ABSTRACT

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The purpose of this study was to determine the effectiveness of bicycle ergometry (30 min per day, 7 days a week, for 12 weeks) on levels of glycosylated hemoglobin, an index of glycemic control, in Type II diabetics. The 28 volunteers were separated into three groups: (1) exercise (n=9), (2) exercise with dietary supplement (n=9), and (3) control (n=10). The purpose of Group 2 was to control for weight loss, thus isolating the physical training effects. The null hypothesis was that there would be no significant differences in glycosylated hemoglobin levels over the treatment period within and between the groups. The mean glycosylated hemoglobin levels in all groups significantly decreased at the p < .05 level; Group 1 (9.17 ± 2.26 to 7.76 ± 2.35 %), Group 2 (9.47 ± 3.03 to 8.62 ± 2.76 %), and Group 3 (10.90 ± 4.88 to 10.27 ± 4.71 %), despite there being no significance between groups. Even though differences between groups were not significant, Group 1 approached the normal range (4.6-7.6 %) set by the Gundersen Clinic blood laboratory. Metabolic complications did not arise in any participant during the study and increased physical training did not produce pronounced weight loss. Therefore, exercise as a supplement to a proper diet could be the most beneficial treatment for Type II diabetics.
Effects of Physical Training on Glycosylated Hemoglobin Levels in Patients with Type II Diabetes Mellitus

A Thesis Presented To The Graduate Faculty University of Wisconsin-La Crosse

In Partial Fulfillment of the Requirements for the Master of Science Degree

by

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We recommend acceptance of this thesis in partial fulfillment of this candidate's requirements for the degree:

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DEDICATION

This work is lovingly dedicated to the six most important people in my life:

My mom, Patti Masotti, whose unending love has inspired me throughout my life.

My dad, Bruno Masotti, for "any man can be a father, but it takes a special father to be a dad."

My brother, Peppi, and my sister, Mari, who have shown me how to care and love.

My best friend, Bridget Broker, whose love has taught me to appreciate the little things in life.

GOD, the author of my life, without whom none of this would have been possible.

I LOVE YOU
CHAPTER I

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by relative or absolute insulin deficiency. It affects about twelve million Americans and along with its complications is the third leading cause of death in the United States (American Diabetes Association, 1985; Duda, 1985). There are two major types of diabetes, Type I or insulin-dependent (IDDM), which accounts for 10 to 20 percent of the known cases in the United States, and Type II or non-insulin-dependent (NIDDM), which accounts for 80 to 90 percent of the known cases (American Diabetes Association, 1984).

Fajans, Cloutier, and Crowther (1978) reported that approximately 80 percent of Type II diabetics are obese. The excess weight affects the glucose metabolism in predisposed individuals and may precipitate overt diabetes. Obesity and NIDDM decrease insulin tissue sensitivity, which is often corrected if weight is lost (Krall, 1978). For this reason, dietary modification is an important treatment for NIDDM. In addition to dietary modification, increased physical activity and, if necessary, oral hypoglycemics or insulin are recommended treatments for Type II diabetics (American Diabetes Association, 1984). Increased physical
training is believed to enhance glucose utilization and decrease insulin requirements (Vranic and Berger, 1979); while endogenous insulin directly increases the amount of insulin in the body and oral hypoglycemics augment insulin production in the pancreas.

Controlling the diet is essential in maintaining blood glucose homeostasis in NIDDM (American Diabetes Association, 1984). Exercise may be as important as diet in the treatment of NIDDM. However, "the actual benefits, actual risks, and guidelines for prescribing exercise are not yet precisely defined" (American Diabetes Association, 1984, p. 33). There is a need to examine how beneficial an exercise program may be for the regulation of Type II diabetes.

**Purpose of the Study**

The purpose of the study was to determine the effectiveness of an aerobic physical training program on levels of glycosylated hemoglobin, an index of glycemic control, in individuals with Type II diabetes mellitus.

**Need for the Study**

Much of the present research in the area of exercise and diabetes mellitus is contradictory. Some studies establish that physical training enhances metabolic control, while others disagree. Those studies stating that increased physical activity is beneficial in the treatment of NIDDM, differ as to the reasons why, and are inconclusive.
"Although it appears sensible to make exercise a part of the treatment plan for a patient with Type II diabetes mellitus, it should be emphasized that more research is needed in this area" (American Diabetes Association, 1984, p.33). The benefits of increased physical activity are: (1) diminished hyperglycemia and (2) reductions of body fat, blood pressure, and lipids (American Diabetes Association, 1984; Bjorntorp & Krotkiewski, 1985; Samsoe, 1984; Vranic & Berger, 1979). A need, therefore, exists to determine whether or not an aerobic physical training program is beneficial in enhancing the control of blood glucose levels in Type II diabetics.

**Hypothesis**

The null hypothesis for this study was that there would be no significant differences in glycosylated hemoglobin levels over the twelve week treatment period within and between the exercise, exercise with dietary supplement, and control groups.

**Assumptions**

The following were basic assumptions made for the purpose of this investigation:

1. The researcher and specific laboratory conditions had no adverse effect on the results of the glycosylated hemoglobin tests and these tests were accurate indications...
of mean blood glucose concentrations over a six to eight week period.

2. The participants exhibited honesty in record keeping of their diet and exercise.

**Delimitations**

The delimitations established for this study were:

1. The subjects selected were consenting volunteers from the La Crosse, Wisconsin, area having diagnosed Type II diabetes mellitus.

2. The physical training program consisted of stationary bicycle ergometry for a target time of 30 minutes per day for the exercise and exercise with supplement groups.

3. The length of the study was 12 weeks.

4. Pre and post measurements of the glycosylated hemoglobin levels were conducted for the purpose of comparison.

**Limitations**

The following were limitations of this investigation:

1. The amount and type of physical activity undergone by the volunteers in the control group could not be rigorously controlled.

2. The amount and type of "extra" physical activity undergone by the participants in the exercise and exercise
with dietary supplement groups could not be stringently controlled.

3. The amount of resistance and the pedal speed could not be rigorously controlled.

4. There was limited control over the diet of all groups. One-day diet histories were recorded at the beginning, middle, and end of the study to monitor the caloric intake of the participants.

5. Strict adherence to the physical training program was restricted due to injuries, illnesses, travel, and other factors.

Definition of Terms

Beta cells - cells from the islets of Langerhans, within the pancreas, which secrete insulin (Thomas, 1977).

Diabetes mellitus - a disorder of carbohydrate, protein, and fat metabolism characterized by hyperglycemia and glycosuria and resulting from inadequate production or utilization of insulin (American Diabetes Association, 1984; Thomas, 1977).

Type I (IDDM) - juvenile-onset or insulin-dependent diabetes mellitus "usually rapid in onset and seems to result from hereditary predisposition to (a) development of antibodies against the beta cells, thus causing autoimmune destruction of these cells, (b) possible destruction of the beta cells by viral disease, or (c) possible simple
degeneration of these cells" (Guyton, 1986, p. 933).

Patients with IDDM are ketosis prone and require insulin.

**Type II (NIDDM)** - adult-onset or non-insulin-dependent diabetes mellitus seems to result from degeneration or suppression of the beta cells as a result of more rapid aging in susceptible persons. Obesity predisposes this type of diabetes in two different ways: (1) the beta cells of the islets of Langerhans become less responsive to stimulation by increased blood glucose and (2) the number of insulin receptors in the insulin target cells are decreased throughout the body (Guyton, 1986).

**Exercise effects** - pertaining to the acute changes occurring during a single exercise session.

**Glycosuria** - the presence of glucose in the urine, possibly resulting from pancreatic (insulin) insufficiency, disorders of the endocrine glands, or reduction of renal threshold (Thomas, 1977).

**Glycosylated hemoglobin** - the glycosylated hemoglobin assay indicates the percentage of total hemoglobin to which glucose is attached (American Diabetes Association, 1984).

**Hyperglycemia** - an increase in plasma glucose levels above normal, as defined by the context (Jones, 1978).

**Hypoglycemia** - a decrease in plasma glucose levels below normal, as defined by the context (Jones, 1978).

**Insulinemia** - insulin in the blood often associated with hyperglycemia or insulin shock (Guyton, 1986).
Insulinopenia - a lack of insulin often leading to hyperglycemia.

Ketoacidosis - as blood glucose levels increase due to the lack of insulin, the body loses large amounts of fluid. The patient becomes dehydrated and because of the insulin lack, a vicious cycle takes place. The body breaks down fats in an attempt to get fuel. Ketone bodies are the end products of improper fat breakdown and these accumulate in the blood and urine, where they are recognized as ketonuria. The patient becomes more dehydrated and an acid condition of the blood develops. In extreme cases or when sufficient insulin or fluids are not given soon enough, coma and unconsciousness occur (Krall, 1978).

Physical training effects - pertaining to chronic changes over an extended period of time due to an exercise training program.
CHAPTER II

REVIEW OF LITERATURE

According to the National Center for Health Statistics (1984), 34,750 people died from diabetes mellitus in the United States in 1981. Approximately twelve million Americans suffer from diabetes, which is the third leading cause of death in the United States (American Diabetes Association, 1985). Although much progress has been made to control diabetes, more research is needed to find a cure. This chapter will examine the physiological characteristics of diabetes mellitus, as well as the possible treatments for this disease, and will be divided into the following sections: (1) definitions, (2) physiology of diabetes, (3) treatments and (4) exercise program.

Definitions

According to Lilly Research Laboratories (1980), diabetes mellitus is a chronic disorder with abnormalities in (1) metabolism of insulin, carbohydrate, fat, and protein, and (2) the structure and function of blood vessels. The criteria for diabetes mellitus is two-fold: (1) classic symptoms of diabetes with an unequivocal elevation of the plasma glucose level and (2) fasting plasma glucose is 140 mg percent or greater (National Diabetes Data
Diabetes mellitus can be categorized into two major classifications: insulin-dependent and non-insulin-dependent.

**Insulin-Dependent**

Insulin-dependent diabetes mellitus (IDDM) accounts for 10 to 20 percent of known cases of diabetes in the United States (American Diabetes Association, 1984; National Diabetes Data Group, 1979). These patients also have severe insulinopenia (American Diabetes Association, 1984; National Diabetes Data Group, 1979). In Type I diabetics, the pancreas fails to produce enough insulin and usually involves rapid onset of symptoms, generally being recognized within four to six weeks (Duda, 1985). The symptoms of this disease are generally present before the age of 40.

**Non-Insulin-Dependent**

In contrast, Type II diabetes usually has its onset after the age of 40 (American Diabetes Association, 1984). Non-insulin-dependent diabetes mellitus (NIDDM) is present in 80 to 90 percent of the known cases of diabetes in the United States (American Diabetes Association, 1984). This accounts for 2.5 percent of the entire American population, or five million persons (American Diabetes Association, 1984). Since many cases are undiagnosed, there are probably about five million more people with Type II diabetes in the United States (American Diabetes Association, 1984).

"Whereas deficiency of endogenous insulin is the primary
defect in Type I diabetic patients, the metabolic disorder in Type II diabetic patients is linked to insulin resistance resulting from a receptor and/or postreceptor defect" (Kemmer, 1985, p. 85). According to the American Diabetes Association (1984), "NIDDM is a heterogeneous disorder characterized by impaired beta cell function and diminished liver and muscle tissue sensitivity to insulin" (p.13). In Type II diabetes, (1) basal insulin secretion is normal, increased, or it may be decreased, (2) insulin resistance usually is present, (3) insulin resistance can lead to the development of a defect in insulin secretion, and (4) impaired beta cell function can lead to a disturbance in insulin action (American Diabetes Association, 1984).

