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THE EFFECT OF EXERCISE INTENSITY
ON POST-EXERCISE INSULIN-BINDING RESPONSES
IN MALE INSULIN-DEPENDENT DIABETICS

By

Gayle Ekstrand

A Thesis Presented to the School of Graduate Studies

in Partial Fullfillment of the Requirements

for the Degree of Master of Science in Kinanthropology

University of Ottawa

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CHAPTER I

THE PROBLEM

Introduction

Growing awareness of physical conditioning and its role in health and disease has rekindled interest in the use of exercise as a tool in the treatment of diabetes. While regular activity has long been encouraged among diabetics, the rationale underlying this recommendation has only come to light in more recent years.

Physical activity has many facets (i.e. intensity, duration, format...) and as a result, there are many varied physiological responses. Generally exercise enhances energy demands, fuel requirements rise, and neuroendocrine processes trigger the adaptations required to maintain overall metabolic homeostasis. Insulin plays a critical role in this regard, for it must balance the consequences of the counter-regulatory or "diabetogenic" hormones. In both healthy and diabetic individuals an appropriate concentration of insulin must be present in order to regulate the fuel fluxes (i.e. hepatic glycogenolysis, gluconeogensis, and lipolysis) and to facilitate muscle uptake of blood glucose. Thus it is not surprising that insulin availability and diabetes control are important determinants of the metabolic outcome of acute exercise in insulin-dependent subjects (Koivisto and Sherwin, 1979).

It is now established that blood glucose concentrations in well-controlled insulin-dependent diabetics fall during exercise, and that physical activity diminishes insulin requirements and improves glucose tolerance (Pedersen et al., 1980; Vranic and Berger, 1979).

These effects have been attributed primarily to an increased uptake of glucose by peripheral tissue (Vranic and Berger, 1979), but the cellular mechanisms are not entirely understood.

Recent advances in the techniques of radioreceptor assays have permitted the study of the insulin receptor on both target (muscle, liver, and adipose) and non-target (lymphocyte, monocyte, and erythrocyte) tissues. Although the receptor for insulin has yet to be fully characterized, it does seem that both target and non-target tissue receptors have many properties in common (Kappy et al., 1979). As a result, much of the human research undertaken has investigated ^{125}I-insulin binding to monocytes or erythrocytes in the hope of establishing some relationship between insulin binding and sensitivity. Indeed, in several clinical experiments, changes in cellular binding of insulin have correlated with alterations in insulin sensitivity and glucose tolerance (Harrison et al., 1976; Olefsky and Reaven, 1977; Defronzo et al., 1978; Beck-Nielsen and Pederson, 1978b). Furthermore, data obtained from healthy subjects (Soman et al., 1978; Koivisto et al., 1979; Polychronakos et al., 1982), and insulin-dependent diabetics (IDD) (Iwasaki et al., 1982; Pederson et al., 1980) suggest that binding to monocytes and/or erythrocytes is altered by acute exercise. Although Michel et al. (1984) have recently examined the role of exercise intensity in the modulation of insulin binding in healthy males, the response of diabetics still warrants investigation (Iwasaki et al., 1982). In addition, the duration of the post-exercise consequences has not been systematically studied in IDD. Soman et al. (1978) reported that insulin binding in healthy individuals returned to

baseline levels by 24 hours post-exercise, but interim values were not available. Detraining effects on binding in athletes have been observed between 12 and 60 hours post-exercise by Polychronakos et al. (1982). Although Caron et al. (1981) did examine the post-exercise fall in glycemia in IDD, they did not study the potential insulin-binding changes. Thus little is known of the post-exercise insulin-binding responses in IDD.

While it is generally agreed that the intensity and duration of physical exertion should be adjusted to the overall health of the diabetic, more precise recommendations must await new developments in our understanding of exercise and diabetes. In particular, there is a need among IDD for a a more systematic comparison of exercise intensity effects, a more physiological approach to studying exercise, an investigation into the duration of the post-exercise consequences and additional research into the exercise-related changes at the level of the insulin receptor.

Purpose

Based on the above mentioned needs, the aim of the present study is to examine and compare the effects of varying exercise intensity on the post-exercise insulin-binding and blood glucose responses in untrained male insulin-dependent diabetics. Ultimately, it is hoped that the knowledge gained may be used to provide more practical exercise guidelines for the active diabetic.

Statement of the Problem

The primary research question posed in this project is whether the insulin-binding responses of controlled insulin-dependent diabetics are different under resting conditions from those responses attained eight hours following exercise of either low or high intensity. An additional sub-problem is whether insulin binding to the entire population of erythrocytes is different from the binding to the youngest (least dense) fraction of these cells.

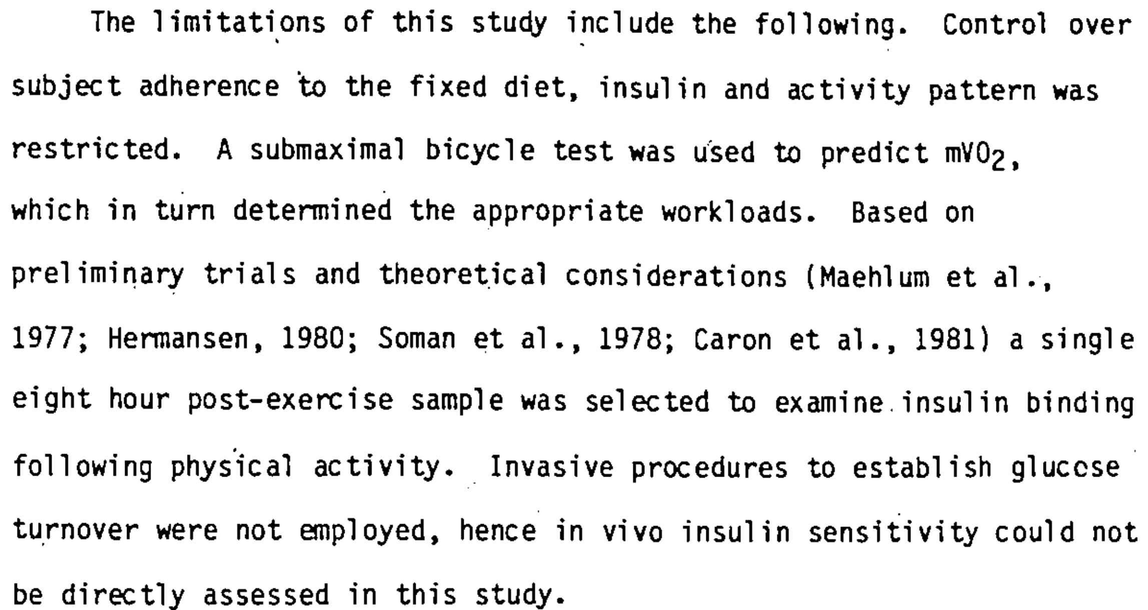
Hypotheses

It is hypothesized that the insulin-binding responses measured eight hours after the low and high intensity exercise protocols will differ significantly from that response observed under the resting condition. It is also hypothesized that the youngest fraction of erythrocytes will demonstrate greater insulin binding than the total erythrocyte population.

Scope of the Study

This study has been delimited in the following aspects. Subjects were eight untrained young adult male insulin-dependent diabetics. The low intensity exercise protocol consisted of one hour of continuous bicycling at an intensity corresponding to 45% of the predicted maximum oxygen consumption (mVO_2). The high intensity workload involved a total of one hour of intermittent two minute work/rest intervals of cycling at an intensity representing 75% mVO_2. Erythrocytes were used to monitor the ^{125}I-insulin-binding responses following physical exertion.

Limitations of the Study



The limitations of this study include the following. Control over subject adherence to the fixed diet, insulin and activity pattern was restricted. A submaximal bicycle test was used to predict mVO_2, which in turn determined the appropriate workloads. Based on preliminary trials and theoretical considerations (Maehlum et al., 1977; Hermansen, 1980; Soman et al., 1978; Caron et al., 1981) a single eight hour post-exercise sample was selected to examine insulin binding following physical activity. Invasive procedures to establish glucose turnover were not employed, hence in vivo insulin sensitivity could not be directly assessed in this study.

Abbreviations Used

The following abbreviations have been used in the text and tables:

AA	Amino acids
BG	Blood glucose
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
FFA	Free fatty acids
F fraction	Fractionated (youngest) erythrocytes
GES	Glucose electrolyte solution
GH	Growth hormone
HbA_1	Glycosylated hemoglobin A
Hmct	Hematocrit
Ht	Height
IBW	Ideal body weight
IDD	Insulin-dependent diabetes (diabetics)
KB	Ketone bodies
$m\dot{V}O_2$	Maximum oxygen consumption
NAD(H)	Nicotinamide adenine dinucleotide, oxidized form, (reduced form)
NIDD	Non-insulin-dependent diabetes (diabetics)
NSB	Non-specific binding
PG	Plasma glucose
SB	Specific binding
SD	Standard deviation
U fraction	Unfractionated erythrocytes
Wt	Weight

CHAPTER II

REVIEW OF LITERATURE

Introduction

The popularity of physical fitness in today's society has generated a parallel interest in the field of exercise and diabetes. Yet, despite ongoing research in this area, much remains to be clarified.

It is the purpose of this review to examine some of the effects of exercise upon insulin-dependent diabetes.

Diabetes

Diabetes mellitus is a disease involving at least one to two percent of the world population (Luft, 1977). In Canada there are approximately 500,000 diagnosed diabetics (Becton Dickinson Canada, 1977).

Diabetes mellitus (or simply diabetes as it will be referred to here) is characterized by deficient insulin activity and hyperglycemia. It does not appear to be a single disease for both the heredity pattern and the clinical manifestations are diverse. Consequently, diabetics are usually classified as one of two types. For those who are insulin-dependent, ketosis-prone and have an early age of onset, the etiology appears to involve a gene-virus interaction causing an autoimmune response which destroys the beta cell (Cahill and McDevitt, 1981). Thus the ability to produce sufficient insulin is lost. This describes the more severe form of the disease and is called type I, juvenile-onset, or insulin-dependent diabetes (IDD). The second category includes

diabetics who are non-insulin-dependent, are often obese, and are usually affected later in life (Cahill and McDevitt, 1981). Premature senescence of the beta cell or altered sensitivity of certain tissues to insulin are considered possible causes. This form of the disease affects roughly 80% of all diabetics (Roth, 1981) and is referred to as type II, obesity- or maturity-onset, or non-insulin-dependent diabetes (NIDD).

Current Therapy

At present there is no cure for diabetes. However, treatment comprises diet, insulin (or perhaps oral medication for NIDD), and exercise. The aim of therapy is to establish a balance of energy availability and expenditure. Metabolic control is usually assessed by the levels of glucose (BG) and ketone bodies (KB) in the blood. Ideally, diabetics should strive to maintain normal values (3.6-6.1mM and 70-250 uM respectively) (McGilvery, 1979; Hultman, 1974), although in practice this is often extremely difficult. The percentage of glycosylated hemoglobin A (HbA_1) is also used to evaluate overall diabetes control, for this measure reflects the average BG concentration during the preceding four to six weeks. Normal values for healthy adults fall between .055 - .085 g/l, whereas those of uncontrolled diabetics are in the .120 - .200 g/l range (Koenig et al., 1976).

The significance of achieving metabolic control is best understood by considering the results of inadequate regulation. Adverse reactions may include — loss of energy and strength, fluctuations in weight, hypoglycemia, hyperglycemia, dehydration, ketoacidosis, coma and

ultimately death. Although absolute proof is lacking, it is generally accepted that "poor" control promotes earlier development of the more chronic complications associated with diabetes — retinopathy, nephropathy, cardiovascular disease, and autonomic neuropathy (Kefalides, 1977). Clearly, the degree of control attained influences the quality of life experienced by the diabetic. For this reason an understanding of diet, insulin, exercise, and their relationships to each other is extremely important.

While acknowledging that all three of these aspects are essential in the treatment of IDD, this review will focus on the role of exercise and its relationship with insulin-receptor adaptation. Exercise is reviewed first, followed by an overview of insulin and insulin receptors. Studies are then presented which specifically relate exercise to insulin-receptor adaptations. The review is concluded by a brief survey of the guidelines presently recommended for the physically active diabetic.

Exercise

Although physical exercise has been considered beneficial in the treatment of diabetes for many years, diet and insulin have, until recently, received the greatest attention (Skyler, 1979). This is now changing for exercise not only promotes general health (improved cardiovascular fitness, flexibility, muscular strength and endurance...), but it specifically benefits the NIDD by favouring weight loss and increased insulin sensitivity, and the IDD, by lowering BG and insulin requirements (Koivisto and Sherwin, 1979). While evidence to support

these and other benefits of exercise for diabetics is growing, research specific to the hormonal and metabolic consequences of different forms of exercise, including appropriate controls, is still scanty (Vranic and Berger, 1979). The following review will summarize those studies of most relevance to this aspect of exercise and diabetes.

Before discussing exercise, it is necessary to distinquish between acute exertion and physical training. Acute exercise refers to short-term or isolated bouts of activity, whereas physical training implies regular activity of a long-term or chronic nature. Although this review is primarily concerned with acute exercise effects, reference to training studies will be made where appropriate.

Fuel Requirements of Acute Exercise

With the onset of acute exercise the metabolism of the active skeletal muscle becomes significant, for the energy demand of this tissue increases dramatically. In fact, muscle fuel consumption can rise seven to forty times the resting rate (Felig and Wahren, 1979). Since the glycogen and lipid stores of muscle are limited, these demands must be met by increased fuel supplies in the blood.

In non-diabetics the general fuel utilization sequence is as follows. During the initial two to three minutes of activity, muscle glycogen serves as the substrate. Then, glucose released from the liver is taken up by the muscle and metabolized either anaerobically (producing lactate), or aerobically. After roughly 10 minutes, glucose uptake by muscle will have increased 15 times above basal, and by 60 minutes it will have increased 25-fold (Hagenfeldt, 1979). As the exercise continues there is greater reliance upon FFA liberated from

adipose tissue, such that after two hours of moderate activity this becomes the major energy-yielding substrate (Vranic and Berger, 1979). Free fatty acids contribute more at the lower work intensities (Vranic and Berger, 1979). Post-exercise ketosis may develop where there is a non-equilibrium situation between FFA release and energy demand. As for the reliance upon KB and AA during exercise, both of these potential fuels play only minor roles, except perhaps under extreme conditions (Vranic and Berger, 1979). Hence BG and FFA are the major blood-borne fuels in healthy individuals.

With training there is a tendency for normal subjects to rely more upon fat than carbohydrate during mild to moderate, or endurance-type activities (Astrand and Rodahl, 1977). Since FFA are metabolized in proportion to their blood concentration, this shift may be partially related to the reduced lactate levels characteristic of trained individuals (Astrand and Rodahl, 1977). Furthermore, it has been suggested that the trained may metabolize FFA more efficiently (Rennie et al., 1974), as they have higher glycerol and lower FFA levels following acute exertion then do the untrained (Krebs et al., 1971). Changes in mitrochondrial enzyme profiles in muscle and reduced diffusion distances between capillaries and muscle cells in the trained support this possibility (Astrand and Rodahl, 1977). Finally, post-exercise ketosis is markedly decreased in the physically trained when compared to sedentary individuals subjected to the same relative workloads (Rennie et al., 1974).

In controlled diabetics (i.e. non-ketotic) the utilization of BG and FFA during moderate exercise is similar to that seen in the

non-diabetics (Wahren et al., 1978). In contrast, insulin-deficient ketotic diabetics depend significantly more upon FFA and KB (Wahren et al., 1978). This difference likely reflects the insulin-deficient state in which FFA are readily mobilized thus increasing plasma levels, and so favouring KB formation and FFA and KB oxidation. Also, the lack of insulin prevents the normal uptake and metabolism of BG. Interestingly, Wahren et al. (1978), as well as Hagenfeldt (1979), have observed that both controlled and uncontrolled diabetics use KB as a fuel during exercise, whereas non-diabetic subjects do not. The reason for this is presently unknown, but perhaps different enzyme profiles are involved.

Unfortunately, little information is available regarding fuel preferences during acute activity in trained versus untrained IDD. However, one study has examined FFA oxidation at rest in the muscle of IDD prior to and following a 10 week running program (Costill et al., 1979). The results demonstrated a 41% increase in FFA oxidation in the trained state -- similar to the findings of a non-diabetic control group. Thus training may produce parallel adaptations in fuel preference in non-diabetics and diabetics alike.

In summary, acute exercise significantly increases energy demands and fuel requirements, thereby altering the metabolic status of the active individual. To accomplish the required balance between energy consumption and fuel supply, both the neural and hormonal systems are called upon. Nervous imput not only controls muscular contraction through the somatic system, but it is also responsible for the "sympathetic drive". Stimulation of the sympathetic component of the autono-

mic nervous system facilitates the adaptation to exercise stress by increasing heart rate, stroke volume, vasodilation of blood vessels to skeletal and cardiac muscle, bronchial dilation and secretion of the catecholamines (Rothe, 1976). This stress adaptation phenomenon is somewhat generalized. Hence the second control system, the hormonal, serves to "fine tune" the balance between metabolic demand and supply by responding to acute exertion in a very precise manner. As it will be pointed out in the discussion on insulin, this can be accomplished either by variations in hormonal concentrations or by adaptations at the receptor level.

General Hormonal and Metabolic Consequences of Acute Exercise

In order to appreciate the impact and role of physical activity in the diabetic, it is important to consider briefly what occurs in the healthy individual.

In non-diabetic subjects the major hormonal alterations seen with the onset of exercise are decreased insulin secretion and increased levels of norepinephrine, epinephrine, growth hormone (GH), glucagon, and cortisol - the latter two particularly during prolonged exercise (Skyler, 1979). In contrast with the "anabolic" nature of insulin, the last five hormones listed are considered "diabetogenic". Norepinephrine enhances lipolysis and FFA mobilization (Skyler, 1979). Epinephrine stimulates muscle glycogenolysis and hepatic gluconeogensis, while also inhibiting insulin release and stimulating glucagon secretion (Skyler, 1979). Growth hormone reduces BG uptake and utilization, increases FFA release and oxidation, and promotes AA uptake and protein synthesis, while reducing protein breakdown (Guyton,

1976). Glucagon increases glycogenolysis and gluconeogensis, as well as promoting ketogensis when insulin is simultaneously decreased (McGarry and Foster, 1976). Finally, cortisol enhances FFA mobilization, KB production, AA mobilization, and gluconeogenesis, but reduces glucose utilization (McGilvery, 1979). Although each of these hormones has specific influences on fuel provision, the final outcome reflects their interaction. Thus while glucagon causes a modest rise in BG and epinephrine exerts a somewhat greater hyperglycemic effect, an increase in both produces an additive though transient rise in BG (Felig et al., 1979). Sustained glucose overproduction occurs when cortisol is added (Felig et al., 1979), or when insulin is lowered sufficiently (Vranic and Berger, 1979).

In the physically trained the hormonal response is similar to that outlined above, with a few important exceptions. Notably, the fall in insulin associated with exercise is blunted, and basal and glucose-stimulated insulin levels are markedly decreased (Vranic and Berger, 1979; Skler, 1979). Trained subjects demonstrate lower norepinephrine (Christensen et al., 1979) and glucagon levels (Astrand and Rodahl, 1977) in response to acute exercise as well.

