance to insulin. Insulin resistance is regarded as the state where a given amount of insulin produces less than a normal biologic effect. The degree of resistance is expressed by the dose-response curve (Olefsky & Kolterman 1981, Rizza et al. 1981) which reflects the concentration of insulin required to metabolize a given amount of glucose by peripheral tissues. Reduction in the number of cellular insulin receptors could lead to insulin resistance. Since cells possess spare receptors, the relationship between insulin receptors and insulin action is not straightforward. The spare-receptor concept is based on in-vitro observation (Olefsky 1976, Olefsky & Kolterman 1981, Rizza et al. 1981) indicating that maximal insulin effect is achieved at an insulin concentration that occupies less than the total number of cell receptors.

In isolated adipocytes, maximal glucose transport occurred when only 10% of insulin receptors were occupied (Kono & Barnham 1971, Gammeltoft & Gliemann 1973-cited by Olefsky & Kolterman 1981) and in soleus muscle glucose uptake was fully stimulated with less than 20% of total receptors (Freychet 1978). Increasing insulin concentration involves an increase in receptor occupancy until the

optimal number of receptors required for a maximal cellular response are occupied. Thus, a decrease in insulin receptors may be overcome by a rise in insulin concentration, yet, achieving the maximal metabolic effect. Only if more than 90% of cell receptors are lost, will maximal insulin response be impaired (Olefsky & Kolterman 1981, Rizza et al. 1981).

When insulin sensitivity is decreased, the amount of insulin necessary for a half-maximal effect is increased, but a normal response to insulin can be achieved. This state is manifested by a rightward shift of the dose-response curve (Fig 3-A). When tissue responsiveness is reduced, the maximal response to insulin can not be reached. This state involves post-receptor defects which can not be overcome by additional amounts of circulating hormone. It is manifested by a downward shift of the curve (Fig 3-B). Abnormalities in receptor and post-receptor sites, are manifested in a right and down shift of the dose-response curve (Fig 3-C), indicating a reduction in insulin sensitivity as well as in insulin responsiveness (Olefsky & Kolterman 1981).

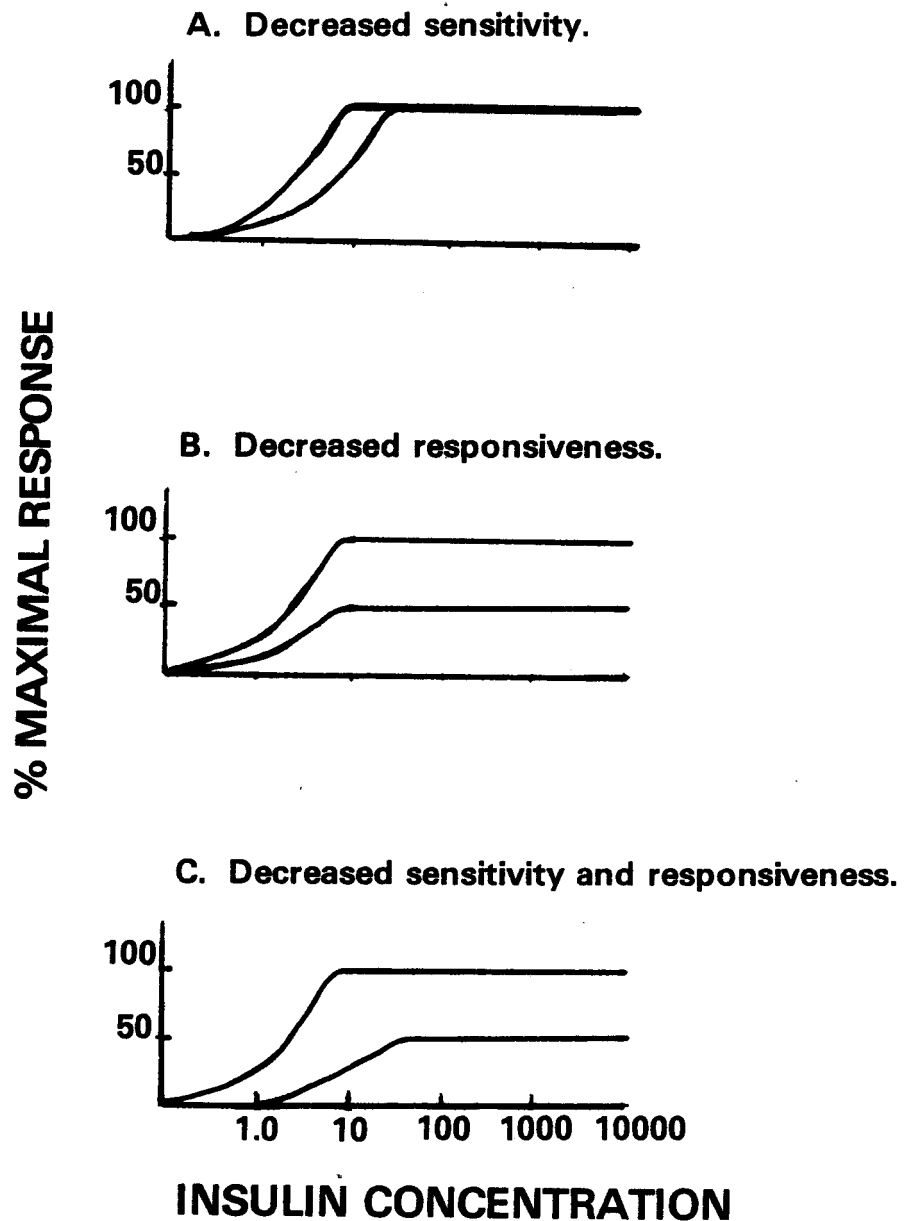


Figure 3: Theoretic insulin biologic action dose-response curve:

A - receptor defect

B - isolated postreceptor defect

C - Combined receptor and postreceptor defects

(From: Olefsky & Kolterman, Am. J. Med 70, 151-168, 1981)

II.2 (2) Impaired Insulin Action in Non-Ketotic Diabetes

The relationship between any defect in the insulin mechanism and glucose intolerance has been widely investigated (Reaven et al. 1976, Olefsky & Reaven 1977, Czech 1981, Olefsky & Kolterman 1981, Rizza et al. 1981).

Insulin deficiency is assumed to be the major cause for impaired CHO homeostasis; however recently, tissue resistance to insulin action has been emphasized in relation to glucose intolerance (Reaven et al. 1976, Rizza et al. 1981). To assess the role of insulin deficiency and insulin resistance in non-ketotic diabetes, Reaven and co-workers (1976) studied a group of normal subjects and four groups of non-obese, non-ketotic subjects with varying degrees of glucose intolerance. Unlike the normal response, glucose uptake did not increase considerably in any of the four groups when plasma insulin was elevated fivefold, suggesting a state of high insulin resistance. During a glucose tolerance test, insulin response in patients with borderline abnormalities of glucose tolerance or chemical diabetes was equal to or greater than that of normal subjects. Glucose intolerance among these patients cannot be attributed to a lack of insulin and may be observed as tissue resistance to the hormonal activity.

Conversely, patients with severe fasting hyperglycemia exhibited minor changes in plasma insulin in response to a glucose challenge, suggesting the existence of both insulin deficiency and insulin resistance. Two hypotheses are proposed by the authors in reference to the relations between insulin deficiency and insulin resistance. Insulin resistance may be the primary lesion in non-

ketotic diabetes. In some patients, a hormone deficiency may develop secondarily to increased insulin secretion leading to pancreatic exhaustion. Alternatively, insulin deficiency could be the primary lesion in patients with severe fasting hyperglycemia, consequently leading to tissue resistance.

The sites causing insulin resistance in non-ketotic diabetes have been further investigated by Olefsky and Reaven (1977). They studied the relationship between plasma insulin, insulin receptors and insulin sensitivity. In patients with chemical-diabetes, insulin binding to monocytes was 45% less than in normals and was associated with a reduction in receptor sites. A significant inverse correlation was found in this group between insulin binding and both fasting plasma insulin and insulin increments during an oral glucose tolerance test.

In patients with fasting hyperglycemia that exhibited hypoinsulinemia in response to a glucose challenge, insulin binding was inversely related to fasting plasma insulin, but not to insulin increments in response to a glucose load. No correlation was found between insulin binding and the degree of insulin resistance in this group. These findings suggest that insulin resistance was independent of changes in cell receptors and that other factors account for most of tissue resistance in patients with fasting hyperglycemia. It was concluded that it is the basal rather than the stimulated levels of insulin that are inversely related to the degree of insulin binding and are associated with regulation of cellular insulin receptors.

II.2 (3) Alterations in Insulin Action with Obesity

Obesity and insulin resistance are often interdependent. The mechanism underlying this hormonal resistance is unknown. One theory is that overeating results in hyperinsulinemia which by down-regulation of insulin receptors may lead to tissue insensitivity (Rizza et al. 1981). To study the sites of insulin resistance, the in-vivo dose-response curve of obese patients has been investigated (Kolterman & Reaven 1979, Kolterman et al. 1980-cited by Rizza et al. 1981, Olefsky & Kolterman 1981). At similar insulin infusion rates, glucose clearance rates were lower in obese compared to normal subjects (Olefsky & Kolterman 1981). As a group, obese subjects exhibited a great variability in their dose-response curve. Some patients showed a normal maximal glucose disposal with elevated insulin concentrations, suggesting a decrease in tissue sensitivity. Others demonstrated a rise in insulin levels with a marked fall in the maximal hormonal activity, indicating a decrease in tissue sensitivity and in tissue responsiveness.

Olefsky and Kolterman (1981) have postulated that the severity of insulin resistance in human obesity is correlated with the degree of hyperinsulinemia. The resistance is progressing from a state of receptor abnormalities to a combination of both receptor and postreceptor defects. These conclusions are in accordance with similar studies (Kolterman & Reaven 1979, Kolterman et al. 1980-cited by Rizza et al. 1981) reporting a rightward shift of the dose-response curve in all obese patients, and a downward shift in some of them, suggesting certain degree of postreceptor defects.

The interrelation between obesity, hyperinsulinemia and insulin resistance has also been investigated by in vitro studies. Olefsky (1976) compared insulin binding to isolated adipocytes from normal and obese patients. Adipocytes from obese subjects bound significantly less insulin than normals. However, when the non-hyperinsulinemic subjects were separated from the obese group, 4 out of 5 showed a normal insulin binding to adipocytes and monocytes. Apparently, obesity does not necessarily lead to hyperinsulinemia, nor to insulin insensitivity. A decrease in insulin binding is likely to be a consequence of obesity when the latter is accompanied by hyperinsulinemia. Amatruda and colleagues (1975) did not find any changes in receptor number of adipocytes from obese patients despite their high fasting insulin levels. Large adipocytes from obese individuals had similar receptor affinity and similar receptor binding to insulin compared to small adipocytes from normal subjects. The authors suggested post-receptor abnormalities to cause resistance to insulin in adipose tissue. It should be noted that in contrast to most studies, Amatruda expressed the number of receptor sites per cell rather than per surface unit. This may partly account for the differences in findings.

Regarding Olefsky's observation (1976) that obesity is not necessarily associated with hyperinsulinemia nor with insulin resistance, the role of hyperinsulinemia per-se in the state of insulin resistance has been evaluated. Whittaker and Alberti (1979) and Kobayashi and Olefsky (1979), studied the effect of insulin administration on glucose tolerance in non-obese rats. After 14 days of subcutaneous insulin injection in gradually increasing

doses, rat adipocytes were isolated and were investigated for their binding to insulin (Whittaker & Alberti 1979). There were no differences between the insulin treated and the sham treated group in body weight, food consumption and weight-gain per day. Fat cell volume increased in the experimental animals and their plasma insulin reached a higher mean value compared to controls. The induced hyperinsulinemia was followed by a 50-60% fall in insulin binding to receptors which was consistent over a wide range of insulin concentration. The decrease in binding seemed to be due to the rise in insulin, causing a down-regulation of hormonal receptors. The change in receptor sites did not affect the maximal glucose transport response. In view of this finding, Whittaker and co-workers suggested that adipose tissue does not directly contribute to a significant decrease in insulin sensitivity.

Using a similar animal model to Whittaker and Alberti, Kobayashi and Olefsky (1979) found no differences in body weight, food intake and weight gain per day, between a non-obese hyperinsulinemic and a control group. The former had greater fat cell size, higher plasma insulin and lower glucose levels compared to controls. Glucose transport, glucose oxidation and glucose conversion into lipids were all greater in the insulin treated rats. The authors attributed the enhanced glucose response in hyperinsulinemic animals to a rise in glucose transport and an increase in intracellular metabolism.

The preceding results are in contrast to other studies (Olefsky 1976, Czech 1976-cited by Kobayashi & Olefsky 1979) that examined hyperinsulinemia associated with obesity and found an

impaired glucose metabolism. Kobayashi and Olefsky proposed that in adipocytes from older obese animals, the inability to properly metabolize glucose is not caused solely by hyperinsulinemia, but may also be induced by other factors related to the state of obesity.

In addition, a time factor may contribute to the differences between induced and chronic hyperinsulinemia. In the study designed by Kobayashi and Olefsky, rats were exposed to high insulin levels for 14 days, whereas spontaneously obese animals experienced hyperinsulinemia for longer time periods. A cumulative effect of high insulin concentrations may lead to some metabolic defects characteristic to obese subjects with chronic hyperinsulinemia. In summary, evidence suggests that neither obesity nor experimental hyperinsulinemia are necessarily associated with insulin resistance. It is the combination between obesity and high insulin concentrations as well as chronic hyperinsulinemia which is more likely to induce tissue resistance to insulin.

II.2(4) Decreased Insulin Activity with Aging

It is known that tissue sensitivity and tissue responsiveness to insulin are altered with aging. Since a gain in body weight is associated with natural development, the relative role that weight and age play in age induced glucose intolerance is unknown.

Weiland et al. (1979) studied insulin removal by the liver in old (1 year) and young (6 weeks) rats. Old rats were characterized by obesity and hyperinsulinemia and their half-time ($t_{1/2}$) of insulin removal by perfused liver was significantly prolonged compared to the young animals. When older rats were treated with streptozotocin to

induce hypoinsulinemia, their $t_{1/2}$ was not decreased. Fourteen days of insulin administration to young rats produced hyperinsulinemia with no prolongation in the $t_{1/2}$ of the hepatic removal of insulin. In accordance with previous findings (Bracho-Romero & Reaven 1977) this study established that the rate of hepatic uptake of insulin declines as rats get older and heavier. This impairment is not a simple function of the insulin level and seems to be affected by other factors related to age and weight.