**Physiology of Diabetes**

The pancreas manufactures, stores, and releases insulin. Another action of the pancreas is the manufacture and release of glucagon; therefore, it appears as though the insulin-glucagon relationship, which is important in maintaining normal blood glucose, is at least partially controlled by the pancreas (Krall, 1978). "A main function of the glucagon is to raise the blood glucose level; thus glucagon has an anti-insulin function" (Krall, 1978, p. 10).

Each normal pancreas has about 100,000 islets of Langerhans, and each islet contains between 80 and 100 beta cells. These cells are capable of measuring the blood glucose level every 10 seconds to within a range of 2 mg percent. Within 60 to 90 seconds, the beta
cells organize themselves to deliver any amount of insulin necessary (Krall, 1978, p. 11).

Even though the pancreas may produce sufficient insulin in Type II diabetics, the islet function has decreased glucose responsiveness (Halter, Beard, & Porte, 1984).

When stimulated by glucose and other foods, the beta cell releases insulin stored in the cells and produces more insulin when the glucose level increases (Krall, 1978). In the normal state, 15 to 20 percent of the ingested glucose is absorbed by muscle tissue, leaving 80 to 85 percent for the liver (Saltin et al., 1979). The normal fasting level of glucose in the blood is 60 to 100 mg percent with glucose appearing in urine between 160 and 180 mg percent (Krall, 1978). "Insulin has a vital role in the maintenance of glucose homeostasis and one of its major biological effects is to promote the metabolism of glucose" (Clark, Rattigan, & Clark, 1983, p. 1236). Patients with NIDDM have beta cell function abnormalities; however, it is unclear whether these are primary or secondary effects (American Diabetes Association, 1984; Reaven, 1984). According to Pollet (1983), insulin resistance, decreased insulin action with its tissue receptors, is probably secondary to downregulation, or diminuation, of the number of cell-surface insulin receptors on target tissues. Lipson and Lipson (1984) stated that with increased insulin levels there is a decrease in the number of insulin receptors.
This abnormal glucose uptake in muscle is a result of diminished oxidative and non-oxidative pathways of glucose utilization (American Diabetes Association, 1984).

Factors affecting insulin resistance are: (1) obesity, (2) diet, (3) physical activity, (4) stress, (5) hormone excess or deficiency, (6) drugs, and (7) aging (Halter et al., 1984).

With 60 to 90 percent of all Type II diabetics being obese in Western societies, weight gain and obesity caused by excessive caloric intake plays an important part in the etiology of NIDDM (National Diabetes Data Group, 1979). In 1985, Bonham and Brock found that black males over 65 are the most obese (20.2% of obese population). Males and females, both black and white, between ages 20 and 44 are the least obese (0.5%). They further stated that the risk of "diabetes generally increased more rapidly with obesity among women than among men" (p. 776). Even though diabetes mellitus is often associated with enlarged adipose cell size (Horton, 1983), the reduction in insulin tissue sensitivity is greater in obese Type II patients than that associated with obese non-diabetics (Hollenbeck, Chen, & Reaven, 1984). Although a majority of Type II diabetics are obese, glucose intolerance is commonly observed in non-obese individuals, especially the aged, (American Diabetes Association, 1984; Elahi et al., 1984).
Muscle glycogen resynthesis after exercise is a major factor affecting glucose tolerance (Bjorntorp & Krotkiewski, 1985). According to Horton (1983), "there is good evidence to show that both strenuous exercise and physical training are associated with increased tissue sensitivity to insulin" (p. 38). The increased insulin sensitivity, occurring when muscle glycogen is resynthesized, will be maintained with an exercise program (Bjorntorp & Krotkiewski, 1985).

Exercise is also known to decrease physiological stress levels, which could increase insulin sensitivity. "Catecholamines and a number of other hormones released during these stress states contribute to the development of hyperglycemia by directly stimulating glucose production and interfering with tissue disposal of glucose" (Halter et al., 1984, p.E47). NIDDM is often preceded by a long period of asymptomatic hyperglycemia (Mohamed, Wilkin, Leatherdale, & Rowe, 1984). Patients with diabetes could develop absolute insulin deficiency and glucagon excess with adrenergic activation during stress (Halter et al., 1984).

Hyperglycemia stimulates insulin secretion while decreasing glucagon secretion, which will diminish the degree of hyperglycemia (Halter et al., 1984). The defects of insulin secretion and insulin reception, found in NIDDM is partially reversible (American Diabetes Association, 1984).

Although Type II patients do not depend on insulin to prevent ketosis, they may require it to help maintain
glucose homeostasis (National Diabetes Data Group, 1979). Other patients may need oral hypoglycemics to control their blood glucose levels by enhancing muscular insulin sensitivity (Samsoe, 1984). Most patients can control their blood glucose levels by an appropriate diet and weight maintenance.

The relationships of impaired pancreatic beta-cell function, decreased effectiveness of insulin on its target tissues, and stress in the development of Type II diabetes have been noted previously in this study. One additional factor is that genetics plays an important role in the etiology of NIDDM (Bonham & Brock, 1985; Horton, 1983; Krall, 1978). Type II diabetes seems to have a stronger familial pattern than does Type I (National Diabetes Data Group, 1979). Results from a study of 200 pairs of identical twins suggests that a genetic predisposition is highly correlated to the development of NIDDM. When one twin developed diabetes, 92 percent of the second twins were diagnosed with the disease within one year, and 100 percent had developed it within three years (Barnett, Eff, Leslie, & Pyke, 1981).

Treatments

According to the American Diabetes Association (1984), the insulin secretory and receptor abnormalities are partially reversible. There are two major goals in
maintaining and controlling Type II diabetes: (1) achieve normal metabolic control, and (2) prevent vascular complications (American Diabetes Association, 1984). In order to achieve these goals, there are three areas of treatment for controlling diabetes: (1) diet, (2) physical training, and (3) medications, either oral hypoglycemics or insulin (American Diabetes Association, 1984; Samsoe, 1984). Until medications became available, the only ways to treat diabetes were diet and exercise. Patients, who cannot control diabetes with just diet and exercise, must incorporate medications into the therapy. In the control of diabetes, if one factor (diet, exercise, or medications) is changed, there must be an adjustment in the other two aspects as well (Samsoe, 1984).

**Diet**

Proper dietary modification is the primary treatment for patients with NIDDM. There is a high correlation between Type II diabetics and obesity, which is the primary problem in these patients (Samsoe, 1984). Therefore, diet modification is the most important aspect in their therapy (American Diabetes Association, 1984; Bogardus et al., 1984). Hyperglycemia increases with the extent of obesity (Clark et al., 1983), and weight reduction lowers fasting serum insulin and glucose concentrations (Horton, 1983; National Diabetes Data Group, 1979). One study gave hypocaloric liquid formula diets to 20 moderately obese
patients with NIDDM. In order to establish the effects of moderate weight loss and sulfonylurea treatment in these individuals, they were divided into two equal groups, one was treated with weight loss alone, while the other group received glipizide in addition to the hypocaloric diet. An average weight loss of 6.5 kg during the four week study showed a significant improvement in hyperglycemia and hyperlipidemia, despite the fact that all subjects were still obese (Liu et al., 1985). Reaven (1985) also demonstrated that an average weight loss of 9 kg dramatically improved glycemic control in elderly insulin-treated patients with NIDDM. "A normalization on the blood glucose concentrations after body weight reduction seems to be associated with normalized serum lipoprotein triglyceride concentrations" (Vessby, Boberg, Karlstrom, Lithell, & Werner, 1984, p. 72).

Total caloric intake must be reduced, dividing the calories proportionally throughout the major meals (American Diabetes Association, 1984), and substituting complex carbohydrates for simple carbohydrates (Lipson & Lipson, 1984). It is most important for these patients to be placed on a closely monitored diet and, even more crucial, that they adhere to the prescribed diet strictly. The purpose of diet in the treatment of Type II diabetes is to control the metabolic abnormalities associated with this disease (Reaven, 1985; Vessby et al., 1984).
Physical Training

"Exercise is a long established part of the treatment of diabetes mellitus. It has been assumed to improve the metabolic balance of the diabetic state, but very few controlled studies have actually been performed to prove this point until recently" (Bjorntorp & Krotkiewski, 1985, p. 3). At this time, the value of physical training in diabetic control is sketchy, a "subject for speculation" (Richter, Ruderman, & Schneider, 1981, p. 206). Even though evidence to support the benefits of a physical training program for the maintenance of glucose homeostasis has been limited (Vranic & Berger, 1979), "the potential and actual benefits of regular exercise far outweigh the potential and actual risks" (American Diabetes Association, 1984, p. 33; Bjorntorp & Krotkiewski, 1985; Richter et al., 1981). Although physical training seems reasonable in the therapy for Type II diabetics, more research is needed to describe the effects of such a program.

Supplement to Diet. Unless contraindicated, a physical training program should accompany a dietary program (American Diabetes Association, 1984; Richter et al., 1981). Regarding NIDDM, "this should be done in addition to--and not in replacement of--diet" (Trovati et al., 1984, p. 419). Bogardus and associates (1984) studied carbohydrate-intolerant and NIDDM subjects, comparing the effects of twelve weeks of physical training and hypocaloric diet
(n = 10) with the effects of diet therapy alone (n = 8) in relation to body composition, carbohydrate tolerance, and insulin secretion and action. While diet therapy alone improved glucose intolerance in Type II diabetics, incorporating exercise with diet in diabetes control significantly increased glucose disposal (Bogardus et al., 1984). Insulin levels during an oral glucose tolerance test (OGTT) decreased for individuals who engaged in a physical training program and received dietary advice (Saltin et al., 1979). Another study examined the metabolic response to exercise in obese postabsorptive Type II diabetics (n = 7) compared to that of obese non-diabetics (n = 7). The results showed that resting hyperglycemia (192 ± 24 mg %) in individuals in a diet group decreased 35 mg percent with exercise. In control subjects, glycemia was 86 ± 4 mg percent and did not change with exercise (Minuk et al., 1981). Even though physical activity by itself does not generally result in significant weight losses, it is recognized as an important aspect of weight reduction (American Diabetes Association, 1984). This is due to the fact that total caloric expenditure is increased (American Diabetes Association, 1984; Samsoe, 1984).

Benefits. Physical training has been advocated as an approach for the treatment of diabetes mellitus (Kemmer & Berger, 1984). Exercise may increase metabolic control in Type II diabetics by decreasing blood glucose levels through
increased muscle absorption (American Running and Fitness Association, 1985; O'Dea, 1984; Samsoe, 1984). It is possible for the blood glucose levels to continue decreasing for up to five hours following exercise (Samsoe, 1984) and remain lowered for 24 to 48 hours (Zinman, Vranic, Albisser, Leibel, & Marliss, 1979). Besides improvements in glucose metabolism, physical training will also benefit carbohydrate and lipid metabolism, blood pressure, and exercise tolerance (Leon et al., 1984; Samsoe, 1984). "It should be stressed from a practical point of view that physical training poses no specific problems in diabetic subjects except those obviously present in terms of poor circulatory responses due to ischemic or hypertensive reactions and tendencies to hypoglycemia" (Bjorntorp & Krotkiewski, 1985, p. 5).