In general, decreased insulin along with increased levels of the "diabetogenic" hormones facilitate the provision of adequate blood-borne fuels (BG, FFA) to the active tissues. The precision of this process in healthy subjects has been demonstrated by Klachko et al. (1972). In their study BG values were followed during mild (4 mph, 2½° slope) and moderate (4 mph, 5° slope) bouts of treadmill walking. During the exercise the BG levels varied minimally (dropping only .3

and .6 mM respectively). With the recovery period, (60 minutes post-exercise), BG values had returned to or slightly exceeded the resting values. Thus it would appear the more intense activity had the greater effect on metabolic homeostasis, although in both situations the BG values were held fairly constant. Hagan et al. (1979) have also confirmed these findings. Their work showed BG values which did not change during light (35% maximum oxygen consumption (mVO_2), 60 minutes) or moderate (55% mVO_2, 60 minutes) treadmill exercise. Only with incremental maximal treadmill workloads did BG rise (by 1.6 mM).

Besides one's state of fitness and the intensity of the activity, it is worth noting that pre-nutritional status also influences the metabolic outcome of acute exercise. For example, healthy individuals on a carbohydrate-rich diet will depend more upon this fuel source due to higher insulin levels, inhibited lipolysis, lower FFA concentrations, and so reduced FFA oxidation (Astrand and Rodahl, 1977).

A final aspect to be considered here and later with respect to diabetics, is the impact of physical activity on glycogen depletion and resynthesis in liver and muscle. Maehlum et al. (1977) found that following bicycle exercise at 70% mVO_2 till exhaustion, the glycogen repletion rate was greatest during the first four hours of recovery. This was paralleled by significantly elevated activity of the I (active) form of glycogen synthetase. By 12 hours of recovery, approximately 83% of the pre-exercise glycogen values had been attained. More recently Calles et al. (1983) have shown that short bouts of acute activity (11 minutes at greater than 85% mVO_2)

produced increased glucose disappearance in healthy subjects 30 minutes post-exercise, even though glycogen depletion did not occur. Thus BG is not only taken up rapidly by muscle during exercise, but during the recovery phase as well.

In concluding this section on the general consequences of acute exercise, insulin is seen to have a significant role. An appropriate insulin response permits both mobilization of fuels from liver and adipose tissue, and uptake of BG by muscle. Diabetics who lack this fine hormonal regulation, frequently experience metabolic consequences seldom encountered by the non-diabetic.

Hormonal and Metabolic Consequences of Acute Exercise In the Diabetic

As in non-diabetics the outcome of acute activity depends upon the extent of training, the duration and intensity of the exercise and the pre-nutritional state. For the diabetic, the degree of metabolic control and insulin availability (i.e. type of insulin, site of injection, time of injection...) also become vital factors.

Klachko et al. (1972) studied the BG values in normal and IDD subjects, (receiving insulin and following a prescribed diet), over a 24 - 30 hour period. During this time the subjects participated in exercise bouts of mild (4 mph, 2½° slope) and moderate (4 mph, 5° slope) treadmill walking. As noted earlier, BG dropped minimally in the controls (.3 and .6 mM respectively), but fell by 1.3 and 1.7 mM in the diabetics. Both groups showed a correlation between the magnitude of the BG drop and the initial BG concentration. Following the exercise, the BG pattern of the IDD returned to the pre-exercise slopes, but at new lower levels. Thus it seems that exercise of

moderate intensity produces a greater hypoglycemic effect than mild work in controlled diabetics.

Pruett and Maehlum (1972) also examined the influence of exercise intensity in IDD, but in this study the subjects were in a post-absorptive state and had not received their insulin. The subjects bicycled at 20, 50, 70 and 90% mVO_2 for three hours or until exhaustion. The results showed variable effects on BG at 20% mVO_2; significantly decreased BG and signs of hypoglycemia at 50 and 70% mVO_2; and elevated BG at 90% mVO_2. In this study the degree of metabolic control was not specified and the amount of residual insulin left from the last injection may have been quite variable. Thus at 50 and 70% mVO_2 the residual insulin may have been just sufficient to control hepatic glucose production, while at the same time permitting muscle uptake. At 90% mVO_2 greater stimulation of the "diabetogenic" hormones may have occurred — liberating more BG and FFA. Since FFA oxidation inhibits glycolysis and since low or negligible amounts of insulin were present, it is not surprising the net effect would be a rise in BG.

Maehlum and Pruett (1972) added to this latter study by examining the glucose disappearance rate in IDD following the three hours of cycling at 20, 50, 70 and 90% mVO_2. Glucose was infused intravenously either 15 or 60 minutes after the cessation of exercise. It was concluded that moderate to severe exercise (50, 70 and 90% mVO_2) enhanced the glucose disappearance rate. This effect persisted for at least one hour into the post-exercise recovery period and has been attributed to enhanced glycogen synthesis in liver and muscle

(Maehlum et al., 1977; Maehlum et al., 1978; Hermansen, 1980).

More recently IDD have been compared on light (35% mVO_2, 60 minutes), moderate (55% mVO_2, 60 minutes) and maximal (incremental) treadmill workloads (Hagan et al., 1979). The subjects had received their insulin eight to ten hours prior to the exercise and had followed their normal diet. Blood glucose fell by 1.6, 3.2 and 7.2 mM for the light, moderate and maximal loads respectively. As in the work of Klachko et al (1972), this experiment suggests the higher the exercise intensity, the greater the BG lowering effect in controlled diabetics.

As seen from these studies, there is only limited research explicitly comparing the acute responses of diabetics to exercise of varying intensity. Moreover, the focus of these studies has been restricted to BG metabolism during or immediately following cessation of the activity. The following investigations, although not specifically designed to compare exercise intensity, do provide some additional insight into the consequences of mild, moderate and strenuous bouts of activity.

Berger et al. (1976) examined the impact of mild endurance exercise in two groups of IDD and a control group. In the first diabetic group adequate control was maintained by providing the last long-acting insulin injection 12 hours prior to the exercise. In the second group insulin was withdrawn for 18 - 48 hours and rises in BG, FFA, KB and branched-chain AA were evident. The results showed a decline in BG in the first group, but a rise in the second — these changes being significantly correlated with the initial BG, FFA and KB levels. (Above 18.3 mM BG, 105 mM FFA, and 1.7 mM KB the bicycling was

associated with an increase in BG, whereas below these limits BG fell with exercise.) Though both diabetic groups demonstrated a rise in KB and glucagon, these increases were greater in the insulin-deficient group. Therefore it was concluded that in insulin-deficient states, exercise has adverse metabolic effects upon IDD. Similar results were obtained by Berger et al. (1977) when they re-examined the effects of prolonged mild exercise in relation to metabolic control in IDD. A moderately-controlled (MC) diabetic group received two-thirds their normal insulin dose 16 hours before exercising, while the ketotic diabetics (KD) had their insulin withdrawn 18 - 48 hours prior to the test. A healthy control group was also included. Bicycling was performed after an overnight fast and lasted three hours at a load requiring 30 - 40% mVO_2. The results revealed a decreased BG after 30 and 60 minutes in the MC and control groups respectively, but a significant rise in the KD group during the first 90 minutes. This datum, like that presented above, demonstrates a rise in BG with exercise when initial BG is high (exceeding 18 mM) and a fall when there is adequate BG control. Glycerol and FFA rose in all groups, but most notably in the KD group. KB rose gradually in the control and MC groups, reaching significant levels only after 150 minutes, whereas the KD group demonstrated a rapid initial increase. Both diabetic groups had higher lactate and lactate-to-pyruvate ratios than did the controls. It was concluded that the hypoglycemic effect of exercise is dependent on the presence of at least some insulin. The hyperglycemia seen in the KD group probably developed because of decreased BG uptake and over-production resulting from a lack of adequate insulin. In the

final analysis; it seems that even mild exercise can be disadvantageous to ketotic "relatively insulin-deficient" IDD.

Although the preceding studies provide information as to the consequences of mild exercise in IDD and they do underline the significance of adequate metabolic control (BG less than 14-17 mM and the absence of ketosis (Skyler, 1979)), the practical implications for active diabetics are limited. This is because typical diabetics do not intentionally withhold their insulin or dietary requirements prior to engaging in physical exertion. One study which has attempted to overcome this situation and still examine the role of metabolic control, had IDD cycle at roughly 33% mVO_2 for 20 minutes, two to three hours after receiving their insulin and breakfast (Locard et al., 1979). Based on pre-exercise BG values, the diabetics were split into high (greater than 7.8 mM) and low (less than 5.6 mM) BG groups. With exercise the high group showed a significant fall in BG during the cycling and up to 60 minutes post-exercise, while the low group showed a non-significant drop. Thus it seems that for diabetics who have received their normal insulin dose, yet still have higher than normal blood sugars, mild exercise has a beneficial hypoglycemic effect. Note this is in marked contrast to diabetics who are insulin-deficient. Tamborlane et al. (1979) account for this differential response to mild exertion by an exaggerated secretion of GH and norepinephrine in uncontrolled diabetes.

Studies which have investigated the outcome of moderate intensity exercise in diabetics are as follows. Sestoft et al. (1977) studied insulin-deprived IDD who bicycled 35 minutes at 50% mVO_2 and found

that glucagon, gluconeogenesis, ketogenesis and lactate-to-pyruvate levels were all elevated compared to a non-diabetic control group. On-the-other-hand, Oberdisse, Woweries and Weber (1977) examined IDD who had received their normal insulin and dietary requirements and who then participated in moderate bicycle exercise (heart rate of 144-150 bpm) for 60 minutes. Blood glucose dropped significantly within 30 minutes, but tended to rise during the recovery period in both the IDD and a non-diabetic control group. The concentration of FFA remained fairly constant in the IDD group, but rose gradually during the exercise in the control group. Both diabetics and controls showed a small FFA rise during the first 10 minutes of recovery. Glycerol levels rose with exercise and then fell during the post-exercise period in both groups, but this pattern was significantly more pronounced among the control subjects. Lactate levels rose initially in both the control and diabetic group and then gradually declined in a parallel fashion. Finally, GH levels rose and peaked at 30 minutes for the diabetics and at 45 minutes for the non-diabetics, with baseline values returning 30 minutes into the recovery phase. The FFA and glycerol results imply reduced lipolysis among the diabetics — perhaps the outcome of peripheral hyperinsulinemia. It was concluded that except for this latter difference, the metabolic changes induced by moderate exercise were comparable for healthy subjects and well-controlled diabetics.

Kemmer et al. (1979) also reviewed the impact of moderate exertion on IDD and control subjects. As in the last study (Oberdisse et al., 1977), the diabetics had received their insulin. Both the IDD and the

control group bicycled intermittently for half-an-hour at a workload sufficient to maintain a heart rate between 120 - 130 bpm. Contrary to the findings of Sestoft's research team (1977), glucagon did not change with exercise in either group. However, in this study the exercise stress was somewhat less than that described in the work of Sestoft et al. (1977). In the present study it was also observed that the KB climbed significantly in the diabetics (by .05 mM in the first 10 minutes) — roughly double that seen in the controls. This may be explained by the fact that although insulin was administered to the diabetics, the control was not ideal (BG values ranged from 3.9 - 15.6 mM). This again underlines the importance of insulin in exercise, especially that involving diabetics.

Investigations of strenuous activity in IDD are not nearly as common as those using mild or moderate workloads due to the ethical considerations, and the result has been a shift towards the use of animal models. Vranic and Kawamori (1979) examined the role of insulin during 75 minutes of strenous treadmill running (100m per minute, 10-12° slope) in intact, depancreatized insulin-deprived and depancreatized insulin-supplied dogs. Glucose turnover was measured by a tracer infusion technique. In the depancreatized dogs maintained on long-acting insulin BG fell in proportion to the pre-exercise plasma concentrations. Plasma insulin increased and was greater than that seen in the intact dogs. This was attributed to rapid mobilization of the injected "depot" insulin. Glucose production rose minimally in the injected dogs, while the metabolic clearance of glucose was similar to that of the intact group. It was concluded that hyperinsulinemia

produces hypoglycemia by inhibiting hepatic glucose production (gluconeogenesis and glycogenolysis) and not by substantially increasing glucose uptake by muscle. Although BG uptake by muscle was dependent upon the presence of insulin, the rate of BG disposal by this means was already near maximal at relatively low insulin concentrations (Vranic and Kawamori, 1979). This effect of insulin mobilization, hyperinsulinemia and resulting hyperglycemia has also been reported by Koivisto and Felig (1977; 1978) who conducted studies examining this same phenomenon in IDD. Using ^{125}I-insulin and external gamma counting over the injection sites, it was found that even moderate (75 - 100 watts) intermittent bicycling was capable of promoting insulin mobilization from the active limb (i.e. leg) and of producing hypoglycemia.

Summary

In view of these studies it is apparent that few researchers have systematically examined the effects of varied exercise intensity. Among those who have (Klachko et al., 1972; Pruett and Maehlum, 1972; Maehlum and Pruett, 1972; Hagan et al., 1979) BG responses suggest that the more intense the exercise the more marked the hypoglycemic effect among well-controlled IDD. However, as little attempt to equate the total energy expenditure of the different workloads was made in these investigations, conclusions regarding the effects of intensity per se must be made with reserve. It should also be recognized that with few exceptions (Klachko et al., 1972; Hagan et al., 1979; Locard et al., 1979; Oberdisse et al., 1977) most of the experimental protocols were atypical of the daily regimen followed by active diabetics - making it

somewhat difficult to assess the practical implications. Despite this, the importance of an appropriate insulin supply certainly stands out. Exercise in an insulin-deficient state encourages uncontrolled catabolism, while the presence of excess insulin inhibits adequate provision of fuels essential to the active tissues. In contrast, responses to acute activity in well-controlled diabetics apparently parallel those of healthy individuals (Oberdisse et al., 1977), with several important differences. These include the fact that in IDD BG fluctuations may be more pronounced, lactate levels for similar relative workloads may be higher (Berger et al., 1977), and KB may be metabolized as fuel even among those who are well-controlled (Wahren et al., 1978). Finally, it is evident from the research presented that there exists a definite lack of post-exercise follow up. Only a minimal amount of data is available regarding how long the hypoglycemic or the other post-exercise consequences may last, or whether they vary with exercise intensity.

In summary, it appears that exercise lowers both BG and insulin requirements of IDD (Vranic and Berger, 1979). Muscle, in turn, plays an important role in this process. Recall that BG uptake during and following exercise is increased to meet the energy demands (Haganfeldt, 1979; Wahren et al., 1978; Maehlum et al., 1977), yet only small quantities of insulin are necessary for this to occur. In fact, in non-diabetic individuals augmented uptake of BG by muscle persists despite a drop in insulin (Skyler, 1979)! The mechanism underlying this process is currently unknown. With rapid advances now being

made in insulin-receptor research, it is possible that exercise may prove to be associated with significant adaptations at the level of this receptor.

Insulin and Insulin Receptors

Characteristics of Insulin

Insulin is composed of two amino acid (AA) chains linked by disulfide bonds. Normally, the biologically active form is produced in the beta cells of the pancreas by progressive modification of a larger precursory molecule - preproinsulin (McGilvery, 1979). Release of insulin into the bloodstream is stimulated primarily by elevated BG or AA concentrations and is inhibited by the catecholamines.

The key target tissues of insulin are skeletal muscle, liver and fat. The actions exerted upon these tissues include -- increased uptake of glucose (muscle and fat), increased glycogen synthesis (muscle and liver), decreased gluconeogenesis (liver) and glycogenolysis (muscle and liver), increased lipogenesis (fat and liver) decreased lipolysis (fat and liver) and increased AA uptake and protein synthesis (muscle) (McGilvery, 1979). It should be noted that the circulating insulin concentration required to elicit these responses varies. For example, peripheral glucose uptake is maximal at an insulin concentration of 1,400 pM (the highest physiological concentration in man), whereas gluconeogensis is suppressed by 700 - 1,400 pM, and glycogenolysis is inhibited at even lower levels (McGilvery, 1979).

Free fatty acid release is suppressed by 200 - 550 pM concentrations and B-hydroxybutyrate disposal requires insulin levels of 350 - 700 pM (McGilvery, 1979). (For comparative purposes, McGilvery (1979) states that non-obese normal individuals have basal insulin levels between 35 - 145 pM).

Theories of Insulin Action

Although a considerable amount of information about insulin is known (i.e. what it is, where it is secreted from and when, what role it plays in metabolism ...), the exact mechanism by which it acts is poorly understood. However, it is generally accepted that insulin initially binds to specific receptors on the target cells and activates some form of "effector system" that has yet to be clearly identified (Porte and Halter, 1981). Unlike most polypeptide hormones, insulin does not stimulate the adenylate cyclase - cyclic adenosine monophosphate (cAMP) pathway (Baxter and Funder, 1979; Roth, 1979). In fact, insulin appears to inhibit cAMP-dependent protein kinase (Walaas and Horn, 1981). Alternatively, it has been suggested that the guanylate cyclase - cyclic guanosine monophosphate (cGMP) system may be involved, but more data is needed before this hypothesis can be fully evaluated (Baxter and Funder, 1979). Calcium fluctuation or translocation has also been considered as a potential mediator of the action of insulin, but this too remains to be established (Baxter and Funder, 1979; Roth, 1979). Though it has been demonstrated that insulin is rapidly internalized by a process of receptor-mediated endocytosis (Schlessinger, 1980), there is little to support the idea that the degraded insulin fragments play the role of the "second

messenger" (Terris, 1978).

In summary, insulin may act by inhibiting the cAMP-dependent kinase, by triggering some other protein kinase, or by modulating calcium levels (McGilvery, 1979). While the "mediator" itself is unknown, the proposed "effector systems" all portray the insulin-receptor interaction as the initial and basic step in eliciting the eventual biological response.

Insulin Receptors

Although the concept of hormones and receptors was introduced in the late nineteenth century, the study of hormones has advanced, while that of receptors has lagged until fairly recently (Kappy et al., 1979).

Structurally the insulin receptor is thought to be a carbohydrate-containing integral membrane protein with a mass in the range of 10^5 to 10^6 daltons (Kappy et al., 1979). Czech and Massague (1982) propose that the receptor is a symmetrical disulfide-linked heterotetramer composed of two α and two B subunits (with apparent molecular weights of 125,000 and 90,000 respectively). In addition, Pilch and Czech (1980) have noted some clustering of insulin receptors — a phenomenon also observed by others (Catt et al., 1979). Though the biological relevance of this is not known, it has been demonstrated that receptors of insulin are mobile (Catt et al., 1979) — suggesting that aggregation may be of significance to the action of insulin. Marshall (1983) has indicated that the time required for de novo insulin-receptor synthesis in fat cells is two to three hours, with a half-life of approximately 24 hours. Others

(Pedersen, 1984) have suggested that the half-life is closer to eight hours.

As previously indicated, for insulin to act it must first bind to a receptor in a target tissue (i.e. muscle, liver, or fat) (Roth, 1979). The function of the receptor is two-fold: (1) it must recognize the insulin molecule from all other hormones and molecules impinging on that cell and (2) it must initiate a series of events which result in a specific and appropriate cellular response (Roth, 1979). As Roth (1979) points out "the hormone molecule is totally dependent on the receptor to express its biological function". This concept has instigated considerable research in the area of receptors. However, it is essential to bear in mind that while insulin-receptor binding is a critical step in producing the desired outcome, it is but one step — a mere starting point for understanding insulin's effects. Thus while post-receptor regulation is entirely plausible, most research is presently aimed at examining the initial binding interaction and what consequences changes at this level may have upon insulin's eventual actions.

