1983

The Relationship Between Fibrinolytic Capacity and Metabolic Control in Insulin-Dependent Diabetes Mellitus.

William Guyton Hornsby Jr

Louisiana State University and Agricultural & Mechanical College

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THE RELATIONSHIP BETWEEN FIBRINOLYTIC CAPACITY AND METABOLIC CONTROL IN INSULIN-DEPENDENT DIABETES MELLITUS

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THE RELATIONSHIP BETWEEN
FIBRINOLYTIC CAPACITY AND METABOLIC CONTROL
IN INSULIN-DEPENDENT DIABETES MELLITUS

A Dissertation
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in
The School of Health, Physical Education,
Recreation and Dance

by
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B.S., University of South Carolina, 1974
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Dedicated to

my mother and father

for their patience and understanding in

the management of my diabetes,

for their encouragement and support of

my professional goals,

and, most of all, for their love and guidance

throughout my life
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ABSTRACT

The purpose of this study was to investigate the relationship between fibrinolytic capacity and metabolic control in insulin-dependent diabetes mellitus. Testing protocol was explained and informed consent was obtained from 26 insulin-dependent diabetics and 20 healthy controls who agreed to serve as subjects. Fibrinolytic capacity (FC) was measured as the change in euglobulin lysis time produced by 10 min of venous occlusion with a sphygmonanometer cuff inflated around the upper arm at a pressure midway between systolic and diastolic blood pressure. Metabolic control was evaluated by measurement of hemoglobin A1 (HbAl) using a minicolumn chromatographic technique. Fasting glucose (GLU), cholesterol (CHO), and triglyceride (TRI) were measured on a Coulter Chemistry Analyzer. Significant differences were found to exist between diabetics and controls, respectively, for FC (46.6 ± 19.3% vs. 64.2 ± 12.1%), HbAl (10.3 ± 2.1% vs. 6.5 ± .9%), GLU (209 ± 102 mg% vs. 95 ± 7 mg%), and CHO (215 ± 56 mg% vs. 186 ± 25 mg%). No differences were found for TRI. Bivariate correlations were calculated between FC and HbAl for diabetics only, for controls only, and for all subjects combined. Significant relationships were found for both the combined sample (r = -.33) and for controls (r = .54), but not for diabetics (r = -.08). Stepwise linear regression revealed that FC
could best be explained in insulin-dependent diabetes by TRI ($p = .03$), and no significance could be attached to HbA1 ($p = .46$). It is concluded that the positive relationship noted between FC and HbA1 for normal subjects is spurious and that the reduced capacity for fibrinolytic activity in insulin-dependent diabetes is not directly related to metabolic control.
CHAPTER I
INTRODUCTION

It has been hypothesized that low fibrinolytic activity could lead to vascular disturbances if the end products of coagulation are allowed to accumulate on vessel surfaces (Astrup, 1956). This premise has been the basis of many investigations which have demonstrated impaired fibrinolysis in a variety of thromboembolic disorders. Insufficient fibrinolytic activity may be involved in the pathogenesis of diabetic vascular disease, but this point has not been conclusively established.

While numerous investigations concerning fibrinolysis in diabetes appear in the literature, they are plagued by problems of methodology. Most researchers have chosen to study spontaneous fibrinolytic activity, but this method is far too insensitive to produce definitive results. More consistent results have been obtained by examining fibrinolytic capacity, but responses appear to vary within groups of diabetic subjects.

Recent evidence indicates that diabetes mellitus is a heterogeneous disorder which contains many different pathophysiological conditions. A new classification scheme has been developed which clearly separates diabetics who have an absolute deficiency of insulin from
those whose deficiency is only relative (National Diabetes Data Group, 1979). The latter division contains a large number of obese individuals, and excess body fat has been shown to be associated with impaired fibrinolytic activity (Almer & Janzon, 1975). The factor of obesity can be eliminated fairly well by studying diabetics who have an absolute insulin deficiency.

Laboratory techniques have been developed for the quantitative determination of diabetic control, and have been applied to investigate the role of glucose homeostasis in the etiology of diabetic vascular disease (Bunn, 1981; Gabbay, Hasty, Breslow, Ellison, Bunn, & Gallop, 1977). Available evidence indicates that poor metabolic control is associated with a state of hypercoagulation (Coller, Frank, Milton, & Gralnick, 1978; Sowers, Tuck, & Sowers, 1980). An important question would, therefore, appear to be: What is the relationship between control of blood glucose and fibrinolytic activity in diabetes mellitus?

**Statement of the Purpose**

The primary purpose of this investigation was to examine the relationship between fibrinolytic capacity and metabolic control in insulin-dependent diabetes mellitus. Secondarily, a number of moderator variables were included to determine their effect on this relationship.
Research Hypothesis

The following research hypothesis was made for this study: Fibrinolytic capacity and metabolic control will be inversely related; that is, those patients with poorest metabolic control will demonstrate lowest fibrinolytic capacity.

Operational Definitions

Fibrinolytic capacity: The ability to increase levels of plasminogen activator above preocclusion values (as judged by a decrease in euglobulin lysis time), in response to 10 min of venous occlusion.

Metabolic control: The percentage of total hemoglobin that is comprised of hemoglobin Al.

Assumptions

The following assumptions were made in this study:

1. All subjects adhered to pretest rules regarding cigarette smoking, vigorous exercise, and ingestion of foods. The pretest Clinical Data Form (Appendix A) accurately documents subject cooperation.

2. Clinical records give an accurate description of the subjects' dependence on exogenous insulin to sustain life.

Limitations

The study is limited as follows:

1. Subjects were volunteers selected from diabetic cases seen at Earl K. Long Memorial Hospital. The sample
is not random.

2. Subjects were type I diabetics and this delimitation restricts application of these results only to other insulin-dependent diabetic individuals.

3. The small sample size employed in this investigation limits the power of the statistical analyses.

Significance

Disorders of large and small blood vessels are associated with increased morbidity and mortality in diabetes mellitus, and have been linked to disturbances of the coagulation-fibrinolysis equilibrium. While there is good evidence of a hypercoagulable state in diabetes, no consensus has been reached concerning fibrinolytic activity. Studies have been plagued by inappropriate experimental procedures and failure to consider pathophysiological differences and coexisting conditions which may affect diabetic fibrinolysis.