Effects. The effects of exercise for patients with Type II diabetes mellitus will be discussed as acute, long-term (training), and cardiovascular.

The acute effects of exercise in Type II diabetics are only understood partially at this time (Minuk et al., 1981). One effect is on the blood glucose levels during and following exercise. Although blood glucose levels vary only slightly in non-diabetics during acute exercise (Richter, et al., 1981; Vranic & Berger, 1979), metabolic and hormonal effects differ with the diabetic's state of metabolic control (Vranic & Berger, 1979). Exercise in NIDDM subjects
results in lowering of plasma glucose, which is related to an inadequate exercise-associated increase in glucose production by the liver (Minuk et al., 1981).

The factor responsible for the increased uptake of glucose by muscular tissues during exercise is not fully known (Vranic & Berger, 1979). Skeletal muscles utilize their own stores of glucose and triglycerides, and free fatty acids that are formed from the breakdown of adipose tissue triglycerides and glucose released by the liver (Richter et al., 1981). Glycogenolysis and gluconeogenesis are two processes related to hepatic glucose production, which meets the increased peripheral carbohydrate requirements during exercise (Vranic & Berger, 1979). Low levels of plasma cortisol could increase glucose uptake in the depleted muscle by enhancing insulin sensitivity, due to an inhibition of insulin secretion (Holm, Bjorntorp, & Jagenburg, 1978; Krotkiewski, 1983). Hepatic uptake of glucose is decreased following exercise as compared to the non-exercise state (Wahren, Felig, & Maehlum, 1977). Prolonged exercise with large muscle groups depletes large glycogen stores which are then replenished during the subsequent days. It is possible for such replenishment to consume 100 grams of glucose per day (Bjorntorp & Krotkiewski, 1985). Normal glucose utilization indicates the possibility that exercise in diabetics may increase insulin sensitivity in the working muscle (Vranic, Steiner,
overproduction of glucose, and hyperglycemia may all contribute to observed differences in glucose flux during exercise in non-insulin-dependent diabetics (Minuk et al., 1981).

During aerobic exercise, blood glucose concentrations decrease (Skyler, 1979). The hepatic glycogen stores are then used leaving the hepatic gluconeogenesis as the next available source of glucose, which is a slower method of generating glucose (Ruderman, Young, & Schneider, 1984). Even though the acute effects of exercise in patients with NIDDM are vague, two effects of physical training are prominent; namely, increased insulin sensitivity and decreased blood glucose levels.

Even though decreases in insulin and glucose are known effects of physical training, exact measurements and causes are unclear. Physical training is generally believed to increase glucose utilization and decrease insulin requirements (Berger, Becker-Zimmerman, & Herberg, 1982; Vranic & Burger, 1979; Soman, Koivisto, Diebert, Felig, & DeFronzo, 1979; Krotkiewski, 1983). Plasma insulin concentrations are increased in obese individuals (Elahi et al., 1984) and are even greater in patients with NIDDM (Hollenbeck et al., 1984). Physical training is important to obese Type II diabetics because it enhances tissue sensitivity to insulin, whether or not it causes weight loss.
(Horton, 1983; Krotkiewski, 1983; Saltin et al., 1979; Soman et al., 1979). This seems to be caused by a factor in the muscle, even though the exact mechanism is unknown (Bjorntorp, de Jounge, & Sjostrom, 1970).

Insulin receptors increase in direct proportion to the improvement in physical fitness (Soman et al., 1979). This might be caused by a change in the secretion of hormones, countering the action of insulin on glucose transport from blood into tissues (Bjorntorp et al., 1970). One study examined the effect of physical training on tissue sensitivity to insulin and on insulin binding to monocytes in six previously untrained healthy adults. Physical training (one hour of cycle ergometry four times weekly for six weeks) resulted in a 30 percent increase (p < 0.01) in insulin-mediated glucose uptake (determined by the insulin clamp technique) (Soman et al., 1979). Bjorntorp (1981) stated that the increase in insulin sensitivity may be due to the "regulating insulin secretion via feedback mechanisms to the beta cell" (p. 125). Physical training is probably associated with augmented sensitivity of the beta- and alpha-adrenergic systems, which helps to decrease insulin levels by decreasing insulin secretion from the pancreas (Bjorntorp, 1981; Bjorntorp & Krotkiewski, 1985; Krotkiewski et al., 1983).

In summary, the decrease of plasma insulin levels after physical training is explained mainly by "a decreased
insulin secretion, but also to a significant degree by an increased peripheral insulin metabolic clearance rate in several peripheral tissues and increased insulin uptake in the liver" (Bjorntorp, 1981, p. 125).

As previously mentioned, blood glucose levels decrease as a result of physical training. A physical training program for patients with Type II diabetes mellitus enhances intravenous glucose disposal (Ruderman, Ganda, & Johansen, 1979) and improves glucose homeostasis (Richter et al., 1981). This is due to increases in insulin sensitivity (Samsoe, 1984). Glycosylated hemoglobin levels reflect long-term diabetic control (Leon et al., 1984) and have been shown to decrease during training (12.2 ± 0.5 to 10.7 ± 0.4 percent; p < 0.02) (Schneider, Amorosa, Kharchadurian, & Ruderman, 1984).

A physical training program has several benefits for the Type II diabetic, provided the patient is disciplined. Within two weeks of stopping exercise, fasting glucose and insulin levels increased significantly, causing the glucose homeostasis to deteriorate (Ruderman et al., 1979; Samsoe, 1984). Another effect of physical training is an increase in total daily glucose utilization, which is oxidized and removed from the body as carbon dioxide and water (Clark et al., 1983). Long-term aerobic programs reduce body lipids, which "may lessen the major biochemical restriction on
normal glucose metabolism" and reduce cardiovascular risks (Clark et al., 1983, p. 1239).

Besides decreasing insulin and blood glucose levels, a physical training program has cardiovascular benefits, which include: (1) decreased resting heart rate and blood pressure, (2) decreased heart rate and systolic blood pressure with submaximal work, (3) increased maximal cardiac output and oxygen consumption, (4) increased myocardial blood flow reserve, (5) faster return to resting heart rate post exercise, (6) increased high density lipoproteins, (7) decreased cholesterol, and (8) decreased triglycerides (Samsoe, 1984). "Because of the two-fold increase in cardiac disease among diabetics, the cardiovascular benefits of exercise are even more important for them than the general population" (Samsoe, 1984, p. 2). Because of this increased incidence of cardiac disease, diabetics generally have lower maximum heart rate and cardiac output at maximal exercise levels than non-diabetics (McMillan, 1979).

McMillan (1979) stated that oxygen exchange is more difficult for diabetics because the basement membranes in the capillary and venous systems are thicker than normal. Physical training will decrease the thickness of the membrane, thus removing some of the stress of the cardiovascular system (Peterson, Jones, Esterly, Wantz, & Jackson, 1980). For these reasons, aerobic physical
training could prove to be of additional value to Type II diabetics.

**Medications**

Yet another benefit of physical training for Type II diabetics is a decrease in insulin or oral hypoglycemic dosages (Vranic & Burger, 1979). "Exercise may spare the beta cells of the pancreas and allow the patient to control the diabetes with just diet and exercising rather than having to resort to using oral hypoglycemic agents or exogenous insulin" (Samsoe, 1984, p. 3). In Type I diabetics, however, Ferstle (1982) states that "careful control of insulin and diet are probably more important in regulating diabetes than exercise" (p. 133).

**Exercise Program**

One final question must be answered regarding physical training: What type of program is used, and how is it implemented? As previously mentioned, aerobic exercise should be used. It should be initiated slowly to prevent injuries and complications (Lipson & Lipson, 1984; Samsoe, 1984). This is due to the prevalence of coronary artery disease, atherosclerosis, and vascular complications in diabetic patients. To further enhance the effects of training, the program should be supervised, at least initially (American Diabetes Association, 1984).
Several factors should be kept in mind before exercising. Individuals could be dehydrated before beginning exercise, which is a characteristic of uncontrolled diabetics (Samsoe, 1984). Therefore, fluids should be appropriately ingested before, during, and after exercise. Foot care is extremely important because of the lack of sensitivity in the extremities of diabetics with peripheral neuropathy. A simple blister can become infected and gangrenous, possibly leading to amputation within 10 days (Samsoe, 1984). A fast acting carbohydrate should be kept with the insulin-using diabetic at all times, in case of an insulin reaction. Exercise should be at approximately the same time of day as many days a week as possible to enhance glucose homeostasis (Samsoe, 1984). Also, the heart rate should be elevated to between 60 and 80 percent of maximum for 20 to 30 minutes (Samsoe, 1984). The program should be individualized to maximize the benefits.

**Summary**

In summary, the etiology of NIDDM is probably multifactorial, with both genetic and environmental factors contributing to varying degrees. Obesity appears to be a determinant of overriding importance, largely to the extent that it confers a state of insulin resistance. The long-term complications of diabetes appear to be linked, ultimately, to the metabolic abnormalities of the disease, and, as such, may be prevented or ameliorated through adequate control (Miles, 1984, p. 61).

NIDDM can frequently be controlled by diet and exercise, and
occasionally with oral hypoglycemics or insulin, whereas insulin-dependent diabetics require insulin to prevent ketosis and death (American Diabetes Association, 1984). The incidence of NIDDM increases with the degree of obesity, therefore weight maintenance is an important element in its treatment. Glucose tolerance is often normalized with weight loss, however, "there is considerable debate as to whether the weight loss per se is the normalizing phenomenon or whether calorie restriction induces both weight loss and improved glucose tolerance" (Sussman, 1985, p. 6).

Incorporating a physical training program with proper dietary maintenance further benefits metabolic control (DeFronzo, Ferrannini, & Koivisto, 1983). The enhanced glucose utilization seems to be associated with increased insulin sensitivity in the muscle (Kemmer, 1985). Exercise may spare the beta cells of the pancreas, thus NIDDM may be treated with just diet and exercising rather than having to resort to using oral hypoglycemic agents or exogenous insulin (Samsoe, 1984). It is not known which of these factors, if any, is the most important in controlling diabetes, or whether it is more beneficial to incorporate all three in a total program. Modern practice tends to lean toward the latter aspect. What if, however, diabetes could be controlled with just diet and exercise? Would it be to the patient's advantage, or does it matter? These are but a few questions that must be answered with further research.
CHAPTER III

METHODS

This study involved patients having Type II diabetes mellitus from the La Crosse, Wisconsin, area. This investigation was conducted to determine the effectiveness of an aerobic physical training program on glycosylated hemoglobin levels in Type II diabetics. The research proposal was accepted by the researcher's thesis committee of the University of Wisconsin-La Crosse and by the Research and Human Investigation committees of Gundersen Clinic, La Crosse, Wisconsin (Appendix A).

The methods utilized in this study are presented in the following sections: (1) subject selection, (2) selection and administration of instrumentation, and (3) statistical treatment of data.