To eliminate the effect of weight on insulin resistance in aging, Bracho-Romero and Reaven (1977) investigated plasma insulin concentration and insulin binding to receptors in young and old weight matched rats. When young (4 week - 150g) and old rats (12 weeks - 400g) were exposed to an oral glucose challenge, insulin concentrations were significantly higher and insulin binding significantly lower in the old compared to the young animals. When rats of similar weight but different age were studied, the older rats exhibited glucose intolerance accompanied by higher insulin levels than did the young animals. It thus appears that both age and weight diminish insulin activity in rats.

Structural changes in the pancreas may partly explain variation in the hormonal function with aging. Reaven and Reaven (1981) studied three groups of aging rats for serum insulin and morphological alterations of β cells. A control group lived under standard laboratory conditions, the second group was involved in daily exercise training and the third group was given a calorie restricted diet to keep the animals weight matched with the exercising ones. Sedentary 12 months old rats were hyperinsulinemic

and demonstrated morphological changes in their pancreatic β cells. The islets showed reduced glucose induced insulin release, compared with islets from young rats. Exercised and calorie restricted rats had low body weight and did not show elevation in insulin levels or any pancreas pathology, in contrast to control animals. However, islets from the calorie restricted group functioned in vitro no better than islets from sedentary rats and the exercised rats were only somewhat improved in this aspect. It was concluded in this study that exercise and weight control diminish the need for insulin, and thus protect the pancreatic islets from significant morphological changes. The decline in the β cells function associated with aging cannot be prevented by these treatments.

It is evident that diabetes, obesity and aging are frequently associated with vary degrees of insulin resistance; however, it is suggested that resistance to insulin often is a reversible state. Human and animal studies have demonstrated that tissue sensitivity and tissue responsiveness to insulin may be altered by physical training in healthy, obese and diabetic patients (Bjorntorp et al. 1977, Owens et al. 1977, LeBlanc et al. 1979, Soman et al. 1979, Koivisto et al. 1979, Wirth et al. 1980, Mondon et al. 1980, Pedersen et al. 1980).

Endurance trained athletes usually present a high tissue sensitivity and tissue responsiveness to insulin. In this aspect they represent the opposite extreme to that found in the state of insulin resistance. Insulin levels are lower, insulin binding to receptors is higher and glucose uptake by the tissues is enhanced in trained compared to untrained individuals (Galbo et al. 1977, Soman

et al. 1979, Koivisto et al. 1979, LeBlanc et al. 1979, Mondon et al. 1980, Galbo et al. 1981).

Improved glucose tolerance in this population will be manifested by a leftward shift of the dose-response curve, indicating high sensitivity and high responsiveness to insulin. Receptor and presumably post-receptor alterations associated with physical exercise promote a maximal glucose response to insulin action despite low levels of circulating hormone.

The relation between exercise training and insulin activity will be discussed in the following section.

II.3 Effects of Exercise on CHO Homeostasis

II.3(1) Effect of Training Programs on Insulin Action

The role of physical activity in enhancing glucose tolerance is well established. It takes place in healthy, obese and diabetic individuals. The extent of the hormonal response to exercise is partly determined by the initial state of the subject (Bjorntorp et al. 1977, Owens et al. 1977, Soman et al. 1979, Koivisto et al. 1979, Pedersen et al. 1980, Krotkiewsky et al. 1980).

In-vivo and in-vitro evidence indicates that training has a diminished effect on insulin secretion at rest as well as during exercise (Gollnick et al. 1970, Bjorntorp et al. 1972, Galbo et al. 1977, Koivisto et al. 1979, Mondon et al. 1980, Galbo et al. 1981, Wirth et al. 1981). At rest, trained rats have been shown to have higher liver glycogen concentrations and lower plasma insulin levels than controls (Galbo et al. 1977). After 90 min. of swimming, plasma glucose was found to increase by 60% in trained and to decrease by 20% in untrained animals. In the former, the rise in glucose was not associated with any changes in insulin or glucagon levels, whereas in the untrained group insulin decreased and glucagon increased markedly during the first 45 minutes of exercise. Liver as well as muscle glycogen concentrations decreased in parallel in trained and control rats. The diminished hormonal response to exercise prevented a decline in blood glucose in trained animals. Since body weight did not differ significantly between the two groups, it is unlikely that different hormonal responses were due to variations in body composition. Galbo and collaborators suggested that sensitivity of adipose tissue to catecholamines was enhanced

with training; thus, marked changes in insulin and glucagon levels were not required for increasing the rate of lipolysis and glycogenolysis during exercise.

In contrast to Galbo's findings Windsor and co-workers (1981) found that endurance trained rats utilize liver glycogen during exercise at a reduced rate compared to non-trained animals. When trained and sedentary rats were exposed to prolonged submaximal exercise, liver cAMP increased by 50% and doubled after 45 and 90 min. of running, respectively, only in the non trained rats. Minor changes in cAMP have been observed in the trained group at the end of the exercise bout. In agreement with Galbo, glucagon and norepinephrine levels were consistently higher in the non-trained compared to the trained group and seem to account for the high cAMP in the former. It is suggested that low cAMP levels are responsible for a slow rate of liver glycogen utilization in endurance trained rats. It thus appears that high liver glycogen stores and a low rate of glycogen utilization, which is often observed in trained animals, may result in a liver glycogen sparing effect during long-term exercise.

In vitro observations have shown adaptations of the secretory organs as well as of peripheral tissues to exercise. Islets of Langerhans were isolated from trained and sedentary weight matched rats (Galbo et al. 1981). When tissues were incubated at various glucose concentrations within the physiological range, insulin release from the islets of trained rats was significantly lower compared to controls. Since there were no changes in the number of β cells, a diminished insulin secretion was assumed to result from

alteration in the "stimulus-secretion coupling" of the β pancreatic cells. A reduced need for insulin (Galbo et al. 1977) may be associated with a decrease in glucose sensitivity of the hormonal secretory mechanism, induced by training.

Physical training also alters tissue sensitivity and tissue responsiveness to insulin. Glucose uptake and glucose oxidation have been found to be higher in fat cells of trained compared to sedentary rats at all insulin concentrations (Craig et al. 1981). When presented with a maximal insulin stimulus, these differences in substrate uptake and oxidation were amplified. Thus, in addition to an increase in insulin receptor number and an enhanced hormonal binding, adaptations distal to the receptor site appear to affect insulin responsiveness in trained rats.

Similar findings have been observed with human studies. Lower insulin concentration has been determined in trained compared to sedentary individuals at rest and during exercise (Koivisto et al. 1979, LeBlanc et al. 1979, Soman et al. 1979, Wirth et al. 1981). In the resting state, both plasma glucose and insulin have been found to be lower in athletes than in controls (Koivisto et al. 1979). Moreover, Soman and colleagues (1979) found insulin binding to monocytes to be 69% higher in trained than in untrained individuals, mainly due to an increased number of receptors in the former. In both groups, insulin concentrations inversely correlated with insulin binding. In previously untrained individuals, physical training resulted in a 35% rise in insulin binding to monocytes (Soman et al. 1979) as well as a 30% increase in insulin mediated glucose uptake (Koivisto et al. 1980). Since insulin binding to

monocytes did not correlate with changes in tissue responsiveness, events occurring beyond receptor binding may have accounted for the enhanced glucose metabolism following training (Soman et al. 1979).

The relationship between physical activity and glucose tolerance are illustrated by Lipman's findings (Lipman et al. 1970, Lipman et al. 1972) of glucose intolerance as a consequence of bedrest (Lipman et al. 1970). Since bedrest involves physical inactivity as well as a change in the gravitation vector, Lipman and associates (1972) examined the individual contribution of each of these factors. Glucose tolerance tests were carried out in subjects exercising at 70% of their maximal aerobic capacity in the supine position while undergoing 35 days of bedrest and in monkeys that were kept immobilized in a vertical position for 6 weeks. Subjects who exercised during bedrest showed an improved glucose tolerance compared to non exercising individuals who were kept in bedrest. The immobilized monkeys exhibited a lower rate of glucose disappearance compared to mobilized animals. None of the treatments caused any changes in body weight. In view of these data, it seems that physical inactivity rather than possible shifts in fluid compartments due to bedrest, is the cause of changes in glucose tolerance. It is possible that the impaired ability to metabolize glucose was secondary to peripheral insulin resistance.

To assess the role of different organs in relation to insulin activity and exercise, glucose uptake by perfused hindlimb muscle and by the liver was studied in rats (Mondon et al. 1980). When trained and age matched controls were compared in a resting state, glucose uptake by hindlimb of the trained group was 17% faster

when no insulin was added and 43% faster when insulin was perfused. After an overnight fast, these differences were even more remarkable. The high glucose clearance rate was observed in trained animals in spite of low insulin levels, indicating an enhanced peripheral sensitivity to insulin. Since the perfused livers from the trained animals did not show any improvement in the rate of glucose clearance, these authors concluded that skeletal muscle rather than the liver is the site of enhanced insulin sensitivity associated with training.

Obese subjects respond to physical training in a way similar to normals. Bjorntorp and associates (1970) investigated the effect of a long term exercise program on CHO metabolism in obese patients. An eight week program resulted in an increased maximal aerobic capacity and a gain in body weight, primarily due to an increase in body fat. Insulin values were markedly decreased with no change in plasma glucose. Similar results were obtained when extremely obese patients underwent physical training for 6 weeks (Bjorntorp et al. 1977).

Krotkiewski and colleagues (1980) studied glucose tolerance in hyperplastic obese subjects. Ten women participated in a training program and an ad-libitum diet to prevent them from losing weight. Before and after measures showed a diminished insulin response during an oral glucose tolerance test as a consequence of training. This effect was much smaller when the women experienced an intravenous glucose administration. The authors explained the differences in the hormonal response by enteric factors that are associated with insulin secretion and which may be affected by training.

Insulin sensitivity had also been investigated in obese, asymptomatic diabetics before and after a combined physical training and diet program (Ratzmann et al. 1981). Four weeks of daily activity on bicycle ergometer and low caloric diet, resulted in a decrease in mean body weight and an increase in power output. Following the experimental periods, the fall in plasma glucose and FFA in response to insulin infusion was significantly greater than the one observed prior to the program. It was concluded that in vivo insulin sensitivity is enhanced in obese asymptomatic diabetics by a combination of exercise training and restricted diet although the relative contribution of each was not known.

Physical exercise is considered to be one of the basic factors in the management of diabetes. Glucose metabolism abnormalities may often be improved as a result of an enhanced insulin sensitivity associated with exercise training.

The ability of diabetic subjects to recover from physical exertion has been investigated. Glycogen resynthesis following exercise was assessed in juvenile-diabetics and non-diabetic males (Hermansen, 1980) and in chronic diabetic compared to normal rats (Hamilton et al. 1981). During recovery from prolonged exercise, muscle glycogen synthesis was comparable in juvenile-diabetic and in healthy subjects (Hermansen, 1980). When skeletal muscles from a rat limb were electrically stimulated for 30 min. glycogen was depleted to similar levels in diabetic and normal muscles (Hamilton et al. 1981 - unpublished data). Repletion showed a biphasic pattern: the initial fast phase was followed by a slower phase lasting from 1 to 8 hours. At 15 minutes of recovery, glycogen was significantly

greater in muscles from normal compared to diabetic rats. In the later phase, repletion patterns were similar in both groups. By 4 hours, glycogen stores of normal muscles were repleted and exceeded the control levels, whereas by 8 hours stores of exercised diabetic muscles were slightly less than those of diabetic control muscles. It appears from both studies that diabetics are able to synthesize glycogen in a pattern similar to normals. The slower rate of repletion may be due to low levels of circulating insulin (Hermansen, 1980).

In order to investigate the effect of exercise training on glucose homeostasis in diabetes, rats were exposed to streptozotocin to induce insulin deficiency (Reaven & Chang 1981). Under these conditions, trained and sedentary young rats were compared as to their plasma glucose, TAG's and insulin concentrations. Physical training attenuated the rise in plasma glucose and TAG's associated with insulin deficiency. Since plasma insulin levels were slightly lower in the trained compared to control animals, tissue sensitivity to insulin rather than endogenous insulin secretion appears to be affected by exercise training.

The relation between physical exercise and the degree of metabolic control in juvenile-diabetics was studied in a large group of diabetic children and adolescents after 1-17 years duration of the disease (Ludvigsson, 1980). The degree of physical activity based on a weekly history was reported and compared to an index of metabolic control (proportion of daily urine tests without glucosuria), while other factors related to metabolic control were kept constant. A significant correlation was found between these two variables, and

was confirmed in a follow up during two more years. However, in single patients a negative correlation between physical activity and the index of metabolic control was reported. The importance of adequate insulin treatment and food intake has been stressed in relation to diabetes and exercise (Vranic & Berger 1979, Ludvigsson 1980, Larsson 1980, Richter et al. 1981).

II.3(2) Effects of Acute Exercise on Glucose Metabolism

During prolonged exercise of moderate intensity (60-70% of maximal aerobic capacity), glycogen breakdown by muscle and liver, glucose transport to muscle cell and FFA release by adipose tissue are markedly increased to match the rate of substrates uptake and utilization by the working muscles (Gynthelberg et al. 1977). Glucose homeostasis during exercise is regulated by an interaction between a number of hormones which involves a decrease in insulin level as the major anabolic hormone and an increase in norepinephrine, epinephrine, glucagon and glucocorticoids. The role of individual hormones varies with the intensity and duration of exercise as illustrated by Richter and co-workers (1981). (Fig. 4).

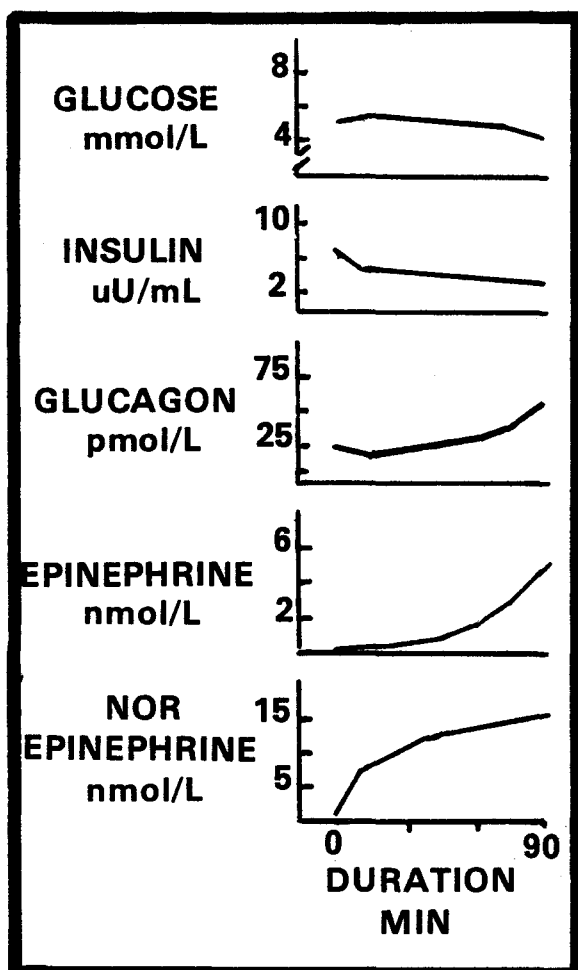


Figure 4: Typical changes in circulating levels of plasma glucose, insulin, glucagon and the catecholamines during prolonged exercise of moderate intensity. (From: Richter et al., Am. J. Med 70, 201-209, 1981)

As previously mentioned, glucose uptake by the cells is enhanced during exercise in spite of low plasma insulin concentration, suggesting temporary changes in tissue sensitivity to the circulating hormone induced by acute exercise. Although differences exist between healthy and insulin resistant subjects in their responsiveness to physical exertion, they all show a relative improvement in glucose metabolism following a bout of exercise.