The capacity for plasminogen activator release in response to a standard stimulus, which is held to be a valid and reliable method of assessing fibrinolytic capacity (Hedner & Nilsson, 1981), was examined. The moderating factor of obesity was controlled by the use of only type I diabetics. This study should provide useful information concerning the relationship between fibrinolytic capacity and metabolic control, and should have application to guidelines for therapeutic management of insulin-dependent diabetes mellitus.
CHAPTER II
REVIEW OF LITERATURE

This review deals with various aspects of fibrinolysis and diabetes mellitus. The section on fibrinolysis includes mechanisms and components of the fibrinolytic enzyme system, available techniques for the assessment of fibrinolytic activity, and a brief review of those studies which have examined both physiological and impaired fibrinolysis. The section on diabetes mellitus encompasses the heterogeneous nature of the disease, methods of measuring metabolic control, and studies of hypercoagulation and fibrinolysis in diabetes.

Fibrinolysis

Human blood contains at least one enzymatic system capable of removing fibrin deposits from the vascular bed. Mechanisms for the enzymatic degradation of fibrin remain the subject of controversy, but it is generally accepted that the plasminogen-plasmin system is the dominant pathway for physiological fibrinolysis (Brozovic, 1977; Collen, 1980; Hedner & Nilsson, 1981). The plasminogen-plasmin system has been well defined and a number of laboratory techniques are available for the quantitative study of the activity and components of this system. Providing that assumptions regarding the importance of plasmin-mediated fibrinolysis are valid, these measures can be used to estimate levels of
fibrinolytic activity.

It has been suggested that an equilibrium normally exists between coagulation and fibrinolysis which provides defense against vascular injury, with the subsequent removal of fibrin which has served its purpose. A shift in this hemostatic balance toward excess fibrin deposition could be an etiological factor in thromboembolic disease (Astrup, 1956). Research in the past three decades has firmly established the fibrinolytic system as an essential factor in normal hemostasis (Kernoff & McNicol, 1977; McNicol & Douglas, 1976). Investigators have studied components of the fibrinolytic system in numerous vascular disorders and have established significant relationships between impaired fibrinolysis and occlusive vascular disease.

Mechanisms and Components of the Fibrinolytic Enzyme System

The classical theory of fibrinolysis involves the basic mechanisms and components introduced by Christensen and Macleod in 1945. This system consists of a number of elements that either promote or inhibit the conversion of a circulating proenzyme, plasminogen, to the active protease, plasmin, which in turn digests fibrin. A simplified scheme of the plasminogen-plasmin system is presented in the Figure.

Plasminogen is present in human plasma in a concentration of 10-15 mg/100 ml (Heimburger, 1971). Plasmin-
Figure. Schematic representation of the plasminogen-plasmin system
ogen levels may be assayed immunologically (Ganrot & Nilehn, 1968), or functionally by lysis of fibrin (Berg, Korsan-Bengston, & Ygge, 1966). Although low levels of plasminogen could potentially be considered a mechanism for reduced fibrinolytic activity, there is relatively little documentation of patients with hypofibrinolysis due to this abnormality. Acute thrombolytic therapy may be associated with drastically reduced plasminogen levels, but synthesis is rapid and levels have been shown to return to normal from zero within 24 hours of stopping therapy (Davidson & Walker, 1979). Levels of plasminogen may also be reduced in hepatic cirrhosis, but fibrinolytic activity is not similarly reduced because of increased availability of activators of plasminogen (Fletcher, Biederman, & Moore, 1964). Deficiencies of plasminogen seem to be rare and are not particularly associated with thromboembolism.

Providing that plasminogen is available in normal concentrations, the critical step in the plasminogen-plasmin system is at the level of plasminogen activation. Activation may occur by three different pathways: an intrinsic or humoral pathway in which all components are found in circulating blood, an extrinsic pathway in which activators are released into the blood from tissues or from the vessel wall, or an exogenous pathway in which
chemical substances are administered for therapeutic purposes.

The intrinsic pathway involves indirect formation of plasminogen activators (PA) by systems consisting of factor XII (Hageman factor), high molecular weight kininogen (Fitzgerald factor), prekallikrein (Fletcher factor), and possibly other components (Revak & Cochrane, 1976). Activation of the fibrinolytic system by this pathway requires the presence of a surface (Ogston & Bennett, 1978). The importance of intrinsic activation in physiological fibrinolysis has not been firmly established and the mechanisms involved are not without controversy (Ratnoff, 1977).

Plasminogen activators are known to be widely distributed throughout the body (Astrup, 1966). These activators are usually named according to the source from which they are isolated: tissue activator from tissue (lung, heart, spleen, intestine, uterus) homogenates, vascular activator released from vessel walls, and urokinase which is present in urine (Rijken, Wijngaards, & Welbergen, 1980, 1981). A small amount of PA can be detected in human blood drawn in the resting state without venous occlusion, and despite avoidance of surfaces that activate factor XII (Fearnley, 1964). The level of PA found at rest is thought to be released by the vascular endothelium and is responsible for sponta-
neous fibrinolytic activity (Nilsson & Pandolfi, 1970; Todd, 1959). This concept is supported by demonstrations of synthesis and release of PA by endothelial cells in culture (Astedt & Pandolfi, 1972; Shepro, Schleef, & Hechtman, 1980).

The use of exogenous activators to enhance fibrinolysis in acute and chronic thrombotic disease has appealed to many investigators. Streptokinase and urokinase are the two substances most widely used in clinical practice, but must be injected and are only useful in major acute thrombotic situations. Davidson and Walker (1979) and Kwaan (1979) have reviewed various aspects of the available synthetic fibrinolytic agents.

Plasmin is produced from plasminogen by the cleavage of a single Arg-Val bond (Robbins, Summaria, Hsieh, & Shah, 1967). The kinetics of this reaction have been recently reviewed by Robbins, Wohl, and Summaria (1981). Plasmin is a rather nonspecific protease capable of hydrolyzing fibrinogen, prothrombin, and factors V and VIII in addition to fibrin. Plasmin is not normally detectable in human plasma, probably because of its association with circulating inhibitors (Ambrus & Markus, 1960).