Quantitative Aspects of Insulin Binding

Typical measurements of insulin binding are carried out in vitro using radioactively labelled insulin (i.e. ^{125}I-insulin), unlabelled insulin and the insulin receptors of interest. This radioreceptor technique is analogous to that used for radioimmunoassay with the exception that the receptor serves as the "binding protein". The tissue with the insulin receptors is incubated with the ^{125}I-insulin in the presence of varying concentrations of

unlabelled insulin until equilibrium is reached. At this point the bound insulin is separated from the free insulin and the radioactivity measured (total binding). That portion of radioactivity remaining in the presence of the excess "cold" insulin is considered non-specific and is subtracted from the total binding to yield the specific binding.

When a series of "cold" insulin concentrations are used the results can be plotted as a competition curve. The abscissa represents the free insulin concentration while the ordinate depicts either the total insulin bound or the ^{125}I-insulin bound. Additional manipulation of the binding data leads to the Scatchard plot (Scatchard, 1949) which is commonly used to predict receptor affinity and number. By plotting the ratio of bound-to-free hormone as a function of the concentration of bound hormone, receptor affinity can be derived from the slope, and receptor number from the X intercept (Baxter and Funder, 1979). This model assumes thermodynamic equilibrium, identical biological activity of labelled and unlabelled hormones, a reversible bimolecular reaction between hormone and receptor and independence of receptor sites (De Meyts et al., 1973). Although this form of analysis is used extensively in receptor work because it tends to linearize the data and simplify the interpretation, the typical Scatchard plot for insulin binding is concave upward rather than linear (Kappy et al., 1979). This has created several possible interpretations. One proposal is that there are two or more independent classes of insulin receptors — each with its own affinity (Kappy et al., 1979). The curvilinear plot would then reflect the sum

of two or more of these straight lines. Alternatively, it has been suggested that there is a single class of receptors capable of negative cooperativity via site-to-site interactions (De Meyts et al., 1973). This implies that as more insulin receptors become occupied, the affinity of the remaining binding sites progressively declines, yielding the typical concave plot. Although the Scatchard plot for insulin binding can be accounted for by either of the two preceding hypotheses, a third option combining these two (i.e. sites with independent affinities and negative cooperactivity between sites), is also possible. Until further evidence becomes available it is difficult to assess which theory best reflects the true situation. For this reason it seems more appropriate to report insulin-binding data in terms of specific binding and to leave predictions regarding receptor affinity and number until a better understanding of the insulin receptor itself is achieved.

Tissues of Interest in Insulin-Binding Research

Having considered the principles of insulin binding and certain quantitative aspects of assessment and interpretation, it is now possible to consider the tissues to which these techniques are applied. It should be pointed out that receptors may be studied on whole cells (freshly isolated or in culture), in membrane-rich fractions from broken cells or in highly purified preparations of plasma membranes (Roth, 1979). However, because the insulin receptor is an integral component of the membrane and because site-to-site interactions appear to exist, research involving intact cells seems to be favoured.

As the target tissues of insulin are fat, liver and muscle, it is

not surprising that these tissues have been used in insulin-binding studies. Kappy et al. (1979) emphasize that insulin binding to muscle is extremely relevant to overall glucose homeostasis in vivo and that studies conducted with this tissue are of particular value. Despite this, much of the work in this field has focused on the properties of animal and human adipocyte receptors (in both crude membrane preparations and intact cells)(Cautrecasas, 1971; Kappy et al., 1979). Briefly, the characteristics of fat cell receptors include — specificity of binding as shown by modified insulins competing for receptors in direct proportion to their biological potencies; binding that is rapid, reversible, saturable, pH dependent (optimum at 7.8-8.0) and temperature dependent (steady-state binding greater at lower temperatures); binding data that is consistent with negative cooperativity (curvilinear Scatchard plot); and a demonstrated affinity of the receptor for insulin (Kappy et al., 1979). Although these general properties are mentioned with respect to adipocyte receptors, they are also shared by insulin receptors in liver (Kappy et al., 1979) as well as in cardiac (Forgue and Freychet, 1975) and skeletal muscle (Le Marchand-Brustel et al., 1978; Kappy et al., 1979).

In contrast to these many similarities, Jarret et al. (1981) have suggested that fat and liver plasma membrane receptors differ morphologically. The adipocyte receptors occur primarily in groups, whereas those of the liver occur singly (Jarret et al., 1981). Also, Pederson et al. (1981) noted a pH optimum of 7.4 - 7.6 for insulin binding to intact human fat cells — a value somewhat lower than that quoted by Kappy et al. (1979). However, overall the characteristics of

insulin receptors in these target tissues appear much the same (Kappy et al., 1979).

Human receptor research has been limited due to ethical considerations for liver, muscle and even fat biopsies are not always readily available. In the early seventies it was noted that human lymphocytes had insulin receptors with the specificity, sensitivity and kinetics of binding very similar to those observed in highly purified plasma membranes of rat liver cells and isolated rat fat cells (Gavin et al., 1972; Archer et al., 1973). This important finding led to the use of the human lymphocyte as a model for studying insulin-receptor interactions (Olefsky and Reaven, 1974a). More recently the monocyte has been proposed as a model for studying insulin receptors in man (Beck-Nielsen et al., 1977; Beck-Nielsen and Pedersen, 1978a). This resulted from the realization that about 90% of the specific insulin binding in a preparation of mononuclear leucocytes was due to the monocyte content (Beck-Nielsen et al., 1977). Although monocytes are not key target tissues for insulin, insulin does stimulate hexose metabolism in these cells (Kappy et al., 1979). More importantly, human monocytes possess the same binding characteristics as those previously listed for fat cells (Kappy et al., 1979). Hence they have proved to be extremely useful for studying human diseases related to insulin binding (Kappy et al., 1979). Despite the fact that monocytes can be obtained safely and rapidly, relatively large quantities of blood are required (anywhere from 50 - 500 ml)(Kappy et al., 1979; Gambhir et al., 1977), thereby limiting the number of possible samples. This particular drawback has been overcome by recent work with

erythrocytes, by far the most abundant cellular component in whole blood.

Early work with turkey erythrocytes revealed similar binding features to those already described for fat, liver and muscle (Ginsberg et al., 1977). Subsequent investigations with human erythrocytes confirmed this (Gambhir et al., 1977; Gambhir et al., 1978). Gambhir et al. (1978) have predicted that there are approximately 2,000 insulin-binding sites per cell — somewhat fewer than the number predicted for monocytes (7,000-28,000 sites per cell) (Beck-Nielsen et al., 1977; Bar et al., 1976). The specific binding to erythrocytes has been assessed as between 7 - 10% in healthy adults (Gambhir et al., 1977; Gambhir et al., 1978). Although the role of the insulin receptor in erythrocyte metabolism is not yet clear, these binding sites have much in common with the monocyte receptors (Gambhir et al., 1978; Wachslicht-Rodbard et al., 1979; Pedersen et al., 1980) which, in turn, are thought to mirror those in the more relevant tissues — fat, liver and muscle (Kappy et al., 1979; Roth and Grunfeld, 1981). As a result, much of the human research in this field is now directed at the more accessible red blood cell.

Although the present trend in human insulin receptor research deals largely with erythrocytes and other circulating cells, this move away from key target tissue sources has not been made without serious thought. Simply because a tissue is more available does not make it a more suitable candidate for valid research. Thus while it is true that erythrocyte receptors are more accessible, they have also demonstrated properties akin to those in the target cells (Gambhir et al., 1978).

However, this does not exclude the possibility of important tissue specific differences and justification of erythrocyte receptor work on this basis alone would be somewhat questionable. Therefore it is essential to realize that there also exists considerable indirect evidence supporting this research approach. There are, for example, many studies showing in vivo insulin sensitivity changes that correlate with changes in insulin receptors located on non-target tissues (Beck-Nielsen and Pedersen, 1978b; Bar et al., 1976; Roth, 1979; Wachslicht-Rodbard et al., 1979; Polychronakos et al., 1982). Since non-target tissue receptors (i.e. those on erythrocytes) are not responsible for in vivo sensitivity changes, they presumably reflect changes also occurring in fat, liver and/or muscle. As this concept of insulin sensitivity and receptor adaptation is extremely important to both healthy and diabetic individuals, this topic is considered in detail below.

Insulin Sensitivity

Insulin sensitivity is said to be low when high concentrations of insulin are required to elicit a normal response. Conversely, heightened sensitivity occurs when the hormone concentrations are diminished, but are still able to produce the expected outcome. These extremes in sensitivity appear to be related to the insulin receptors — in both normal and disease states.

Considering first healthy adults, Beck-Nielsen and Pedersen (1978a) observed a diurnal variation in insulin binding to monocytes that parallelled the daily variations in insulin sensitivity and glucose tolerance. Binding reached a minimum in the afternoon,

increased during the evening and peaked in the early morning. This variation may have been related to food intake as the pattern was over-ridden when there was total fasting. Fluctuations in binding were attributed to changes in binding affinity and not receptor concentration. These investigators interpreted the simultaneous decrease in insulin binding and insulin sensitivity as evidence that varied insulin sensitivity might be the result of altered insulin binding in the target tissues.

In a related study, Beck-Nielsen and Pedersen (1978b) measured the glucose disappearance rate in healthy adults after intravenous glucose and insulin injections were administered. Insulin binding to monocytes was again monitored. Significant positive correlations were observed between the binding data and the glucose disappearance rate after both glucose and insulin infusions. It was concluded that a close relationship exists between the insulin receptor and glucose disappearance rate in normal man.

Among receptor diseases, insulin resistance in obesity is the most common hormone-resistant state (Roth, 1979). Harrison et al. (1976) examined the relationship between receptor binding to fat cells and sensitivity in obese human subjects and found that the number of binding sites was correlated directly with insulin sensitivity (as measured by the rate of fall in BG after intravenous insulin infusion). They also observed that receptor number was inversely correlated with fasting plasma insulin levels. These findings have been further supported by the work of Olefsky (1976). In this study insulin binding to both adipocytes and monocytes from obese subjects was significantly

reduced when compared to normal weight individuals. Furthermore, there was a strong positive correlation between the binding data from the adipocytes and monocytes — suggesting similar receptor adaptations in both tissues.

Bar et al. (1976) examined binding to monocytes in obese and normal subjects and again confirmed a reduced level of binding in the obese hyperinsulinemic individuals. This was attributed to a decrease in binding sites in the obese (6,000-13,000 sites per monocyte versus 15,000-28,000 sites per monocyte in the normals). Following 48-72 hours of fasting the circulating insulin levels dropped and binding rose in conjunction with increased receptor affinity. Refeeding after acute fasting produced a return to the pre-fasting metabolic status, whereas chronic dieting restored insulin levels, insulin binding and receptor concentration to normal, but receptor affinity was unaltered. In summary, these researchers demonstrated the versatility of the monocyte insulin receptor in its ability to alter both its affinity and concentration in obese insulin-resistant subjects.

Insulin binding to monocytes and insulin's actions in obese humans under conditions of starvation and refeeding have also been examined by De Fronzo et al. (1978). In this research a euglycemic insulin clamp technique was used to raise the insulin level, while at the same time infusing glucose so as to maintain a constant BG level. The amount of glucose infused represented the tissue sensitivity to insulin. For normal subjects 15.8 mM/m2/min of glucose was required compared with 7.6 mM/m2/min for the obese, thereby indicating reduced sensitivity

in the overweight subjects. Binding to monocytes was 8.3% in controls and 4.6% in the obese. There was a significant positive correlation between insulin sensitivity and monocyte binding — once again confirming earlier observations.

In review, obese and hyperinsulinemic states in man show reduced ^{125}I-insulin binding in both adipocytes and monocytes as a consequence of diminished receptor number. It is assumed the monocyte adaptations parallel those in the important target tissues, since the severity of the receptor defect correlates well with in vivo measurements of insulin resistance (Roth, 1979). It is interesting to note that the elevated insulin levels in obesity may cause a "down-regulation" of the number of receptors. However, it is not certain which occurs first — the heightened insulin levels "down-regulating" the receptors, or the reduction in binding sites necessitating higher insulin levels.

In contrast to these studies dealing with obesity, untreated patients with anorexia nervosa demonstrate greater than normal binding (12.2% versus 6.8%) in both monocytes and erythrocytes (Wachslicht-Rodbard et al., 1979). This difference has been attributed to an increased number of receptors per cell with little or no change in receptor affinity. Restoration of normal food intake and body weight corrected this abnormality. Interestingly, in the anorexic state plasma insulin levels were lower than normal, while those of cortisol and growth hormone were elevated. It is suggested that perhaps these endocrine abnormalities, along with the restricted diet, contribute to the greater insulin sensitivity and receptor number

associated with anorexia nervosa.

Although insulin sensitivity and obesity (as well as anorexia) have been researched fairly extensively in humans using adipocytes and circulating non-target tissues, there has been limited research focusing on muscle receptors. That which does exist has involved mainly animal models. Forgue and Freychet (1975) have investigated the effects of obesity on insulin binding to cardiac muscle from obese hyperglycemic mice. Their results indicated reduced binding similar to that observed for fat, lymphocyte and liver tissues from this species. This pattern was again attributed to a decrease in binding sites. Work undertaken by Le Marchand-Brustel et al. (1978) also examined insulin binding and the biological effects in muscle of obese and lean mice. In this case skeletal muscle, (the soleus), was used. The results demonstrated a decrease in receptor number and insulin sensitivity (as noted by reduced 2-deoxyglucose uptake, glycolysis and glycogen synthesis). In both this study and that of Forgue and Freychet (1975) acute fasting was accompanied by increased binding and sensitivity. These findings indicate that striated muscle, like fat and other tissues, is capable of insulin sensitivity and binding changes.

From the preceding discussion it appears insulin binding and insulin sensitivity are closely related. Several factors have already been mentioned as influencing insulin binding — time of day, degree of obesity, dietary intake and hormonal status (insulin, cortisol, growth hormone levels). Other "biologically relevant" regulators of affinity and/or concentration of insulin receptors have also been established. Apparently antireceptor antibodies (Kappy et al., 1979), acidic pH

(Fantus et al., 1981) and aged cells (Eng et al., 1980; Sinha et al., 1981) reduce insulin binding, whereas sulfonylureas (Roth, 1981), cAMP (Roth, 1979), ketone bodies (especially B-hydroxybutyrate) (Roth, 1981; Fantus et al., 1981) and exercise (Roth, 1981) seem to enhance it.

Both affinity and receptor number affect total insulin binding. Affinity appears to be intrinsic to the receptor itself, but adaptation of receptor concentration seems to require cellular processes beyond the binding site (Kappy et al., 1979). This distinction is of interest since it has been suggested that short-term or acute regulation of insulin binding (within 24-48 hours) is the result of changes in affinity, whereas alterations in receptor number require several days to develop in man and are of a more chronic nature (Bar et al., 1976; Insel et al., 1980).

Realizing that an insulin receptor occupancy rate of only 20% elicits a maximal response (Le Marchand-Brustel et al., 1979), one might question the impact of increased receptor concentration on biological sensitivity. Despite this so-called "spare receptor" phenomenon, the effect can be significant. This is due to the fact that for a given quantity of insulin, the rate of insulin-receptor complex formation will be greater the more sites available. Thus it can be argued that these "spare" receptors are not spare at all, but rather enhance the response of the target cell by allowing a faster insulin response and by increasing the cellular sensitivity to low concentrations of insulin.

Roth (1981) has neatly summarized the value of receptor regulation as follows. He stresses that in modulating hormone action in vivo the

only variable of the hormone is its concentration, but with the receptor both number and affinity are adaptable. Furthermore, receptor affinity itself is subject to separate control through both the association and dissociation rates. Roth (1981) concludes that "the receptor, which is a much larger and more complex molecule than the complementary hormone, is capable of a much wider range of regulatory events." The effects of such receptor regulation on cell function apply to both normal and disease states, as seen in the case of insulin sensitivity. Beck-Nielsen and Pedersen (1978a; 1978b) demonstrated a positive correlation between binding and sensitivity in healthy adults, while many others (Harrison et al., 1976; Olefsky, 1976; Bar et al., 1976; Defonzo et al., 1978; Wachslicht-Rodbard et al., 1979) have remarked upon this same relationship in disease states, especially those related to obesity.

Insulin Sensitivity and Binding in Diabetes

Since NIDD is frequently characterized by obesity and/or insulin resistance, it is only natural that receptor work has been extended to include this particular group. Olefsky and Reaven (1974b) noted that in 20 untreated hyperglycemic non-ketotic non-obese NIDD there was a 50% reduction in binding to lymphocytes when compared to normals. This difference was attributed to fewer receptors and was not considered to be the result of the hyperglycemia. In a later study investigating insulin sensitivity and binding among NIDD (Olefsky and Reaven, 1977) these same authors found that patients with chemical diabetes (abnormal oral glucose tolerance test) had a 45% decrease in insulin binding to monocytes. Binding was inversely correlated to both the degree of

insulin resistance and to the fasting insulin level. De Pirro and associates (1980) studied insulin binding to erythrocytes in non-obese NIDD and again observed significantly lower binding when compared to normals (5.39% specific binding versus 6.39%). This discrepancy was accounted for by differences in receptor concentration. As in the preceding study (Olefsky and Reaven, 1977), binding was inversely correlated to fasting insulin levels. Hence, in all three investigations (Olefsky and Reaven, 1974b; Olefsky and Reaven, 1977; De Pirro et al., 1980) the relationship between insulin sensitivity and binding persists.

When it comes to IDD it is clear that the primary metabolic problem is the absence of the hormone insulin. However as Roth (1981) emphasizes "even in conditions in which hormone secretion represents the major problem, receptors may play a key role". Accordingly, Fantus et al. (1981) examined insulin binding to the monocytes of eight poorly controlled non-obese IDD. The results indicated heterogeneous binding that was normal or slightly elevated, suggesting that the degree of hypoinsulinemia or ketonemia might be determinants of increased binding. However there were no significant correlations between binding, receptor number or affinity and fasting BG or degree of ketonemia. The fact that insulin binding decreased with improved metabolic control may have been the consequence of peripheral hyperinsulinemia and subsequent "down-regulation". This idea has been supported by Brown et al. (1982) who found the binding response of typical IDD to be reduced when compared to that of healthy controls. Recently Turco et al. (1984) have further added to our interpretation of binding in IDD by

suggesting that modulation of insulin receptors may be less evident in those who require only moderate daily doses of insulin (i.e. mean of 33 units).

Thus even in IDD where insulin deficiency is dominant, the receptor may influence the clinical state of the insulin-dependent diabetic. In fact, it is quite conceivable that many of the acute "unexplained" alterations in metabolic control of the IDD may be related to changes at the level of the insulin receptor. Further research in this area is required before a definitive answer can be reached.

Summary

In concluding this section on insulin it should be apparent that both insulin and the insulin receptor are important in maintaining metabolic homeostasis. In the past greater emphasis has been placed upon the regulatory role of the hormone. Now the adaptability of the receptor and the role it may play in health and disease is also being recognized. This has come about largely because of the development of radioreceptor assays. Both animal and human target and non-target tissues have been studied. Overall the results indicate many common properties among receptors originating from very different tissues (i.e. liver, fat, muscle, monocytes, erythrocytes). Furthermore, it appears that many of the receptor changes which occur in non-target tissues (i.e. erythrocytes or monocytes) parallel the observed changes in sensitivity. This has been interpreted as suggesting that binding alterations in non-target tissues likely mirror the more important alterations which occur in liver, fat and muscle. This situation

occurs in both insulin-resistant obesity and NIDD. For IDD the main problem remains a lack of insulin, yet it is entirely possible that alterations at the receptor level may play a significant role in both the clinical state and the therapy of this disease.