Ivy and Holloszy (1981) studied the effect of exercise on glucose uptake and glycogen synthesis in skeletal muscles. Rats were subjected to one bout of moderate swimming that resulted in a reduction of 50% of muscle glycogen concentration. Glucose uptake and glycogen resynthesis were measured in perfused hindlimb muscles during 30 min. starting 60 min. following the exercise. In the absence of insulin, the rate of glucose uptake was tenfold higher in hindlimbs of exercised animals than in controls. The differences in glucose uptake in the presence of insulin still existed, but were less pronounced. The main pathway for glucose disposal was glycogen synthesis, thus, the rate of glycogen accumulation was greater in the exercised than in control muscles.

To investigate the site(s) of the hormonal mechanism affected by exercise, insulin binding to receptors has been studied following a bout of moderate activity. Three hours of exercise at approximately 40% of $\dot{V}O_2$ max. has been shown to lead to an increase of 36% in insulin binding to monocytes in healthy, untrained subjects (Soman et al. 1978, Koivisto et al. 1979). This rise in binding was due to an increased receptor affinity and was not accompanied by a change in receptor number, nor did it correlate with the fall in

plasma insulin. At the same relative exercise intensity, trained athletes exhibited a fall of 31% in binding to monocytes compared to their resting state (Koivisto et al. 1979). The different response of the two groups to acute exercise may be explained by a lesser dependence on CHO and a greater usage of lipids during exercise in the athletes as substantiated by their lower respiratory exchange ratio (R value).

Studies from the same laboratory reported a change in insulin binding to monocytes in obese patients after acute exercise (Koivisto et al. 1980). Obese and control subjects performed three hours of cycle ergometry at a steady heart rate of 120 beats/min. Insulin binding to monocytes increased by a mean of 13% in the obese and of 36% in the non-obese group. In accordance with previous studies (Soman et al. 1978, Koivisto et al. 1979), the enhanced insulin binding induced by acute exercise was attributed mainly to a change in receptor affinity. Few explanations are proposed by the investigators concerning the different responses of the two groups. Although the relative workloads were similar, obese subjects exercised at an absolute workload that was 25% lower compared to controls. The lower muscular work may partly explain a smaller change in receptor binding among overweight patients. In addition, elevated insulin concentrations that were observed in the obese patients with cessation of exercise, are known to cause down-regulation of insulin receptors and may explain the variation in the hormonal binding between the groups.

In agreement with Soman's and Koivisto's findings, Ravussin and co-workers (1980) reported an improved CHO utilization in obese

patients during exercise. Obese and control subjects performed 2 hours of bicycle ergometry 1 hour after ingestion of 100g glucose. In the basal state, the obese individuals displayed a lower rate of CHO utilization as suggested by the difference in R value. With exercise, both groups exhibited a similar rate of CHO oxidation. The enhanced CHO utilization during exercise may be explained by a decrease in peripheral resistance to insulin which facilitates substrate uptake.

According to the previous studies, acute exercise appears to have a positive effect on glucose utilization in healthy and in obese individuals. This change is partly due to a rise in insulin binding to receptors. However, factors distal to receptor site may be temporary altered and affect tissue responsiveness during exercise. (Amatruda et al. 1975, Kalant et al. 1978).

In the state of diabetes, the metabolic response to an acute exercise bout is largely determined by the initial state of the subject. It varies from a possible hyperglycemia in patients with basal plasma glucose of above 300mg/100ml (Allen et al. 1916-cited by Richter et al. 1981), to hypoglycemia among insulin dependent diabetics (Richter et al. 1981).

Studies have been carried out to assess the insulin secretory mechanism as well as peripheral tissue response to exercise in diabetic patients (Heding & Ludvigsson 1980, Pedersen et al. 1980, Martin et al. 1981). β cell response to exercise was examined in insulin dependent diabetics (IDD) and non-diabetic children (Heding & Ludvigsson 1980). The two groups performed 20 min. of bicycle ergometry followed by a 10 min. rest. Plasma glucose, insulin, C-

peptide and proinsulin were determined before and immediately after exercise as well as after 10 min. of recovery. In the non-diabetic subjects plasma glucose did not change, but insulin, proinsulin and C-peptide decreased during exercise. Ten min. after completing the exercise, glucose and all three β cell products increased markedly. The IDD children, did not present significant changes in glucose or insulin. Endogenous insulin in this group showed no change both during exercise or during recovery. The lack of increased insulin secretion during recovery was suggested to be due to hyperinsulinemia during exercise, suppressing the β pancreatic cells.

Physical exercise is also known to improve glucose tolerance and to diminish insulin requirements in well controlled diabetics. To examine whether these effects of exercise are associated with adaptations in insulin receptors, insulin binding to erythrocytes and monocytes were studied in IDD young men during exercise (Pedersen et al. 1980). One group of patients was subjected to three hours of bicycle exercise following a standard breakfast and a daily injection of insulin. A second group exercised for two hours at a fasting state with no insulin injection. Insulin binding to erythrocytes and monocytes increased by 30% with exercise in both experimental groups. Since possible effects of food intake and insulin injection on insulin receptor binding was eliminated in fasting subjects, the significant change in insulin binding was attributed to exercise.

Although insulin receptors on monocytes are not the main target for insulin binding, they have been found to correlate with glucose uptake and tissue sensitivity (Beck-Nielsen & Pedersen 1978 - cited by Pedersen 1980) and thus, alterations in insulin binding to

monocytes are assumed to reflect changes in other target tissues. It has been suggested by Pedersen and associates that working muscle cells undergo variations similar to monocytes and thus contribute to the effects of exercise on glucose homeostasis in both healthy subjects and patients with various degrees of insulin resistance.

There is evidence to suggest that physical training as well as acute exercise improve glucose tolerance. The mechanism responsible for this change and the duration of the metabolic adaptation are not yet fully understood.

II.4 Effects of Food Restriction and Diet Composition on Glucose Metabolism

Caloric balance as well as the composition of the diet have been found to influence glucose tolerance in healthy and insulin resistant patients. The relationship between these factors has been widely investigated in human and animal models (Bar et al. 1976, Weiland et al. 1979, Richard et al. 1980, LeBlanc et al. 1981).

Bar and associates (1976) studied insulin concentrations and insulin binding to receptors in obese patients before and after 6-12 weeks of dieting. Food restriction resulted in a fall in plasma insulin level and normalization of insulin binding to monocytes. Circulating insulin was inversely related to receptor concentration and the rise in binding was associated with an increase in receptor number with no change in receptor affinity.

Similar results were observed when obese diabetic patients were exposed to a ten day fast (Greenfield et al. 1978). The treatment induced a reduction in body weight and a significant decrease in plasma glucose and insulin. Upon refeeding, glucose level rose to a level about half way between fasting and prefeeding values where it remained for several months.

Weiland and co-workers (1979) investigating caloric restriction in rats, found decreased half-time of insulin removal by perfused liver when weight gain was prevented. Since the enhanced rate of insulin removal was not accompanied by a fall in the hormonal concentration, it was attributed to alterations in the target cell.

Glucose homeostasis in rats has been assessed after food restriction, training and ad-libitum food intake (Wirth et al. 1980,

Richard et al. 1980, Richard & LeBlanc 1980). Wirth and colleagues (1980) compared insulin concentration, insulin binding to monocytes and glucose metabolic clearance rate in three animal groups: sedentary food restricted (SFR), sedentary free eating (SFE) and a trained group(T). There were no differences in body weight between SFR and T animals which were both lower compared to SFE. Insulin concentration and glucose metabolic clearance rate (MCR) were comparable in the two sedentary groups but differed significantly from the trained troupe. The trained animals also showed the lowest insulin-glucose ratio; however, no changes in insulin binding were induced by any of the treatments.

A similar animal model was studied by Richard and LeBlanc (1980) who reported a decrease in basal and glucose stimulated insulin levels with no change in glucose tolerance in T and SFR rats. These groups had lower body weight and less adipose tissue compared to SFE. Since physical training and food restriction caused similar changes in insulin homeostasis, the authors suggested that a reduction in body weight and adiposity induced by these treatments improved glucose tolerance in the experimental rats. This hypothesis was confirmed by an additional study from the same laboratory (Richard et al. - unpublished data), which reported an impaired glucose metabolism in rats that were simultaneously exposed to physical training and a high caloric diet. Diminished insulin levels were observed in all trained animals but only when trained rats were fed standard chow, the drop in insulin was accompanied by an improved glucose tolerance. Conversely, when trained rats were fed "cafeteria diet" they increased their weight and adiposity and exhibited an

impaired glucose tolerance compared to sedentary chow fed rats. In view of these findings, the authors suggested that energy balance rather than training per-se determines glucose homeostasis in rats.

In humans, caloric restriction has been found to have similar effects on insulin secretion (LeBlanc et al. 1981). When trained athletes refrained from physical activity for 3 days while eating ad-libitum, their insulin response to a glucose load was increased. However, when food intake was restricted during detraining, the diminished insulin response to a glucose load was retained, suggesting that caloric balance is an important modulator of insulin action.

Evidence suggests that fasting for short periods of time (24-48 hours) may also affect glucose uptake and insulin binding to cell receptors (Bar et al. 1976, Olefsky et al. 1976, Goodman & Ruderman 1980). Goodman and Ruderman (1980) reported that glucose uptake by skeletal muscles from starving rats was twice as great as the uptake by controls, when tissue was perfused with varying insulin concentrations within the physiological range. When young rats were compared with old animals in the fed state, the former took up four times as much glucose per gram muscle as older rats. However, with starvation, glucose uptake was comparable in both groups, suggesting that starvation counterbalanced the effect of aging on glucose metabolism.

Changes in insulin binding to receptors have been shown to contribute, at least in part, to an improved glucose homeostasis following fasting (Bar et al. 1976, Olefsky 1976). Bar and associates (1976) compared insulin binding to monocytes in obese and

normal subjects before and after fasting. In the basal state, monocytes of obese patients bound less insulin compared to normals, mainly due to lower concentration of receptor sites per cell. Following a 48-72 hours fast, circulating insulin decreased in the obese group and insulin binding to monocytes reached a normal value. It appears that insulin receptors on adipocytes undergo similar alterations, since Olefsky (1976) reported an increase in insulin binding to isolated rat adipocytes after a 24 hour fast. In both cases the high hormonal binding was attributed to changes in receptor affinity since no change in receptor number was observed.

The mechanism by which fasting alters receptor affinity has been investigated by Olefsky and Kobayashi (1978). Studying the kinetics of insulin binding, these authors suggested that fasting leads to a decrease in the rate of insulin dissociation from its receptors. They reported a two-fold prolongation of the time required for 50% of the bound ^{125}I -insulin to dissociate from receptors and this correlated with a two-fold increase in binding affinity. The rate of insulin association with its receptors was not altered by fasting.

In addition to caloric balance, the actual composition of the diet has also been found to affect insulin activity and glycogen synthesis. The ratio between CHO and fat intake and its relationship to glucose metabolism has been examined by several authors (Kolterman et al. 1980, Costill et al. 1981, Grundleger & Thenen 1982).

Saekow and Olefsky (1980) studied the effect of a high CHO diet on glucose metabolism in adipocytes from obese rats and from rats treated with streptozotocin to induce insulin deficiency. Each

group of animals was divided into two subgroups that were kept on a high CHO diet or a control diet for 10 days. A high CHO diet led to an increase in insulin secretion in the obese animals, but did not alter insulin levels in the diabetic, insulin deficient rats. Glucose transport and glucose oxidation increased markedly only in the obese and did not change in the diabetic group. The authors concluded that high CHO intake induces increments in insulin secretion which mediate the changes in transport and intracellular metabolism of glucose. In the absence of those increments, glucose metabolism is unchanged.

Kolterman and associates (1979) investigated adaptations in insulin activity following a short (5 days) and a long period (14 days) of high CHO feeding in healthy non-obese subjects. Plasma glucose and insulin levels, insulin binding to adipocytes and glucose MCR were observed. In agreement with the previous study (Saekow & Olefsky 1980), high CHO intake induced hyperinsulinemia in all subjects. Insulin binding to adipocytes decreased after 5 and 14 days primarily due to a reduced receptor affinity after the short-term treatment and a change in receptor number after the longer treatment. With both dietary periods, in-vivo insulin action as reflected by the rate of glucose removal from plasma was enhanced and may be related to the low fat content in diet. The increased insulin action despite a decrease in the hormonal binding, may be explained by alterations of intracellular events and suggests that changes in insulin binding do not necessarily reflect the overall state of glucose metabolism (Kolterman et al. 1979).

A high fat diet appears to have an opposite effect on insulin activity than a high CHO diet. Grundleger and Thenen (1982) examined the relationship between diet composition and insulin resistance in an in-vitro study of isolated rats' muscles. Ten days of high fat diet (67%) induced a rise in insulin concentrations and a reduction in insulin binding to receptors compared to rats fed a high CHO diet (67%). This change was attributed to a fall in receptor number. In-vivo observations revealed that total glucose utilization was significantly lower in the fat fed compared to the CHO fed animals. However, glycogen synthesis was not affected by dietary conditions in this study. Since maximal glucose uptake occurred when only 44% of total receptors were occupied and since the decrease in receptor concentrations did not exceed 35%, alterations at steps distal to receptor interaction should be considered. This conclusion is in agreement with the study previously reported (Kolterman et al. 1979).