A complex system of fibrinolytic inhibitors is believed to play a major role in the regulation and control of fibrinolysis. Antiplasmins, such as alpha₂-
plasmin inhibitor and alpha$_2$-macroglobulin, are known to react with plasmin to form enzymatically inactive complexes (Harpel, 1976; Mullertz & Clemmenson, 1976). Methods have been presented for the quantitative determination of these antiplasmins (Harpel, 1981). The presence in plasma of antiactivators of plasminogen has been described by several investigators (Crutchley, Conanan, & Maynard, 1981; Gallimore, 1975; Hedner, 1973), but a hypothesis of specific inhibitors of activation has not been clearly established (Collen, 1979). Antiplasmins, and possibly antiactivators, prevent widespread proteolysis and direct the action of plasmin specifically to deposits of fibrin.

Although it is generally held that physiological fibrinolysis is primarily mediated by the plasminogen-plasmin system, it should be recognized that other mechanisms independent of plasmin have been postulated (Moroz & Gilmore, 1976). Lysis of fibrin by alternate proteolytic enzymes such as those in the complement system (Goodkofsky & Lepow, 1971) and in the granules of leukocytes (Plow & Edgington, 1975), has been described, but the importance of non-plasmin-mediated fibrinolysis has not been determined.

**Measurement of Plasminogen Activator (PA)**

The estimation of fibrinolytic activity is primarily based on measurement of PA. As fibrinolysis is the net
effect of PA and inhibitors, functional assays for the determi-
nation of PA should attempt to eliminate the effect of inhibi-
tors. The most widely used procedures for the measurement of PA are: the diluted clot lysis
time, the euglobulin lysis time (ELT), and the fibrin plate method.

Methods of the whole blood clot lysis time are sometimes used as a screening test for hyperfibrinolysis
(Conrad, 1976), but the presence of inhibitors makes this assay far too insensitive and nonspecific to detect impaired fibrinolytic activity. Fearnley, Balmforth, and Fearnley (1957) have described a method in which fibrino-
lytic inhibitor effects are reduced by dilution. Whole
blood is diluted in buffer at pH 7.4, clotted with throm-
bin, and incubated at 37 °C. The diluted clot lysis time is shorter than that of undiluted blood, and the assay is considered to be more specific for measurement of PA.

Greater sensitivity is gained by precipitating the euglobulin fraction of plasma, which contains plasminogen,
plasmin, fibrinogen, and PA (Buckell, 1958). Most fibrinolytic inhibitors remain in the supernatant and are eliminated. The euglobulin fraction is dissolved in buffer, coagulated with calcium, and the time for clot lysis is recorded. The ELT is the most commonly used assay for PA and is reasonably sensitive and rapid (Nordby, Arnsen, Anderson, & Godal, 1980). This method
does, however, vary with the amount of fibrinogen and plasminogen in the plasma sample.

Levels of fibrinogen and plasminogen may be standardized by using a prepared plate of plasminogen-rich fibrin as the substrate (Astrup & Mullertz, 1952). After placing the euglobulin fraction of the sample on the fibrin plate, fibrinolytic activity is read as the circular area of lysis which occurs after incubation. The fibrin plate method is the most precise of the available functional assays, but it is technically more complex and time consuming than the ELT.

A histochemical film technique has been described which allows for a direct measure of the vessel PA reserve (Pandolfi, 1972). In this method, a biopsy specimen is taken, usually from the superficial veins of the hand, and the PA content of the vessel wall is quantitatively determined.

The capacity of an individual to release PA from the vascular endothelium may be assessed by measuring the response to various stimuli such as injection of nicotinic acid (Robertson, 1971), infusion of vasopressin (Aberg & Nilsson, 1975), exercise (Cash, 1968, Cash & Woodfield, 1967), or venous occlusion (Clarke, Orandi, & Cliffton, 1960; Holemans, 1963; Pandolfi, 1972). Some people respond weakly or not at all to such stimuli. These individuals are referred to as poor responders and
may be at greater risk for thromboembolic disease (Cash, 1975).

The measurement of fibrinolytic capacity is believed to be a more appropriate technique for studying the fibrinolytic enzyme system than simple determinations of spontaneous fibrinolysis because of the dynamic state of the system and because resting levels of PA must be extreme and prolonged to detect subject differences (Cash, 1968). Of the available PA release stimuli, venous occlusion would appear to be most suitable for routine clinical use because it offers highly reproducible stress and low risk to subjects (Robertson, Pandolfi, & Nilsson, 1972). In addition, the ELT has been shown to be as reproducible and probably even more sensitive than the fibrin plate method after the use of venous occlusion (Nordby et al., 1980).

**Physiological Fibrinolytic Activity**

The view that the fibrinolytic system is continuously active is supported by the finding of PA activity in normal blood (Mullertz, 1953; Sawyer, Fletcher, Alkjaersig, & Sherry, 1960). Levels of spontaneous PA are known to fluctuate in any one person from day to day and throughout the same day (Fearnley, Balmforth, & Fearnley, 1957; Rosing, Brakman, Redwood, Goldstein, Beiser, Astrup, & Epstein, 1973). Levels of lowest activity are found at 8:00 a.m., with peak activity between 5:00 and 8:00 p.m.
(Rosing, Brakman, Redwood, Goldstein, Beiser, Astrup, & Epstein, 1970). The diurnal variation may be substantially reduced in older individuals (Rosing et al., 1973).

Release of vascular PA can be dramatically increased in response to several naturally occurring events such as exercise (Biggs, MacFarlane, & Pilling, 1947; Ogston & Fullerton, 1961; Sherry, Lindemeyer, Fletcher, & Alkjaersig, 1959) and mental stress and anxiety (Cash & Allen, 1967; MacFarlane & Biggs, 1946). The mechanism controlling the enhanced release of PA from the endothelial cells has been recently reviewed (Cash, 1978). The two main hypotheses are: the action of adrenoceptor stimulation (Gader, Da Costa, & Cash, 1974) and a change in vascular motility (Nilsson & Pandolfi, 1970). These theories have been challenged by findings that the rise in PA can be only partially prevented by adrenergic beta-blockade (Cash, Woodfield, & Allen, 1970) and that several potent vasoactive drugs have no effect on PA release (Weiner, de Crines, Redisch, & Steele, 1959). On the basis of this and other evidence, Cash (1978) has suggested that PA release may be under neurohormonal control and that endothelial cells may be sensitive to a yet unidentified plasminogen activator releasing hormone.