Exercise, Insulin Sensitivity and Insulin-Receptors

Exercise and Preliminary Sensitivity Studies

Interest in potential insulin receptor modulation with exercise has recently grown. This trend has no doubt evolved from earlier work based on altered glucose tolerance and insulin sensitivity following chronic exercise programs. For example, in the early seventies Bjorntorp et al. (1970; 1972) suggested that regular physical activity might produce adaptations in glucose tolerance. Although their studies confirmed this, the mechanism was not entirely clear.

Berger et al. (1979) re-examined the effects of training on glucose tolerance in normal anaesthetized rats and found both lower BG and insulin levels in the trained animals at rest and during an intravenous glucose tolerance test. Significant increases in insulin-stimulated glucose uptake (55%) and lactate oxidation (78%) were also noted in perfused skeletal muscle isolated from the trained rats. These researchers concluded that mild physical training could improve glucose tolerance and insulin sensitivity in normal rats due, at least in part, to an increase in insulin sensitivity of skeletal muscle glucose metabolism. Mondon et al. (1980) arrived at a similar conclusion after comparing the sensitivity of muscle and liver of sedentary and exercise trained rats. Once again insulin levels were

lower for the trained rats following glucose ingestion. Glucose uptake was 17% faster in muscle from the trained group when perfused without added insulin and 43% faster when exogenous insulin was included. In contrast, glucose clearance by liver in the trained rats was less than one-half that of the controls. Therefore skeletal muscle and not liver was established as the organ primarily responsible for the increased insulin sensitivity to glucose uptake with regular physical training. In a third study, Wirth et al. (1980) compared trained and sedentary rats and noted that both the plasma insulin concentration and the peripheral insulin/glucose ratio were lower in the trained group, again suggesting an increased sensitivity to peripheral BG utilization. Interestingly, insulin binding to adipocytes in this group was no different from that of the controls. As the only other peripheral tissue significantly involved in BG metabolism is muscle, it is unfortunate that binding to this tissue was not examined.

Exercise-Related Insulin-Receptor Adaptations in Non-Diabetics

Research into both the chronic and acute exercise-related changes in insulin sensitivity and insulin binding in healthy human subjects has also been conducted. Though muscle, liver and fat biopsies have not been readily available for study, recall that a number of investigators (Olefsky and Reaven, 1977; Defronzo et al., 1978; Beck-Nielsen and Pederson, 1978b) have demonstrated that insulin binding to monocytes is correlated with glucose tolerance and insulin sensitivity.

Soman et al. (1979) examined insulin binding to monocytes in healthy adults prior to and following a six week training program (one

hour of cycling at 70% mVO_2, four times per week). At the conclusion of the training period there was a 20% improvement in mVO_2, a 30% increase in insulin-mediated glucose uptake and a 35% elevation in insulin binding at rest (from 9.4 to 12.8%). As there was no change in body weight, it was felt that physical training might play a role in the management of insulin-resistant states (i.e. obesity and NIDD) which would be independent of its influence upon obesity.

With regard to acute exercise effects, Soman et al. (1978) found that bicycling for three hours (at a workload requiring a heart rate of 120 bpm) led to a 36% increase in insulin binding to monocytes at the completion of activity (from 6.6 to 9.0%). This was attributed to an increase in receptor affinity. Binding returned to basal levels by 24 hours. Although the elevated binding correlated with the fall in BG (13%), it did not correlate with either the drop in plasma insulin (35%), or the rise in KB (five to six-fold above baseline). The authors concluded that acute exercise increases insulin binding which in turn may contribute to the augmented sensitivity associated with exercise.

Koivisto et al. (1979) investigated insulin binding to monocytes in trained athletes and sedentary controls in the resting state and after three hours of bicycling at 40% mVO_2. At rest specific binding was 69% higher in the athletes (14% versus 8%), while BG and insulin levels tended to be lower. During acute exercise specific binding rose by 35% in the controls (to 11%), but fell by 31% in the athletes (to 9%). Insulin levels declined in both groups. For the athletically trained this pattern fits in with the heightened insulin sensitivity

noted at rest, as well as with the shift from carbohydrate to fat as the preferential exercise fuel (Astrand and Rodahl, 1977). The work of Leblanc et al. (1979) has produced similar results in that the more physically fit subjects showed significantly lower insulin and BG levels in response to an intravenous glucose tolerance test. Moreover, insulin binding to monocytes at rest was greater among the more highly trained subjects.

Michel et al. (1984) recently investigated the role of exercise intensity and duration on insulin-binding responses in trained healthy males. It was found that following 15 minutes of exhaustive bicycle exercise insulin binding to moncytes decreased, whereas 90 minutes of cycling at 50% mVO_2 produced an increase in receptor binding. The authors hypothesized that the bidirectional binding responses reflected cellular changes in insulin sensitivity mediated by different serum components (ketone bodies, free fatty acids, lactate...) resulting from the different exercise conditions.

Polychronakos et al. (1982) have contributed to our awareness of post-exercise insulin sensitivity and binding changes in healthy athletes by examining the effects of detraining. Insulin sensitivity (as assessed by the euglycemic clamp technique) and binding to erythrocytes decreased in parallel between 12 and 60 hours after the last exercise regimen. Thus results from this study demonstrate the adaptability of and the close relationship between in vivo insulin sensitivity and insulin binding to erythrocytes.

In summary, insulin sensitivity and binding adaptations associated with exercise among healthy individuals appear to be related. For the

untrained, acute exercise promotes both elevated sensitivity and binding (Soman et al., 1978). Similar changes are observed among athletes under resting conditions (Koivisto et al., 1979; Leblanc et al., 1979; Soman et al., 1979). On-the-other-hand, physical exertion among the highly trained may be accompanied by diminished binding and so a favourable shift towards preferential fat metabolism (Koivisto et al., 1979; Leblanc et al., 1979; Soman et al., 1979; Astrand and Rodahl, 1977; Michel et al., 1984).

Exercise-Related Insulin Sensitivity and Receptor Adaptations in Diabetics

In addition to the above research conducted with healthy subjects, diabetics have also come under scrutiny. Saltin et al. (1979) studied middle-aged men with pathological oral glucose tolerance test responses (mild NIDD) to see if they might benefit from physical training. Following three months of activity (one hour, twice a week) both mVO_2 and glucose tolerance values improved. In fact, glucose tolerance was normalized in a group of subjects receiving both the training program and dietary advice. Insulin levels were also reduced in this group. Despite the association between insulin resistance and obesity, the subjects who had improved glucose tolerance in this study did not undergo any significant weight loss. Ruderman et al. (1979) also examined glucose tolerance in NIDD following moderate activity (30 minutes, five times a week, for six weeks). Training improved mVO_2 values by 15% and increased the glucose disappearance rate following an intravenous glucose tolerance test without any change in insulin response. Thus a greater sensitivity to insulin is suggested.

However, this effect was short-lived for it deteriorated within two weeks of the end of the program.

Iwasaki et al. (1982) have investigated the acute exercise effect on insulin binding to erythrocytes in NIDD and normal controls after 30 minutes of jogging (approximately 30 - 40% mVO_2). In the control subjects binding was elevated immediately post-exercise, but fell to baseline values by 30 minutes. In contrast, binding in NIDD tended to decrease at five minutes post-exercise and demonstrated a significant reduction by 30 minutes. Iwasaki et al. (1982) concluded that the control mechanism of insulin receptor adaptation to exercise may be different in NIDD and normal subjects.

At present limited research has been done with regard to the potential effects of exercise on insulin sensitivity or binding in IDD. Brassard and Taylor (1980) did find that both endurance and power training programs diminished insulin requirements in streptozotocin-induced diabetic rats — suggesting heightened sensitivity. This finding has been supported more recently by the work of Yki-Jarvinen et al. (1984) involving IDD. A six week training program consisting of cycle ergometer exercise (one hour per day, four times a week) resulted in an enhanced rate of glucose uptake and a reduction in insulin requirements. Insulin binding to erythrocytes remained unchanged. However, it is important to note that the binding technique employed removed the uppermost 5 to 10% of the erythrocyte pellet, thereby eliminating the youngest and perhaps most adaptable cells.

Caron et al. (1981) have examined the fall in glycemia in the

hours following acute exercise in IDD. Thirty minutes after breakfast subjects began a bout of moderate bicycle activity (50% mVO_2) that lasted 45 minutes. In four of the five diabetics studied the post-lunch glycemia (several hours later) was significantly lower than values obtained on a control day. No difference in the level of free insulin was observed. Therefore these researchers concluded that post-prandial exercise produced a delayed effect in lowering BG that appeared to be the result of enhanced insulin sensitivity. Unfortunately binding characteristics were not investigated in this particular study.

Currently, there is only one study available which specifically examined the effects of acute exercise on insulin sensitivity and binding adaptations in IDD (Pedersen et al., 1980). These researchers attempted to determine whether the improved glucose tolerance and diminished insulin requirements following exercise in well-controlled IDD, were linked to alterations at the receptor level. Binding to erythrocytes and monocytes was studied in athletically untrained diabetic men treated with insulin during three hours of post-prandial bicycle exercise (33% mVO_2). Compared with a control period, exercise significantly increased insulin binding (by 30%) to both erythrocytes and monocytes. Actually, diabetics show a diurnal variation in binding capacity, with the low occurring before noon (Beck-Nielsen and Pedersen, 1978a). When exercise was implemented at this point, the binding simply did not decline as occurred in the control study. This was attributed to changes in binding affinity as receptor number remained constant. During the three hours of

post-prandial exercise reductions were observed in BG, FFA and cortisol, while elevations were found in insulin (site of injection was in the leg), glucagon and lactate. The duration of these adaptations into the recovery period was not specified. Overall, these findings in well-controlled IDD parallel those obtained for healthy untrained subjects (Soman et al., 1978; Koivisto et al., 1979).

Although Pedersen et al. (1980) acknowledge that insulin, KB and steroids have been shown to affect binding under various conditions, their study failed to show any relation between changes in insulin, glucagon, cortisol, BG, FFA, KB or lactate and cellular binding of insulin. Hence, the factors operating in this exercise-induced enhancement of insulin-receptor binding are unknown. While it could be argued that exercise causes a mobilization of erythrocytes and monocytes with greater binding capacities than those in circulation at rest, Pedersen et al. (1980) did not see any differences following exercise in the reticulocyte concentration of the erythrocyte preparation. In conclusion, Pedersen, Beck-Nielsen and Heding (1980) state that the increased insulin binding to monocytes and erythrocytes associated with acute exercise probably reflects a similar adaptation in muscle tissue, ultimately contributing to the improved glucose tolerance seen in healthy and IDD subjects.

In reviewing this section, it is evident that many times the exercise-related adaptations in glucose tolerance and insulin sensitivity are parallelled by modifications at the receptor level. While this does not necessarily imply a causal relationship, this possibility does exist. As emphasised earlier by Roth (1981), the receptor is capable

of an extremely large range of regulatory events! The observed sensitivity and receptor adaptations are not limited to healthy individuals, but also apply to disease states such as insulin-resistant obesity, NIDD and IDD. While stressing that insulin deficiency is the basic abnormality in IDD, receptor adaptation in this group may still play an important role. In fact, the clinical state of these individuals may be greatly influenced by receptor changes, as suggested by the exercise-enhanced glucose tolerance and binding results of Pedersen et al. (1980). Additional research in this area will be required in order to clarify the precise significance of these recent receptor findings.

Present Guidelines for the Active Diabetic

At present only a handful of practical guidelines exist for the active diabetic. These are presented below.

Food should be provided before or during the activity to avert hypoglycemia due to increased energy consumption and insulin mobilization. Additional food may also be required up to 24 - 48 hours following exercise to replenish depleted fuel stores. Some authorities suggest reducing insulin dosage rather than increasing food intake. However, if the subjects are not overweight an increased dietary intake to balance the caloric expenditure seems preferable, particularly in light of the faster insulin mobilization during activity. As a generalization Flood (1980) has proposed that brief vigorous exercise should entail an increase in food intake; prolonged exercise, a decrease in insulin and prolonged vigorous activity (i.e. marathon events), a combination of the two. More specific guidelines concerning

changes in insulin dose are not available — perhaps reflecting large inter-subject variability.

From the studies concerning the role of metabolic control it should be clear that insulin-deficient diabetics should not undertake exercise as it leads to a further deterioration of the diabetic state. It should be kept in mind though, that studies describing insulin-deficient diabetics usually involve subjects whose insulin has been partially or totally withheld — an unrealistic situation for most exercising diabetics. Thus it seems important here to distinguish between "insulin-deficient" and poorly controlled diabetics who are simply not achieving an optimal balance between diet and insulin. Contrary to the impression left by the statement that "uncontrolled diabetics should not exercise", it seems reasonable that diabetics who overeat in relation to their normal diet and insulin requirements (and so have elevated BG levels) might benefit from acute exercise. Whether a better metabolic balance is attained will depend largely on the type of activity, hormonal response and degree of insulin insufficiency.

Although some controversy concerning the impact of active versus non-active site of injection still exists, it is usually recommended that a non-active site be selected prior to exercise (Koivisto and Felig, 1978).

While activity is generally considered beneficial for diabetics, there are several risks or considerations that must be taken into account. As already noted subjects must take precautions to avoid hypoglycemia, while those whose insulin is withheld should avoid physical exertion. Diabetics with microangiopathy may have reduced

vascular dilation and impaired exercise tolerance. In addition, because exercise decreases blood flow to the kidneys and increases proteinuria, diabetic nephropathy may be aggravated (Skyler, 1979). Similarly, exercise may worsen proliferative diabetic retinopathy by increasing blood pressure and the risk of vitreous or preretinal hemorrhage (Skyler, 1979). Finally, for diabetics with ischemic heart disease there is the risk of angina pectoris or myocardial infarction. In such individuals exercise is best initiated in formal classes where trained personnel are available.

In general, diabetics are encouraged to follow these guidelines and include regular exercise in their therapy. Unfortunately, until more is known, additional recommendations can not be offered.

General Summary

Exercise has long been recommended and considered beneficial in the treatment of diabetes. Acute activity alters the energy demands, fuel requirements, hormonal pattern and metabolic status of the individual depending upon such factors as exercise intensity, duration and level of fitness. In the case of the insulin-dependent diabetic insulin availability is also critical. To date, research explicitly comparing the effects of activity of varying intensity in stable IDD is scarce and quite limited with regard to the post-exercise consequences.

With the rapid expansion of receptor research the adaptability of the insulin receptor in both health and disease is being recognized. Consequently, acute exercise benefits to the diabetic such as lower

glycemia, reduced insulin requirements and improved glucose tolerance and sensitivity are being re-examined in light of the recent knowledge of insulin-receptor modulation. Unfortunately such research specific to the acute exercise effects in the IDD is still limited and additional investigation is warranted if better guidelines are to be developed for today's active diabetic.

This review chapter points out that our present understanding of exercise and diabetes could be considerably enhanced by research aimed at systematically comparing exercise intensity effects upon IDD; by using more physiological, or typical, exercise conditions for IDD; by investigating the duration of the post-exercise consequences and by further examining the phenomenon of insulin-receptor adaptation. Accordingly, this study will examine and compare the effects of varying exercise intensity on the post-exercise insulin-binding and blood glucose responses in untrained male insulin-dependent diabetics.

CHAPTER III

RESEARCH METHODOLOGY

This chapter presents the methods used to investigate the post-exercise effects of exercise intensity on ^{125}I-insulin-binding and plasma glucose responses in male insulin-dependent diabetics.

Subjects

Participants in the study were eight untrained male IDD between 23 and 32 years of age. Subjects were within +/- 21% of their recommended ideal body weight (Metropolitan Life Insurance Company, 1983) and were receiving insulin by injection one to two times a day. None of the volunteers were being treated with continuous subcutaneous insulin infusion (i.e. the insulin pump). The subjects were not involved in extensive physical training programs, although some were participating in light to moderate activities on a semi-regular basis. Females were excluded from the study on account of the wide variation in insulin binding during the menstrual cycle (De Pirro et al., 1981). Participants were in good health and had no serious diabetic complications. Informed written consent was obtained from all volunteers prior to the start of the study (Appendix E).

Experimental Design

Due to large inter-subject variability in diabetic status, subjects acted as their own controls. The design was such that each participant was evaluated under three distinct conditions. These

consisted of a control day and two "eight hour post-exercise days". On the control day the subjects merely reported to the laboratory to provide a morning blood sample. However, the two "eight hour post-exercise days" required that the volunteers participate in an exercise protocol on the evening immediately preceding their morning blood sample. It was felt that by having the subject exercise in the evening (21:30 to 22:30) the subsequent overnight eight hour period would be less affected by factors such as stress, physical activity and diet. The order in which the three treatment conditions were performed was random for each volunteer. For any given subject all three sessions were completed over a two week period, with a minimum of three days between each.

Details of the exercise protocol (intensity, duration, format) were discussed with each participant in advance so that insulin and diet could be adjusted according to individual needs. Subjects were informed that they were to establish and maintain exactly the same insulin, diet and activity patterns (excluding the imposed exercise protocol) under all three experimental conditions. The importance of this was stressed and participants were given diary sheets (Appendix F) and asked to record the above parameters on the day prior to each test day when the morning blood samples were drawn (three days in total). This procedure was included to encourage adequate diabetic control, to provide a record of the regimen followed initially so that this could be duplicated in the two subsequent protocols and to encourage compliance on the part of the volunteers. Participants were requested to refrain from smoking and consuming caffeine prior to both evening

exercise sessions. It was also requested of the volunteers that they avoid extra physical exertion 24 hours prior to providing their morning blood samples.

On the first exercise evening (high or low intensity) the subject reported to the Ottawa Civic Hospital Diabetes Research Laboratory at 20:30. A capillary blood sample was subjected to an immediate BG estimate (using the commercially available "Dextrostix" and Glucometer system) to ensure adequate control on each of the two exercise evenings. When BG values exceeded 15 mM the subjects were rescheduled. At approximately 20:45 a submaximal predictive mVO_2 test was begun using a stationary Monarch bicycle ergometer. The protocol used was that outlined by Astrand and Rodahl (1977). After the application of chest electrodes, the subject cycled for six minutes at a workload of 300 kgm/min (50 watts) at a set pedal frequency of 50 rpm. The load was then progressively increased by 300 kgm/min every six minutes. The heart rate was monitored on a Cambridge VS4 recorder during the last two minutes of each load. When the heart rate reached 130-150 bpm the test was stopped at the end of the sixth minute. Based on this heart rate and the last load, the subject's mVO_2 was predicted using the nomogram of Astrand and Rhyming (1954) (Appendix D). A correction factor for age was applied when necessary (Astrand and Rodahl, 1977). From the predicted mVO_2 value, 75% mVO_2 was determined and the corresponding high intensity workload derived from the nomogram (Astrand and Rhyming, 1954). The total amount of work to be completed under the high intensity condition was then calculated, as the subject would pedal intermittently in two minute work/rest intervals for the

duration of one hour, (30 minutes of actual cycling time.). Based on this, the low intensity workload was determined as that intensity which would enable the same total amount of work to be completed during 60 minutes of continuous cycling. This represented approximately 45% $m\dot{V}O_2$. The amount of work completed during the two exercise protocols was matched in order that the total energy and fuel requirements would be comparable. (When the subject returned for the second exercise session the submaximal bicycle test was repeated to ensure that the total amount of work completed would be identical on both occasions. However, all workload calculations were derived from the initial ergometer test.).