Subject Selection

Subjects selected for this study were individuals with Type II diabetes mellitus. The participants were originally contacted by Gundersen Clinic staff (Appendix B) and asked to contact the clinic to be included in the study. A statement by Gundersen Clinic (Appendix C) was released seeking more volunteers for the study. The researcher then telephoned these individuals to confirm their interest in
Selection and Administration of Instrumentation

This study examined the effects of physical training on the metabolic homeostasis of Type II diabetics. The instrumentation of the study was necessary to isolate the participating, followed by formal correspondence (Appendix D) describing the time and location of the introductory research procedures. The initial communication by Gundersen Clinic staff was to preserve the identity of those patients who did not wish to participate. A total of 36 participants were included in the study and were placed into one of three groups: (1) control (n=14), (2) exercise (n=12), and (3) exercise with dietary supplement (n=10). These groups were established to isolate the effects of physical training on blood glucose levels in the patients. The control group was asked to continue their usual daily activities during the twelve week study (Appendix E). The group was allowed to exercise, but not any more than they would typically do. An aerobic physical training program was implemented for the exercise group (Appendix D). This program consisted of cycle ergometry exercise performed for 30 minutes, 7 days a week. The participants executed the exercise at home, keeping records of their exercise. The exercise with dietary supplement group was prescribed the same exercise program with the added dimension of supplementing their diet to control for weight loss (Appendix E).
effects of physical training on the glycosylated hemoglobin levels. The patients reported to Gundersen Clinic to sign the "Informed Consent" form (Appendix H) and the following procedures were implemented.

**Glycosylated Hemoglobin Test**

The Glycosylated Hemoglobin Test (Appendix I) was administered to all patients at the beginning (January 2 and 3) and end (March 31 and April 1) of the study. No restrictions were placed on the participants prior to the tests because "measurement of glycosylated hemoglobin (GHB) provides an accurate, virtually tamperproof index of average blood glucose concentration during the previous 2-4 months" (Goldstein, 1986, p. 1). The tests, which were simple blood draws, were administered and analyzed by the Gundersen Clinic staff and the results were given to the researcher at the end of the study.

**Daily Exercise Record**

The Daily Exercise Record (Appendix J) was administered to the participants of the exercise and exercise with dietary supplement groups. The purpose of the records was to monitor and verify the cycle ergometry exercise performed by these individuals. The volunteers of the control group were asked not to alter their exercise habits. On the other hand, the participants of the exercise and exercise with dietary supplement groups were placed on a physical training
regimen in an effort to increase their exercise habits to bicycling 30 minutes, 7 days a week, for 12 weeks.

A bicycling program was begun at whatever level the person was currently exercising. The exercise session consisted of a 5-minute warm-up with light resistance, followed by 20 minutes of exercise with increased tension, concluding with a 5-minute cool-down of decreased pressure. The participants increased their exercise until they attained a target time of a total of 30 minutes. Once this level had been attained, increases in resistance and pedal speed followed. These latter adjustments were made individually in conjunction with the researcher through weekly telephone conversations. These contacts, as well as the mailing of the Daily Exercise Records to the researcher, assisted in monitoring adherence to the prescribed program.

One-Day Diet History

All participants recorded their caloric intake on the One-Day Diet History sheets (Appendix K). Three diet history days, January 5, February 18, and March 25, were established to monitor adherence to the participants' normal eating habits by comparing their caloric intakes over the three days. The form "Instructions For Keeping Good Food Records" (Appendix L) was given to the participants to assist in accurate dietary recalls. Participants of the control group, as well as the exercise group, were asked to maintain their normal eating habits throughout the study.
The participants in the exercise with dietary supplement group were expected to increase their caloric intake by approximately 200 calories every day they bicycled. Examples of such food items were given to these volunteers to assist with this aspect of their program (Appendix M). The exercise with dietary supplement group was necessary in order to isolate the variable of physical training on glycosylated hemoglobin levels, by controlling for weight loss. The One-Day Diet Histories were mailed to the researcher following the designated dates.

**Statistical Treatment of Data**

Basic descriptive statistics, including means, standard deviations, and ranges of age, body weight changes, caloric intake, amount of daily exercise, and changes in glycosylated hemoglobin levels, were reported. A one-way analysis of variance (ANOVA) was used for within-group and between-group comparisons of pre and post testing of glycosylated hemoglobin levels. A level of significance was set at .05.
CHAPTER IV

RESULTS AND DISCUSSION

This study was conducted to establish the effectiveness of an aerobic physical training program on glycosylated hemoglobin levels in Type II diabetics. Certain variables were selected to be examined before, during, and after the twelve week research period. These variables were as follows: glycosylated hemoglobin, body weight, caloric intake (three one-day diet histories), and exercise time. A one-way analysis of variance was conducted to determine if there were statistically significant differences between pretest and posttest measures.

This chapter presents and analyzes the data collected throughout the course of this study. These findings are then discussed relative to their statistical significance, their effects on the subjects, and their importance to the outcome of the study.

Demographic Data of Subjects

Twenty-eight of the original 36 subjects successfully completed the requirements of the study; only their data are included. Four subjects were excluded from the statistical treatment of data due to insufficient exercise time (<20 min
Demographic data related to the remaining 28 subjects are presented in Table 1 and include: age and sex.

Table 1

Demographic Data of Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>56.33</td>
<td>+8.37</td>
<td>47-76</td>
</tr>
<tr>
<td>(M=6)</td>
<td>55.33</td>
<td>+4.68</td>
<td>48-60</td>
</tr>
<tr>
<td>(F=3)</td>
<td>58.33</td>
<td>+12.66</td>
<td>47-76</td>
</tr>
<tr>
<td>Exercise &amp; Diet</td>
<td>64.44</td>
<td>+7.48</td>
<td>52-76</td>
</tr>
<tr>
<td>(M=6)</td>
<td>69.00</td>
<td>+4.32</td>
<td>62-76</td>
</tr>
<tr>
<td>(F=3)</td>
<td>55.33</td>
<td>+2.49</td>
<td>52-58</td>
</tr>
<tr>
<td>Control</td>
<td>55.70</td>
<td>+12.39</td>
<td>40-80</td>
</tr>
<tr>
<td>(M=5)</td>
<td>57.60</td>
<td>+14.97</td>
<td>40-80</td>
</tr>
<tr>
<td>(F=5)</td>
<td>53.80</td>
<td>+8.70</td>
<td>47-71</td>
</tr>
</tbody>
</table>

Note: * M = Males

F = Females
Glycosylated Hemoglobin Results

Tables 2 and 3 report the pretest and posttest values for the glycosylated hemoglobin of all three groups. These tables were included to determine whether differences existed between sexes, however, no statistical differences were exhibited between the sexes at the $p < .05$ level of significance.

Table 2

Pretest Glycosylated Hemoglobin Values

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>8.93</td>
<td>± 2.08</td>
<td>6.60-12.30</td>
</tr>
<tr>
<td>Females</td>
<td>9.63</td>
<td>± 3.03</td>
<td>7.50-13.10</td>
</tr>
<tr>
<td>Exercise &amp; Diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>9.77</td>
<td>± 0.89</td>
<td>8.50-10.90</td>
</tr>
<tr>
<td>Females</td>
<td>8.87</td>
<td>± 5.83</td>
<td>5.40-15.60</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>11.28</td>
<td>± 3.09</td>
<td>6.90-15.00</td>
</tr>
<tr>
<td>Females</td>
<td>10.52</td>
<td>± 6.61</td>
<td>4.70-21.70</td>
</tr>
</tbody>
</table>
Table 3

Posttest Glycosylated Hemoglobin Values

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>7.10</td>
<td>± 1.76</td>
<td>4.40-9.40</td>
</tr>
<tr>
<td>Females</td>
<td>9.07</td>
<td>± 3.23</td>
<td>7.20-12.80</td>
</tr>
<tr>
<td>Exercise &amp; Diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>9.27</td>
<td>± 1.18</td>
<td>7.80-11.10</td>
</tr>
<tr>
<td>Females</td>
<td>7.33</td>
<td>± 4.82</td>
<td>4.50-12.90</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>10.82</td>
<td>± 3.91</td>
<td>5.50-14.20</td>
</tr>
<tr>
<td>Females</td>
<td>9.72</td>
<td>± 5.82</td>
<td>4.30-19.30</td>
</tr>
</tbody>
</table>

The glycosylated hemoglobin levels decreased an average of one percent after the experimental period. Figure 1 illustrates the pretest and posttest values for the three groups.

Utilizing the one-way analysis of variance, the observed F-ratio did not indicate a significant difference at the p < .05 level between the pretest and posttest values. This is presented in Table 4.
Figure 1. Mean glycosylated hemoglobin levels.

Table 4
ANOVA Results for Glycosylated Hemoglobin

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sum of Squares</th>
<th>Degrees Freedom</th>
<th>Means Square</th>
<th>F Ratio</th>
<th>Sig. of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4897.73</td>
<td>1</td>
<td>4897.73</td>
<td>201.09</td>
<td>0.00</td>
</tr>
<tr>
<td>Group</td>
<td>46.22</td>
<td>2</td>
<td>23.11</td>
<td>0.95</td>
<td>0.40</td>
</tr>
<tr>
<td>Error</td>
<td>608.89</td>
<td>25</td>
<td>24.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rows</td>
<td>12.92</td>
<td>1</td>
<td>12.92</td>
<td>13.14</td>
<td>0.00</td>
</tr>
<tr>
<td>Rows/Group Interaction</td>
<td>1.52</td>
<td>2</td>
<td>0.76</td>
<td>0.77</td>
<td>0.47</td>
</tr>
<tr>
<td>Error</td>
<td>24.59</td>
<td>25</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Body Weight Changes

The data presented in Table 5 is indicative of the body weight of the subjects before beginning and after completing the program. The study controlled for weight loss in the exercise with dietary supplement group. The participants of the exercise and control groups were to continue with their current diets.

Table 5

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>107.92</td>
<td>+34.23</td>
<td>83.20-180.00</td>
</tr>
<tr>
<td>Post</td>
<td>107.06</td>
<td>+32.61</td>
<td>82.30-173.20</td>
</tr>
<tr>
<td><strong>Exercise &amp; Diet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>96.06</td>
<td>+23.15</td>
<td>58.70-134.90</td>
</tr>
<tr>
<td>Post</td>
<td>96.41</td>
<td>+23.58</td>
<td>58.00-136.00</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>85.14</td>
<td>+15.04</td>
<td>64.00-116.20</td>
</tr>
<tr>
<td>Post</td>
<td>85.35</td>
<td>+14.91</td>
<td>63.30-116.50</td>
</tr>
</tbody>
</table>
Exercise

Table 6 presents the daily exercise time for the exercise and exercise with dietary supplement groups. The training program assigned was 30 minutes per day, every day, for 12 weeks.