The distribution of vascular activator throughout the body is reviewed by Pandolfi (1972). Vessel walls of
small arteries and veins, some large veins, such as the renal vein and vena cava, and the lymphatics are found to contain PA. Significant venous-arterial differences suggest that fibrinolytic activity is considerably higher in the microcirculation than in the systemic circulation (Holemans, Mann, & Cope, 1967). The activity of the microcirculation probably plays an important physiological role in maintaining the patency of the capillary bed.

Circulating levels of PA should not be interpreted as direct evidence of ongoing fibrinolytic activity. Physiological mechanisms must be operating to prevent widespread proteolysis. Three theories have been proposed for the limitation of plasmin activity specifically to fibrin: plasminogen may be preferentially adsorbed to fibrin where the effect of inhibitors is weak (Alkjaersig, Fletcher, & Sherry, 1959), plasmin may have a greater affinity for fibrin than for antiplasmins (Ambrus & Markus, 1960), or PA may be selectively bound to fibrin to contact circulating plasminogen away from inhibitors (Chesterman, Allington, & Sharp, 1972). These mechanisms, independently or in combination, could be responsible for the localized digestion of fibrin deposits by plasmin in the absence of circulating inhibitors. If deposits of fibrin are not in the vascular tree, no fibrinolysis will occur despite significant release of PA.
Impaired Fibrinolytic Activity

More than a century ago, von Rokitansky (1854) proposed that deposits of fibrin were the initiating lesions of arteriosclerosis. This concept was reviewed by Duguid in 1946 when he observed that many of the lesions which were classified as atherosclerotic were actually the result of excess arterial fibrin deposition. Astrup (1956) expanded this idea by suggesting the existence of a dynamic equilibrium between coagulation and fibrinolysis.

Investigators have studied components of the fibrinolytic system to determine if impaired fibrinolytic activity might be an etiological factor in occlusive vascular disease. The largest body of evidence concerns relationships between low fibrinolysis (as judged by low spontaneous PA levels, reduced capacity for release of PA, or low vessel wall PA content) and the presence of venous and arterial thromboembolic complications. Many studies have demonstrated impaired fibrinolytic activity in patients with venous thrombosis (Clayton, Anderson, & McNicol, 1976; Gordon-Smith, Hickman, & Le Quesne, 1974; Isacson & Nilsson, 1972; Mansfield, 1972). Reduced fibrinolysis is also associated with occlusive arterial disease (Mettinger, Nyman, Kjellin, Siden, & Soderstrom, 1979; Nestel, 1959).

There is available evidence in support of the

Several investigations have produced evidence which is more indicative of a causal relationship between impaired fibrinolysis and vascular disease than the associational studies mentioned above. Pharmacological enhancement of fibrinolysis has been shown to improve the clinical condition of patients which an increased tendency toward thromboembolic disorders (Bielawiec, Mysliwiec, & Perzanowski, 1978; Cunliffe, Roberts, & Dodman, 1973; Hedner, Nilsson, & Isacson, 1976; Nilsson, Nilsson, & Hedner, 1981). Experimental studies have shown that vascular complications can be produced by
decreasing fibrinolytic activity with diet in the rat (Naimi, Goldstein, Nothman, Wilgram, & Proger, 1962) and by pharmacologically increasing levels of inhibitors in the rabbit (Kwaan & Astrup, 1964).

Summary

Components and mechanisms for the enzymatic removal of fibrin have been reviewed. Although several mechanisms have been identified, it is generally accepted that the extrinsic pathway of the plasminogen-plasmin system is the dominant mechanism in physiological fibrinolysis. Synthesis and release of PA by the vascular endothelium appears to be the critical step in this pathway. It was concluded that fibrinolytic activity could best be estimated by the measure of PA levels.

Available techniques for PA determination were presented. Fibrinolysis can be assessed by measures of spontaneous fibrinolysis, fibrinolytic capacity, or vessel wall PA content. The ELT assay of the PA response to venous occlusion is rapid, simple, reasonably sensitive, and appears to be particularly well suited for clinical investigations of fibrinolytic capacity.

Various aspects of physiological and impaired fibrinolytic activity were discussed. Physiological fibrinolysis demonstrates diurnal variation and may be affected by exercise and emotional stress. It has been postulated that a balance must be maintained between
coagulation and fibrinolysis to avoid pathological changes associated with excess fibrin deposition. The importance of normal fibrinolytic activity has been inferred from relationships between impaired fibrinolysis and thrombotic or atherosclerotic disease.

**Diabetes Mellitus**

Vascular disease plays a major role in the morbidity and mortality of diabetes mellitus. The classification of diabetes mellitus indicates that blood glucose concentrations are above acceptable standards, but heterogeneous metabolic and biochemical abnormalities may be present in a population identified solely on the basis of hyperglycemia. For decades, researchers have argued the importance of strict blood glucose control in the delay or prevention of diabetic vascular complications, but until the recent availability of glycosylated hemoglobin, this controversy has been plagued by the lack of a reliable index of metabolic control (Raskin, 1978).

Disturbances in the systems regulating hemostatic balance are among the possible etiological factors in the development of diabetic angiopathy. A number of investigations support the hypothesis that diabetic patients face an increased tendency for hypercoagulation (Jones & Peterson, 1981a, 1981b). Studies of fibrinolytic activity in diabetes mellitus have yielded conflicting reports. Preliminary evidence indicates that vascular complications
may be reduced by improved metabolic control. It has also been suggested that variation in diabetic fibrinolysis may be explained by differences in control of blood glucose (Gunnarson, Nyman, Walinder, & Ostman, 1980).

**Heterogeneity of the Syndrome**

Diabetes mellitus affects more than 10,000,000 Americans and is the third ranking cause of death in this country (Scope and Impact of Diabetes, 1976). Recent research has clearly indicated that diabetes is not one disease, but a heterogeneous group of syndromes with a variety of genetic and environmental causes (Fajans, Cloutier, & Crowther, 1978; Nerup & Lernmark, 1981; Rotter & Rimoin, 1981). The diagnosis of diabetes mellitus implies the expression of hyperglycemia, but reveals nothing about the metabolic and biochemical abnormalities that may be present.