With the respective workloads established and after a brief rest, the subject began pedalling at 21:30 and continued until 22:30 following either the high intensity intermittent or low intensity continuous format of exercise. The heart rate was recorded every five minutes. Capillary BG values were obtained at the start and then every 20 minutes until completion of the exercise. Where BG values fell below 4mM carbohydrate was given orally to avoid hypoglycemia.

Under the control condition no evening exercise was performed. However, the same insulin and dietary patterns were adhered to as on the other two occasions.

After each of the three experimental protocols the subject returned to the laboratory the next morning at 6:30 (eight hours post-exercise) following an overnight fast and prior to injecting his morning dose of insulin. A blood sample from the cubital vein (20 ml) was collected in heparinized vaccutainers. All samples were assayed

for immediate BG (as previously described), hematocrit, plasma glucose, HbA_1, and ^{125}I-insulin binding to erythrocytes.

For each 20 ml of blood collected approximately 1.3 ml of whole blood was immediately removed for the determination of immediate BG (.1 ml), hematocrit (.2 ml) and HbA_1 (1 ml). The remainder was centrifuged at 2500 rpm, 4°C for 10 minutes. The plasma was allocated for the plasma glucose assay and the cells, for the erythrocyte binding assay as described below. All metabolic assays were carried out in duplicate. (All centrifugation processes under 3500 rpm were perfomed on Damon/IEC division centrifuges. Ultra centrifugation was used for the dextran density separation of the erythrocytes (Beckman L8-70, 18,000 rpm)).

Subject Analysis

Percent Ideal Body Weight

The 1983 Metropolitan Life Insurance Company Height and Weight Table for Men was used as a basis for calculating the percent ideal body weight (Appendix C). Frame size was not estimated, therefore calculations used the mean of the entire range of weights corresponding to a particular height, as depicted in the table. Thus % IBW was determined as follows

$$\% \text{ IBW} = \frac{\text{Subject Wt} - \text{Mean Wt of small to large frame size limits for the appropriate subject Ht}}{\text{Mean Wt of small to large frame size limits for the appropriate subject Ht}} \times 100\%$$

Blood Analysis

Hematocrit

Since exercise tends to cause hemoconcentration, the hematocrit (Hmct) was determined for each of the three samples in the event that corrections for the plasma glucose concentrations might be necessary. Plasma volume changes based on differences between the control day Hmct and post-exercise Hmct were calculated according to the method of Beaumont (1972).

$$\%\ \text{Plasma Volume Change} = \frac{100}{100 - \text{Control Hmct}} \times (-\%\ \text{Hmct Change})$$

Plasma Glucose

Plasma glucose determination was carried out on plasma frozen immediately after collection and stored at -20°C for up to five weeks. An automated ABA-200 Abbott Analyzer and the associated "A-Gent Glucose-UV" reagent kit were used for the assay (Abbott Laboratories, 1979). This reagent kit is an optimized formulation of hexokinase (833 U/l), glucose-6-phosphate dehydrogenase (1667 U/l), sodium adenosine triphosphate (1.8 mM), magnesium aspartate (1.2 mM), nicotinamide adenine dinucleotide (NAD) (1.5 mM), triethanolamine hydrochloric acid (50 mM), sodium carbonate (16.5 mM) and potassium oxalate (9 mM). The principle of this technique is based upon the phosphorylation of glucose by hexokinase in the presence of magnesium ions and excess adenosine triphosphate to yield glucose-6-phosphate and adenosine diphosphate. Glucose-6-phosphate is then oxidized in the presence of glucose-6-phosphate dehydrogenase and NAD producing

6-phosphogluconate and the reduced nucleotide (NADH). When 50 ul of plasma was reacted with .5 ml of the "A-Gent Glucose-UV" reagent the degree of reduction of NAD to NADH was linearly related to the amount of glucose present and could be measured spectrophotometrically at 340 nm, thus providing a quantitative measure of plasma glucose.

Glycoslyated Hemoglobin A

Samples were analysed for HbA_1 to determine the degree of glucose control and to verify similar status throughout the duration of the study. HbA_1 was measured using the "Quik-Sep" fast hemoglobin test system (Isolab Inc., 1981). This involved mixing 50 ul of whole blood with 200 ul of the Fast Hb Sample Preparation Reagent. This was shaken and then let sit for 10 minutes. Fifty microlitres of the resultant hemosylate was added to a prefilled ion-exchange column. The total HbA_1 fraction was eluted by adding 200 ul of the Fast Hb Elution Solution. The remaining hemoglobins were collected separately with 4 ml of a second eluting agent. After diluting the latter fraction with 16 ml of water the absorbances of both fractions were read at 415 nm on a Bausch and Lomb Spectronic 710 spectrophotometer. The percentage HbA_1 was calculated as

$$HbA_1 = \frac{\text{Absorbance of the Fast Hb Fraction}}{\text{Abs of the Fast Hb Fraction} + 5\ (\text{Abs of the Other Hb Fraction})}$$

Whole blood was stored at 4°C for no longer than 24 hours prior to undergoing this analysis.

^{125}I-Insulin-Binding Analysis

Iodination Procedure

^{125}I-insulin was supplied by the Polypeptide and Hormone Research Laboratory at McGill University. All assays were performed with the same batch of ^{125}I-insulin within four weeks of its preparation. A modified version of the chloramine T method described by Greenwood, Hunter and Glover (1963) was used as this has been shown to be suitable for preparing labelled insulin with the same biological activity as the cold hormone (Schneider et al., 1976). Briefly, 10 ul of phosphate buffer (.5 M, pH 7.4) was reacted with 10 ul of ^{125}I (New-England Nuclear) (1 mCi/10 ul of .5 M phosphate buffer), 10 ul of porcine insulin (Sigma) (5 ug/10 ul of .01 N hydrochloric acid) and 25 ul of chloramine T (Sigma) (1 mg/ml of .05M phosphate buffer). The reaction proceeded for 30 seconds and was terminated by the addition of 100 ul of sodium metabisulfite (.35 mg/ml of .05M phosphate buffer). To this, .245 ml of 2.5% bovine serum albumin (BSA) in 25 mM Tris buffer (pH 7.4) was added to yield a final volume of .5 ml of dilute reaction mixture (D). Trichloroacetic acid precipitation was used to assess the percentage ^{125}I incorporated with the hormone. Provided this was greater than 85%, solution D was applied to a Sephadex column (G-50 fine, 70 cm x 1 cm) pretreated with 1 ml of 2.5% BSA in 25 mM Tris buffer. The eluate was collected 1 ml at a time in glass tubes containing .1 ml of 2.5% BSA in 25 mM Tris buffer. The tubes were counted to determine the iodinated protein peak which was then pooled and frozen in small aliquots. Specific activity of the ^{125}I-insulin was estimated based on the percent incorporation and

the quantity of insulin and ^{125}I reacted.

Insulin-Binding Assay

The technique used was based on the work of Polychronakos et al. (1981). After centrifuging the whole blood specimen (approximately 18 ml) at 2500 rpm, 4°C for 10 minutes, the leucocytes and platelets were removed from the erythrocytes by pouring the cells over a column composed of a 50% mixture by weight of α cellulose and Sigma cell type 50 cellulose (Beutler et al., 1976). Saline was run over the column to facilitate filtration. The saline and red blood cell suspension was centrifuged at 2500 rpm, 4°C for seven minutes in 50 ml plastic conical centrifuge tubes. The supernatant was aspirated and the cells resuspended with room temperature saline and then left to sit for 30 minutes before recentrifuging. This last sequence of steps was repeated — the purpose being to remove insulin and insulin antibodies that might be present in high concentrations in IDD. This mixture was spun down once again (2500 rpm, 4°C, seven minutes) and the saline removed before adding a few millilitres of buffer GES 300 to the cells and mixing thoroughly. (GES 300, a glucose electrolyte solution, contained 6.75 g/l sodium chloride, 4.85 g/l dihyrous sodium dihydrogen phosphate, .824 g/l potassium dihydrogen phosphate and 2.00 g/l glucose).

Dextran density centrifugation was employed to separate the youngest (least dense) erythrocytes (Danon and Marikovsky, 1964) from the entire erythrocyte population. This aspect was included in the study as insulin binding declines in erythrocytes as they age (Eng et al., 1980). Since exertion may stimulate release of immature

erythrocytes (Guyton, 1976), examination of binding within a given age fraction of cells would avoid possible biasing. It was decided to investigate the youngest fraction of cells since erythrocytes lose their nuclei with maturation. Should change at the receptor level involve some form of protein adaptation, it would thus seem most likely to appear in the younger cells. An "enriched solution" of the youngest erythrocytes was isolated by placing several millilitres of the GES-erythrocyte mixture onto 2 ml of a 1.095 g/ml dextran gradient, and centrifuging at 18,000 rpm, 18°C for 10 minutes. (The remainder of the GES-erythrocyte mixture was returned to the fridge to be used for the unfractionated analysis). The upper layer or "enriched solution" was then removed and placed onto a 1.092 g/ml dextran gradient and recentrifuged at 18,000 rpm, 18°C for 45 minutes. At the end of this time the upper fraction (F) was removed and washed with saline, as were the temporarily stored unfractioned cells (U). Buffer G (11.915 g/l HEPES, 6.057 g/l Tris, 2.033 g/l heptahydrous magnesium chloride, .584 g/l EDTA, 1.80 g/l dextrose, 1.47 g/l dihydrous calcium chloride, 2.925 g/l sodium chloride, .373 g/l potassium chloride and 1.00 g/l BSA) was then added, mixed, centrifuged at 3000 rpm, 4°C for three minutes, and the supernatant aspirated. The cells (both unfractionated and fraction F) were transfered to separate smaller graduated tubes, resuspended in buffer G and recentrifuged at 3000 rpm, 4°C for five minutes. Enough supernatant was aspirated to establish a 50% hematocrit and the cells were then well mixed in the remaining buffer G. Thirty microlitres of this suspension were transfered to a Coulter cup containing 10 ml of isoton. Evaluation of the number of

erythrocytes in this volume was carried out on a Coulter counter. Binding data was later standardized to a cell concentration of 4.0 x 10^9 erythrocytes/ml.

Specific insulin binding was examined by incubating 30 ul of each of the erythrocyte fractions (unfractionated and F fraction) with 10 ul of the tracer and 10 ul of either buffer G or excess unlabelled insulin (500,000 ng/ml). (The tracer contained 2 ml of buffer G, 2 mg bacitracin and approximately 20 ul of ^{125}I-insulin or enough to contain roughly 2,000,000 cpm). Both erythrocyte fractions were incubated in quadruplicate at the tracer and excess insulin concentrations. The assay tubes were sealed and allowed to incubate for 20 hours at 4°C, set in a shaker. After this time 3.5 ml of chilled saline was added to each tube (excluding those containing tracer only). The tubes were then centrifuged at 3000 rpm, 4°C for three minutes to separate the free insulin from bound insulin. This was followed by the addition of 1.5 ml of chilled 37% formaldehyde. After 10 minutes the tubes were inverted, drained and the radioactivity counted (10 minutes per tube).

To determine specific binding the average amount of radioactivity remaining in the presence of excess cold insulin (non-specific binding) was subtracted from the mean total binding at the tracer concentration. Therefore percent specific binding (%SB) was calculated as

$$\%SB = \frac{\text{Total Binding} - \text{Non-Specific Binding}}{\text{Total Binding}} \times 100$$

Statistical Analysis

Analysis of Subject and Exercise Data

Descriptive statistics were used to characterize subject and exercise parameters. Two-way repeated measures univariate analysis of variance was employed to examine both BG and carbohydrate intake over time during the two exercise sessions.

Analysis of Hematocrit, Plasma Glucose and Glycosylated Hemoglobin

Hematocrit, plasma glucose and glycosylated hemoglobin were each examined for differences between control, low and high days using a one-way repeated measures analysis of variance.

Analysis of Binding Data

Assessment of the percent specific binding between the U and F fractions under the three experimental conditions involved a two-way repeated measures analysis of variance. Non-specific binding was evaluated in the same manner. A one-way repeated measures analysis of variance was used to ensure that the % F recovery did not vary significantly with experimental treatments.

Correlational Analysis

Pearson's correlation coefficient was determined for the relationship between specific insulin binding and age, body weight, insulin dose, mVO_2, total work performed, plasma glucose and glycosylated hemoglobin.

An α level of .05 was selected for all significance testing. Post hoc procedures were performed as required.

Non-Diabetic Comparative Data

A review of the literature on insulin binding indicates a range of reported normal values. The nature of the present design was selected to specifically examine binding responses among a diabetic population. However, it was felt that by providing baseline non-diabetic binding values results derived from the assay described in this paper would be made more meaningful. It was not the intention of the present research to compare these groups directly. Rather, the purpose of including non-diabetic data was to provide a basis of interpretation for those readers interested in relating the findings of this work to that of others in the field. For this reason all results derived from the five non-diabetic volunteers are presented separately in Appendix B.

CHAPTER IV

RESULTS

The results of this study are presented as follows: Subject Data, Exercise Parameters (including the acute exercise effects monitored), Post-Exercise Plasma Glucose Responses (including HbA_1), Post-Exercise Insulin-Binding Responses and Correlations. Original data not presented in this chapter can be found in Appendix A. An α level of .05 was selected for all statistical analyses. All significance tests were two tailed, except where specifically reported as being one tailed.

Comparative results concerning the non-diabetic subjects appear separately in Appendix B.

Subject Data

Descriptive characteristics of the eight diabetic subjects are shown in Table 1.

TABLE 1

SUBJECT CHARACTERISTICS

SUBJECT	AGE (yrs)	HT (cm)	WT (kg)	% IBW (%)	DIABETES DURATION (yrs)	INSULIN (units)	INSULIN/KG (units/kg)	[illegible]	[illegible]
[illegible]	24	[illegible]	[illegible]	21	[illegible]	54	[illegible]	[illegible]	[illegible]
[illegible]	26	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2	[illegible]	[illegible]	[illegible]	[illegible]
5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	57.5	-16	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
8	[illegible]	172	[illegible]	-4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
MEAN	25	175	70.8	-4	[illegible]	55	[illegible]	[illegible]	[illegible]
SD	[illegible]	5	[illegible]	[illegible]	[illegible]	21	[illegible]	[illegible]	[illegible]

Mean subject age was 25 years with a range of 23 to 32 years. The average duration of insulin-dependent diabetes was 11 years, with a range of 2 to 22 years. Body weight varied from 57.5 to 91.2 kg, with a mean value of 70.8 kg. Percent ideal body weight was calculated from the height and weight measurements as described in Chapter III. Five of the eight subjects fell within their recommended ideal body weight ranges (Metropolitan Life Insurance Company, 1983). However, subject 1 exceeded the upper limit of his range by 7.2 kg, while subjects 2 and 6 were 1.5 and 4.7 kg below the lower limits of their respective weight ranges.

The mean insulin dosage was 55 ±21 units or .78 ±.29 units/kg/day (mean ±SD). Three of the volunteers (subjects 4, 5 and 8) received their insulin as two daily injections (before breakfast and dinner), while the remainder received only a single

morning dose. Most of the subjects were using a combination of short- and intermediate-acting insulins.

Based on the submaximal bicycle ergometer test the predicted mVO_2 values fell between 2.1 and 3.3 l/min with a mean of 2.7 l/min. The values expressed in ml/kg/min represented a range from 25 to 46 ml/kg/min with an average mVO_2 of 38 ml/kg/min.

Exercise Parameters

A summary of the mean values and standard deviations of the low and high exercise intensity conditions are presented in Table 2.

TABLE 2

SUMMARY OF THE LOW AND HIGH EXERCISE INTENSITY CONDITIONS (MEAN VALUES +/- SD)

PARAMETER	LOW EXERCISE INTENSITY	HIGH EXERCISE INTENSITY
WORKLOAD (w)	69 +/- 12	136 +/- 24
TOTAL WORK (kj)	250 +/- 42	245 +/- 43
%mVO2 (%)	45 +/- 1	74 +/- 3
WORKING HR (bpm)	122 +/- 8	155 +/- 10

The workload performed under the high intensity condition was approximately twofold greater than that of the low intensity condition (136 ±24 versus 69 ±12 w). However, the total amount of work completed under both exercise protocols was relatively constant — the difference being only 2% (245 ±43 versus 250 ±42 kj). The relative workload intensities were 74 ±3 and 45 ±1% mVO_2 for

the high and low conditions respectively. Only subject 7 was unable to maintain the workload corresponding to 75% mVO_2, so a reduction in load to that representing 67% mVO_2 was required during the first 10 minutes. The resultant mean heart rate for the more intense protocol was 155 ±10 bpm, while that of the low intensity format was 122 ±8 bpm. Thus the basic conditions of the design, (exercise of two different physiological intensities and constancy of total work performed), were met.

Table 3 presents the mean blood glucose values and quantities of carbohydrate administered during each of the two exercise sessions.