Table 6
Average Daily Exercise

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>29.31</td>
<td>+0.81</td>
<td>27.74-30.00</td>
</tr>
<tr>
<td>Exercise &amp; Diet</td>
<td>26.56</td>
<td>+2.25</td>
<td>22.75-30.00</td>
</tr>
</tbody>
</table>

Caloric Intake

In Table 7, the caloric intakes from the three diet histories were compared between groups. The diet histories were at the beginning, middle, and end of the study. The purpose of the diet histories was to help control for weight loss in the exercise with dietary supplement group.
### Table 7

**Caloric Intake**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beg.*</td>
<td>1777.13</td>
<td>±392.58</td>
<td>1358.70-2532.10</td>
</tr>
<tr>
<td>Mid.</td>
<td>2385.82</td>
<td>±1090.16</td>
<td>1242.00-4273.30</td>
</tr>
<tr>
<td>End</td>
<td>1918.86</td>
<td>±671.38</td>
<td>1005.00-2784.40</td>
</tr>
<tr>
<td><strong>Exercise &amp; Diet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beg.</td>
<td>1954.02</td>
<td>±680.50</td>
<td>643.90-3290.80</td>
</tr>
<tr>
<td>Mid.</td>
<td>1893.03</td>
<td>±408.26</td>
<td>1182.30-2781.80</td>
</tr>
<tr>
<td>End</td>
<td>1772.58</td>
<td>±503.74</td>
<td>807.40-2492.70</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beg.</td>
<td>2127.27</td>
<td>±699.73</td>
<td>1390.50-3543.90</td>
</tr>
<tr>
<td>Mid.</td>
<td>2395.34</td>
<td>±821.83</td>
<td>1325.30-3635.20</td>
</tr>
<tr>
<td>End</td>
<td>1890.19</td>
<td>±344.18</td>
<td>1480.10-2272.10</td>
</tr>
</tbody>
</table>

Note: * Beg. = Beginning of study  
Mid. = Middle of study  
End = End of study
Discussion

The null hypothesis was accepted for the variables measured throughout the study. Statistically significant differences (p < .05) were not observed between groups in glycosylated hemoglobin levels over the twelve week treatment period.

Tables 2 and 3 illustrated the pretest and posttest glycosylated hemoglobin levels in the three groups. Although the glycosylated hemoglobin levels significantly (p < .05) decreased in all groups, significance was not found between groups. According to the Gundersen Clinic blood laboratory norms, the exercise group closely approached the expected normal range (4.6-7.6 %) of percent glycosylated hemoglobin. The decrease (9.17 ± 2.26 to 7.76 ± 2.35 %; p < .05) observed in the exercise group is comparable to results found by Schneider and associates (1984). Glycosylated hemoglobin levels decreased (12.2 ± 0.5 to 10.7 ± 0.4 %; p < .02) in 20 sedentary Type II diabetics after six weeks of thrice weekly training (Schneider et al., 1984). Leon and associates (1984) also found that exercise training failed to significantly alter glycosylated hemoglobin levels, although there was a downward trend in the group which exercised 60 minutes, 4 times a week at 70 percent of their HR reserve (0.70 (HR max-HR rest) + HR rest).

As presented in Figure 1, the lower the initial glycosylated hemoglobin level, the greater was the decrease
following treatment. Schneider and associates (1984) observed that patients with plasma glucose levels < 11.1 mmol/l had a three-fold greater decrease in these amounts over the patients with initial measurements > 11.1 mmol/l. In contrast, another study showed that the higher the fasting plasma glucose (FPG) level before training, the greater was the improvement in FPG after the program (Reitman, Vasquez, Klimes, & Nagulesparan, 1984). Due to the lack of control on intensity levels, the larger decrease seen by the exercise group over the exercise with dietary supplement and control groups could be due to differences in exercise intensities. Leon and associates (1984) found more pronounced glycosylated hemoglobin declines in the group which exercised at the highest intensity (70 % HR reserve), with the longest duration (60 min/session), and the largest frequency (4 sessions/week).

The results shown in Table 7 illustrated that weight loss was not responsible for any of the decreases observed in the three groups. Saltin and associates (1979) demonstrated that there was distinct improvement in oral glucose tolerance test (OGTT) with three months of training in spite of no body weight changes. Reitman and associates (1984) demonstrated that after 6 to 10 weeks of intensive aerobic training while maintaining body weight, FPG declined in all subjects (avg = -33 mg/dl) and OGTT improved in five of the six (avg = -74 mg(3 h/dl)). A possible reason for
the decreases in our three groups could be the dates of the blood draws. The glycosylated hemoglobin levels from the first blood draws were affected by the caloric intake during the Halloween, Thanksgiving, and Christmas seasons, whereas the second blood draws were not. This could be a reason for the significant decreases observed in all three groups. Another possible explanation for the diminished values was the weekly telephone contacts with the participants. These conversations could have unintentionally influenced the participants to observe their proper diets, thus affecting the results.

Even though significant decreases in glycosylated hemoglobin levels were not observed between groups, increased insulin sensitivity might have increased if it had been measured. Horton (1983) found that a 12-week physical training program increased insulin concentrations despite no change in OGTT or body composition. Bjorntorp and associates (1970) found that oral glucose tolerance tests with plasma radioimmunochemically determined insulin showed no changes in blood glucose values after training, but exhibited a marked decrease in insulin values.

**Summary**

In conclusion, even though exercise did not produce significant differences between the groups, there was a definite trend toward glucose homeostasis in the exercise
groups. According to Leon and associates (1984),

the inconsistencies among studies are likely accounted for by the heterogeneity of diabetes; by baseline differences in disease severity, relative weight, body fatness, and fitness levels; by whether or not weight was lost during training; by the intensity, frequency, and duration of exercise and types of exercise used; and by the amount of improvement in aerobic power with training (p. 285).
CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This study was conducted in an effort to establish whether or not an aerobic physical training program would be beneficial in the maintenance of metabolic homeostasis in patients with Type II diabetes mellitus. Thirty-six volunteers from the La Crosse, Wisconsin, area were selected to participate in one of three groups: (1) exercise, (2) exercise with dietary supplement, and (3) control. Data of eight participants was excluded from analysis due either to insufficient daily exercise or medication changes during the study. Measurements in glycosylated hemoglobin, exercise, body weight, and caloric intake were recorded throughout the research period.

Participants in both the exercise and exercise with dietary supplement groups were asked to bicycle 30 minutes a day, seven days a week, for 12 weeks on a stationary bicycle. The control volunteers maintained their current level of activity, not increasing or decreasing. The exercise and control groups were also instructed not to change their dietary habits. The patients in the exercise with dietary supplement group increased their caloric intake
by approximately 200 calories every day that they exercised to control for weight loss. All participants were contacted weekly to assure adherence to their prescribed programs. The volunteers of the exercise and exercise with dietary supplement groups mailed exercise records to the researcher weekly to assist in maintaining compliance. Also, all participants recorded their caloric intake on three separate days during the study to monitor their diets.

A one-way analysis of variance was used to make within group and between group comparisons. The glycosylated hemoglobin levels in all groups significantly decreased at the .05 level, however no significance was found between groups at the same level. Even though there were no significant differences, the exercise group approached normal glycosylated hemoglobin levels. The results suggest that aerobic physical training might be beneficial in the regulation of Type II diabetes.

Conclusions

Within the limitations and boundaries of this study, the following conclusions can be made:

1. Glycosylated hemoglobin levels significantly decreased in all groups, although there was no significant difference between groups.

2. Increased physical training did not produce pronounced weight loss.
3. Metabolic complications did not arise in any participant during the training program.

Recommendations for Future Studies

To further evaluate the effectiveness of aerobic training on the maintenance of metabolic homeostasis in Type II diabetics, the following studies are recommended:

1. A similar study with a larger population, increased control, longer duration.

2. A similar study dividing the participants into the groups after the first blood draw.

3. A comparison of the glycosylated hemoglobin levels between three groups: (1) exercise, (2) exercise with weight reduction, and (3) control.

4. A psychological profile of participants to assess self-image/self-concept benefits of physical training.
REFERENCES CITED


September 5, 1985

To: Alexander Masotti

From: Martin J. Smith, M.D.
Director of Research

Dear Mr. Masotti:

You will be pleased to know that the Research Committee approved your protocol entitled "Effects of Physical Training on Glycosylated Hemoglobin Blood Levels in Patients with Non-Insulin Dependent (Type II) Diabetes Mellitus" at its meeting of August 30, 1985.

Please proceed to the Human Investigation Committee under the Chairmanship of Rev. Daniel Vinge.

I wish you success in your project.

Sincerely,

Martin J. Smith, M.D.

cc: Dr. Pehling
    Rev. Vinge
November 13, 1985

Dear,

We are privileged to have a graduate student at the University of Wisconsin-La Crosse, Alex Masotti, who has attained approval to do a research study on the "Effects of Physical Training on Blood Sugar (glycosylated hemoglobin or hemoglobin Alc) in patients with Type II diabetes (controlled with just diet or diet plus oral medications)." He will be working with Dr. Gregory Pehling (Gundersen Clinic endocrinologist) and me. The process of getting approval for a study like this is very detailed and we are pleased that he can do it. There is no cost to you for participating in the study and you will receive some free blood tests and counseling.

The study will begin in January and continue through March. The participants will be selected to participate in one of three groups: exercise, exercise with food supplement, or continuing present diet and activities. The exercise will be on a stationary bicycle. Further explanation of this and participation in the groups will come at a later date. Adherance to the programs will be a must for the best possible results. Two blood tests at the beginning and end of the study, are necessary to measure the glycosylated hemoglobin levels. The results of these tests will be sent to your physician at no cost. Also, three-day diet histories will need to be taken every month. Participating in the study will require a slight change in your daily activities, but it could prove very valuable.

The results of this study could prove to be significant in controlling your diabetes, as well as the diabetes of other patients. Your participation in the study would be much appreciated, and would be very valuable to the future of diabetic treatment.

In order to get the necessary number of people for the study, I will be contacting many of you in the near future by phone. If you want to be sure to be included in the study, please call me at your earliest convenience. If I am not available, a message may be left with my secretary. Alex and I look forward to working with you on this exciting study.

Sincerely,

Marge Samsoe, M.S.
Director of Exercise Physiology
FOR IMMEDIATE RELEASE

Volunteers sought for diabetes research study

Persons with Type II diabetes are needed to participate in a special research study to be conducted at Gundersen Clinic, La Crosse, from January through March.

Goal of the project is to determine the effects of physical training on blood sugar in persons with Type II diabetes. This form of diabetes can be controlled with just diet, or diet and oral medications.

"The results of this study could be significant in helping participants control their own diabetes as well as the diabetes of other individuals," said Marge Samsoe, M.S., director of exercise physiology and cardiac rehabilitation at Gundersen. "Volunteer participants thus could be making a valuable contribution to the future of diabetic treatment."

The study will be conducted by Samsoe, diabetes specialist Gregory B. Pehling, M.D., and UW-La Crosse graduate student Alex Masotti, under the auspices of Gundersen Medical Foundation.

85-39

Peter King, extension 2743

-MORE-
Volunteers selected to participate in the study will be placed in one of three groups. There will be an exercise group, an exercise with food supplement group, and a group that will continue its present diet and activities. Exercises will be done on a stationary bicycle.

Blood tests will be done on participants at the beginning and end of the three-month study to measure their blood sugar levels. Results of these tests will be sent to each participant's personal physician at no cost.

One-day diet histories will be taken each month. Participants also will be asked to make minor changes in their daily activities during the study. All exercises and testing will be done under professional supervision.

"Adherence to the study's guidelines will be a must for the best possible results," Samsoe said. "We want only volunteers who are sure they will stay with the study for the entire three months."

Persons with Type II diabetes who would like to volunteer for the study or get more information should call Samsoe at (608) 782-7300, extension 2673. If she is not available to talk, a message can be left with her secretary.

###
December 18, 1985

Dear

Please allow me to introduce myself. My name is Alex Masotti, a graduate student in the Adult Fitness/Cardiopulmonary Rehabilitation Master of Science Degree program at the University of Wisconsin-La Crosse. I have one semester of courses, a three-month internship, and my thesis remaining to fulfill the degree requirements. I would like to thank you in advance for your enthusiasm and willingness to participate in my thesis study.