The heterogeneous nature of diabetes mellitus has led to confusion and inconsistencies in the reporting of diabetes research. The National Diabetes Data Group (1979) has done much to solve this problem by reaching an international consensus regarding the clinical and diagnostic classification of glucose intolerance disorders. This workgroup has made specific recommendations for the collection of clinical and research data.

Historically, diabetes has been thought to be a
disease of insulin deficiency. The two major subgroups of clinical diabetes mellitus have been classified by the National Diabetes Data Group (1979) primarily on their dependence on exogenous insulin to sustain life. Insulin-dependent diabetes mellitus (IDDM, type I, formerly juvenile-onset) is characterized by an absolute deficiency of insulin with an essential dependence on injected insulin and a proneness to ketosis. Non-insulin-dependent diabetes mellitus (NIDDM, type II, formerly maturity-onset) is characterized by significant insulin production, but in quantities insufficient to maintain normal glucose homeostasis. Research has shown that IDDM and NIDDM are different pathological entities, rather than simple gradations of insulin deficiency.

Endogenous insulin is negligible in the type I diabetic. Insulin secretion, as determined by C-peptide measurements, is generally not detectable in individuals with IDDM who have received insulin therapy for more than five years (Block, Mako, Steiner, & Rubenstein, 1972). Because insulin is the primary factor which controls the storage and metabolism of ingested nutrients, an absolute deficiency of insulin results in inadequate disposal of foodstuffs (glucose intolerance) and excessive mobilization or production of endogenous fuels (fasting hyperglycemia, hyperaminoacidemia, and elevated free fatty acids). In its most severe form, there is a marked
acceleration of all catabolic processes, which produces diabetic ketoacidosis (DKA).

Attention to diet and exercise, with the systematic injection of insulin, are the standard therapeutic measures taken to normalize blood glucose levels in IDDM (Treatment of Diabetes, 1976). In recent years, several innovative programs of insulin administration have reported close approximation of physiological homeostasis (Skyler, Seigler, & Reeves, 1982; Skyler, Skyler, Seigler, & O'Sullivan, 1981; Tattersall & Gale, 1981), but true euglycemia has only been achieved with special research instruments capable of infusing insulin on the basis of feedback from glucose sensors (Botz, Marliss, & Albisser, 1979; Service, Rizza, Westland, Hall, Nelson, Haymond, Clemens, & Gerich, 1980). Conventional therapy consists of single or multiple injections of insulin on a daily basis. The matching of injected insulin to metabolic need is a mere approximation, even with the newer modes of therapy, and intermittent hyperglycemia is relatively unavoidable (Peterson, 1982).

NIDDM accounts for more than 80% of the cases of diabetes in the United States, and the large majority of these patients are obese at the time of their diagnosis (Bennett, 1981). Obesity is thought to contribute to a state of insulin resistance often seen in the type II diabetic (Kolterman, Reaven, & Olefsky, 1979; Olefsky,
Basal insulin levels are typically normal or even increased in NIDDM, but glucose stimulated insulin secretion is generally diminished (Olefsky & Reaven, 1977). Glucose homeostasis cannot be maintained with the insulin resistance of NIDDM, but there are generally less marked fluctuations in blood glucose levels than are seen in IDDM, and DKA is very rare.

Therapy for the obese diabetic usually involves a diet restricted in calories and an increase in physical activity (Treatment of Diabetes, 1976). These measures have been shown to improve insulin sensitivity (Davidson, 1976; Soman, Koivisto, Diebert, Felig, & DeFronzo, 1979). If diet and exercise are not effective in maintaining metabolic control in NIDDM, it may be necessary to prescribe oral hypoglycemic drugs or insulin.

**Glycosylated Hemoglobin and Metabolic Control**

It has been argued for years that insulin deficiency and its metabolic consequences are the fundamental etiological factors in the pathogenesis of diabetic complications (Raskin, 1978). The major problem in attempting to relate hyperglycemia to the development of long-term complications has been the availability of a valid and reliable method of assessing chronic metabolic control. Glucose values are found to undergo wide fluctuations from hour to hour and from day to day in
diabetes (Molnar, Taylor, & Ho, 1972). Little or no correlation may exist between blood glucose concentrations obtained during a clinic visit and values obtained throughout a diabetic's normal day (Rifkin, 1978; Tchobroutsky, 1978). Monitoring of glycosuria, fasting blood glucose, and glucose tolerance tests cannot be used as valid estimates of long-term control, and serial measures of blood glucose concentrations have been plagued by problems of sampling frequency (Molnar, Marien, Hunter, & Harley, 1979).

Glycosylated hemoglobin is a variant of normal adult hemoglobin (HbA1). It has been shown to be elevated with chronic hyperglycemia and correlates closely with the average blood glucose concentration over several weeks (Gonen, Rubenstein, Rochman, Tanega, & Horowitz, 1977; Paisey, MacFarlane, Sherriff, Hartog, Slade, & White, 1980). Total HbA1 is composed of several minor variants (HbAa1, HbAa2, HbAb, and HbAc) which are formed slowly and continuously throughout the life span of the red cell (Bunn, Haney, Kamin, Gabbay, & Gallop, 1976). Thus, levels of HbA1 should provide a measure of the mean blood glucose level over the preceding two to three months (Gabbay et al., 1977).

Allen, Schroeder, and Balog (1958) first identified the minor components of normal adult hemoglobin as the fast-moving fraction in a chromatographic system using a
cation-exchange resin. Total HbAl was found to comprise about 7% of total hemoglobin, with HbAlc, the major component, representing 5-6% of the total. Hemoglobin Alc is formed by the attachment of glucose to the N-terminal valine of the beta chain (Bookchin & Gallop, 1968) and the chemical steps involved in this synthesis have been well defined (Higgins & Bunn, 1981).

A variety of techniques are available for the determination of HbAl (Bunn, 1981). The original technique of isolation by column chromatography is recognized as the standard and is most widely used (Allen et al., 1958). Metabolic control of diabetes may be reported as HbAlc, or as total HbAl. While HbAlc can be elevated as much as three fold in diabetic red cells (Huisman & Dozy, 1962; Paulsen, 1973), the concentrations of the remaining components appear to be normal in diabetes (McDonald, Shapiro, Bleichman, Solway, & Bunn, 1978). Thus, elevations in HbAl are thought to reflect changes in HbAlc.