TABLE [illegible]

MEAN BLOOD GLUCOSE VALUES AND QUANTITIES OF CARBOHYDRATE ADMINISTERED DURING THE LOW AND HIGH EXERCISE INTENSITY PROTOCOLS

(MEAN VALUES +/- SD)

	LOW EXERCISE INTENSITY		HIGH EXERCISE INTENSITY	
TIME (min)	BG (mM)	CARBOHYDRATE (g)	BG (mM)	CARBOHYDRATE (g)
[illegible]	5.8 +/- [illegible]	5.2 +/- 5.1	5.8 +/- [illegible]	[illegible]
20	[illegible] +/- [illegible]	5.5 +/- 4.3	[illegible]	[illegible]
40	[illegible] +/- [illegible]	4.4 +/- [illegible]	[illegible]	[illegible]
[illegible]	[illegible] +/- [illegible]	[illegible] +/- [illegible]	[illegible]	[illegible]

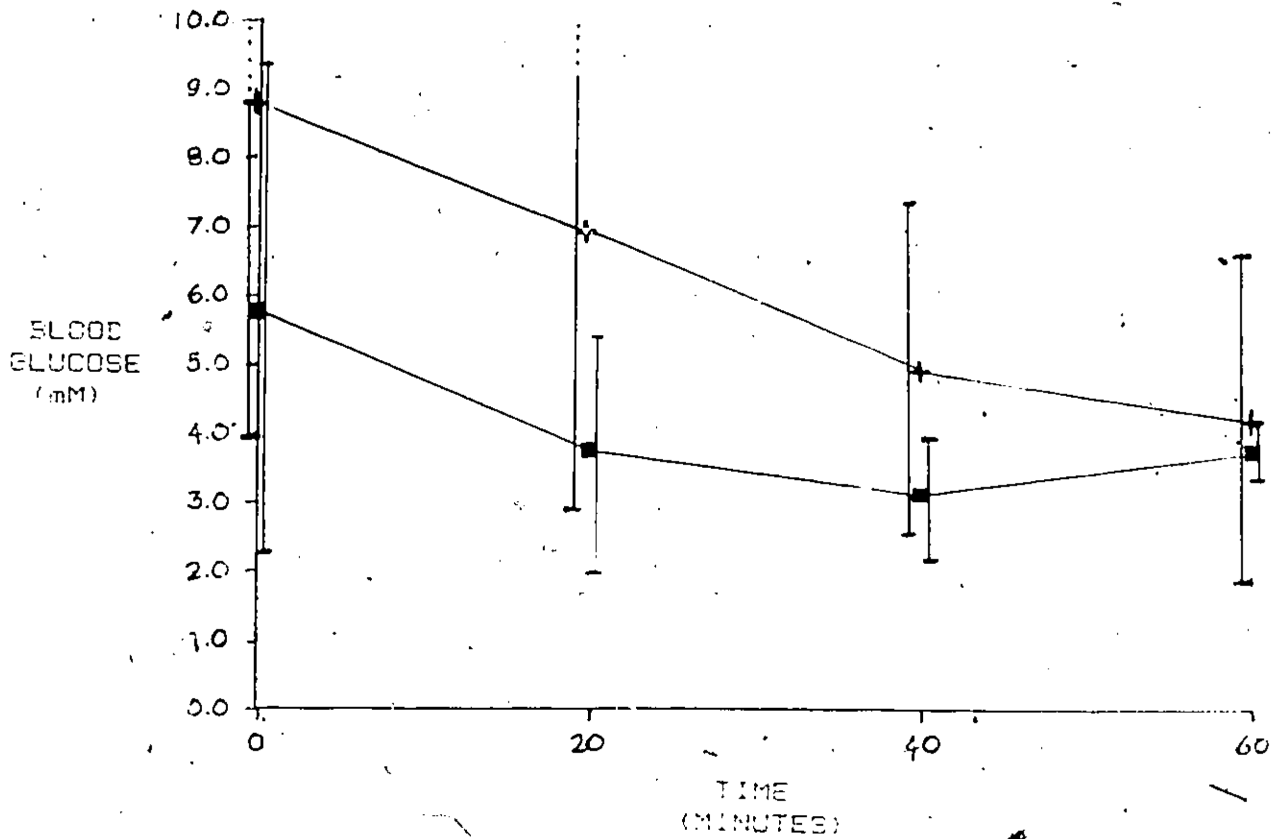
Two-way repeated measures analysis of variance indicated a significant difference in blood glucose over time ($F(3,21) = 10.92$; $p = .0002$). No such difference was noted between the overall BG values of the low and high intensity protocols ($F(1,7) = 3.39$; $p = .11$). There was however, a significant interaction between time and intensity ($F(3,21) = 3.48$; $p = .03$).

Subsequent Scheffé post hoc analysis of BG over time revealed that the values measured at 20 minutes (mean of 5.3 ±3.5 mM), 40 minutes (mean of 4.0 ±2.1 mM), and 60 minutes (mean of 4.0 ±1.8 mM) were all significantly lower (α = .05) than those recorded at zero time (mean of 7.3 ±4.3 mM).

Examination of the time-intensity interaction entailed the comparison of the mean low and high intensity BG results at each of the four time periods. For this purpose Scheffé post hoc simple effects testing was carried out (α = .05). Significant differences between the low and high intensity BG means were noted at zero, 20 and 40 minutes, but not at 60 minutes. These results are represented graphically in Figure 1.

FIGURE 1

ACUTE GLUCOSE RESPONSE UNDER
LOW AND HIGH EXERCISE INTENSITY CONDITIONS
(MEAN VALUES +/- SD)



■ LOW EXERCISE INTENSITY

+ HIGH EXERCISE INTENSITY

Two-way repeated measures analysis of variance of the amount of carbohydrate administered during the two exercise sessions indicated a significant difference over time only ($F(3,21) = 3.13$; $p = .047$). The main effect of low versus high exercise intensity on the amount of carbohydrate consumed to maintain the BG above 4mM was not significant ($F(1,7) = .95$; $p = .36$), nor was the time-intensity interaction ($F(3,21) = .65$; $p = .59$).

Scheffé post hoc analysis ($\alpha = .05$) of the amount of carboydrate administered across time demonstrated that more carbohydrate was provided in response to dropping BG at 20 and 40 minutes, than at 60 minutes.

Post-Exercise Plasma Glucose Responses

Table 4 summarizes the blood values monitored under each of the three treatment conditions.

TABLE 4

SUMMARY OF THE CONTROL AND EIGHT HOUR POST-EXERCISE BLOOD VALUES: HEMATOCRIT, PLASMA GLUCOSE, AND GLYCOSYLATED HEMOGLOBIN MEAN VALUES (+ - SD)

BLOOD PARAMETER	CONTROL	LOW	HIGH
HMCT (%)	54.4 + - 2.8	55.7 + - 4.[illegible]	53.[illegible] + - [illegible]
PG (mM)	[illegible] + - [illegible]	4.[illegible] + - [illegible]	[illegible] + - [illegible]
[illegible]	[illegible] + - [illegible]	[illegible] + - [illegible]	[illegible] + - [illegible]

Mean hematocrit values for the control, low and high days were 54.4 ±2.8, 55.7 ±4.0 and 53.1 ±3.3% respectively. Since

these did not vary significantly (F(2,14) = 3.55; p = .06), it was considered unnecessary to alter the plasma glucose results to account for plasma volume changes. Accordingly, the mean plasma glucose values for the control, low and high days were 7.3 ±4.8, 4.8 ±1.8 and 6.6 ±4.6 mM. These differences were not statistically significant (F(2,14) = 1.06; p = .37). Similarily, it was found that glycosylated hemoglobin did not differ significantly on the three experimental days (.089 ±.014, .090 ±.016 and .089 ±.014; F(2,14) = .26; p = .77).

Post-Exercise Insulin-Binding Responses

The specific ^{125}I-insulin-binding responses to the three treatment conditions are shown in Table 5.

TABLE 5

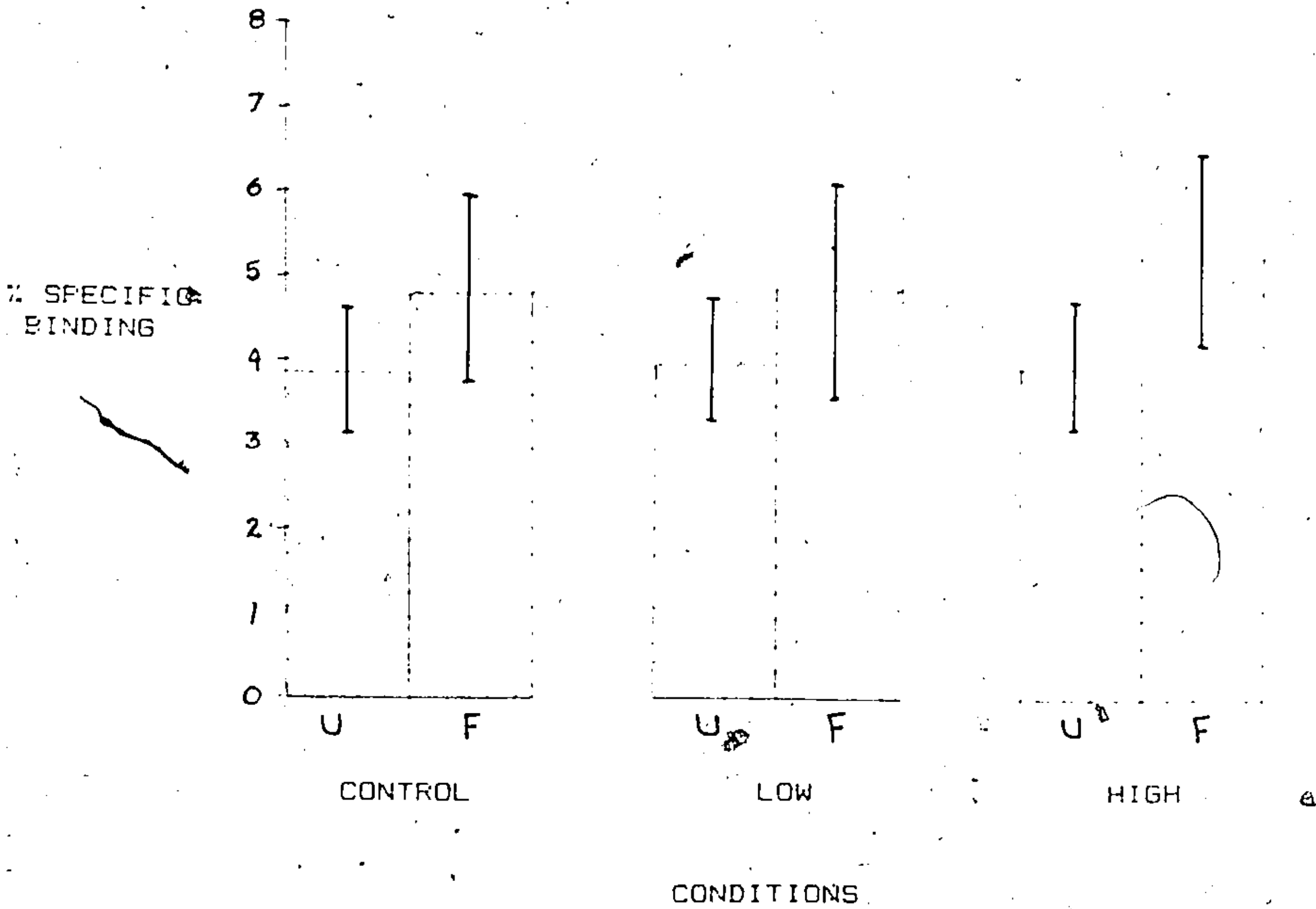
SPECIFIC INSULIN BINDING UNDER CONTROL AND EIGHT HOUR POST-EXERCISE CONDITIONS FOR UNFRACTIONATED AND FRACTIONATED ERYTHROCYTES

SUBJECT	CONTROL DAY		LOW DAY		HIGH DAY	
	U	F	U	F	U	F
1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
MEAN	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Mean specific binding values for the unfractionated red blood cells (U) were 3.85 $\pm$.80, 3.95 $\pm$.90 and 3.92 $\pm$.87% for the control, low and high days respectively. Corresponding figures for the fractionated series (F) were 4.78 $\pm$1.13, 4.85 $\pm$1.28 and 5.25 $\pm$1.15%. A two-way repeated measures analysis of variance revealed no significant differences across the three treatment conditions ($F(2,14) = .95$; $p = .41$). However, the fractionated samples proved to have significantly higher specific insulin binding than the unfractionated erythrocytes ($F(1,7) = 8.77$; $p = .02$). The sole exception to this overall trend was subject 3 who consistently demonstrated higher specific binding in his unfractionated samples. These findings are illustrated in Figure 2.

FIGURE 2

MEAN INSULIN BINDING RESPONSES
UNDER CONTROL AND EIGHT HOUR POST-EXERCISE CONDITIONS
(MEAN VALUES +/- SD)



U UNFRACTIONATED ERYTHROCYTES

F FRACTIONATED ERYTHROCYTES

The percent F fraction recovered on each of the three experimental days averaged 4.6 ±4.1, 4.7 ±3.8 and 5.4 ±3.5% (control, low and high days respectively). These did not differ significantly ($F(2,14) = .47$; $p = .63$). Thus the F fraction comprised the least dense (i.e. youngest) 5% of the total erythrocyte population.

Non-specific binding (NSB) had a mean value of 2.34 ±.50% for the U series and 2.62 ±.66% for the F series. Using a two-way repeated measures analysis of variance it was shown that NSB did not vary with treatment conditions ($F(2,14) = .25$; $p = .78$) or with the fraction of cells assayed ($F(1,7) = 3.53$; $p = .10$).

Correlations

Since the F fraction of red blood cells bound significantly higher than the U fraction, the former results were used in all correlations.

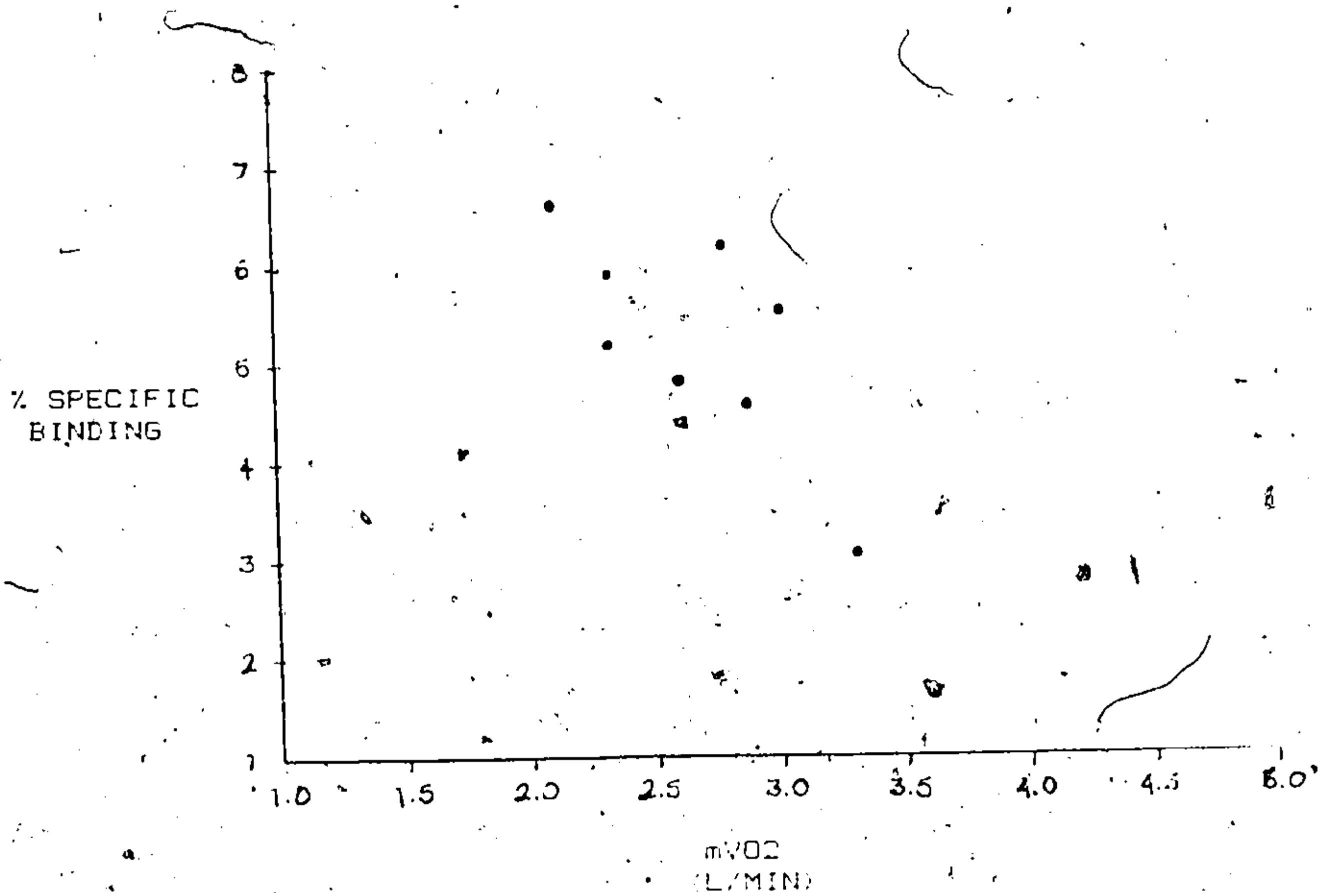
Pearson correlation coefficients for the control day insulin binding (F fraction) and age ($r = -.10$; $p = .41$), weight ($r = -.24$; $p = .29$), and insulin dose expressed both in absolute units ($r = -.47$; $p = .13$) and units/kg ($r = -.41$; $p = .16$) were all non-significant ($\alpha = .05$, one-tailed significance testing).

Maximum oxygen consumption expressed in l/min and ml/min/kg were each correlated separately to the F fraction binding data on the control, low and high days. Results from the control day binding and mVO_2 in l/min ($r = -.65$; $p = .08$) and ml/min/kg ($r = -.39$; $p = .36$) both proved to be non-significantly correlated. Insulin binding following the low exercise intensity correlated poorly with mVO_2 when

expressed either as l/min ($r = -.58$; $p = .14$) or as ml/min/kg ($r = -.10$; $p = .82$). In contrast, high intensity binding and mVO_2 expressed in l/min did show a significant correlation ($r = -.76$; $p = .03$). This relationship is depicted in Figure 3. The analogous correlation between high intensity binding and mVO_2 as ml/min/kg proved to be non-significant ($r = -.58$; $p = .15$).

FIGURE 3

RELATIONSHIP BETWEEN mVO2 (L/MIN)
AND PERCENT SPECIFIC BINDING OF THE F FRACTION
EIGHT HOURS FOLLOWING HIGH INTENSITY EXERCISE



When the total amount of work performed under low and high exercise conditions was correlated to the respective F binding fraction, the following results were obtained: $r = -.59$; $p = .13$ (low day) and $r = -.68$; $p = .06$ (high day).

Correlations between plasma glucose and F fraction binding over all three experimental conditions produced a non-significant $r = -.22$; $p = .31$. Similarly, glycosylated hemoglobin measured under control and post-exercise conditions and F binding correlated non-significantly with an $r = .19$; $p = .38$.

CHAPTER V

DISCUSSION

The discussion is divided into five sections corresponding to the results as presented in Chapter IV.

Subjects

Most subjects were within or slightly below (subjects 2 and 6) their ideal body weight range, with the exception of subject 1 (Metropolitan Life Insurance Company, 1983). This latter individual exceeded his proposed upper limit by 7.2 kg. Despite this tendency towards obesity, subject 1 did not demonstrate any apparent differences in his responses to the treatment conditions when compared with his leaner counterparts. Since it is recognized that obesity is associated with reduced insulin binding and sensitivity (Harrison et al., 1976; Olefsky, 1976; Bar, 1976; DeFronzo et al., 1978), perhaps the extent to which subject 1 was overweight was below that required to elicit any noticeable differences.

The insulin dose among participants varied from 32 to 90 units/day with a mean of .78 units/kg/day. These values correspond with the approximate dose of .5 to 1.0 units/kg/day recommended by Skyler et al. (1981). Interestingly, the two underweight subjects (numbers 2 and 6) were on the lowest insulin doses (.49 and .52 units/kg/day respectively). While this might suggest that both participants were receiving suboptimal quantities of insulin, their

respective HbA_1 levels were within normal limits and did not support this hypothesis.

Maximum oxygen consumption estimates (mean of 38 ±7 ml/min/kg) were somewhat lower than those reported by Astrand and Rodahl (1977) for a comparable group (47 ±5 ml/min/kg). It is feasible that the diabetic group may have been less prone to engage in regular fitness activities due to difficulties arising from hypoglycemic episodes and the changes in insulin and diet required.

Acute Exercise Responses

The inability of subject 7 to complete the 75% mVO_2 workload was rather unexpected as his predicted mVO_2 was one of the higher values at 42 ml/min/kg, and similar problems were not experienced by any of the other volunteers. The most likely explanation is that his predicted mVO_2 was overestimated since his mean working heart rate, after the exercise intensity was reduced to 67% mVO_2, was still 166 bpm compared with the group average of 155 ±10 bpm. Therefore despite the reduction in relative intensity, the heart rate implies that a high level of exertion was maintained.

Mean heart rates for the low (122 ±8 bpm) and high exercise bouts (155 ±10 bpm) were comparable to those reported in earlier exercise studies (Oberdisse et al., 1977; Kemmer et al., 1979).