The study will begin on January 2nd and 3rd. Please report to Gundersen Clinic, Exercise Physiology Lab (3rd Floor), between 9:00am and 4:00pm on either of these two days. If for some reason you cannot make it at these times or these days, please contact me at 785-7191 on December 31st or January 1st. We will discuss your participation in the study, as well as complete the preliminary paperwork. You will then be directed to the laboratory for the blood draw. Your total time at the Clinic should not exceed 30 minutes.

If I have enclosed a consent form in your letter, please have it signed by your physician and bring it with you to Gundersen Clinic. If a consent form was not included, I am having it signed by your physician at Gundersen Clinic to facilitate the procedure.

Thank you again, for your spirited interest to participate in the study. I look forward to meeting with you on January 2nd or 3rd.

Sincerely,

M. Alexander Masotti

MAM/daw
Welcome to the "Effects of Physical Training on Glycosylated Hemoglobin Levels in Patients with Type II Diabetes Mellitus" thesis study. Again, I would like to thank you in advance for your participation in this research project.

Following is a list of dates during the study:

First Blood Draw --------------------------------- January 2, 3 (Thur. & Fri.)
Start Program ------------------------------------------- January 5 (Sun.)
First Diet History --------------------------------- January 7 (Tues.)
Second Diet History --------------------------------- February 18 (Tues.)
Third Diet History ----------------------------------- March 25 (Tues.)
Second Blood Draw—End of Study ------------------ March 31, April 1 (Mon. & Tues.)

Your participation in the study will be three-fold:

(1) Have blood draws done at Gundersen Clinic between 9:00 a.m. and 4:00 p.m. on the dates indicated.

(2) Do One-Day Diet Histories and mail them to me the next day.

(3) Live as you normally do. DO NOT increase or decrease your present exercise or dietary intake levels.

If you have any questions or problems that arise during the study, please do not hesitate to contact me.

Alex Masotti
2611 Jackson St., Apt. #1
La Crosse, WI 54601

(608) 785-7191
EXERCISE GROUP

Welcome to the "Effects of Physical Training on Glycosylated Hemoglobin Levels in Patients with Type II Diabetes Mellitus" thesis study. Again, I would like to thank you in advance for your participation in this research project.

Following is a list of dates during the study:

First Blood Draw ----------------------------- January 2, 3 (Thur. & Fri.)
Start Program ------------------------------- January 5 (Sun.)
First Diet History ----------------------------- January 7 (Tues.)
Second Diet History --------------------------- February 18 (Tues.)
Third Diet History ---------------------------- March 25 (Tues.)
Second Blood Draw—End of Study -------------- March 31, April 1 (Mon. & Tues.)

Your participation in the study will be four-fold:

(1) Have blood draws done at Gundersen Clinic between 9:00 a.m. and 4:00 p.m. on the dates indicated.

(2) Exercise Program.
   (a) 30 minute program.
       — 5 minute warm-up (light resistance).
       — 20 minute exercise (increased resistance).
       — 5 minute cool-down (light resistance).
   (b) Exercise every day.
   (c) Mail Daily Exercise Record to me at the end of each week.

(3) Do not change your diet. Do not increase or decrease your dietary intake level.

(4) Do One-Day Diet Histories and mail them to me with that week's Daily Exercise Record.

If you have any questions or problems that arise during the study, please do not hesitate to contact me.

Alex Masotti
2611 Jackson St., Apt. #1
La Crosse, WI 54601
(608) 785-7191
EXERCISE WITH DIETARY SUPPLEMENT GROUP

Welcome to the "Effects of Physical Training on Glycosylated Hemoglobin Levels in Patients with Type II Diabetes Mellitus" thesis study. Again, I would like to thank you in advance for your participation in this research project.

Following is a list of dates during the study:

First Blood Draw ----------------------------- January 2, 3 (Thur. & Fri.)
Start Program -------------------------------- January 5 (Sun.)
First Diet History ------------------------------- January 7 (Tues.)
Second Diet History ----------------------------- February 18 (Tues.)
Third Diet History ----------------------------- March 25 (Tues.)
Second Blood Draw—End of Study ---------------- March 31, April 1 (Mon. & Tues.)

Your participation in the study will be four-fold:

(1) Have blood draws done at Gundersen Clinic between 9:00 a.m. and 4:00 p.m. on the dates indicated.

(2) Exercise Program.
   (a) 30 minute program.
       —5 minute warm-up (light resistance).
       —20 minute exercise (increased resistance).
       —5 minute cool-down (light resistance).
   (b) Exercise every day.
   (c) Mail Daily Exercise Record to me at the end of the week.

(3) Eat approximately 200 more calories than normal every day that you exercise. If you do not exercise, DO NOT eat 200 extra calories that day. We are trying to maintain your current weight.

(4) Do One-Day Diet Histories and mail them to me with that week's Daily Exercise Record.

If you have any questions or problems that arise during the study, please do not hesitate to contact me.

Alex Masotti
2611 Jackson St., Apt. #1
La Crosse, WI  54601
(608) 785-7191
INFORMED CONSENT FOR THE EXPERIMENTAL STUDY OF THE
"EFFECTS OF PHYSICAL TRAINING ON GLYCOSYLATED HEMOGLOBIN
LEVELS IN PATIENTS WITH TYPE II DIABETES MELLITUS"

I desire to engage voluntarily in this experimental study in order to fulfill its purpose, to determine the effectiveness of an aerobic physical training program on glycosylated hemoglobin levels in individuals with Type II diabetes mellitus, and to enhance the advancement of medical knowledge in this area. My refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled, and I may discontinue participation at any time without penalty or loss of benefits to which I am otherwise entitled. My participation in this study has been approved by my physician, Dr. __________________________.

I will be administered a Glycosylated Hemoglobin Test by Gundersen Clinic staff at the beginning of the study. I will, then, be selected to participate in one of three groups: (1) Control, (2) Exercise, or (3) Exercise with Dietary Supplement. The mode of exercise for the Exercise and Exercise with Dietary Supplement groups will be bicycle ergometry for a target time of 30 minutes per day, 7 days a week, for 12 weeks. There will be no added costs to the participants for stationary bicycles. The subjects of the control group are expected to maintain their present level of exercise, not to increase or decrease. Except for the dietary supplement, all participants will maintain their current diets.

I may be exposed to a few risks during this study. These may include problems involving infections from the blood draws, as well as foot or cardiac problems. I also understand that Gundersen Clinic, the University of Wisconsin-La Crosse, and the researchers may not be held responsible for injuries or complications resulting from the study. Although these risks are minimal, they still exist. The possible benefits to Type II diabetics are: (1) regulation of glucose metabolism, (2) reductions of body fat, blood pressure, and lipids, (3) improvement in cardiovascular and respiratory functions, and (4) increases in high-density lipoprotein cholesterol levels.

The information which is obtained during the laboratory evaluations and exercise sessions of the experimental study will be treated as privileged and confidential, and will not be released or revealed to any non-medical person, without my expressed written consent. The information obtained, however, may be used for a statistical or scientific purpose with my right of privacy retained. I also approve of periodic forwarding to my physician of data relative to the laboratory evaluation(s), and my involvement in the exercise sessions.

I have read the foregoing and I understand it. Any questions which have arisen or occurred to me have been answered to my satisfaction. If I have any further questions during the study, I may contact the researcher, M. Alexander Masotti, at 785-7191.

Signed:

(Participant) ____________________________ (Researcher) ____________________________

I affirm that ____________________________ has read and signed this consent in my presence today and that there were no questions that had not been answered by the researcher. This person understood and willingly gave consent to participate in this study.

(Witness) ____________________________ (Dated) ____________________________

(Physician) ____________________________ (Dated) ____________________________
An affinity chromatography test kit for the quantitative determination of Glycosylated Hemoglobin in whole blood hemolysates or red blood cell hemolysates.

PRODUCT USE AND DESCRIPTION

The Pierce GlycoTest™ in vitro diagnostic kits are an affinity chromatography based method for the separation and quantitation of glycosylated hemoglobin from whole blood hemolysates or red blood cell hemolysates. The speed of this method and the significant differences in percent glycosylated hemoglobin observed between normal and diabetic individuals make this affinity procedure a convenient patient screening and monitoring system.

The kits consist of GLYCO-GEL™ Analytical Columns, a Sample Preparation Reagent, an Equilibration/Wash Buffer and a Glycosylated Hemoglobin Elution Buffer.

INTRODUCTION

Hemoglobin has been shown to form a stable covalent derivative with glucose without the aid of an enzyme. Methods currently in use for measuring glycosylated hemoglobin are often tedious and difficult or very sensitive to small changes in temperature and/or pH. In an attempt to obviate the difficulties inherent in currently used methodologies, a method has been developed which utilizes reversible affinity chromatography to provide a specific interaction with the glycosylated fraction.

Affinity chromatography is a powerful technique for the separation of biomacromolecules due to their biological activity or chemical structure. The separation media consists of a ligand attached to an insoluble, inert matrix such as agarose. The ligand-matrix affinity chromatographic support will reversibly bind and thus retain substances which interact with the ligand. Elution of the retained substances can be achieved by altering the buffer composition.

CLINICAL SIGNIFICANCE

The measurement of glycosylated hemoglobin has been shown to provide a convenient indicator for the diagnosis and management of diabetes mellitus. Elevated levels of glycosylated hemoglobin are observed in uncontrolled diabetics. An elevated value may also indicate this disease in a previously undiagnosed diabetic. In addition, the assessment of long term control can be ascertained in the diabetic individual. Values lower than the normal range as described for this method are of uncertain clinical significance.

PRINCIPLE OF THE PROCEDURE

Recent studies have delineated the chemical steps involved in the post-translational non-enzymatic glycosylation of hemoglobin in red blood cells. As shown in Figure 1, the aldehyde group of glucose forms a reversible Schiff base linkage with the N-terminus of the α- and β-chains and certain lysine residues of the hemoglobin molecule. This labile adduct then undergoes an Amadori rearrangement to the stable ketoamine, commonly referred to as glycosylated hemoglobin. These resultant coplanar cis-diol groups of glycosylated hemoglobins can interact with GLYCO-GEL B (see Kit Contents, Reagents Supplied section) to form a reversible five-member ring complex. This complex can be dissociated by sorbitol.

Glycosylated hemoglobin can be separated and quantitated with the Pierce GlycoTest kits. In practice, the sample containing a mixture of glycosylated and non-glycosylated variants is applied to the Pierce pre-packed GLYCO-GEL Analytical Columns. Glycosylated variants will be retained by the column whereas non-glycosylated entities will pass through. After appropriate washing, the bound glycosylated species are eluted with a sorbitol containing buffer. Relative ratios of glycosylated and non-glycosylated fractions are quantitated by measuring the absorbance at an appropriate wavelength.
KIT CONTENTS

The GlycoTest Kit contains 12 GLYCO-GEL B Analytical Columns and sufficient reagents to perform 120 determinations. Each column is used, regenerated and re-used for a total of 10 analyses per column.

GlycoTest 25 contains 25 GLYCO-GEL B Analytical Columns and sufficient reagents to perform 25 determinations. Each column is used only once and discarded.

GlycoTest 100 contains 100 GLYCO-GEL B Analytical Columns and sufficient reagents to perform 100 determinations. Each column is used only once and discarded.