Recently, a labile form of glycosylated hemoglobin has been identified which is chromatographically indistinguishable from HbAlc (Goldstein, Peth, England, Hess, & Da Costa, 1980). If this labile fraction is not removed, the concentration of glycosylated hemoglobin may change significantly over a period of hours. To use HbAl as an accurate index of long-term glucose control, it is necessary to remove the labile material by incubation of
erythrocytes in saline at 37°C prior to column chromatography (Compagnucci, Cartechini, Bolli, De Feo, Santeusanio, & Brunetti, 1981; Goldstein, Parker, England, Wiedmeyer, Rawlings, Hess, Little, Simonds, & Breyfogle, 1982).

Hypercoagulation in Diabetes Mellitus

Available therapeutic measures have not prevented the development of chronic diabetic complications affecting the eyes, nerves, kidneys, and arteries. Vascular pathology is the central focus for many of these complications and is generally divided into two major categories; microangiopathy, which is an alteration of the small blood vessels; and macroangiopathy, which is an involvement of the large vessels. Extensive reviews are available for the clinical manifestations of diabetic microvascular (McMillan, 1975) and macrovascular (Ganda, 1980) disease. The role of hyperglycemia in the biochemical alterations is presented by Brownlee and Cerami (1981).

Although it should be recognized that diabetic angiopathy is probably multifactorial in origin, increasing attention is being paid to abnormal hematological alterations in the pathogenesis of micro- and macrovascular disease (Jones & Peterson, 1981a, 1981b). Histological studies show that vascular lesions in diabetes (Davies, Woolf, & Carstairs, 1966; Farquhar, MacDonald, &
Ireland, 1972) and atherosclerosis (Haust, Wyllie, & Moore, 1965) contain large deposits of fibrin. Findings of increased platelet adhesion (Fuller, Keen, Jarrett, Omer, Meade, Chakrabarti, North, & Stirling, 1979; Sharma, 1981), increased coagulation factors (Gensini, Abbate, Favilla, & Serneri, 1979; Fuller et al., 1979; Mayne, Bridges, & Weaver, 1970), and elevated fibrinogen levels (Coller et al., 1978; Wardle, Piercy, & Anderson, 1973) suggest the possibility of a hypercoagulable state in diabetes. This theory is further supported by reports of increased fibrinogen turnover (Bannerjee, Sahui, & Kumar, 1973; Ferguson, Mackay, Phillip, & Sumner, 1975).

Recent investigations indicate that hypercoagulation is related to levels of HbA1 (Coller et al., 1978; Sowers et al., 1980). Clinical studies demonstrate that platelet function (Peterson, Jones, Koenig, Melvin, & Lehrman, 1977) and fibrinogen turnover (Jones & Peterson, 1979) can be normalized by reduction in HbA1. These findings suggest that increases in blood glucose concentrations are associated with states of hypercoagulability and thus, increased fibrin deposition.

**Fibrinolysis in Diabetes Mellitus**

Unneeded deposits of fibrin are normally removed from the vascular tree by activity of the fibrinolytic enzyme system. A hypercoagulable state should, therefore, be compensated for by enhanced fibrinolytic activity.
Although investigations consistently demonstrate increased coagulation in diabetes mellitus, there is no such agreement concerning fibrinolysis. Findings have been plagued by inconsistent methodologies and inadequate classification of diabetic subjects.

Single sample analysis techniques of spontaneous PA levels have been criticized for their inability to identify states of impaired fibrinolysis because of the normal variation in fibrinolytic activity found both within and between subjects (Cash, 1968). Investigations of spontaneous fibrinolysis in diabetes are so controversial that it would be difficult to base conclusions on the available reports. Increased (Bern, Cassani, Horton, Rand, & Davis, 1980; Cash & McGill, 1969), normal (Denborough & Paterson, 1962; MacKay & Hume, 1964), and decreased (Fearnley, Chakrabarti, & Avis, 1963; Badawi, El-Sawy, Mikhail, Nomeir, & Tewfik, 1970) spontaneous fibrinolysis is reported in the literature.

Several investigators have examined differences in spontaneous fibrinolysis within subgroups of diabetic patients. Sharma (1981) found activity to be significantly increased in type I diabetics and decreased in type II diabetics when compared to controls. Almer and Nilsson (1975) reported no differences in type I and type II diabetics, but there was a correlation between impaired fibrinolysis and obesity. Almer, Pandolfi, and Nilsson
(1975) found the spontaneous fibrinolytic activity to be decreased in diabetics with retinopathy compared to those who had not developed retinopathy. Bern et al. (1980) found essentially no differences between nonobese groups of type I diabetics without retinopathy, type I diabetics with retinopathy, and type II diabetics with atherosclerosis, although all values were higher than in controls.

Studies of PA release capacity and vessel PA content have provided more consistent results. The diabetic response to exercise (Cash & McGill, 1969; Seth, 1973) and venous occlusion (Almer & Nilsson, 1975) is depressed and the PA content of the vessel wall is lower in diabetics than in controls (Almer et al., 1975). No significant difference was found, however, in the fibrinolytic response to adrenaline in diabetics and nondiabetics (Tanser, 1967). The inability to detect differences in this investigation may have resulted from the use of the less sensitive diluted clot lysis assay.

Almer et al. (1975) found differences in fibrinolytic capacity to be related to the presence of retinopathy, and Almer and Nilsson (1975) found a significant correlation between depressed capacity for fibrinolysis and obesity. Diabetic subjects were not examined on the basis of insulin dependency. Cash and McGill (1969) and Seth (1973) examined "young" diabetics (19-30 yr) and newly diagnosed maturity-onset diabetics (26-55 yr)
respectively, and both studies produced similar data. No attempt was made to explain variations found within the diabetic groups.

Gunnarson et al. (1980) established a relationship between HbA1 and fibrinolytic capacity, but a very heterogeneous group of diabetic subjects, and two healthy controls, were used in this investigation. Diabetics ranged in age from 18 to 75 yrs and therapy consisted of diet only, diet and oral hypoglycemics, or insulin. It is possible that variation in fibrinolytic capacity could have been explained by a variety of factors such as age, obesity, etc., but no systematic attempt to examine these variables was reported.