Comparison of the BG responses to the two different exercise protocols demonstrated a significant drop across time ($p = .0002$). Mean values at 20 (5.3 ±3.5 mM), 40 (4.0 ±2.1 mM) and 60 (4.0 ±1.8 mM) minutes were each significantly lower than the pre-exercise

BG value (7.3 ±4.3 mM). This trend occurred despite the fact that carbohydrate was administered to prevent BG from falling below 4mM. Such a response has been well documented (Klachko et al., 1972; Pruett and Machlum, 1972; Oberdisse et al.; 1977; Hagan et al., 1979) and is not surprising in light of the fuel requirements. Insulin release from depot sites during exercise may also have contributed to this BG decline by inhibiting liver glycogen breakdown and release. The fact that greater quantities of carbohydrate were necessary at 20 and 40 minutes compared to 60 minutes ($p = .047$) supports the findings of Oberdisse et al. (1977) where BG fell most dramatically in the first 30 minutes of activity — analagous to the pattern seen here.

No difference was observed in the overall BG means between the low and high exercise intensities ($p = .11$). This would appear to contradict the research of Klachko et al. (1972) and Hagan et al. (1979) whose studies have suggested that the higher the exercise intensity, the greater the BG lowering effect. However, interpretation of the present result is complicated by the fact that the two initial pre-exercise BG levels differed (significant time-intensity interaction, $p < .05$), and that carbohydrate was provided to prevent risk of hypoglycemia. Considering these circumstances it would be unwise to attempt to draw any conclusions concerning intensity effects per se on the acute BG responses.

Hematocrit, Plasma Glucose and Glycosylated Hemoglobin

The hematocrit values on the control and two post-exercise days did not differ significantly ($p = .06$). If any hemoconcentration did occu[illegible]t was likely corrected by fluid intake before the subsequent

eight hour post-exercise blood sample was drawn. Since hematocrit was consistent, no adjustment of plasma glucose was made.

The resultant PG levels did not show any statistical differences ($p = .37$), though both post-exercise means (4.8 ±1.8 mM for the low and 6.6 ±4.6 mM for the high condition) were slightly lower than the control day mean (7.3 ±4.8 mM). The rather large variance in PG between subjects may have contributed to the lack of significance reported.

Glycosylated hemoglobin did not differ across the three treatment conditions ($p = .77$), substantiating that the degree of long-term diabetes control was stable throughout the experimental period. Values for the control day averaged .089 g/l with a range from .072 to .110 g/l. These were slightly higher than the corresponding figures quoted by Koenig et al. (1976) for non-diabetics (.055 to .085 g/l), but less than those reported for uncontrolled diabetics (.120 to .200 g/l) (Koenig et al., 1976). Since different laboratories have different norms, the diabetic HbA_1 results were compared with those from five non-diabetics evaluated at the same laboratory (Appendix B). A significant difference was noted ($p = .002$), confirming that the eight participants were not in "ideal" control (i.e. equivalent to non-diabetics), but were within the usual diabetic range (.077 to .120 g/l) (Ottawa Civic Hospital, personal communication).

Insulin-Binding Responses

Comparison of insulin binding following the three treatment conditions revealed no significant differences ($p = .41$). Therefore,

the original hypothesis that insulin-binding responses measured eight hours after the low and high exercise conditions would differ significantly from that noted under resting conditions was not supported.

Although a number of researchers have demonstrated enhanced insulin binding immediately post-exercise (Soman et al., 1978; Koivisto et al., 1979; Pedersen et al., 1980), few have examined how long this effect persists. Soman et al. (1978) reported a return to baseline values by 24 hours, but interim measures were not reported. The present study suggests that such potential receptor adaptations (as monitored by the erythrocyte assay) are not demonstrable at eight hours post-exercise. The differences in the control (4.78 ±1.13% SB), low intensity (4.85 ±1.28% SB) and high intensity binding responses (5.25 ±1.15% SB) did show an increasing trend, but failed to reach significance. The reason for this may be that the effects induced by exercise are relatively short-lived. Support for this comes from glycogen studies which have demonstrated approximately 83 to 91% repletion during the first 12 hours of recovery from exercise, but with the maximal rate occurring during the first four hours (Maehlum et al., 1977; Hermansen, 1980).

The F fraction of cells proved to have significantly higher specific insulin binding than the unfractionated cells (p = .02). This confirms the earlier work of Polychronakos et al. (1981; 1982) and supports the second hypothesis stated in Chapter 1. This higher binding among the younger fraction of cells may reflect a greater protein adaptibility which is reduced in the more mature cells lacking nuclei.

It is peculiar that subject 3 consistently demonstrated greater specific binding in his unfractionated samples — contrary to all other subjects. Nothing unusual appeared in his non-specific binding values. Perhaps in this individual the younger cells were somehow altered, damaged or generally less receptive to the insulin molecule. As these cells were not specifically examined and such a finding has not been reported elsewhere, a clear explanation of this phenomenon is lacking.

The percent of F fraction recovered on each of the three experimental days did not vary significantly ($p = .63$), implying a consistency in the red cell density (age) distribution (Danon and Marikovsky, 1964).

Values for the non-specific binding revealed no statistical difference between the U ($2.34 \pm .50\%$) and F series ($2.62 \pm .66\%$) ($p = .10$), indicating that the separation technique did not disrupt the integrity and binding characteristics of the younger cells. Comparison of NSB across treatment conditions also proved to be consistent, as expected ($p = .78$).

A final point worth discussing is the interpretation of the absolute binding values obtained. Reports from the literature quote values for healthy male adults of approximately 7-10% per 3.52×10^9 erythrocytes/ml (Gambhir et al, 1978; Gambhir et al., 1979). Diabetic values have been variously reported as either higher (Fantus et al., 1981) or lower (Brown et al., 1982) than normal controls. This heterogeneity appears to stem from the degree of metabolic control attained. When diabetics are insulin-deficient and ketotic the binding may be enhanced (Fantus et al., 1981), whereas adequately controlled

diabetics seem to undergo a down-regulation phenomenon related to peripheral hyperinsulinemia (Brown et al., 1982). Results from the diabetics in this study (4.78 ±1.13% SB per 4.0 x 10^9 erythrocytes/ml) suggest that down-regulation has occurred, since comparative values from both the literature (Gambhir et al., 1978) and from the five non-diabetic volunteers (7.14 ±1.60% SB, Appendix B) are substantially greater ($p = .009$).

Correlations

Correlations between F fraction binding and the subject characteristics of age, weight and insulin dose all proved to be non-significant. While others have found important negative relationships between these parameters (Pedersen, 1984), the above results may reflect the limited ranges of the variables (i.e. a span of only 10 years for age), as well as the small number of subjects studied. Despite this lack of significance, each of the above factors was related in a negative sense to insulin binding. It has been hypothesized that a reduction in receptor number may be responsible for these trends (Harrison et al., 1976; Bar et al., 1976; Brown et al., 1982).

Correlations involving F fraction binding and mVO_2 data produced rather interesting findings. Whenever the weight factor was removed from the mVO_2 expression (mVO2 in l/min versus mVO2 in ml/min/kg) the correlation coefficient was reduced. This suggests that body weight somehow contributes to the binding response. It is known that elevated levels of body fat are associated with reduced binding

(Harrison et al., 1976; Bar et al., 1976). Therefore it may be this particular aspect of the body weight term that maintains a slightly stronger correlation when mVO_2 is expressed in l/min.

All correlations of binding with mVO_2 were negative and non-significant with the exception of F fraction binding following high intensity exercise and mVO_2 in l/min ($r = -.76$; $p = .03$). This indicates that the more aerobically fit the individual, the lower the receptor binding eight hours after the intense exercise bout (or conversely, the less fit the individual the higher the binding eight hours post-exercise). Although Koivisto et al. (1979) have reported a similar inverse relationship between mVO_2 and immediate post-exercise binding values in athletes, subjects in the present study were untrained. Why this relationship should prove to be significant following the high intensity activity only is not entirely clear. Factors known to influence binding, (ketone bodies, hormones, PH etc.), may have varied as a result of the different treatment conditions. For example, the more fit individuals may have experienced less post-exercise ketosis under the high intensity conditions than their less fit counterparts. Since ketone bodies induce elevated receptor binding (Pedersen, 1984) this could theoretically account for the negative correlation seen. Finally, this correlation may have been due to chance. Additional research involving a larger sample size might help to clarify this relationship.

Although PG was negatively correlated with binding as previously reported in some sensitivity studies (Soman et al., 1978), it proved to be non-significant ($r = -.22$; $p = .31$). Pedersen et al. (1980) also

failed to demonstrate any relationship between glucose levels and erythrocyte binding. Therefore despite the presumed link between binding and tissue sensitivity to glucose uptake, circulating PG alone cannot be expected to predict binding changes.

The last correlation considered was that of HbA_1 and binding. A lower HbA_1 indicates better control which is usually maintained by peripheral hyperinsulinemia. Down-regulation of receptors would be expected with high insulin levels. The positive correlation obtained ($r = .19$; $p = .38$) supports this line of reasoning, however the small range among HbA_1 values may have precluded the demonstration of a significant correlation.

CHAPTER VI

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The purpose of this study was to determine whether or not exercise of varying intensity would alter the post-exercise insulin-binding responses in male insulin-dependent diabetics eight hours after the completion of activity. It was also the intent to compare whether or not insulin-receptor binding to the entire erythrocyte population would differ from binding to the youngest (least dense) fractions of these cells. It was reasoned that the fall in glycemia during the post-exercise hours among insulin-dependent diabetics (Caron et al., 1981) would be related to an enhanced insulin sensitivity (Maehlum et al., 1977; Hermansen, 1980) reflected in binding adaptations in the erythrocyte model.

Eight adult male insulin-dependent diabetics were monitored under three treatment conditions — a control day (no exercise), a low intensity exercise day (60 minutes of continuous cycling at a workload corresponding to 45% mVO_2) and a high intensity exercise day (60 minutes of intermittent work/rest intervals at a workload representing 75% mVO_2). Post-exercise blood samples were drawn eight hours after the exercise was completed.

The acute response to both exercise protocols was a lowering of blood glucose, but this effect did not remain in evidence when followed

up eight hours later. No significant differences in insulin binding were found between the control and two exercise conditions. However, binding to the youngest fraction of erythrocytes did prove to be significantly greater than binding to the entire blood cell population. Finally, a significant negative correlation was demonstrated between mVO_2 expressed in l/min and binding to the fractionated erythrocytes measured eight hours after high intensity exercise.

Conclusions

Based on the findings of this research the following may be concluded:

1. A significant decline in blood glucose occurs in reasonably well-controlled insulin-dependent diabetics during exercise of 45% mVO_2 (60 minutes of continuous activity) and 75% mVO_2 (60 minutes of intermittent work/rest activity, 30 minutes total work). Furthermore, this drop can be detected as early as 20 minutes into the activity.
2. Plasma glucose values measured in insulin-dependent diabetics eight hours following low intensity (45% mVO_2, 60 minutes) and high intensity exercise (75% mVO_2, 30 minutes) tend not to differ significantly from each other or from control day values.
3. Exercise of either 45% mVO_2 (60 minutes) or 75% mVO_2 (30 minutes) does not significantly alter the insulin-binding responses on erythrocytes in male insulin-dependent diabetics

eight hours following completion of activity. Since glucose turnover was not assessed in the present study, broader interpretations concerning post-exercise insulin sensitivity can not be made.

4. The youngest (least dense) fraction of erythrocytes demonstrates markedly higher insulin-receptor binding as compared with the entire erythrocyte population. Though not definitive, this pattern accomodates the idea that change at the receptor level may involve some form of protein adaptation.

5. Fractionated cell binding in insulin-dependent diabetics measured eight hours after a bout of high intensity exercise (75% mVO_2, 30 minutes) correlates significantly, in a negative sense, with mVO_2 expressed in l/min. This inverse relationship associates lower binding at eight hours post-exercise with higher physical fitness levels. The significance of this is currently unknown.

Recommendations

In view of the outcome of this study, the following recommendations are proposed for further research:

1. If a similar study were to be undertaken it is suggested that the measurement of insulin-receptor binding to erythrocytes be accompanied by a technique directly evaluating in vivo insulin sensitivity (i.e. the euglycemic clamp technique). The

alternative of obtaining muscle samples for glycogen analysis and insulin-receptor studies would likely be difficult to accomplish on ethical grounds.

2. Expansion of the experimental design to include serial time samples from completion of the exercise until baseline values were re-established could systematically determine the duration of post-exercise receptor and sensitivity changes.
3. In light of the unexplained significant correlation between post-high exercise intensity binding and mVO_2 (l/min), additional research specific to these variables may be warranted. Obtaining a larger sample size with a greater range of mVO_2 values might help to elucidate this relationship.

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APPENDIX A

EXPERIMENTAL DATA

Table A1 Low Exercise Intensity Conditions

Table A2 High Exercise Intensity Conditions

Table A3 Control and Eight Hour Post-Exercise Blood Values

Table A4 Insulin-Binding Data

TABLE A1

LOW EXERCISE INTENSITY CONDITIONS

SUBJECT	LOAD (w)	TOTAL WORK (J)	% $\dot{V}O_2$ (%)	HEART RATE (bpm)	TIME (min)	BLOOD GLUCOSE (mM)	CARBOHYDRATE RECEIVED (g)
1	60	216000	46	130	0	3.4	3.2
					20	2.2	9.6
					45	2.1	9.6
					60	3.7	
2	50	180000	46	130	0	6.1	
					30	2.9	6.4
					50	2.8	
					60	3.8	
3	85	306000	42	113	0	9.8	
					20	7.3	
					40	5.3	
					60	3.9	
4	60	216000	46	123	0	3.1	9.6
					20	2.3	9.6
					40	2.8	6.4
					60	3.9	
5	90	238000	44	123	0	2.9	6.4
					20	2.5	9.6
					40	2.9	9.6
					60	4.1	
6	70	252000	46	116	0	2.8	12.8
					20	4.1	
					30	3.7	
					40	2.8	
					60	3.6	9.6
7	75	270000	44	121	0	11.9	
					20	5.6	
					40	3.7	9.6
					60	3.7	
8	75	270000	45	109	0	6.2	9.6
					20	2.8	9.6
					40	2.7	
					60	3.4	
MEAN	69	249750	45	122		4.1	8.7
SD	12	40419	1	8		2.1	2.2

TABLE A2

HIGH EXERCISE INTENSITY CONDITIONS

SUBJECT	LOAD (W)	TOTAL WORK (J)	% $\dot{V}O_2$ (%)	TIME (min)	MEAN HEART RATES WORK (bpm)	MEAN HEART RATES REST (bpm)	TIME (min)	BLOOD GLUCOSE (mM)	CARBOHYDRATE RECEIVED (g)
1	115	207000	75	30	148	105	0	2.9	6.4
				60	149	107	20	3.1	6.4
							40	2.9	6.4
							60	2.7	6.4
2	100	180000	75	30	144	101	0	12.2	
				60	150		20	7.8	
							40	4.7	
							60	3.4	
3	175	315000	75	30	158	111	0	14.2	
				60	172	129	20	11.9	
							40	8.1	
							60	6.0	
4	120	216000	75	30	157	104	0	8.0	
				60	167	130	20	5.8	
							40	3.8	6.4
							60	2.6	
5	155	279000	75	30	151	95	0	14.4	
				60	152	96	20	12.7	
							40	9.5	
							60	9.8	
6	140	252000	75	30	150		0	7.9	
				60		105	20	4.5	3.2
							40	3.8	6.4
							60	4.2	
7	133	239400	67	30	169		0	9.0	
				60	162	100	20	6.6	6.4
							30	6.1	9.6
							40	3.6	
							60	2.2	
8	150	270000	75	30		92	0	1.7	12.8
				60	138		20	2.0	12.8
							40	2.8	9.6
							60	2.5	
MEAN	136	244800	74		155	105		6.2	7.7
SD	24	43542	3		10	12		3.8	2.9

TABLE A3

CONTROL AND EIGHT HOUR POST-EXERCISE BLOOD VALUES: GLYCOSYLATED HEMOGLOBIN, HEMATOCRIT, PLASMA VOLUME CHANGES FROM CONTROL, AND UNCORRECTED AND CORRECTED GLUCOSE VALUES

SUBJECT	HbA1 (g/1%)			HMCT (%)			PLASMA VOLUME CHANGE (%)		UNCORRECTED PLASMA GLUCOSE (mM)			CORRECTED PLASMA GLUCOSE (mM)		
	C	L	H	C	L	H	L	H	C	L	H	C	L	H
1	0.076	0.070	0.075	58.3	57.0	52.7	5.4	25.4	3.7	2.7	7.7	3.7	2.8	9.6
2	0.073	0.080	0.083	52.3	56.5	50.9	-15.5	5.8	3.7	4.1	3.2	3.7	3.5	3.4
3	0.089	0.086	0.075	54.8	54.[illegible]	55.7	-0.4	-3.6	14.2	3.8	11.4	14.2	3.8	[illegible]
4	0.101	0.105	0.102	49.1	51.2	48.3	-8.0	3.2	3.8	7.6	[illegible]	3.8	7.0	3.1
5	0.110	0.116	0.107	55.1	54.7	53.5	1.6	6.6	14.8	3.6	15.4	14.8	3.5	16.4
6	0.072	0.072	0.072	53.8	50.3	50.1	15.0	16.0	6.0	6.0	3.7	6.0	6.9	4.2
7	0.093	0.093	0.099	56.3	57.7	54.9	-5.5	5.8	3.3	7.0	4.9	3.3	6.6	5.2
8	0.097	0.101	0.101	55.6	63.1	58.3	-26.7	-10.4	8.8	3.5	3.3	8.8	2.6	[illegible]
MEAN	0.089	0.090	0.089	54.4	55.7	53.1	-4.3	6.1	7.3	4.8	6.5	[illegible]	4.6	[illegible]
SD	0.014	0.016	0.014	2.8	4.0	3.3	17.9	11.0	4.8	1.8	4.6	4.8	1.9	4.9

C = CONTROL CONDITION
L = LOW INTENSITY EXERCISE CONDITION
H = HIGH INTENSITY EXERCISE CONDITION

TABLE A4

INSULIN-BINDING DATA

DAY	SUBJECT	F RECOVERY (%)	NON-SPECIFIC BINDING (%) U	NON-SPECIFIC BINDING (%) F	SPECIFIC BINDING (%) U	SPECIFIC BINDING (%) F
CONTROL DAY	1	3.0	1.40	2.00	3.40	3.91
	2	1.3	1.72	1.55	4.39	5.77
	3	2.8	2.92	2.45	3.94	3.18
	4	13.5	2.38	2.91	4.33	6.09
	5	2.0	2.39	3.54	2.75	5.47
	6	2.5	2.40	2.90	3.20	4.66
	7	7.5	2.62	2.47	3.34	3.88
	8	3.8	2.70	2.70	4.92	4.67
	MEAN	4.6	2.27	2.57	3.85	4.78
	SD	4.1	0.59	0.69	0.80	1.13
LOW EXERCISE DAY	1	4.5	1.27	1.24	3.91	3.21
	2	5.0	1.41	1.51	3.73	7.05
	3	2.5	2.62	3.74	3.95	3.66
	4	13.3	2.74	2.31	5.31	5.59
	5	2.5	2.51	3.51	2.80	4.23
	6	1.5	2.15	3.94	4.71	5.19
	7	5.5	3.13	2.31	3.44	4.10
	9	2.5	2.55	2.98	5.04	5.54
	MEAN	4.7	2.30	2.63	3.95	4.85
	SD	3.8	0.65	0.92	0.90	1.29
HIGH EXERCISE DAY	1	3.8	2.51	2.47	3.31	5.25
	2	10.5	2.63	3.12	3.89	5.69
	3	3.8	2.21	1.94	3.75	3.35
	4	11.3	2.49	2.65	4.54	5.96
	5	2.0	2.21	3.41	3.62	5.33
	6	3.3	2.74	2.57	2.99	4.85
	7	5.0	2.29	2.86	4.29	4.57
	8	3.5	2.48	2.39	5.55	6.33
	MEAN	5.4	2.45	2.69	3.92	5.25
	SD	3.5	0.19	0.46	0.87	1.15

APPENDIX B

NON-DIABETIC COMPARATIVE DATA

Since insulin-binding values can vary considerably from one research group to another, the following data have been included so that the results based on the technique used in this study would be available for both diabetic and non-diabetic populations. Table B1 represents the individual subject characteristics, means and standard deviations obtained under resting conditions from five non-diabetic volunteers.