Reagents Supplied:

1. Prepacked GLYCO-GEL Analytical Columns
   - Each column contains 0.5 ml GLYCO-GEL B for the separation of glycosylated hemoglobin from non-glycosylated hemoglobin in whole blood hemolysates or red blood cell hemolysates.
   - Active Ingredients: GLYCO-GEL B, immobilized m-amino-phenylboronic acid on a support of cross-linked 6% beaded agarose in an aqueous medium 0.02% sodium azide as a preservative.

2. Equilibration/Wash Buffer (EWB)
   - For the elution of the non-glycosylated hemoglobin fraction from the glycosylated hemoglobin fraction.
   - Active Ingredients: Ammonium acetate (0.25 mol/L), magnesium chloride (0.05 mol/L), and sodium azide (0.02%) as a preservative, pH 8.0.

3. Glycosylated Hemoglobin Elution Buffer (GlyHB-EB)
   - For the elution of GLYCO-GEL B, bound glycosylated hemoglobin.
   - Active Ingredients: Sorbitol (0.2 mol/L), tri(hydroxymethyl)aminomethane (0.1 mol/L), and thimerosal (0.01%) as a preservative, pH 8.5.

4. Sample Preparation Reagent
   - For preparation of hemolysates from whole blood specimens.
   - Active Ingredients: Octylphenoxypolyethoxy ethanol (0.1%) and sodium azide (0.02%) as a preservative.

Materials Supplied:

1. White Strip Labels - Supplied with GlycoTest (product 42000) only. Not supplied with GlycoTest 25 or GlycoTest 100.

White strip labels are supplied as aids for monitoring column regeneration. After regeneration of a GLYCO-GEL Analytical Column and equilibration with Column Storage Solution, replace the top and bottom column stoppers. Place a label horizontally on the top column approximately one inch from the top of the gel bed. Write 1x, 2x, etc. on the label to indicate “1 time regenerated,” “2 times regenerated” etc. GLYCO-GEL Analytical Columns can be regenerated 9 times, therefore 10 determinations can be performed per analytical column.

Materials Required but not Supplied:

1. Pipettes capable of accurately dispensing 50 μl, 0.5 ml, 2.0 ml, 3.0 ml and 5.0 ml.
2. Collection tubes, 16 x 125 mm test tubes are recommended for collection of the fractions.
3. Spectrophotometer capable of measuring absorbance (A) at 414 nm. The instrument should have a narrow bandpass of 20 nm or less.
4. Column Regeneration Solution: Required for GlycoTest (product 42000) only. Not required for GlycoTest 25 or GlycoTest 100. Prepare 1000 ml 0.1 N HCl by diluting 8.3 ml concentrated hydrochloric acid to 1000 ml with deaerated, deionized water.
5. Column Storage Solution: Required for GlycoTest (product 42000) only. Not required for GlycoTest 25 or GlycoTest 100. Prepare 1000 ml 0.001 N HCl. Dilute 10 ml Column Regeneration Solution to 1000 ml with deaerated, deionized water.

NOTES AND PRECAUTIONS:

For in vitro Diagnostic Use

1. Prepacked GLYCO-GEL Analytical Columns
   a. When handling capped columns, it is very important to follow the instructions in the MAKE READY section accurately. If the bottom tip stopper is not in place when the top stopper is removed, air may be drawn in through the bottom of the gel. This will cause bubbles to form in the gel and render the column unsuitable for use. IMPORTANT: Remove the top stopper first!
   b. When the columns are out of 4°C storage and at the bench, avoid direct sunlight for prolonged periods as the gel may discolor. The gel on occasion may develop a slight pink cast which will not interfere with its performance. If the gels should become dark colored (red-purple) through excessive exposure to direct sunlight or prolonged storage at elevated temperatures, they should be discarded. Do not use after the expiration date shown.

2. Equilibration Wash Buffer (EWB) and Glycosylated Hemoglobin Elution Buffer (GlyHB-EB)
   a. The Equilibration/Wash Buffer (EWB) contains 0.02% sodium azide. Do not allow sodium azide solutions to come in contact with metal plumbing systems.
   b. Buffers supplied should not be used after expiration date. Do not use buffers if they appear discolored or if precipitates are present. If there is any evidence of fungal or microbial growth, discard buffer.
   c. Store all buffers at 4°C and tightly capped when not in use.
   d. The buffers included in the Pierce GlycoTest Kits are sufficiently stable so that pH will not be substantially altered by any properly prepared hemolysate. Variations in pH of ± 0.25 pH units will not significantly affect the results of this test.
   e. Column bubbles. A few small bubbles (with a diameter of less than 2 mm) in the column bed or under the bottom frit do not impair the performance of the column. Columns which contain many small bubbles or a single, very large bubble should not be used since they can alter column flow and contribute to channelling.

   To prevent or minimize the formation of bubbles always allow all components of the kit to come to complete room temperature before use. Also, use only degassed, deionized water to prepare the regeneration and storage solutions. It is best to prepare these at least one day before use.

3. Equilibration Wash Buffer (EWB) and Glycosylated Hemoglobin Elution Buffer (GlyHB-EB)
   a. The Equilibration/Wash Buffer (EWB) contains 0.02% sodium azide. Do not allow sodium azide solutions to come in contact with metal plumbing systems.
   b. Buffers supplied should not be used after expiration date. Do not use buffers if they appear discolored or if precipitates are present. If there is any evidence of fungal or microbial growth, discard buffer.
   c. Store all buffers at 4°C and tightly capped when not in use.
3. Specimen Storage
   a. Whole blood can be stored at 4°C for 1 week.
   b. Hemolysate of red blood cells may be stored for up to one month at -20°C or up to six months at -70°C to -90°C.
   c. Hemolysates of whole blood may be stored up to 3 months at -70°C.

4. Affect of Temperature
   Ambient temperature changes have a minimal effect on affinity glycosylated hemoglobin values in both the normal and diabetic range. Ambient changes from 20°C to 25°C will result in a decrease in glycosylated values of less than 2.5% absolute in the diabetic range and correspondingly less in the normal range. This negligible difference eliminates the need for any temperature control of the Glyco-Gel columns or temperature correction factors. It is recommended, however, that laboratories doing long term longitudinal studies on individual patients perform each assay at the same ambient temperature, ± 1°C. This will assure the best assay to assay accuracy.

5. Affect of “Labile” or “Pre” HbAlc
   Glycosylated hemoglobin values obtained by this affinity method are unaffected by the presence of “labile” or “pre” HbAlc. These species are not retained on the gel but elute with the non-bound, non-glycosylated fraction. Pretreatment of samples to remove the “labile” or “pre” HbAlc is not necessary.

6. Polycythemia and Hemolytic Anemia
   Polycythemia and Hemolytic Anemia are conditions which often result in a significant decrease in the life span of red blood cells. Clinically significant data relies upon the normal 120-day life span of red blood cells. Therefore values obtained from individuals with these disorders may be difficult to properly evaluate.

7. Affect of Hemoglobin Variants
   Hemoglobin variants such as S, C, and L do not interfere since the specificity of this method is with the cis-diaxid on glucose modified hemoglobin.

8. Affect of Hyperlipidemia
   If hyperlipidemia is suspected or if the plasma appears cloudy, use procedure 2 in the “Specimen Collection, Preparation and Storage” section to prepare the hemolysates. Elevated lipids could cause the not bound fractions to become cloudy. Turbidity will affect the measurement of absorbance in any colorimetric procedure.

9. Affect of Column Regeneration
   Diabetic and nondiabetic (normal) hemolysates were assayed on fresh and regenerated (up to 9 times) columns. The assays were done over a period of several days and five replications of each sample were run at each regeneration step. For the normal samples a standard deviation for percent Glycosylated Hemoglobin of 0.17 (CV = 3.0%, n = 50) was obtained. The diabetic samples gave a standard deviation of 0.28 (CV = 1.8%, n = 50).

10. Affect of Metal Contaminates
    DO NOT pipette or dispense buffers and column regeneration solutions through devices containing metal parts. Metal contamination can cause a shift in the absorbance of hemoglobin at 414 nm resulting in erroneous values.

SPECMEN COLLECTION, PREPARATION, AND STORAGE

Avoid highly lipemic specimens by not drawing blood immediately after a meal. Do not obtain blood from a limb which is also receiving an infusion. Draw venous blood in standard blood collection tubes containing EDTA or fluoride as the anticoagulant (“purple or grey tops”). Mix all specimens well prior to preparation of hemolysate by either procedure below:

1. Whole Blood Procedure for Preparation of Hemolysates
   This procedure can be used to prepare hemolysates from all specimens except those that are known or suspected to be lipemic (See Notes and Precautions).

   Prepare a 1:10 hemolysate by mixing 1 part Whole Blood with 10 parts Sample Preparation Reagent. For example, add 50 μl of Whole Blood to 0.5 ml of Sample Preparation Reagent in a small vial or test tube. Swirl or vortex to mix well and let stand 5 minutes. Use the hemolysate immediately.

2. Packed Cell Procedure for Preparation of Hemolysates
   This procedure is to be used if the specimens appear cloudy (plasma fraction) or if lipemia is suspected.

   Centrifuge the Whole Blood to separate plasma from red blood cells (RBC's). Remove the plasma and white blood cells (“buffy coat”) by aspiration. Wash the packed cells by adding 3 parts saline (0.9% sodium chloride) to 1 part RBC's, mix by inversion, centrifuge and aspirate the top layer. Prepare a 1:20 hemolysate by mixing 1 part washed RBC's with 20 parts distilled or deionized water. (For example, add 100 μl of packed RBC's to 2.0 ml of deionized water in a 13 x 100 mm test tube.) Vortex the hemolysate for 15-20 seconds and centrifuge to remove cell debris and incompletely lysed cells. The clear hemolysate may be used immediately or may be stored at -70°C pending analysis.

MAKE READY: Equilibration of Columns

1. Bring the columns and buffers out of 4°C column storage and allow them to come to room temperature.
2. Remove the top column stoppers only, then pour off and discard the storage solution. Now remove the bottom caps and place the columns in a suitable rack.
3. Equilibrate the columns by adding 2.0 ml of the Equilibration/Wash Buffer (EBW); allow the buffer to flow through the columns, discarding the effluent.
4. The columns are now ready for use.
PERFORMANCE OF TEST:

percent Glycosylated Hemoglobin Assay

A pictorial diagram of the procedures is shown below. The step-by-step procedure is described thereafter.

**SEPARATION**

Sample Application:

- **Elution Of Glycosylated Hemoglobin**
- **Change Collection Tubes**
- **3.0 ml GlyHb Elution Buffer**

**QUANTITATION**

- **Hemoglobin**
- **Spectrophotometer**

**CALCULATION**

- **% GlyHb = 3.0 x 0.110 x 100 / (5.55 x 0.850 + 3.0 x 0.110)**
- **% GlyHb = 6.5%**

**COLUMN REGENERATION**

Required for GlycoTest (product 42000) only. Not required for GlycoTest 25 or GlycoTest 30.

GLYCO-GEL Analytical Columns can be regenerated nine (9) times without affecting the accuracy of the results. To regenerate GLYCO-GEL Analytical Columns follow the steps below:

1. As soon as possible after the columns have been used, add 5 ml of Column Regeneration Solution (0.1 N HCl) to each column and allow to flow through; discard the effluent.
2. Next add 5 ml of Column Storage Solution (0.001 N HCl) to each column and allow about 3 ml to flow through; discard the effluent.
3. When about 2 ml solution remains, pick up the column and place the bottom stopper over the column tip. Now insert the top stopper.
4. Regeneration is complete. Using the supplied labels properly mark each regenerated column whether it is the first, second, etc. regeneration.