Summary

The heterogeneous nature of diabetes mellitus has been reviewed. Two main subclasses, IDDM and NIDDM, have been identified by the National Diabetes Data Group (1979). Patients with type I and type II diabetes differ primarily in production of endogenous insulin. Type II diabetes is closely associated with obesity, and a study which contains subjects with NIDDM may be affected by a variety of factors commonly found in normal obese populations, in addition to chronic hyperglycemia.

The metabolic basis of diabetic vascular disease has been difficult to investigate because of a lack of a reliable index of longterm blood glucose control. The
recent availability of HbA1 assays now provides a means to establish relationships between metabolic control and various hematological abnormalities and complications of diabetes.

Studies of the hemostatic mechanisms in diabetes have been reviewed. Evidence favors the hypothesis of a hypercoagulable state which is associated with chronic hyperglycemia. Studies of the fibrinolytic system in diabetes are very controversial. Conflicting reports have come from investigations which have looked at spontaneous fibrinolysis. Studies which have used the ELT, the fibrin plate method, or determination of vessel wall PA content, agree that the capacity for release of PA from the endothelium is reduced in diabetes mellitus, but levels of activity are found to vary among diabetic patients. Obesity and diabetic vascular disease may account for some of the differences in fibrinolytic capacity. A preliminary investigation suggests that different levels of metabolic control may also be a source of fibrinolytic variation, but further study is needed.
CHAPTER III

METHODS

Subjects

Twenty-six (nine males, 17 females) subjects with IDDM, and 20 (nine males, 11 females) healthy controls were examined. Diabetic subjects were selected from cases seen in the Diabetes Management Clinic at Earl K. Long Memorial Hospital, Baton Rouge, Louisiana. Documentation of IDDM was based on criteria developed by the National Diabetes Data Group (1979). The subjects were informed of the nature of the study before commencement of testing and were asked to sign a consent form in accordance with the Human Use Committee at Louisiana State University (Appendix B). Clinical and anthropometric data for subjects by group are presented in the Results.

Procedures

All subjects were tested between the hours of 7:00 and 9:00 a.m., following an overnight fast. Diabetic subjects were instructed to follow their usual schedule for insulin injection. No subject had smoked cigarettes or engaged in vigorous exercise the morning of the investigation.

Samples of venous blood were collected before and after venous occlusion and were subsequently assayed for ELT, HbAl, serum glucose (GLU), cholesterol (CHO), and
Diabetic subjects were tested at Earl K. Long Memorial Hospital under the supervision of Glen Johnson, M.D., with technical assistance by Phyllis Sauchier, R.N.

**Blood Collection**

Approximately 8 ml of blood were withdrawn by direct venepuncture into chilled 10 ml plastic syringes for pre- and post-occlusion samples. The pre-occlusion sample was taken with a minimum of stasis (pressure below diastolic for less than 30 sec) from an antecubital vein in one arm. A sphygmanometer cuff was placed around the contralateral arm and blood pressure was determined. The cuff was then inflated to a pressure midway between systolic and diastolic blood pressure and was maintained for 10 min. At the end of minute 10, the post-occlusion sample was collected from an antecubital vein distal to the cuff, and the cuff was deflated.

**Euglobulin Lysis Time (ELT)**

Immediately following blood collection, 2.25 ml of the sample were transferred to a 15 ml polypropylene centrifuge tube containing 0.25 ml sodium oxalate, 1.34%, that had been previously chilled to 5°C. The tubes were then placed in melting ice to await centrifugation. Plasma was separated by centrifugation at 3,000 x g for 15 min within 30 min of collection.

Two 15 ml polypropylene centrifuge tubes per sample
were prepared the day before testing for subsequent
dilution and acidification of plasma. The tubes,
containing 9 ml distilled water and 0.1 ml 1% acetic
acid, had been chilled overnight in a refrigerator at
5°C.

Following centrifugation, 0.5 ml of plasma were
pipetted into the prepared tubes. The tubes were covered
with Parafilm and placed in a refrigerator at 5°C for 30
min to allow precipitation of the euglobulin fraction.
The tubes were then centrifuged for 5 min at 3,000 x g.
The supernatant was decanted by inverting the tubes and
draining on filter paper.

The precipitate was resuspended in 0.5 ml borate
solution (0.9 g sodium chloride + .1 g sodium borate made
to 100 ml with distilled water) and stirred gently with a
glass rod. The tubes were then placed in a water bath at
37°C for 5 min. Calcium chloride (0.025M), 0.5 ml, was
then added and the time was recorded.

The tubes were inspected at regular intervals until
complete clot lysis had occurred. The ELT was recorded
as the difference in minutes from addition of CaCl₂ to
complete lysis. The mean for the duplicate
determinations was calculated. These procedures, with
minor modifications, were described by Buckell (1958).

The ELTs were used to compute FC using the formula:

\[ \frac{(A - B)}{A} \times 100 = FC \]

where A and B respectively represent pre- and post-
occlusion ELT's.

Glycosylated Hemoglobin (HbAl)

For analysis of HbAl, 2.5 ml of the pre-occlusion sample were emptied into a chilled tube of 3% EDTA and stored at 5°C. Samples were assayed in duplicate within two days of collection. A Glycosylated (Fast Fraction) Hemoglobin Quick Column Kit (Helena, cat. no. 5340) was used to measure HbAl, and the procedures of the Total Fraction Method were followed. For quality control purposes, Lyophilized G-Hb Quick Column Control (Normal) (Helena, cat. no. 5347) and Lyophilized G-Hb Quick Column Control (Abnormal) (Helena, cat. no. 5348) were assayed with each determination.

Prior to performing column chromatography, the following procedures were utilized to remove the labile fraction of HbAl. The red blood cells were washed three times with saline (0.85%), and the saline was decanted. The washed packed cells were then incubated at 37°C for four hours. Samples were then prepared by placing 310 μL of Hemolysate Reagent-C into a small test tube containing 10 μL of packed red blood cells. Sample preparations were shaken to produce complete hemolysis and were allowed to stand for 5 min. All columns and reagents were at room temperature and this temperature was recorded.

Glycosylated hemoglobin was determined by placing
100µL of sample preparation in a Helena Quick Column and an additional 100 µL of the same preparation in a large collection tube. A small collection tube was placed under the Quick Column. Following absorption of the sample into the resin bed of the column, 15 ml of Fast Fraction Developer were added to elute the HbA1. The eluted fraction was referred to as the fast fraction (FF). The FF was made to 3 ml in the small collection tube with purified water. The 100 µL sample in the large collection tube was made to 15 ml with purified water and was referred to as the total fraction (TF).