Fell and associates (1980) examined muscle glycogen resynthesis in rats, following exhaustive exercise that caused muscle and liver glycogen depletion. The largest increase in glycogen concentrations took place during the first 3 hours following exercise, in the presence of high glucagon and epinephrine and low insulin levels. This phenomenon may be explained on the basis that low muscle glycogen concentrations stimulate glycogen synthesis and override the inhibitory effect of catecholamines on glycogen repletion (Conlee et al. 1978, Fell et al. 1982). In addition, Fell and co-workers suggested that exercise has an insulinlike effect on muscle that results in an enhanced glucose uptake following exercise.

When rats were fasting or fat fed after exhaustive exercise, their muscle glycogen levels reached 50-100% of the values found in CHO fed animals. In contrast to the rapid rate of liver glycogen repletion in the latter, liver glycogen in fasting rats was still depleted 24 hours after exercise. It has been concluded that in the absence of CHO feeding, muscle glycogen stores are preferentially repleted in exhausted animals. Since liver stores were essentially depleted, it appears that glycerol derived from TAG's and gluconeogenic a.a. derived from muscle protein were the primary substrates for gluconeogenesis.

Costill and colleagues (1981) examined the effects of the amount and frequency of CHO intake on glycogen synthesis, after exercise that induced a significant reduction in muscle glycogen. Muscle biopsies from trained males were studied before as well as 24 and 48 hours after strenuous running that was followed by a number of dietary treatments. The frequency of feeding (2 vs. 7 meals a day), did not affect glycogen resynthesis when total CHO intake was kept constant. A high CHO intake of 525g/day resulted in a significant gain whereas a low CHO intake of 188g/day, led to reduced glycogen stores compared to the mixed diet (375g/day). These findings are in contrast to Fell's study (1980) that observed normal muscle glycogen repletion in rats, in spite of CHO starvation.

Glycogen concentration in muscle has been studied in relation to glucose tolerance and glucose uptake by the tissues (Ivy et al. 1982, Fell et al. 1982). Recently, Ivy and co-workers (1982) investigated the influence of muscle glycogen stores on glucose tolerance and insulin sensitivity in untrained subjects. Glucose

tolerance tests were administered after 3 days on a mixed diet (muscle glycogen 79.6 mmol/kg WW) and again following exhaustive exercise and 1 day on a high fat-protein diet (muscle glycogen 49.1 mmol/kg WW). In spite of the low glycogen concentration following exercise, glucose tolerance was not improved. The authors suggested that high plasma levels of FFA and ketones due to high fat intake, caused tissue resistance to insulin which overrode the effect of exercise on the rate of glucose uptake.

Fell and associates (1982) studied the effect of muscle glycogen content on glucose uptake in rats. Following exhaustive exercise, rats were kept on either a low or a high CHO diet to produce significant differences in muscle and liver glycogen concentrations. When muscles were perfused with glucose and insulin, rats that were fed a low CHO diet took up 60-80% more glucose compared to animals that were exposed to a high CHO diet. The rate of glucose conversion into glycogen was also enhanced in the former. Both the rate of glucose transport and the rate of glycogen synthesis increased when glycogen was kept low, following exhaustive exercise.

It is evident that diet plays a major role in determining insulin activity by altering insulin secretion and insulin binding to its target cells. Receptor affinity and receptor concentration are susceptible to dietary conditions and account in part, for changes in glucose transport and glucose uptake by the tissues. Alterations distal to receptor interaction are also considered to affect glucose metabolism; however, the mechanism for this is still unknown.

II.5 Mechanisms Which Might Alter Insulin Resistance

The effects of physical exercise and food restriction upon insulin activity have been reviewed in the previous chapter. Evidence suggests that with both treatments insulin resistance is diminished and glucose metabolism is enhanced (Bar et al. 1976, LeBlanc et al. 1979, Mondon et al. 1980). Insulin secretion, insulin binding to receptors and steps distal to receptor interaction are altered and affect tissue sensitivity and tissue responsiveness to insulin.

Several explanations have been suggested regarding the mechanisms responsible for the metabolic adaptations. Among these are changes in maximal aerobic power, adiposity level, enzymatic activity associated with glucose metabolism and muscle glycogen concentration.

Adiposity: LeBlanc and co-workers (1979) assessed aerobic capacity, adiposity and insulin activity in trained and sedentary subjects. Significant differences were found between the groups in all three variables; however, when partial correlation was calculated to evaluate the individual contribution of aerobic capacity and adiposity to the degree of insulin binding to receptors, body fat rather than maximal oxygen consumption was found to affect plasma glucose, plasma insulin and insulin binding to monocytes. The authors suggested the low body fat of the well trained subjects to be the primary cause for their higher glucose tolerance.

To further investigate this hypothesis, a study from the same laboratory (Richard & LeBlanc 1980) attempted to differentiate between the role of physical activity and weight loss in determining

glucose tolerance. Trained (T), sedentary pair weighted (PW), sedentary free eating (SFE) and sedentary food restricted (SFR) rats were examined. Basal as well as glucose stimulated insulin levels were diminished by training with no change in glucose tolerance; however, similar results were obtained with the PW and SFR groups. T, PW and SFR rats had all lower body weight and less adiposity compared to SFE. According to this study, body weight was highly correlated with glucose stimulated insulin response in all groups. Since food restriction and physical training caused similar changes in glucose tolerance, Richard and LeBlanc suggested that the reduction in body weight and adiposity due to exercise, rather than training per-se, enhanced glucose metabolism.

The effect of caloric balance on insulin action has been emphasized in a recent study (LeBlanc et al. 1981), that reported a rise in insulin levels in response to a glucose challenge in endurance trained athletes, 3 days after cessation of training. This augmented response to a glucose load was eliminated when detraining was associated with food restriction to maintain caloric balance.

Obesity is often associated with high levels of circulating insulin and an impaired glucose tolerance, mainly due to a decreased number of insulin receptors (Bar et al. 1976, Greenfield et al. 1978). With a loss in body weight and adiposity in obese patients due to diet restrictions, insulin levels were diminished and receptor concentration was increased leading to an improved glucose tolerance (Bar et al. 1976, Rizza et al. 1981). Similarly, when weight gain was prevented from sedentary rats, their half-time of

insulin removal by perfused liver was shortened (Weiland et al. 1979).

It is accepted that body weight and body composition partly determine insulin resistance in both humans and animals. However, the concept that alterations in insulin activity induced by training are due to changes in body composition and energy balance appears unjustified (Owens et al. 1977, Bjorntorp et al. 1977, Soman et al. 1979).

Maximal Aerobic Power: Bjorntorp and associates (1970, 1977) reported an improved glucose tolerance in obese patients after a long-term training program. Maximal aerobic power increased together with an unexpected increase in body weight and body fat, thus, the improved insulin action observed in this group cannot be due to reduction in adiposity. Similarly, a moderate exercise program that was designed to eliminate changes in body composition (Owens et al. 1977), resulted in a reduction in lipolysis and glucose conversion to CO_2 . Since changes in adiposity due to the exercise program were minimal, alterations in lipids and glucose metabolism were considered to be directly attributable to physical training.

In healthy, untrained subjects, a physical training program increased maximal aerobic power proportionate with the rise in glucose uptake by the tissue (Soman et al. 1979). Insulin binding to monocytes was also found to have increased due to an increase in receptor number. Since the exercise training program did not induce any decreases in adiposity, it is unlikely that insulin sensitivity in this study was affected by body composition and suggests that other factors within the target cell may have been responsible for

the changes in tissue sensitivity. Furthermore, insulin binding to monocytes did not correlate with the change in insulin sensitivity, thus, events occurring distal to receptor interaction were suggested to contribute to a decreased insulin resistance with physical training.

These results are in agreement with those of Mondon et al. (1980) who studied glucose uptake by perfused hindlimb muscle and liver in trained and untrained rats. Differences in body weight induced by the training period were eliminated by comparing the trained rats to younger weight matched controls. The trained animals had low plasma insulin values and a significantly higher glucose uptake by the hindlimb compared to controls. These differences were obtained when no insulin was added and increased almost three-fold when insulin was perfused.

With regard to the site of enhanced insulin sensitivity, it was suggested (Mondon et al. 1980) that since glucose uptake by perfused liver of trained was comparable to that of untrained animals, the muscle rather than the liver was the site affected by training. The insulin secretory mechanism was also studied by investigating the pancreatic β cells in trained and untrained rats (Galbo et al. 1981). Islets from trained rats were found to release less insulin than untrained rats in response to a glucose load; however, glucose disappearance rate was faster in the former. Since there were no differences in the number of cells between the two groups, it was suggested that physical training affects the stimulus-secretion coupling in the islets and diminishes both the need for insulin and the glucose sensitivity of the insulin secretory mechanism. In

accordance with other investigations (Bjorntorp et al. 1970, 1972, Soman et al. 1979), the changes in insulin secretion observed were attributed to physical training and not to a loss in body weight and fat. This conclusion was made in view of the findings that insulin secretion of islets from trained rats was lower not only when compared to sedentary free eating, but also when compared to weight matched controls. Differences in adiposity between the groups did not consistently correlate with the differences in insulin secretion.

Mondon and co-workers (1980) discussed the possible mechanisms that may alter insulin activity. A reduction in body weight gain with training may partly account for the enhanced clearance rate of glucose. However, since variations in glucose disposal were observed when differences in body weight were eliminated, other factors were suggested to play a role in determining insulin sensitivity.

Muscle Enzyme Activity: An increase in muscle enzyme activity such as hexokinase and glycogen synthetase was found to be associated with physical training (Holloszy et al. 1976) and may affect glucose transport and glucose oxidation. Recently, exercise training has been shown to enhance intracellular activity of hexokinase, citrate synthase and succinate dehydrogenase in normal, diabetic and insulin treated rats (Noble et al. 1982).

Although Mondon and co-workers (1980) did not obtain any differences in hepatic glucose uptake between trained and sedentary animals, Galbo and colleagues (1981) reported high hepatic glycogen synthetase activity and low phosphorylase activity in trained versus untrained rats. This was accompanied by a high liver weight and high glycogen concentration as well as low blood glucose levels during a

glucose tolerance test. These authors suggested that physical training enhanced hepatic capacity for glycogen synthesis and storage in rats.

Galbo et al. (1981) also proposed that exposure to high plasma catecholamine levels with repeated exercise may induce long lasting changes within the pancreatic β cells. The interdependence between catecholamines and insulin has been previously reported by Rousseau-Migneron et al. (1976), who observed low basal and glucose stimulated insulin levels in rats chronically treated with adrenaline. When rats were adrenalectomized, training failed to decrease basal insulin concentrations (Richter et al. 1979).

Muscle Glycogen Content: Muscle glycogen concentration has recently been found to influence the rate of glucose uptake by the tissue (Fell et al. 1982). When rats were fed a CHO restricted diet following exhaustive exercise to maintain low muscle glycogen levels, glucose uptake by perfused muscle was significantly greater compared to muscles with a high glycogen concentration. Most of the glucose taken up by the tissue was synthesized into glycogen. At similar submaximal insulin concentration, glycogen synthesis was higher in muscles containing low compared to those containing high glycogen stores. Likewise, in similar studies, MacDougall et al. (1977) and Piehl et al. (1974) demonstrated in human subjects that the rate of muscle glycogen resynthesis following exercise depletion, is most rapid when muscle glycogen levels are low and is most gradual as they approach pre-exercise values.

These studies confirm reports by Cohen and colleagues (1979) who found glycogen content to play a role in regulating its own

synthesis, by altering intracellular activity of glycogen synthase I. Thus, it may be suggested that the ability of the muscle to take up glucose and synthesize glycogen in an enhanced rate following a bout of exercise, persists as long as glycogen concentration remains low. Moreover, a high glucose clearance rate observed with trained individuals may simply be due to low muscle glycogen content induced by the last exercise session and not a chronic effect of continuous physical training.

Summary: The present study was designed to investigate possible changes in insulin resistance upon cessation of training, by assessing glucose uptake in endurance athletes during training and within 7 days of detraining. It was hypothesized that if insulin activity and glucose metabolism are altered during this experimental period, the enhanced tissue sensitivity and tissue responsiveness to insulin that is usually seen in highly trained subjects, may be interpreted as a transient effect of the last bout of exercise.

CHAPTER III

METHODS

III.1 Experimental Design

The present study was designed to confirm a high tissue sensitivity and a high tissue responsiveness to insulin in endurance trained individuals and to observe any changes in insulin activity upon cessation of training.

Using a glucose clamp technique, insulin resistance was examined in seven well trained subjects within 12-16 hours of their last training session and at various times during detraining, for comparison with results in untrained controls.

III.2 Subjects

The experimental group consisted of 7 highly trained individuals, 4 females and 3 males. In addition, 3 untrained females served as controls. A description of the physical characteristics of both groups is given in Table 1.

The subjects volunteered to take part in the present study and were informed of the purpose, the experimental details and the possible complications associated with this research. A consent form was signed by each subject prior to commencement of testing. (Consent form in Appendix I).

For the purpose of this study, an individual was considered trained if he had been involved in intensive physical training on a regular basis for at least one year prior to the study. Six of the trained subjects ran from 60-100 miles per week and the seventh was a national level swimmer who trained for 12-15 hours per week. The

Table 1: Characteristics of subjects.

	Trained Group (N=6)	Untrained Group (N=3)
Age (years)	30.4 <u>±</u> 3.9	24.7 <u>±</u> 0.7
Weight (kg)	63.3 <u>±</u> 4.0	60.8 <u>±</u> 1.9
Height (cm)	174.1 <u>±</u> 4.1	173.7 <u>±</u> 4.4
Body surface area (m ²)	1.76 <u>±</u> 0.07	1.72 <u>±</u> 0.05
Body density (g/cc)	1.0563 <u>±</u> 0.0087	1.0418 <u>±</u> 0.0019
$\dot{V}O_2$ max. (ml·kg ⁻¹ ·min ⁻¹)	57.2 <u>±</u> 3.5	43.4 <u>±</u> 1.3*

Each value represents the mean±S.E.

* Denotes a significant difference ($p < 0.02$) between groups.

control subjects did not participate in any physical training program during the previous year.

III.3 Insulin Resistance Test

Insulin resistance was determined on either two or three different occasions with each of the trained subjects. Insulin resistance was assessed initially 12-16 hours after the last training session and this value was considered as baseline. It was then reassessed after 60 hours and/or seven days of detraining. All subjects were instructed to refrain from any food intake for 12 hours prior to each test and to refrain from physical training during the experimental period following the initial test. In addition, they were asked to maintain regular daily activities and to avoid any significant changes in their diet. Recording of food intake was completed by each individual starting 24 hours previous to the first assessment.

A control group of 3 subjects was tested on two different occasions to establish normal values for glucose metabolic clearance rate (MCR) in untrained individuals. Controls followed the same testing protocol as was previously described for the experimental group.