**CONTROLS**

The Pierce Glycosylated Hemoglobin Controls have been prepared for use with GlycoTest and other quantitative kits. Not all controls are suitable for use with these affinity kits.

Each lab can also prepare and retain their own controls from blood samples obtained from known normals and diabetics.

**EXPECTED VALUES:**

**Normal Range**

The expected normal range for percent Glycosylated Hemoglobin using this procedure was determined by assaying samples from 92 presumably healthy non-diabetic volunteers and found to be 6.2 - 8.0.

**PERFORMANCE CHARACTERISTICS**

**Accuracy**

This affinity chromatographic procedure for determining percent glycosylated hemoglobin has been shown to give a correlation coefficient of 0.97 when compared to an HPLC ion exchange reference method. The same data used to generate this linear regression correlation is further shown below in Table 1.

**Table 1**

<p>| %GlyHb (Affinity) vs %HbA1c (Ion-exchange) |
|-----------------------------|-----------------------------|</p>
<table>
<thead>
<tr>
<th>%HbA1c</th>
<th>%GlyHb</th>
<th>n</th>
<th>Average %HbA1c</th>
<th>Average %GlyHb</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>6%</td>
<td>14</td>
<td>4.99</td>
<td>3.85</td>
<td>1.08</td>
<td></td>
</tr>
<tr>
<td>6-8%</td>
<td>10</td>
<td>7.23</td>
<td>8.48</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>8-10%</td>
<td>16</td>
<td>8.85</td>
<td>11.43</td>
<td>1.29</td>
<td></td>
</tr>
<tr>
<td>10-12%</td>
<td>11</td>
<td>10.71</td>
<td>15.25</td>
<td>1.42</td>
<td></td>
</tr>
</tbody>
</table>
The data shows that as the level of glycosylation increases, the affinity method gives progressively higher values when compared to HPLC ion exchange. This is due to the affinity method assaying all glycosylated hemoglobins, not just the β-terminal modified species (HbA₁c) that is assayed by HPLC ion exchange.

Another correlation study compared the whole blood procedure with the packed cell procedure. A total of thirty four (34) specimens (10 normals and 24 insulin dependent diabetics) were assayed using the Pierce GlycoTest™ kit. An excellent correlation coefficient of 0.998 was obtained. Y = 0.98X + 0.1, where Y = whole blood procedure and X = packed cell procedure.

**Precision**

The GlycoTest Kit was evaluated for precision by performing the assay on hemolysate pools from normoglycemic and diabetic volunteers. Hemolysates were prepared as described above and aliquots were stored at 4°C until the assays were run. All buffers and columns were at room temperature.

The following procedure was employed on both the whole blood hemolysate pools and on the packed cell hemolysate pools. Five (5) replications were done on each hemolysate pool starting with fresh columns and going through 9 column regenerations. A complete duplicate set of assays was performed using fresh columns and 3x, 6x, and 9x column regenerations. No significant differences were seen between fresh and regenerated columns. The overall results for the hemolysate pools are given below in Table II.

**BIBLIOGRAPHY**


**OTHER SELECTED REFERENCES**


<table>
<thead>
<tr>
<th>Table II</th>
<th>Precision of the GlycoTest Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole Blood Hemolysates</strong></td>
<td><strong>Packed Cell Hemolysates</strong></td>
</tr>
<tr>
<td><strong>Normoglycemic Volunteer</strong></td>
<td></td>
</tr>
<tr>
<td>No. of replications</td>
<td>50</td>
</tr>
<tr>
<td>Mean % Gly-Hb</td>
<td>5.7%</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.17</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>3.0%</td>
</tr>
<tr>
<td><strong>Diabetic Volunteer</strong></td>
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</tr>
<tr>
<td>No. of replications</td>
<td>50</td>
</tr>
<tr>
<td>Mean % Gly-Hb</td>
<td>15.4%</td>
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<tr>
<td>Standard deviation</td>
<td>0.28</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>1.8%</td>
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</table>
# Daily Exercise Record

<table>
<thead>
<tr>
<th>DATE</th>
<th>MINUTES EXERCISING</th>
<th>RESTING PULSE (OPTIONAL)</th>
<th>EXERCISING PULSE (OPTIONAL)</th>
<th>EXERTION LEVEL/ COMMENTS</th>
</tr>
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NAME ___________________________ WEEK # __________________

WEIGHT __________________

MINUTES EXERCISING: Amount of time spent exercising.

RESTING PULSE: Heart rate measured before exercising.

EXERCISING PULSE: Heart rate measured during exercise.

EXERTION LEVEL: Level of exertion during exercise.

COMMENTS: Additional information or notes about the exercise session.
# ONE-DAY DIET HISTORY

NAME ___________________________ DATE __________________

MEDICATIONS ______________________ VITAMINS __________________

COMMENTS _________________________

<table>
<thead>
<tr>
<th>FOOD ITEM</th>
<th>PORTION SIZE</th>
<th>ID CODE #</th>
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</table>
INSTRUCTIONS FOR KEEPING GOOD FOOD RECORDS

Please record everything you eat and drink from the time you get up in the morning until you go to bed for three days, one of which is a weekend day. Be sure to mention everything you eat or drink at home, at work, or away from home. Include snacks and beverages. You can write amounts in pieces (1 slice bread, 1 small banana, 1 egg), or approximate serving sizes in household measures (½ cup cooked carrots, 4 ounces 2% milk, etc.). Appropriate measurements which you should use will be listed in each food group in parentheses ( ).

Milk and Milk products (ounces or measuring cups)

- Kind of milk - whole, 2%, skim, etc.
- Cheese-type of fat it contains - e.g., low fat cottage cheese with 2% butter fat.
- "Hard" cheese made from corn and safflower oils
- Ice cream, ice milk or sherbet - specify which

Fruit (piece or measuring cup)

- Specify fresh, frozen or canned fruit (heavy or light syrup) or juice whether sweetened or unsweetened.

Vegetable (measuring cup)

- Specify kind and record amount of butter, margarine, sauce or gravy added to or included in vegetables (canned, fresh or frozen).

Breads and Cereals (piece or measuring cup) - Brand name

- Bread or sandwich
  - Type of bread - whole wheat, rye, white, etc.
  - Butter, margarine, peanut butter or jelly added
  - Thickness, if from unsliced loaf.

Cereals
- Kind of cereal, and don't forget any milk, sugar, honey and amounts!

Meats, Poultry, Fish, Eggs, and Main Dishes (ounces or portion - e.g., 1 egg)

- Kind - beef, pork, chicken, etc.
- How it is prepared - baked, fried, broiled, etc.
- Amount and type of fat, if any, used in preparation
- Cut of meat, if known - sirloin tip roast, round steak, etc.
- Mixed dishes - amounts and type of meat, macaroni, sauce, fat, soup and vegetables in recipe.

Sandwich
- Type of bread
- Number of slices of bread
- Amount of meat, cheese or filling
- Butter, margarine, mayonnaise

Refer to size sheet attached
Desserts

Cake
- dimensions of piece (e.g. 2" x 2") or proportion of total (give size of pan, e.g. 9" x 13")
- Any frosting - kind

Pie
- Type of crust - graham cracker, pastry, etc.
- Kind of fat or oil used in making crust
- Type of pie - apple coconut cream, etc.
- Diameter of pie
- Proportion of total pie
  - For example: 1/6 of 8" pumpkin pie - crust made with corn oil

Cookie
- Diameter
- Type of cookie

Other desserts
- Dimension of piece e.g., 2"x3"
- Any topping - whipped cream, "Dream Whip"

Fats (teaspoon or tablespoon)
- Type of spread used
  - butter
  - margarine - kind of oil(s) it contains - list oil(s) in order as given on label (Brand Name)
- Don't forget to include any fats or oils used in cooking

Beverages (ounces or measuring cup)
- Coffee, tea, Postum, Sanka
- Any cream, sugar, lemon added
- Pop (ounces - not "one bottle or can")
  - regular or low calorie
  - preferably give brand name

Mixed drinks
- kind and amount of mix
- kind and amount of alcohol

Beer
- type and amount

Snack foods (ounces or number of pieces)
- 12 peanuts
- 3 cups popcorn

Below is a sample food record for one day:
Sample food record:

BREAKFAST:
- 4 ounces frozen orange juice - unsweetened
- 2 slices whole wheat with 1 teaspoon corn oil margarine and 2 tsp. honey
- 1 egg fried in "Pan" aerosol
- ½ cup oatmeal with 1 tbsp raisins and ½ cup skim milk
- 1 cup black coffee

SNACK:
- ¼ cup pineapple packed in its own juice

LUNCH:
Sandwich made of:
- 2 slices white bread
- 2 ounces salami
- 1 ounce Swiss cheese
- 2 teaspoons butter
- 1 teaspoon mustard
- 1 leaf of lettuce

1 carrot stick - large
Macaroni Salad
- 2½ cup macaroni
- 2 Tbsp. seas
- 1 Tbsp. mayonnaise
- 1 Tbsp. celery
- 15 potato chips (all of 12 ounce bag)
- 1 cup chocolate drink, made with skim milk

SNACK:
- 16 ounces iced tea with 2 teaspoons sugar
- 3" x 3" piece of chocolate cake with chocolate frosting

SUPPER:
Blood Mary - made with 1½ ounces vodka and 6 ounces tomato juice
- 6 ounces broiled t - bone steak
- 20 french fries - fried in Crisco Shortening
- 1 cup lettuce salad, ¼ of 3" tomato, 3 cucumber slices, with 2 Tbsp. Creamy Italian Dressing
- ½ cup cauliflower with 2 Tbsp. cheese sauce
- 1 cup black coffee
- Grasshopper made with ½ cup ice cream, 1 ounce creme de cocoa and 1 ounce creme de menthe

SNACK:
- 12 ounces regular (not diet) 7-Up
MEAT LOAF

Two slices this size: 3 ounces

ROUND STEAK (lean only)

One piece this size: 3 ounces

HAMBURGER (lean)

One patty this size: 3 ounces

ROAST BEEF ROUND (lean only)

Two slices this size: 3 ounces

ROAST TURKEY

2 slices this size = 3 ounces

Two slices of meat this size: 3 ounces
PORK CHOP (lean only)

Two chops this size (fat removed): 3 ounces

HAM (lean only)

Two slices this size: 3 ounces

VEAL CUTLET (trimmed)

One cutlet this size: 3 ounces
EXAMPLES OF FOOD SUPPLEMENTS

1 bagel & 1 Tbsp. cream cheese
1 bagel & 1 slice of cheese
1 bagel & 1 slice of cold cut
2 pieces bread & 1 slice of cheese
2 pieces bread & 1 slice of cold cut
2 cups skim milk
1 cup 2% milk & ½ bagel
1 cup 2% milk & 1 slice cheese
1 cup 2% milk & 1 biscuit
1 cup 2% milk & 1 piece bread
1 apple & 1 banana
1 apple & 1 cup orange juice
1 banana & 1 cup orange juice
1 small pear & 1 cup grape juice
10 Tbsp. raisins
2 Tbsp. peanut butter & 1 cup celery