TABLE B1

NON-DIABETIC COMPARATIVE SUBJECT DATA
(RESTING CONDITION)

SUBJECT	AGE (yrs)	HT (cm)	WT (kg)	+/- %IBW (%)	HMCT (%)	HbA1 (g/[illegible])	PLASMA GLUCOSE (mM)	F RECOVERY (%)	NON-SPECIFIC BINDING U (%)	NON-SPECIFIC BINDING F (%)	SPECIFIC BINDING U (%)	SPECIFIC BINDING F (%)
1	26	177	85.5	14	55.0	0.057	4.9	4.0	2.91	2.88	[illegible]	5.34
2	25	170	66.0	-7	53.2	0.071	4.7	5.0	2.41	2.45	4.46	[illegible]
3	23	176	67.5	-9	48.1	0.064	4.6	2.5	2.46	2.05	[illegible]	[illegible]
4	28	182	73.4	-5	51.0	0.071	5.1	7.5	2.68	[illegible]	[illegible]	9.20
5	25	173	69.5	-3	57.0	0.060	4.9	1.5	[illegible]	[illegible]	[illegible]	7.39
MEAN	25	176	72.4	-2	52.9	0.065	4.8	4.1	2.57	2.28	4.46	[illegible]
SD	2	4	7.8	9	3.5	0.006	0.2	[illegible]	0.23	[illegible]	[illegible]	1.60

Selected statistical analyses were carried out on the above insulin-binding data for the purpose of comparison with that presented

in Chapter IV.

As with the diabetic group the F fraction of erythrocytes from the non-diabetics bound significantly higher insulin (7.14 ±1.60% SB) than the corresponding unfractionated red blood cell samples (4.46 ±.85% SB) ($t_{(4)}$ = 5.36; p = .003; one-tailed test). In addition, the non-diabetics proved to have significantly higher F fraction binding under control conditions (7.14 ±1.60% SB) than did the diabetics (4.78 ±1.13% SB; $t_{(11)}$ = 3.14; p = .009). Under the same resting conditions the unfractionated binding samples did not differ significantly between the two groups (4.46 ±.85% SB for the non-diabetics and 3.85 ±.80% SB for the diabetics, $t_{(11)}$ = 1.32; p = .21). The percent F recovery was similar between both groups (4.1 ±2.3% for the non-diabetics and 4.6 ±4.1% for the diabetics, $t_{(11)}$ = .22; p = .83), as was the non-specific binding (2.52 ±.34% for the non-diabetics and 2.42 ±.60% for the diabetics, $t_{(11)}$ = .59; p = .56).

Of all the other parameters in Table B1, only the HbA_1 values differed significantly (α = .05) between the non-diabetic (.065 ±.006 g/l) and diabetic groups (.089 ±.014 g/l) ($t_{(11)}$ = 3.60; p = .002).

APPENDIX C

METROPOLITAN LIFE INSURANCE COMPANY HEIGHT AND WEIGHT TABLE FOR MEN 1983

1983 Metropolitan Height and Weight Table for Men

Weights at ages 25-59 based on lowest mortality. Weight in pounds according to frame (in indoor clothing weighing 5 lbs., shoes with 1" heels).

Source of basic data: *1979 Build Study*, Society of Actuaries and Association of Life Insurance Medical Directors of America, 1980.

Height Ft.	Height Inch.	Small Frame	Medium Frame	Large Frame
5	2	128–134	131–141	138–150
5	3	130–136	133–143	140–153
5	4	132–138	135–145	142–156
5	5	134–140	137–148	144–160
5	6	136–142	139–151	146–164
5	7	138–145	142–154	149–168
5	8	140–148	145–157	152–172
5	9	142–151	148–160	155–176
5	10	144–154	151–163	158–180
5	11	146–157	154–166	161–184
6	0	149–160	157–170	164–188
6	1	152–164	160–174	168–192
6	2	155–168	164–178	172–197
6	3	158–172	167–182	176–202
6	4	162–176	171–187	181–207

Weights at ages 25-59 based on lowest mortality. Weight in kilograms according to frame (in indoor clothing weigbing 2.3 kgs., shoes with 2.5 cm heels).

Source of basic data: *1979 Build Study*, Society of Actuaries and Association of Life Insurance Medical Directors of America, 1980.

Height Cms.	Small Frame	Medium Frame	Large Frame
158	58.3–61.0	59.6–64.2	62.8–68.3
159	58.6–61.3	59.9–64.5	63.1–68.8
160	59.0–61.7	60.3–64.9	63.5–69.4
161	59.3–62.0	60.6–65.2	63.8–69.9
162	59.7–62.4	61.0–65.6	64.2–70.5
163	60.0–62.7	61.3–66.0	64.5–71.1
164	60.4–63.1	61.7–66.5	64.9–71.8
165	60.8–63.5	62.1–67.0	65.3–72.5
166	61.1–63.8	62.4–[illegible]	65.6–73.2
167	61.5–64.2	62.8–68.2	66.0–74.0
168	61.8–64.6	63.2–68.7	66.4–74.7
169	62.2–65.2	63.8–69.3	67.0–75.4
170	62.5–65.7	64.3–69.8	67.5–76.1
171	62.9–66.2	64.8–70.3	68.0–76.8
172	63.2–66.7	65.4–70.8	68.5–77.5
173	63.6–67.3	65.9–71.4	69.1–78.2
174	63.9–67.8	66.4–71.9	69.6–78.9
175	64.3–68.3	66.9–72.4	70.1–79.6
176	64.7–68.9	67.5–73.0	70.7–80.3
177	65.0–69.5	68.1–73.5	71.3–81.0
178	65.4–70.0	68.6–74.0	71.8–81.8
179	65.7–70.5	69.2–74.6	72.3–82.5
180	66.1–71.0	69.7–75.1	72.8–83.3
181	66.6–71.6	70.2–75.8	73.4–84.0
182	67.1–72.1	70.7–76.5	73.9–84.7
183	67.7–72.7	71.3–77.2	74.5–85.4
184	68.2–73.4	71.8–77.9	75.2–86.1
185	68.7–74.1	72.4–78.6	75.9–86.8
186	69.2–74.8	73.0–79.3	76.6–87.6
187	69.8–75.5	73.7–80.0	77.3–88.5
188	70.3–76.2	74.4–80.7	78.0–89.4
189	70.9–76.9	74.9–81.5	78.7–90.3
190	71.4–77.6	75.4–82.2	79.4–91.2
191	72.1–78.4	76.1–83.0	80.3–92.1
192	72.8–79.1	76.8–83.9	81.2–93.0
193	73.5–79.8	77.6–84.8	82.1–93.9

APPENDIX D

NOMOGRAM USED FOR THE DERIVATION OF mVO_2 AND EXERCISE WORKLOADS

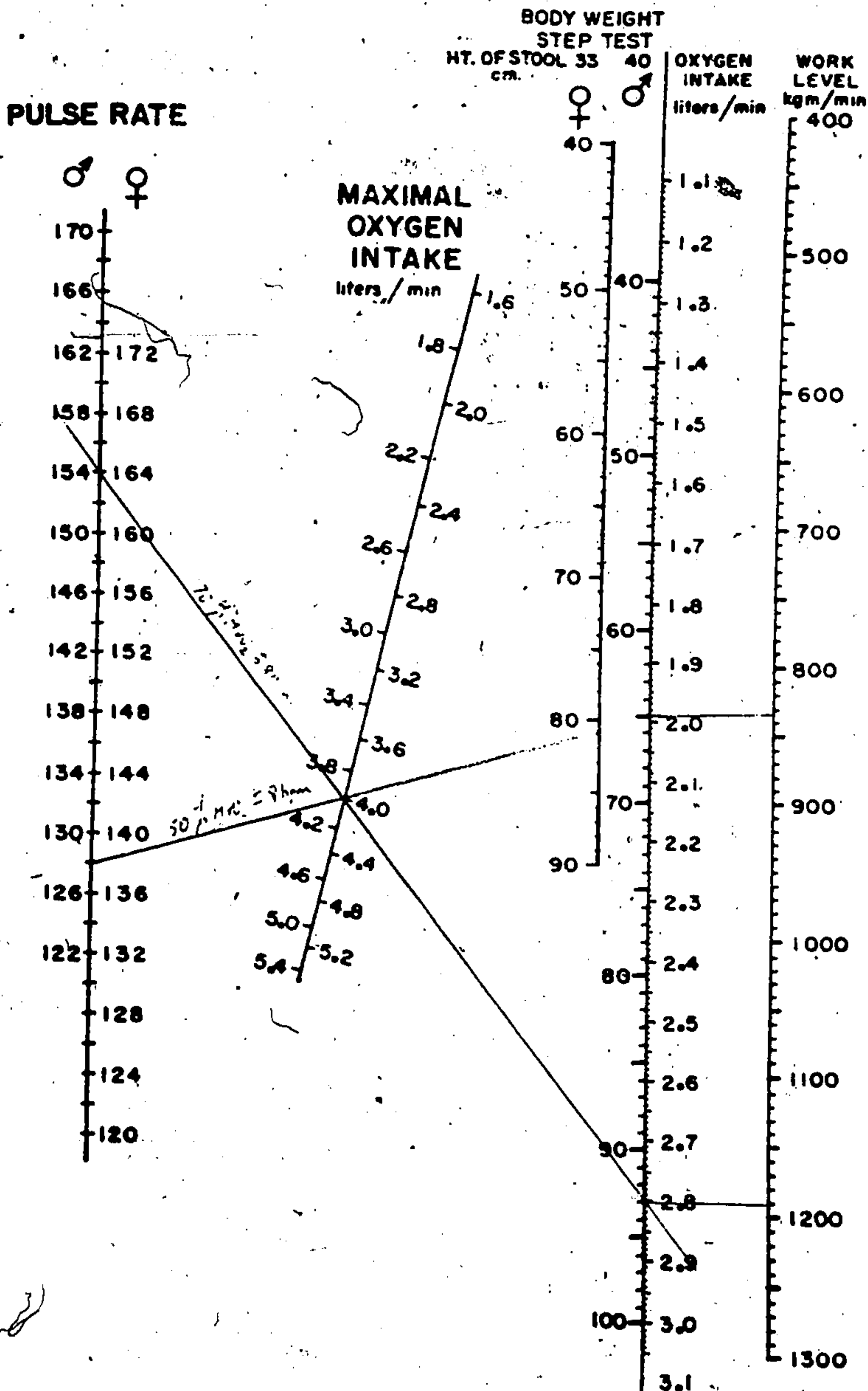

Fig. 10-3. Calculation of aerobic capacity during submaximal work. [*Åstrand and Ryhming* (1954)]

APPENDIX E

SUBJECT AND PHYSICIAN CONSENT FORMS

STATEMENT OF INFORMED CONSENT

PROJECT TITLE: The Effect of Exercise Intensity on Metabolic and Insulin-Binding Responses in Male Insulin-Dependent Diabetics.

RESEARCHERS: Gayle Ekstrand, Graduate Student
Michael Booth, Ph.D., Researcher Advisor

This thesis project will investigate the duration, and compare the metabolic and insulin-binding responses of male insulin-dependent diabetics following activity of low and moderately high intensity. The anticipated research benefits include increased knowledge of the effects of varied exercise intensity on post-exercise diabetic control, and insight into the possibility of acute exercise-related insulin receptor adaptation. Ultimately it is hoped that such information can be used to provide more practical guidelines regarding exercise for the active diabetic.

I understand that in volunteering for this study I will submit to the following procedures. On three separate days I will report to the Diabetes Research laboratory at 6:30 (fasting) at which time a blood sample will be drawn from the antecubital vein (20 ml). On two of the evenings immediately preceding these morning blood samples, I will participate in a bicycle exercise protocol. A submaximal bicycle test will be performed on both these occasions to predict the maximal oxygen consumption. This will then be followed either by the low intensity workbout continued for 1 hour at approximately 40% maximal oxygen consumption (roughly corresponding to a heart rate of 120 bpm), or by the high intensity workload performed in work/rest intervals over a 1 hour period. (This latter load will represent 75% of maximal oxygen consumption and will correspond to a heart rate of approximately 160 bpm). The total amount of work completed on both evenings will be the same. The order of the exercise and control (non-exercise) evenings will be randomized. ECG heart rate monitoring will be carried out during all activity sessions. It is anticipated that I may wish to reduce my normal insulin dose, or increase my diet on the exercise days. The manner in which insulin and diet are manipulated is best discussed with a medical doctor as the regime established on the first day should be followed on both subsequent experimental days.

As with other forms of physical activity there are certain risks inherent in this study. Infection, bruising, muscle soreness, muscle strain, hypoglycemic reactions, hyperglycemia, and exercise-induced cardiovascular complications are possible. However precautions will be taken to minimize these risks.

I realize that in volunteering for this project all personal data will be kept confidential. General findings of the study will be provided upon request, and an opportunity to discuss personal results will be offered on an individual basis.

I, ________________________________, have read this document and understand the nature and risks involved in this project. I have had the opportunity to discuss any questions with the researcher, Gayle Ekstrand, and agree to participate in the study, I am aware that I may withdraw my consent or discontinue participation in this research at any time.

Signature of Volunteer Subject ______________________________

Signature of Witness ______________________________

Date ____________________

Dear Doctor,

I have recently expressed interest in participating in a graduate research project being conducted by Gayle Ekstrand, Department of Kinanthropology, University of Ottawa. The study will investigate the duration, and compare the metabolic and insulin-binding responses in male insulin-dependent diabetics following acute exercise of varying intensity.

The design of the study is such that I will report to the Diabetes Research laboratory at 6:30 on three mornings at which time a fasting blood sample will be drawn. On two of the evenings immediately preceding these morning blood samples, I will participate in a bicycle exercise protocol. A submaximal bicycle ergometer test will be performed on both these occasions to predict maximal oxygen consumption. This will then be followed either by the low intensity workbout continued for 1 hour at approximately 40% maximal oxygen consumption (roughly corresponding to a heart rate of 120 bpm), or by the high intensity workload performed in work/rest intervals over a 1 hour period. (This latter work load will represent 75% of maximal oxygen consumption and will correspond to a heart rate of approximately 160 bpm). The total amount of work completed on both evenings will be the same. The order of the exercise and control (non-exercise) evenings will be randomized. ECG heart rate monitoring will be carried out during all activity sessions. Fasting morning blood specimens will be analyzed for glucose, glycosylated hemoglobin A, and specific insulin receptor binding to erythrocytes.

It is recognized that exercise usually necessitates a reduction in insulin dose, an increase in dietary intake, or both. Accordingly it is hoped that medical advice can be offered regarding modification of the normal diet and insulin regimen so as to prevent possible episodes of hypoglycemia following the two exercise evenings. (Although it is preferable to avoid this situation, glucose will be readily available should hypoglycemic symptoms or blood glucose values of 60 mg% or less occur.)

In order that I may be considered eligible for this research project I would greatly appreciate it if you could complete the enclosed form.

If you should have any questions please feel free to contact Gayle Ekstrand(home: 235-8660/Lab: 231-6543.)

Sincerely,

Volunteer Subject: ______________________________

Having acquainted myself with the nature of this study, and being familiar with the medical status of __________________________ I consider this individual to be in adequate diabetic control, without serious diabetic or other complications, and to be capable of participating in the described project without undue risk. Modification of the normal insulin and dietary pattern in light of the exercise involved in this project has been discussed with the potential volunteer.

Witness ____________________ Physician __________________________

Date _______________________ Date ________________________

APPENDIX F

SUBJECT DIARY SHEET

RECORDING SHEET

Name ______________________________ Date ______________

- [x] Day Immediately Prior to Day 1
- [ ] Day Immediately Prior to Day 2
- [ ] Day Immediately Prior to Day 3

Please check off the appropriate box above. and complete this form as accurately as possible - include all relevant details. Note that on Days 1, 2, and 3 you are to come to the laboratory fasting and without having injected your insulin dose. You are requested to refrain from smoking and consumption of caffeine on the mornings of Days 1, 2, and 3. In addition, you are requested not to participate in any physical exertion on the day immediately preceding each experimental day. Your cooperation in these matters is very much appreciated.

INSULIN REQUIREMENTS

Time of insulin injection(s) ______________________________

Site of insulin injection(s) ______________________________

Type of insulin(s) ______________________________

Dose of insulin(s) ______________________________

DIETARY AND ACTIVITY PATTERNS

	Time	Description	Quantity	Activity
Breakfast				
a.m. Snack				
Lunch				

2.

DIETARY AND ACTIVITY PATTERNS

	Time	Description	Quantity	Activity
p.m. Snack				
Dinner				
p.m. Snack				

List below any OTHER FACTORS that you feel may have affected your diabetic control today - (i.e. medication other than insulin, emotions, illness, infection ....)

ABSTRACT

Despite the growing interest in physical activity and insulin-receptor research, little is known of the post-exercise insulin-binding responses in insulin-dependent diabetics.

To study this, eight untrained male insulin-dependent diabetics (mean age of 25 years) participated in three treatment conditions — a control day and two post-exercise days. The exercise protocols consisted of either low intensity (60 minutes of continuous cycling at 45% mVO_2) or high intensity bicycle activity (60 minutes of intermittent work/rest cycling at 75% mVO_2). Post-exercise blood samples were drawn eight hours after completion of the exercise.

It was hypothesized that the post-exercise insulin-binding responses would differ significantly from values obtained on the control day. In addition, it was postulated that binding to the youngest fraction of erythrocytes would be greater than that involving the entire erythrocyte population.

The results indicated no differences in receptor binding across treatment conditions, although the youngest fraction of cells did demonstrate higher specific binding than did the total red blood cell population. A significant negative correlation between mVO_2 expressed in l/min and receptor binding following the high intensity protocol was noted. In addition, exercise caused a significant drop in blood glucose during the 60 minutes of activity, however this effect did not persist when re-evaluated eight hours later.

It was concluded that exercise of the intensities examined does not cause notable changes in insulin-receptor binding to erthrocytes eight hours post-exercise in insulin-dependent diabetics. The significance of this to potential insulin sensitivity changes cannot be assessed from the present study. Binding to the youngest erythrocytes is significantly greater than to the entire red blood cell distribution — perhaps reflecting a greater adaptability. Finally, the unexplained correlation between mVO_2 (l/min) and the binding response following high intensity exercise emphasises the need for additional research in this field.