A modification of the glucose clamp technique described by Greenfield et al. (1981) was used to determine insulin resistance.

The technique involves maintaining plasma insulin at a constant level by continuous infusion of insulin, while at the same time infusing glucose at a rate which will maintain plasma glucose at the initial level. The required rate of glucose infusion is a direct reflection of the degree of insulin resistance and is inversely related to the latter. Insulin resistance is expressed in terms of glucose metabolic clearance rate ($\text{MCR ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). It is calculated by dividing the amount of glucose metabolized (mean glucose infused per min. per kg body weight) by the initial plasma glucose concentration. (For application of MCR to different populations see Appendix II).

Experimental details were as follows: on arrival at the laboratory, intravenous catheters (IV) were inserted into a peripheral vein in each arm. One arm was used for insulin and glucose infusion and the opposite arm for blood sampling (Illustration in Appendix III). The IV's were kept patent with normal Sodium Chloride solution (0.9% - ABBO-VAC*) during 120 min. of testing. A primary insulin infusion of $80 \mu\text{U} \cdot \text{min}^{-1}$ during 5 min. was followed by a constant infusion rate in the required concentration to produce a total of $40 \mu\text{U} \cdot \text{m}^2 \cdot \text{min}^{-1}$ (or $2.4 \text{ U} \cdot \text{m}^2 \cdot \text{h}^{-1}$) for 115 min. (Insulin-Toronto, Beef and Pork Connaught Laboratories Limited). Glucose infusion commenced 4 min. (T_4) after insulin infusion was initiated (Dextrose 50% - ABBO-VAC*) at a precalculated rate to supply $0.48 \text{ g glucose kg}^{-1} \cdot \text{h}^{-1}$. Two adjustable Harvard pumps were used to infuse insulin and glucose (Infusion/Withdrawal pump model. Harvard Apparatus Co., INC Millis, Mass.). One ml of blood was withdrawn every 5 minutes and was analyzed for glucose concentration by a Reflomat Reflectometer (Boehringer Mannheim GmbH, Mannheim, W. Germany). The rate of

glucose infusion was adjusted according to changes in blood glucose level in order to maintain the initial concentration.

In addition, blood samples were collected at T_0 (fasting sample prior to insulin infusion), T_{30} , T_{60} , T_{90} and T_{120} for serum insulin (10ml), C-peptide (10ml) and serum glucose (7ml) determinations. Glucose was assessed by an automated electrolytes system. Insulin was determined by a radioimmunoassay method (Hales & Randle, 1963). The RIA Kit had been purchased from Amersham Cor. Canada. C-peptide was measured by a radioimmunoassay (Kabayashi 1975) as an indirect method to assess the endogenous insulin level during insulin infusion. The C-peptide II Kit was purchased from Daiichi Radioisotope Labs., Ltd., Japan.

III.4 Calculations of Insulin Binding

Analysis of insulin binding to erythrocyte receptors was carried out in order to investigate possible changes in the hormonal binding upon cessation of training.

Blood samples (20ml) were collected from trained individuals, at 12 hours and at 60 hours after their last exercise session. Fresh blood samples were fractionated by density gradient centrifugation into two fractions of defined density. Since red blood cells density correlates well with cells' age, cells with a density below 1.089 were defined as young and cells with a density between 1.089 - 1.092 were considered as whole cells.

Percentage of specific binding to receptors of young and whole cells was measured and compared at the two time points.

Insulin binding was expressed as bound to free ratio (b/F) versus bound hormone (B). The following formula was used:

$$B = \frac{F \cdot r_1 \cdot k_1}{1 + F \cdot k_1} + \frac{F \cdot r_2 \cdot k_2}{1 + F \cdot k_2}$$

where r_1 and r_2 are the molar concentrations and k_1 and k_2 are the corresponding affinity constants of the two noncooperative sites.

All binding analysis was performed at McGill University, Montreal, in collaboration with the Polypeptide Hormone Laboratories.

III.5 Measurements of Maximal Aerobic Power

Maximal oxygen consumption ($\dot{V}O_2$ max.) was measured during a progressive treadmill (Quinton, Model 2472) running test. The test was performed at a constant speed (7mph for females and 8mph for males) and stepwise grade increments of 2% every 2 minutes until exhaustion. Heart rate was continuously monitored as was $\dot{V}O_2$ by means of an open circuit gas analysis system. Inspired air volume was constantly measured on a Hewlett Packard 4000 VR Pneumotachograph and mixed expired CO_2 and O_2 by means of a Godart Capnograph and Rapox O_2 analyzer. All data were recorded on a 4 channel Narco-Physiograph (E & M instruments Company, Houston, Texas) and the highest $\dot{V}O_2$ achieved selected as $\dot{V}O_2$ max.

III.6 Measurements of Body Density

Body density was estimated by hydrostatic underwater weighing at full inspiration. Vital capacity was measured by a dry gas flow meter (American Meter Company #802) and residual volume was evaluated

by the helium dilution technique using a Collins 9 liter respirometer and a helium meter (Warren E. Collins Inc., Braintree, Mass.). Body density was calculated by using the following formula:

$$D_b = \frac{\frac{Ma}{Ma - M_w}}{D_w} - (VC + RV) \quad (\text{Brozek et al. 1963})$$

where Ma is body mass in air (g)
 Mw = body mass in water (g)
 Dw = water density at a given temperature
 VC = vital capacity (cc)
 RV = residual volume (cc)

III.7 Validity of Equipment

The validity of using the Reflomat Reflectometer for blood glucose determination was previously assessed by comparing the results reported by the clinical chemistry laboratory MUMC, with those read by the Reflomat Reflectometer. Eighteen samples were validated over a wide range of glucose concentration. (See Appendix IV).

III.8 Statistical Analysis

Differences in mean values for MCR, insulin concentration and percentage of specific insulin binding to erythrocytes were tested for significance using a matched pairs t-test (Klugh, 1970). Differences in glucose MCR, serum insulin and serum glucose were studied between the following time points:

1) To (baseline, 12-16 hours after the last exercise) and T_I (60 hours after the last exercise)

2) T_O and T_{II} (7 days after the last exercise)

3) T_I and T_{II}

Differences in insulin binding were observed between T_O and T_I .

An independent t-test was used for comparison of $\dot{V}O_2$ max., glucose MCR and blood parameters between the trained and the untrained groups.

The level of probability was set at $p < 0.05$.

CHAPTER IV

Results

IV.1 Physical Characteristics of Subjects

As indicated by Table 1, no differences were found in any of the physical parameters between trained (T) and untrained (UT) individuals. The two groups differed however in their maximal aerobic power, presenting mean values of 43.4 ± 1.3 and 57.2 ± 3.5 ml·kg⁻¹·min⁻¹ for the UT and T group, respectively ($p < 0.02$).

Fasting values for serum glucose and serum insulin were comparable in the two groups. Mean glucose concentrations were 86.4 ± 3.6 and 89.1 ± 3.8 mg/100ml and mean insulin concentrations were 5.3 ± 0.7 and 6.5 ± 0.8 μ U/ml for the T and UT group, respectively. (For initial values see Table 2).

IV.2 Glucose MCR

Glucose MCR was determined by a glucose clamp technique during which plasma insulin was elevated to a range of 55.8-61.1 μ U/ml. Simultaneously, C-peptide level decreased in 3 out of the 9 subjects; indicating a diminished endogenous insulin concentration. In one subject, C-peptide increased and in the others, the pre-infusion and during infusion values were all below 100pmol/L. Due to the low levels of C-peptide, the exact concentration could not be determined.

Table 2: Fasting serum glucose and serum insulin values for trained and untrained subjects.

Trained Group	Serum Glucose (mg/100 ml)	Serum Insulin (μ U/ml)
C.C.	84.6	4.2
P.M.	82.8	4.2
R.W.	104.4	3.5
H.W.	82.8	5.5
W.B.	82.8	7.6
B.C.	82.8	6.9
Untrained Group	$\bar{x} \pm \text{S.E. } 86.4 \pm 3.6$	$\bar{x} \pm \text{S.E. } 5.3 \pm 0.7$
A.J.	84.6	4.9
J.D.	93.6	7.6
C.B.	79.2	6.9
	$\bar{x} \pm \text{S.E. } 89.1 \pm 3.8$	$\bar{x} \pm \text{S.E. } 6.5 \pm 0.8$

One of the trained subjects B.H. was excluded from the study due to an experimental error that caused insulin concentration on the second assessment to be 41% lower compared to his initial test.

The initial values for glucose MCR achieved by each of the trained subjects, are given in Table 3. At T_0 (12-16 hours after the last exercise session), glucose MCR of the T group was 15.6 ± 1.8 ml·kg⁻¹·min⁻¹ (N=6) compared with 7.8 ± 1.2 ml·kg⁻¹·min⁻¹ (N=3) for the UT group ($p < 0.01$). At T_I (60 hours after the last exercise session), glucose MCR dropped by 35% to 10.1 ± 1.0 ml·kg⁻¹·min⁻¹ (N=4) and at T_{II} , after 7 days of detraining it decreased significantly to 8.5 ± 0.5 ml·kg⁻¹·min⁻¹ (N=5) ($p < 0.05$), a value which was indistinguishable from sedentary controls. The changes in metabolic clearance rate of glucose between the three time points are illustrated in Fig 5.

IV.3 Basal Serum Glucose and Insulin

A description of changes in blood parameters (serum glucose, serum insulin and insulin binding to erythrocytes) for the trained subjects is given in Table 4. A comparison of fasting glucose and insulin concentrations in T subjects at T_0 and T_I revealed no significant differences in these variables. However, insulin level increased by 53% during 7 days of detraining and at T_{II} it reached a mean value of 8.1 ± 1.5 μ U/ml compared with 5.3 ± 0.7 μ U/ml on the initial test.

Table 3: Glucose MCR ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) of trained athletes at 12 hours (T_0) 60 hours (T_I) and 7 days (T_{II}) after the last exercise session.

Subjects	T_0	T_I	T_{II}
H.W.	16.8	7.9	8.5
P.M.	22.4	12.7	9.8
R.W.	9.7	10.5	9.5
C.C.	16.5	9.3	---
B.C.	12.4	---	7.4
W.B.	15.9	---	7.3

$\bar{X}=15.6 \pm 1.8$

N=6

$\bar{X}=10.1 \pm 1.0$

N=4

$\bar{X}=8.5 \pm 0.5^*$

N=5

Bottom values represent the mean $\pm$ S.E.

* Denotes a significant difference ($p < 0.05$) from T_0 .

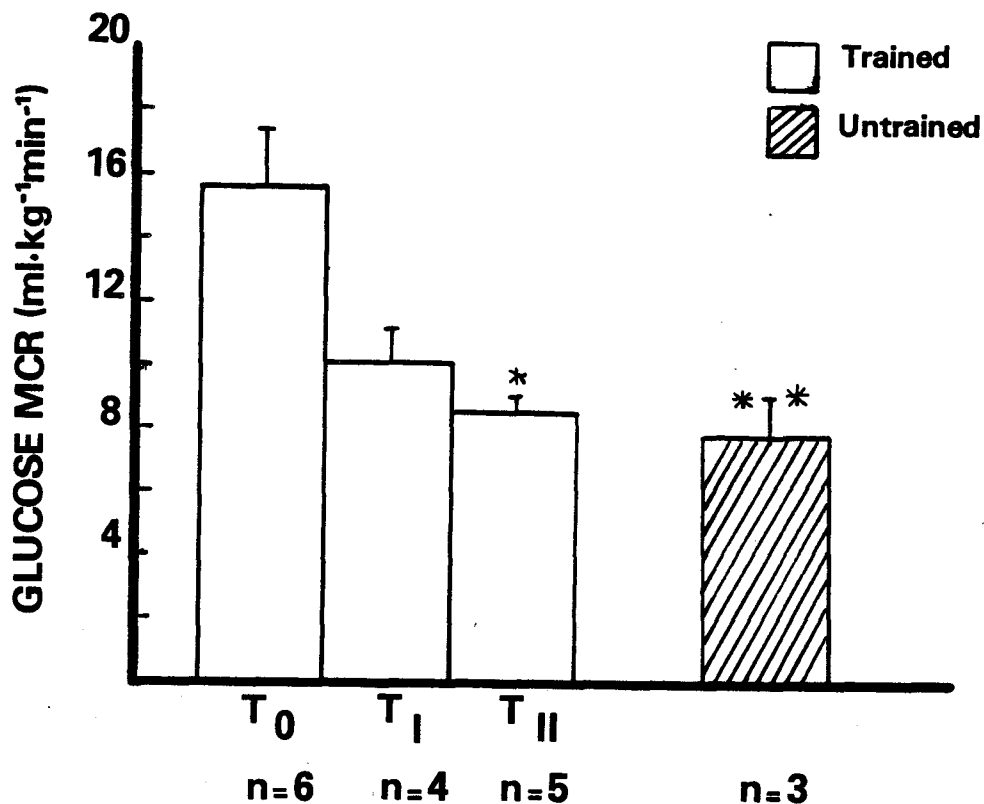


Figure 5: Glucose MCR in $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for trained athletes at 12 hours (T_0) 60 hours (T_I) and 7 days (T_{II}) after the last exercise session and for untrained controls.

Values represent the mean $\pm$ S.E.

* denotes a significant difference ($p < 0.05$) from T_0

** denotes a significant difference ($p < 0.01$) between trained and untrained groups.

Table 4: Fasting values for serum glucose, serum insulin and insulin binding to erythrocytes for trained athletes at 12 hours (T_0) 60 hours (T_I) and 7 days (T_{II}) after the last exercise session.

Blood Variables	T_0	T_I	T_{II}
Serum Glucose (mg/100 ml)	86.4 $\pm$ 3.6	91.8 $\pm$ 5.8	88.2 $\pm$ 2.2
Serum Insulin (μ U/ml)	5.3 $\pm$ 0.7	4.9 $\pm$ 0.6	8.1 $\pm$ 1.5
Insulin/Glucose	.0613	.0534	.0918
Insulin Bound (% /4x10 ⁹ cells)	10.4 $\pm$ 0.9	8.12 $\pm$ 0.7*	-----

Values represent the mean $\pm$ S.E.

* Denotes a significant difference ($p < 0.01$) from T_0 .

IV.4 Insulin Binding

Insulin binding to fractionated young erythrocytes was $10.4 \pm 0.9\%$ / 4×10^9 cells at T_0 ($N=7$) and decreased significantly to $8.12 \pm 0.7\%$ / 4×10^9 cells at T_I ($p < 0.001$). The changes in insulin binding to erythrocyte receptors are illustrated in Fig 6. (For initial values of insulin binding see Table 5).

When insulin resistance was reassessed on two different occasions in UT subjects, the plasma clearance rate of glucose was within a mean of 12% of their initial value.

Changes in insulin binding and insulin concentrations within 7 days of detraining are illustrated in Fig. 7.

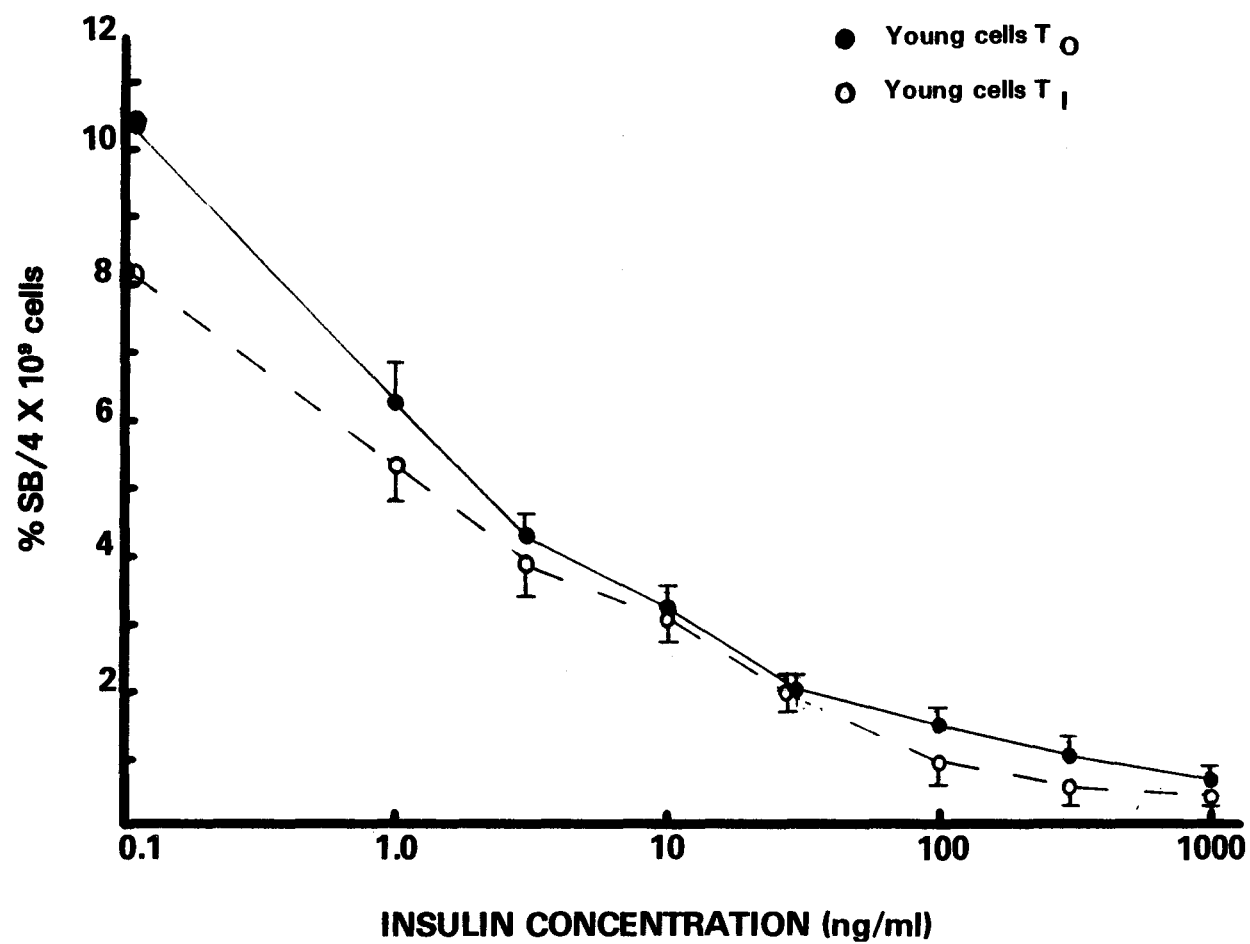


Figure 6: Insulin-binding to erythrocytes at 12 (T_0) and 60 hours (T_1) after the last exercise session.

Table 5: Insulin-binding to erythrocytes in trained athletes 12 (T_0) and 60 hours (T_1) after the last exercise session.

Insulin specific binding to erythrocytes
(% / 4×10^9 cells)

Subject	Young Cells		Whole Cells		% Drop from T_0	
	T_0	T_1	T_0	T_1	Young	Whole
+ R.W.	6.8	5.5	4.8	4.1	19.6	14.7
R.L.	12.3	8.7	5.8	5.1	29.2	11.6
+ C.C.	14.3	11.0	7.9	7.8	22.8	1.2
+ W.B.	8.8	6.6	3.9	3.5	25.2	9.6
B.H.	10.3	8.3	6.3	5.4	20.1	14.8
+ B.C.	9.5	8.0	5.7	5.7	15.7	0.3
R.C.	10.7	8.8	5.8	4.6	17.4	20.9

10.4 $\pm$ 0.9 8.12 $\pm$ 0.7** 5.8 $\pm$ 0.5 5.2 $\pm$ 0.5* 21.4 $\pm$ 1.8 10.5 $\pm$ 2.8

Bottom values represent the mean $\pm$ S.E.

* Denotes a significant difference ($p < 0.02$) from T_0 .

** Denotes a significant difference ($p < 0.001$) from T_0 .

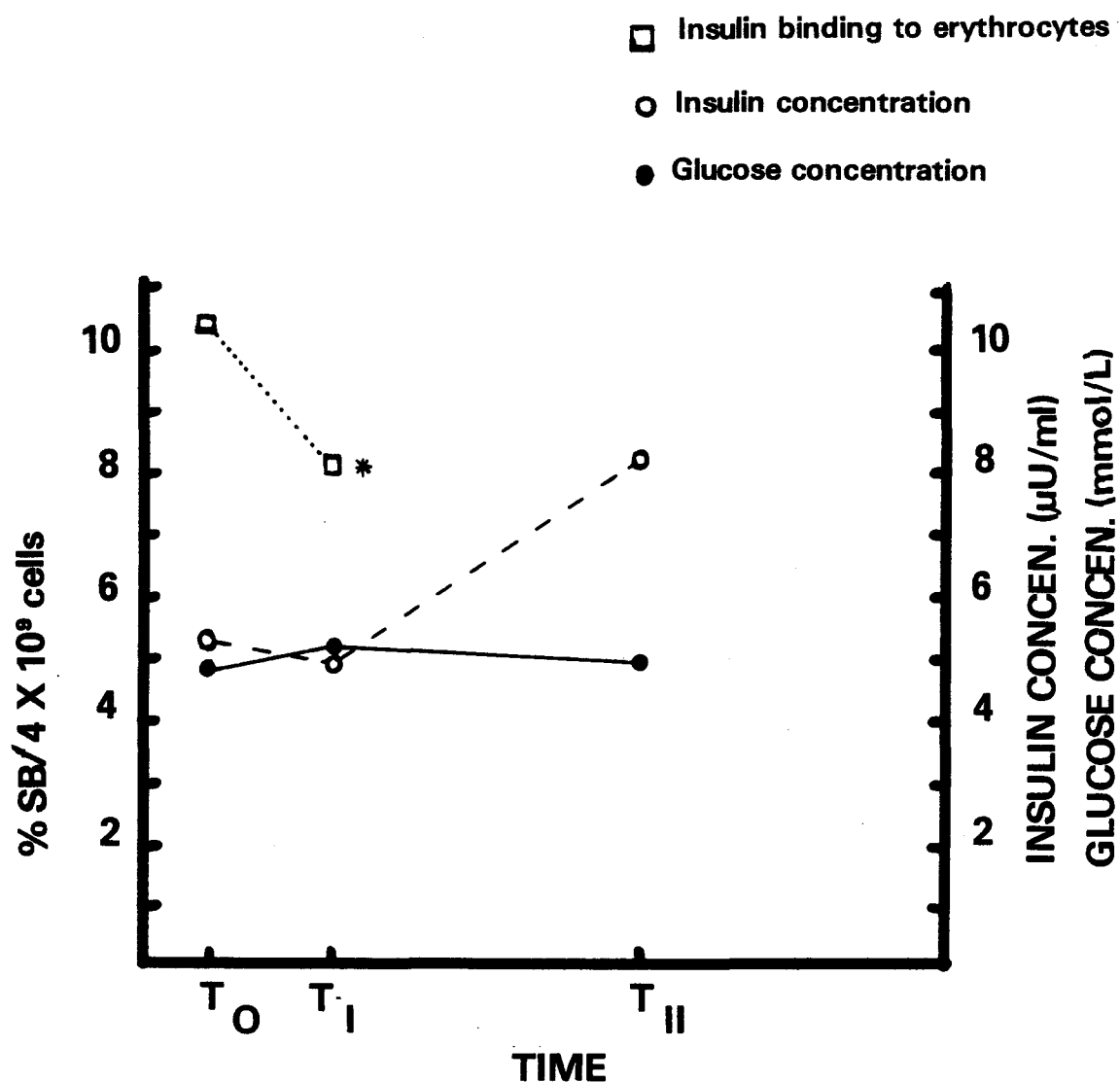


Figure 7: Fasting values for serum glucose, serum insulin and insulin-binding to erythrocytes for trained athletes at 12 hours (T₀) 60 hours (T_I) and 7 days (T_{II}) after the last exercise session.

Values represent the mean of the group

* denotes a significant difference ($p < 0.01$) from T₀

CHAPTER V

Discussion and Conclusions

V. The present study was designed to confirm previous findings suggesting an enhanced insulin action and glucose metabolism in endurance trained athletes (LeBlanc et al. 1979, Soman et al. 1979, Koivisto et al. 1979) and to examine possible changes in these variables upon cessation of training.

A comparison of insulin resistance between trained and untrained individuals, as reflected by their plasma clearance rate of glucose, revealed significantly higher clearance rates in the trained group. Possible mechanisms which may affect insulin resistance have been previously discussed. (See II.5: Possible mechanisms which might alter insulin resistance). In brief, four major factors might be implicated in relationship to changes in glucose metabolism with physical training:

- 1) Body composition (LeBlanc et al. 1979, Richard and LeBlanc 1980).
- 2) Maximal aerobic power (Soman et al. 1979, Galbo et al. 1981).
- 3) Intracellular enzymatic activity (Holloszy & Booth 1976, Ivy et al. 1982).
- 4) Glycogen concentrations in skeletal muscle and liver (Galbo et al. 1981, Richter et al. 1981, Fell et al. 1982).

The present study failed to observe any differences in body weight or body density between the trained and untrained subjects,

thus; variations in body composition cannot explain the differences in the clearance rate of glucose between these groups.

Differences in the state of training between endurance athletes and untrained controls as reflected by their maximal aerobic power, may be partly responsible for the variations observed. No correlation was found between maximal aerobic power and glucose MCR among the trained individuals. However, other factors such as the activity of enzymes involved in glucose metabolism and the concentration of muscle and liver glycogen, are affected by training and should be considered in relation to insulin and glucose activity.

The in-vivo insulin resistance as reflected by the tissues' ability to take up glucose, could be affected at three sites of the hormonal mechanism: secretion of insulin, insulin interaction with receptors on the target cell and at steps distal to receptor binding. Changes in any of these sites will alter the rate of glucose uptake by the tissue.

When trained individuals were studied 12 hours after their last exercise session, their fasting values for glucose and insulin as well as their insulin glucose ratio, were indistinguishable from sedentary controls. However, during a glucose clamp technique, they exhibited a significantly higher ability to metabolize glucose compared to untrained individuals (Fig. 5). This indicates that tissue sensitivity and/or tissue responsiveness to insulin rather than the secretory mechanism, underly the enhanced glucose MCR in endurance athletes.

Thus, receptor and/or post-receptor events affected by physical training are the probable source of changes of insulin resistance in this group.

By 60 hours after the last training session, trained athletes lost about 50% of their initial capacity to take up glucose at the same insulin concentration and by 7 days, glucose MCR decreased significantly to values comparable to controls (Fig. 5). Insulin concentration did not change in any of the trained subjects by 60 hours of detraining, indicating that the insulin secretory mechanism did not respond to cessation of training at that phase. Insulin binding to erythrocytes decreased significantly within this time period and may partly explain the diminished clearance rate of glucose. Although erythrocytes are not the major target for insulin to bind, they are considered to reflect variations in other target tissues, thus, representing the overall changes in insulin binding (Pedersen et al. 1980).

By 7 days of detraining basal insulin concentration increased considerably in 3 out of 5 subjects resulting in a 53% increase in the mean plasma insulin in this group. This change suggests that certain adaptations of the β cells may have taken place upon cessation of training. The slow rate of insulin secretion which has been found to result from physical training (Richard & LeBlanc 1980, Galbo et al. 1981), was altered in some of the trained individuals within 7 days of detraining.

Since a fall in percentage of insulin binding to erythrocytes was observed in all subjects by 60 hours of detraining, whereas a change in insulin concentration was observed only in some

athletes by 7 days of detraining (Fig. 7), it is suggested that changes in the secretory mechanism are secondary to peripheral adaptations which involve receptor and post-receptor steps. The rise in insulin levels may be interpreted as a β cell response, compensating for a loss in receptor number. Alternatively, it may be induced by a change in the CHO balance due to a low energy expenditure upon cessation of training. An increased insulin secretion in response to a high CHO diet has been reported by Saekow and Olefsky (1980) however, it was not accompanied by an impaired glucose tolerance.

According to the present study, no conclusions can be made regarding events distal to receptor interaction, but combining the data associated with pre-receptor and receptor alterations, the following assumptions can be made: glucose MCR decreased by 64% within 48 hours whereas the fall in insulin receptor binding did not exceed a mean value of 21%. Knowing that a maximal intracellular response to insulin is achieved when only 10-20% of cell receptors are occupied (Olefsky and Kolterman 1981, Rizza et al. 1981) and that insulin concentration remained stable during this time period, intracellular changes may have contributed to the diminished rate of glucose clearance by 60 hours after cessation of training. Alternatively, this fall in MCR may be explained by an unlinear relationship between receptor binding and the rate of glucose clearance.

Both muscle and hepatic cells may respond to physical training by an enhanced ability to take up glucose. Galbo and associates (1981) found liver weight and liver glycogen concentration to be higher in trained compared to sedentary rats. Mondon and co-workers (1980), studying glucose uptake by perfused muscle and liver,

suggested the muscle rather than the liver to be the site of enhanced insulin sensitivity in trained animals.

Considering the obvious limitations of studying liver adaptations to exercise in man and the variability that may exist between the human and animal models, final conclusions can not be made, as to whether it is the muscle or the liver that mainly responds to physical training. However, knowing that the daily intensity and duration of training of the subjects in the present study was approximately 1 - 1-1/2 hour at 75% $\dot{V}O_2$ max. it is likely that muscle and not liver glycogen stores are reduced at the end of each training bout. Thus, it is assumed that in these athletes, muscle rather than hepatic tissue was the site of enhanced insulin sensitivity and responsiveness.

The present study reported changes in insulin activity in endurance athletes upon cessation of training and indicated the sites of the hormonal mechanism that underwent alterations within 7 days of detraining.

The mechanism(s) responsible for the changes observed is still unknown but possible explanations will be discussed in the following section:

- (a) Maximal aerobic power - The possibility that the reduction in glucose MCR in trained athletes by 60 hours and 7 days of detraining was due to changes in their maximal aerobic power can be excluded in view of evidence showing a decrease of only 6% in $\dot{V}O_2$ max. in young healthy subjects following a complete bed rest for 7 days (Friman 1979). Conversely, subjects in the present study refrained from training, yet maintained their daily

activities during the experimental period, thus, any change in aerobic power in their case would be negligible.

- (b) Body composition - Minor alterations in body composition may have occurred with detraining, but since none of the methods for determining body fat is sensitive enough, these changes could not be detected; thus, the possibility that adiposity changes did affect insulin action can not be entirely excluded. However, since body weight did not change within the 7 days of detraining and since body composition was not considered as a key factor that determined a high MCR in the trained group on their initial test, it was concluded that changes in insulin activity were not due to a rise in either body weight or adiposity.

In view of the findings that the high insulin sensitivity and responsiveness of endurance trained athletes disappeared shortly after the last exercise session, it may be related to physiological parameters that undergo rapid changes with varying treatments. Among these, activity of enzymes which are involved in glucose metabolism as well as glycogen synthesis should be considered.

- (c) Enzymatic activity - Since changes in enzymatic activity were not examined in this study, no suggestions can be made with regard to this possibility. Other studies reported higher glycogen synthase in trained compared to untrained rats (Galbo et al. 1979) and an increase in the activity of glycolytic enzymes (hexokinase, phosphofructokinase) after exercise training in normal and diabetic animals (Ianuzzo et al. 1982). According to these observations, enzymatic

activity may be partly responsible for the variability in plasma glucose clearance rate observed in trained individuals.

- (d) Glycogen concentrations - It is well known that glycogen concentration is considerably higher in endurance trained athletes than in controls (Gollnick et al. 1973) and may be a key factor responsible for the rapid changes in clearance rate of glucose in the former. Since it appears that glycogen level affects its own rate of synthesis (Cohen et al. 1979, Fell et al. 1982), glycogen depletion could be expected to result in an enhanced rate of glucose uptake.

The initial determination of insulin resistance in the trained individuals was carried out 12 hours after a bout of exercise, which was of sufficient intensity and duration to reduce muscle glycogen considerably and was followed by an overnight fast. Evidence suggests that within 24 hours, muscle glycogen stores are repleted (MacDougall et al. 1977) when liver stores remain intact. If both muscle and liver stores are depleted, recovery will be completed by 48 hours (Piehl 1974, Costill et al. 1981). Thus, the high MCR of glucose observed 12 hours after an exercise session may be explained by low glycogen concentration that enhanced glucose uptake by the tissue. Forty eight hours later, when glycogen stores were recovered, the high tissue capacity to take up and metabolize glucose was diminished and by 7 days of detraining it achieved values similar to controls.

In order to examine the hypothesis that glycogen levels determined glucose uptake, changes in glucose MCR with detraining were studied in one subject while maintaining a regular diet and

again while CHO content in the diet was minimal. The change in diet was in an attempt to keep muscle glycogen stores depleted by 60 hours of detraining. Under these conditions, the initial assessment of MCR was carried out 12 hours after a bout of exercise (a 10 mile run) and was followed by an overnight fast and a low CHO diet (50g/day) for 48 hours, when glucose MCR was reassessed. In order to maintain an equal caloric intake with low CHO and with regular diet, the content of fat and protein in the diet was increased (For diet content see Appendix V).

Hypothesizing that muscle glycogen content affects glucose uptake by the tissue, glucose MCR was expected to remain high when cessation of training was accompanied by CHO restriction.

Surprisingly, the rate of glucose clearance decreased to about 25% of the initial value and presented the greatest change obtained among the trained athletes. However, this observation does not necessarily exclude glycogen content from being responsible for changes in glucose uptake. A number of explanations may be given for these results:

- (a) Studying muscle glycogen repletion with different diets, Fell and co-workers (1980) reported glycogen accumulation in muscles of exhausted rats in spite of CHO starvation. Thus, the assumption that glycogen concentration remained low at the second assessment in the present study may have been incorrect. However, if this was the case, there is no explanation for a greater fall in MCR with a low CHO compared to a regular diet with detraining.

- (b) With the glucose clamp technique on the initial test, glucose was infused at an average rate of 0.57 ml per min. during 116 minutes. The total of 66 ml glucose infused may supply part of the CHO requirements for glycogen recovery. However, under fasting conditions it is unlikely that the muscle rather than the brain will be the preferable organ to consume the glucose infused. In addition, the 66g CHO supplied during the test brought the total CHO consumption on the first day of diet restriction to about 120 grams. Since brain metabolizes 150g glucose/day (Costill et al. 1981, Stryer 1981), the glucose pool would still have remained below basal requirement and therefore complete glycogen repletion is unlikely to occur.
- (c) As previously mentioned, fat content in the diet was increased in order to maintain an isocaloric diet. A high fat diet is known to induce tissue resistance to insulin (Saekow and Olefsky 1980) and thus may override the enhancing effect of glycogen depletion on glucose uptake and result in a decrease rather than an increase in glucose clearance rate.

On the grounds of one subject tested and with no direct determination of glycogen levels by a muscle biopsy, no definite answer can be given for the results observed. For reasons previously discussed, the first two explanations for a high insulin resistance when detraining was accompanied by a low CHO diet, can be eliminated. Alternatively, it is suggested that the high fat content in the diet caused tissue resistance to insulin, overriding the effect of low glycogen concentrations on glucose uptake.

Summary:

Results of the present study indicate an enhanced clearance rate of glucose in endurance trained athletes compared to sedentary controls. Their high capacity to metabolize glucose appeared to be due to high tissue sensitivity and tissue responsiveness to insulin, since neither insulin concentration nor plasma glucose differed significantly between the groups.

When trained individuals refrained from training for 60 hours or for 7 days, their enhanced capacity to take up glucose diminished and their values for glucose clearance rate were indistinguishable from untrained controls.

The change in glucose uptake was accompanied by a significant decrease in insulin binding to erythrocytes, possibly due to a loss of receptor number. A rise in basal insulin concentration was observed in a number of subjects by 7 days of detraining and is suggested to appear secondary to peripheral alterations.

Although this study did not aim to examine the mechanisms underlying the changes observed, it is suggested that muscle and perhaps liver glycogen levels play a major role in determining glucose uptake by the tissues. Further investigation is required to confirm this hypothesis.

While the mechanism is still unclear, it is indicated by the present results that high tissue sensitivity and responsiveness induced by physical training disappear shortly after cessation of training; thus it is suggested to be an acute effect of the last exercise bout rather than a chronic effect of training.

Recommendations

- 1) In order to assess the acute effect of exercise on insulin sensitivity, sedentary subjects should be tested for glucose MCR at 12 - 16 hours after an exhaustive bout of exercise, for comparison with highly trained athletes.
- 2) To investigate the role of muscle glycogen concentration on insulin sensitivity, glucose MCR should be assessed 12 - 16 hours after an exhaustive exercise. Direct measurements of glycogen levels by a muscle biopsy are required to confirm glycogen depletion. During an experimental period of 48 hours, subjects should refrain from training while they keep a low CHO diet to maintain glycogen stores depleted. At 60 hours of detraining, both glucose MCR and muscle glycogen concentration should be reassessed.

If muscle glycogen level is a key factor in determining insulin sensitivity, subjects will maintain a high rate of glucose clearance on the second test regardless of their state of training.

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CONSENT FORM - INSULIN INFUSION STUDIES

Purpose: The goal of the study is to see if insulin works more efficiently in aerobically trained subjects, and if the apparent intensified effect disappears abruptly after detraining.

Experimental Protocol: The more detailed aspects are outlined in the protocol titled "Insulin Resistance Test". In essence, insulin will be infused intravenously at a steady rate, and glucose will then be given to maintain the blood glucose concentration at its starting level. The quantity of glucose infused per minute is directly related to the insulin resistance. Blood glucose will be measured every 5 minutes.

Adverse Effects: There is a remote chance that the site of the I.V. may become infected or obstructed. A low blood sugar may occur but the likelihood is very low because glucose is infused, glucose is measured every 5 minutes, and a physician will be continually present.

The details of the procedure have been explained to my satisfaction by _____.

I understand that other than the honorarium offered, I stand to gain nothing from this experiment. My current or future medical care will not be affected by refusal to participate in this experiment which I may do at any time.

No details of this study will be released, except in an anonymous way in the form of a scientific publication.

I hereby agree to be a subject in this study.

Date

Signature

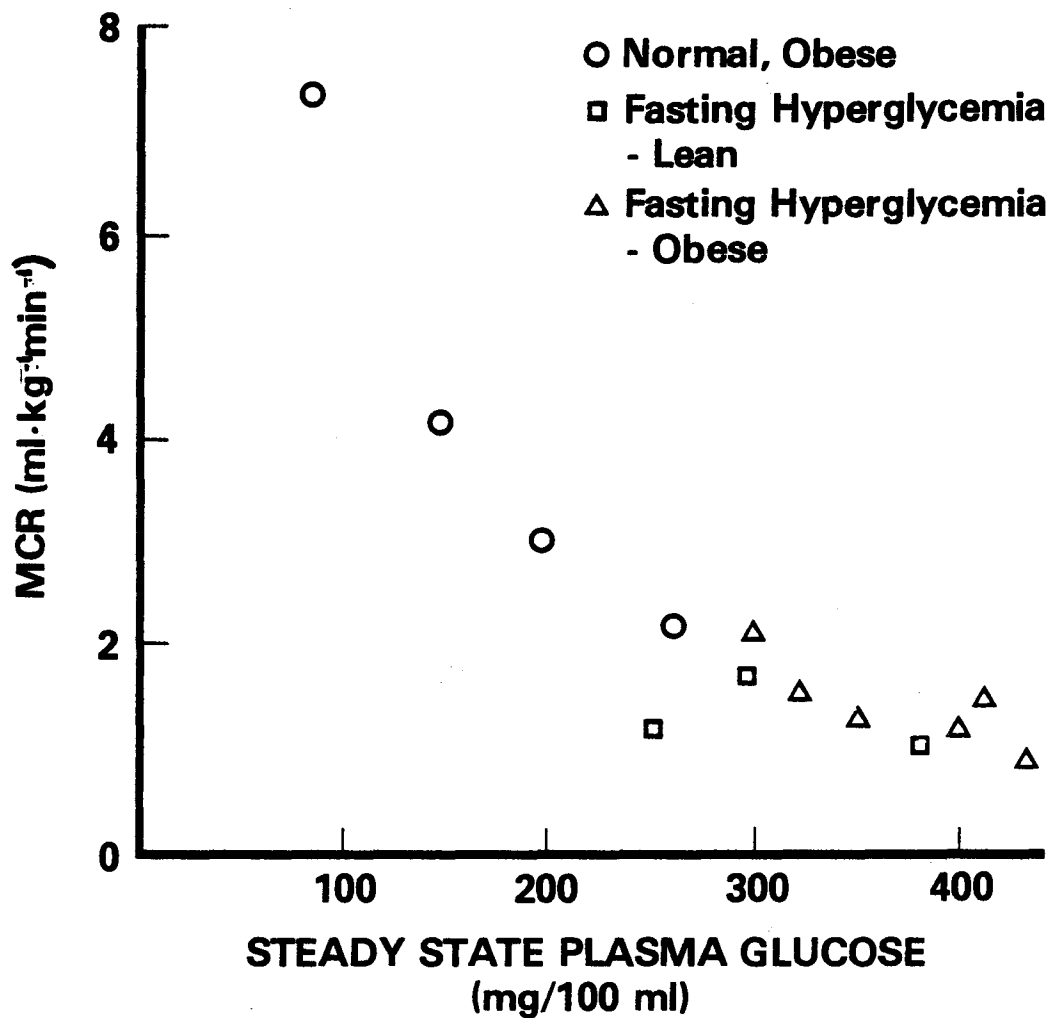
Witness

I have explained the nature of the study to the patient and believe he/she has understood it.

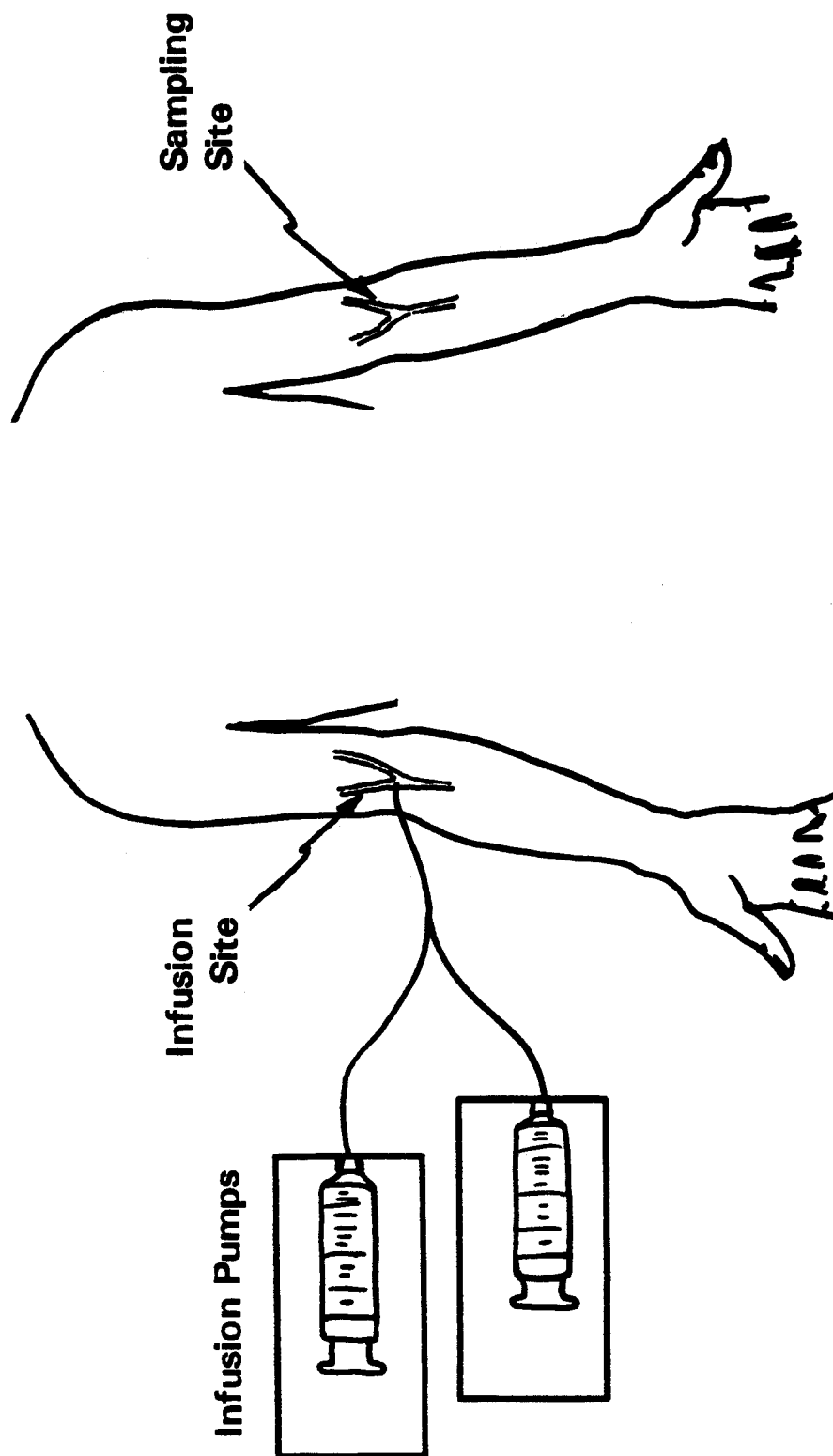
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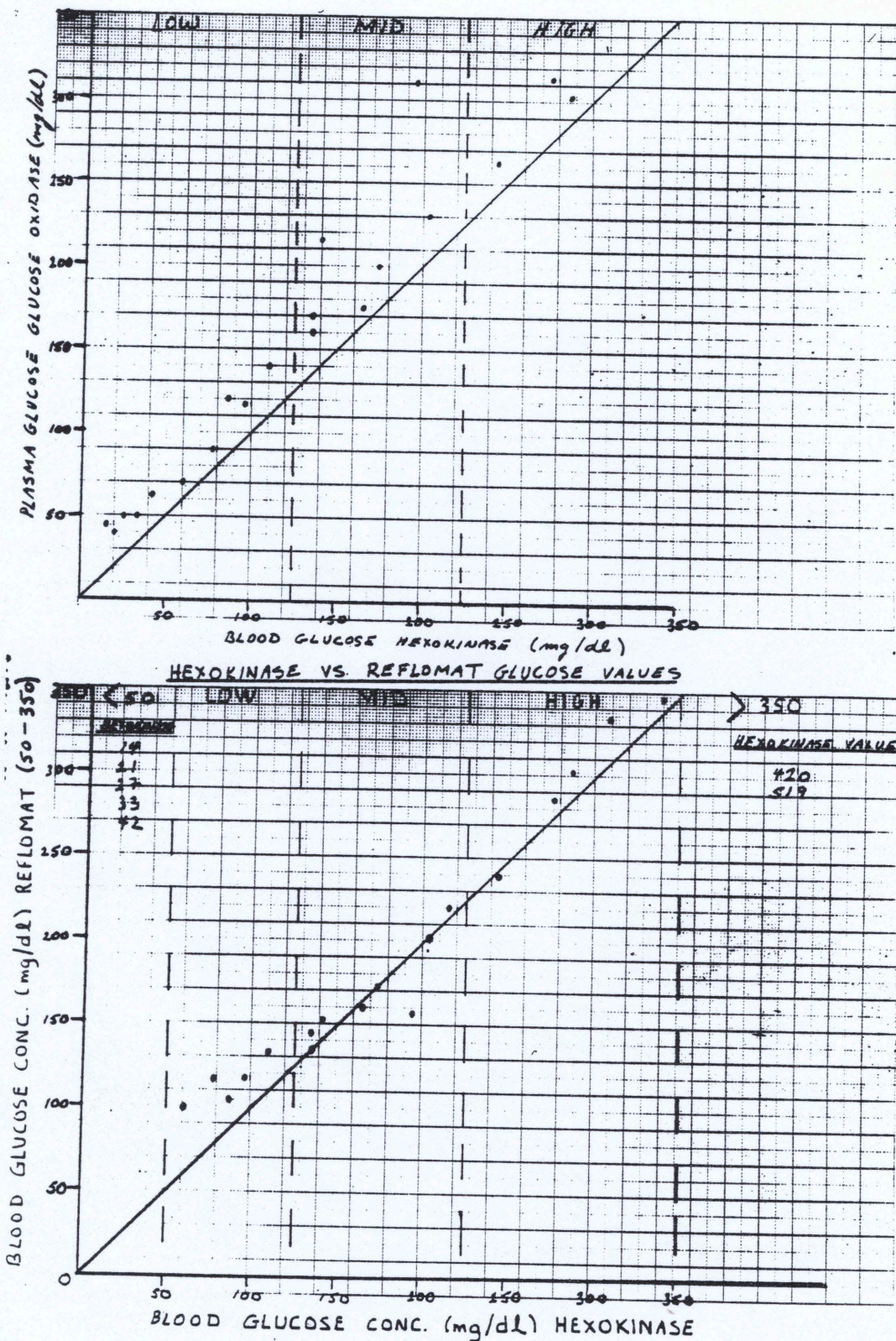
APPENDIX II: Values for glucose MCR in different populations
(From: Greenfield et al., Diabetes 30, 387-392, 1981)



APPENDIX III: Illustration of glucose clamp technique.



APPENDIX IV: Validity of Equipment.



APPENDIX V: Typical daily caloric intake and dietary composition for trained athletes during training (Tr) and detraining (Dtr) period.

Subject	kcal		Protein(g)		Fat(g)		CHO(g)	
	Tr.	Dtr.	Tr.	Dtr.	Tr.	Dtr.	Tr.	Dtr.
W.B.	2370	1745	97	65	92	52	279	260
P.M.	1891	1778	91	101	97	89	197	165
H.W.	4570	2130	217	95	241	95	324	258
B.C.	2012	2013	100	113	90	65	244	245
C.C.	1505	1600	52	49	32	88	270	163
x+S.E.	2469 ₊₅₅₁	1853 ₊₉₇	111 ₊₂₈	84 ₊₁₂	110 ₊₃₅	78 ₊₈	263 ₊₂₁	218 ₊₂₃
C.C.*		1827		105		124		43

* Denotes a low CHO diet during detraining period.

APPENDIX VI: Blood glucose, glucose infusion rate*, MCR, insulin and C-peptide values at a steady state phase during the glucose clamp technique.

Subject	Time (min)	Blood glucose (mg/100 ml)	Infusion rate (ml/min)	MCR (ml/kg/min)	Insulin (μ U/ml)	C-peptide (pmol/L)
Trained group						
H.W. (T_0)	T_0	60			5.5	186
	T_{80}	61	0.429			
	T_{90}	50				
	$T_{90:30}$		5.73			
	$T_{92:30}$		0.573			
	T_{98}	50			50.7	<100
	T_{103}		0.862			
	T_{108}	45				
	T_{114}		1.15			
	T_{116}	50	1.75			
	T_{117}		5.73			
	T_{122}	73			50.7	<100
			$\bar{x} = 1.582$	16.8		
H.W. (T_I)	T_0	59			6.2	122
	T_{90}	78	1.15		51.4	<100
	T_{96}		0.862			
	T_{100}	72				
	T_{104}		0.573			
	T_{108}	72				
	T_{112}		0.429			
	T_{114}	65				
	T_{119}	65			50.7	<100
			$\bar{x} = 0.738$	7.9		
H.W. (T_{II})	T_0	55			8.3	186
	T_{101}	62	0.862		56.2	167
	T_{114}	70				
	T_{117}		0.573			
	T_{125}	65				
	T_{129}	50			52.1	<100
			$\bar{x} = 0.738$	8.5		

Subject	Time (min)	Blood glucose (mg/100 ml)	Infusion rate (ml/min)	MCR (ml/kg/min)	Insulin (μ U/ml)	C-peptide (pmol/L)
P.M. (T_0)	T_0	50			4.2	<100
	T_{30}	45	0.229		52.1	<100
	T_{38}		0.573			
	T_{45}	45				
	T_{49}		1.15			
	T_{63}	45				
	T_{67}		1.72			
	T_{73}	45			56.9	<100
	T_{80}		2.29			
	T_{92}	50			54.2	<100
			$\bar{x} = 1.274$	22.4		
P.M. (T_I)	T_0	61			3.5	<100
	T_{55}	50	0.429			
	T_{56}		0.573			
	T_{67}	50				
	T_{73}		0.862			
	T_{74}	45			56.9	<100
	T_{79}		1.15			
	T_{90}	45				
	T_{93}		1.72			
	T_{95}	45				
			$\bar{x} = 0.872$	12.7		
P.M. (T_{II})	T_0	75			4.2	<100
	T_{22}	61	0.573			
	T_{29}	57			50	<100
	T_{37}	45				
	T_{40}		0.862			
	T_{47}	50				
	T_{50}		1.15			
	T_{58}	45				
	T_{65}	50	1.72		59.7	<100
			$\bar{x} = 0.837$	9.8		

Subject	Time (min)	Blood glucose (mg/100 ml)	Infusion rate (ml/min)	MCR (ml/kg/min)	Insulin (μ U/ml)	C-peptide (pmol/L)
R.W. (T_0)	T_0	75			3.5	<100
	T_{100}	90	1.15			
	T_{105}		0.862			
	T_{112}	69			50.0	<100
	T_{116}		1.15			
	T_{123}	88				
	T_{126}		0.862			
	T_{132}	75				
	T_{137}	75			59.7	<100
			$\bar{x} = 0.979$	9.7		
R.W. (T_I)	T_0	70			4.2	<100
	T_{35}	70	0.766			
	T_{51}	74				
	T_{62}	60			59.7	<100
	T_{70}		0.958			
	T_{90}	61				
	T_{106}	78				
	T_{118}	78				
			$\bar{x} = 0.997$	10.5		
R.W. (T_{II})	T_0	75			9.7	<100
	T_{34}	65	0.573			
	T_{39}		0.766			
	T_{44}	65				
	T_{53}	65				
	T_{57}		0.862			
	T_{61}	60			77.0	<100
	T_{67}		1.15			
	T_{72}	67				
	T_{80}	65				
	T_{89}	70			73.6	<100
			$\bar{x} = 0.919$	8.8		
C.C. (T_0)	T_0	58			4.2	<100
	T_{87}	52	1.15			
	T_{104}	60			41.0	<100
	T_{121}	60			41.0	<100
			$\bar{x} = 1.15$	16.5		

Subject	Time (min)	Blood glucose (mg/100 ml)	Infusion rate (ml/min)	MCR (ml/kg/min)	Insulin (μ U/ml)	C-peptide (pmol/L)
C.C. (T_I)	T_0	61			5.5	246
	T_{33}	45	0.429		37.0	249
	T_{37}		0.573			
	T_{43}	50				
	T_{52}	59				
	T_{60}	45				
	T_{64}		0.862			
	T_{70}	45			47.9	< 100
	T_{74}		1.15			
	T_{86}	45				
			$\bar{x} = 0.682$	9.3		
B.C. (T_0)	T_0	60			6.9	< 100
	T_{26}	55	0.429			
	T_{30}		0.573			
	T_{31}	50			50.7	< 100
	T_{38}		0.862			
	T_{40}	55				
	T_{43}		1.15			
	T_{45}	50				
	T_{49}		1.71			
	T_{50}	55				
			$\bar{x} = 0.795$	12.4		
B.C. (T_{II})	T_0	65			5.5	< 100
	T_{60}	52	0.429			
	T_{71}		0.573			
	T_{73}	63				
	T_{65}					
	T_{97}	58			60.4	< 100
			$\bar{x} = 0.53$	7.4		
W.B. (T_0)	T_0	60			7.6	< 100
	T_{30}	60	0.46			
	T_{44}	45				
	T_{50}		0.92			
	T_{51}	50				
	T_{55}	48				
	T_{58}		2.29			
	T_{60}	50				
	T_{65}	60			55.5	< 100
			$\bar{x} = 0.878$	15.9		

Subject	Time (min)	Blood glucose (mg/100 ml)	Infusion rate (ml/min)	MCR (ml/kg/min)	Insulin (μ U/ml)	C-peptide (pmol/L)
W.B. (T_{II})	T_0	60			12.5	<100
	T_{48}	70	0.429			
	T_{54}	70				
	T_{60}	60			66.6	126
	T_{66}		0.573			
	T_{69}	68				
	T_{75}	60				
	T_{80}	68				
	T_{85}	65				
			$\bar{x} = 0.503$	7.3		
Untrained group						
J.D. (T_0)	T_0	62			7.6	132
	T_{24}	62	0.573			
	T_{37}	45			52.7	159
	T_{45}		0.764			
	T_{49}	50				
	T_{54}		1.432			
	T_{62}	50			70.1	160
			$\bar{x} = 0.792$	10.2		
J.D. (retest)	T_0	65			6.2	180
	T_{40}	48	0.382		57.6	
	T_{44}		0.764			
	T_{50}	45				
	T_{53}		1.432			
	T_{67}	51			65.3	
			$\bar{x} = 1.034$	12.6		
A.J. (T_0)	T_0	74			4.8	
	T_{100}	70	0.143			
	T_{105}	50				
	T_{109}		0.382			
	T_{113}		0.286			
	T_{115}					
	T_{119}		0.764			
	T_{123}	50				
	T_{132}				44.4	
			$\bar{x} = 0.509$	5.9		

Subject	Time (min)	Blood glucose (mg/100 ml)	Infusion rate (ml/min)	MCR (ml/kg/min)	Insulin (μ U/ml)	C-peptide (pmol/L)
A.J.(retest)	T ₀	65			6.2	
	T ₆₄	86				
	T ₆₈		0.509			
	T ₈₁	89			50.0	
	T ₈₅		0.382			
	T ₉₅	70				
	T ₁₀₉	80				
			$\bar{x} = 0.447$	6.0		
C.B. (T ₀)	T ₀	65			6.9	290
	T ₂₂	76	0.764			
	T ₂₆		0.573			
	T ₃₄	71				
	T ₄₈	68			54.2	240
			$\bar{x} = 0.602$	7.3		

* refers to total solution containing 50% Dextrose in water.