A spectrophotometer set at 415 nm was used to determine the optical density (OD) of both the FF and TF. The following formula was used to calculate the percentage of HbA1 for each sample:

\[
\text{OD of FF} \times 100 = \text{HbA1%}
\]

Values obtained were adjusted with the Helena Temperature/G-Hb Conversion Chart if room temperature was not 22°C at the time of HbA1 determination. The mean for duplicate determinations was calculated.

Serum Glucose (GLU), Cholesterol (CHO), and Triglyceride (TRI)

Approximately 3 ml of the pre-occlusion sample were emptied into a chilled glass tube, placed on ice, and allowed to clot. Serum was removed by centrifugation for 10 min at 3,000 x g. Determinations of GLU, CHO, and TRI
were performed on a Coulter Chemistry Analyzer using standard Earl K. Long Clinical Chemistry Laboratory procedures.

**Statistical Analysis**

Three major approaches for data analysis were utilized. First, *t*-tests were made between the diabetic and control groups for FC, HbAl, CHO, TRI, GLU, and other variables. Secondly, simple bivariate correlations between variables were calculated for diabetic subjects only, for normal controls only, and for the entire subject cohort. Finally, multiple linear regression with a stepwise solution was utilized to examine the contribution of HbAl, CHO, TRI, and GLU as explanatory variables for FC. Each independent variable was forced into the analysis during separate runs to examine their importance when entered first, and also with other variables already in the statistical model. A calculated probability of .05 or less was taken as the index of statistical significance.
CHAPTER IV
RESULTS

Characteristics of Subjects and Group Differences

Clinical and anthropometric data for subjects by group are presented in Table 1. No significant differences were found to exist between diabetics and the normal controls for age ($t = 1.661, p = .104$), height ($t = 1.479, p = .148$), or weight ($t = 1.549, p = .130$), which supports the assumption that groups were appropriately matched for these variables. Raw data for diabetics and controls are included in Appendices C and D, respectively.

**TABLE 1**
Clinical and Anthropometric Data for Subjects by Group

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age</th>
<th>Height</th>
<th>Weight</th>
<th>Duration of Diabetes</th>
<th>Insulin Dose (U/24 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>26</td>
<td>25.3</td>
<td>165.7</td>
<td>59.9</td>
<td>9.4</td>
<td>48.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±6.8</td>
<td>±8.0</td>
<td>±11.8</td>
<td>±4.3</td>
<td>±21.9</td>
</tr>
<tr>
<td>Non-Diabetic</td>
<td>20</td>
<td>28.4</td>
<td>169.7</td>
<td>65.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td>±5.4</td>
<td>±9.5</td>
<td>±14.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note.** Values are $M \pm SD$.

Mean values for FC, HbA1, GLU, CHO, and TRI for diabetics and nondiabetics are given in Table 2. Significant differences were found between groups for FC
(t = 3.786, p = .0005), HbAl (t = 8.305, p = .0001), GLU (t = 5.710, p = .0001), and CHO (t = 2.325, p = .026). No significant differences were seen for TRI (t = 1.615, p = .116). Raw scores for diabetics and controls are included in Appendices E and F, respectively.

### TABLE 2

Fibrinolytic Capacity (FC), Glycosylated Hemoglobin (HbAl), Serum Glucose (GLU), Cholesterol (CHO), and Triglyceride (TRI) for Diabetics and Nondiabetics

<table>
<thead>
<tr>
<th>Group</th>
<th>FC (%)</th>
<th>HbAl (¢)</th>
<th>GLU (mg%)</th>
<th>CHO (mg%)</th>
<th>TRI (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>46.6</td>
<td>10.3</td>
<td>209</td>
<td>215</td>
<td>78</td>
</tr>
<tr>
<td>SD</td>
<td>±19.3</td>
<td>±2.1</td>
<td>±102</td>
<td>±56</td>
<td>±60</td>
</tr>
<tr>
<td>n</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>Nondiabetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>64.2</td>
<td>6.5</td>
<td>95</td>
<td>186</td>
<td>56</td>
</tr>
<tr>
<td>SD</td>
<td>±12.1</td>
<td>±.9</td>
<td>±7</td>
<td>±25</td>
<td>±24</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>19</td>
<td>19</td>
<td>20</td>
<td>19</td>
</tr>
</tbody>
</table>

**Bivariate Correlations**

Bivariate correlations between variables for diabetic subjects are presented in Table 3. A significant inverse correlation was found to exist between FC and TRI (r = -.45, p = .029), but FC and HbAl (r = -.08, p = .713)
were not statistically related. A positive relationship was found for HbAl and CHO ($r = .59$, $p = .002$), but correlations were not significant for HbAl and GLU ($r = .35$, $p = .084$) or HbAl and TRI ($r = .24$, $p = .249$). No significant relationship was found for FC and CHO ($r = .24$, $p = .883$).

**TABLE 3**

Bivariate Correlations for Diabetic Subjects

<table>
<thead>
<tr>
<th></th>
<th>FC</th>
<th>HbAl</th>
<th>GLU</th>
<th>CHO</th>
<th>TRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-.08</td>
<td>.32</td>
<td>.03</td>
<td>-.45</td>
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<td></td>
</tr>
<tr>
<td>.71</td>
<td>.11</td>
<td>.88</td>
<td>.03</td>
<td></td>
<td></td>
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<td>26</td>
<td>26</td>
<td>24</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbAl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.35</td>
<td>.59</td>
<td>.24</td>
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<tr>
<td>.08</td>
<td>.002</td>
<td>.25</td>
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<td>26</td>
<td>25</td>
<td>24</td>
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<td></td>
</tr>
<tr>
<td>GLU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.13</td>
<td>-.25</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>.54</td>
<td>.25</td>
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<td>25</td>
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<tr>
<td>CHO</td>
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<td>.39</td>
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<tr>
<td>23</td>
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<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Note.** Values are $r$, bivariate correlation; $p$, calculated probability; $n$, sample size.

For nondiabetic subjects (Table 4), FC was again inversely related to TRI ($r = -.51$, $p = .027$). Unlike
diabetic subjects, the normal controls showed a significant positive relationship between FC and HbAl ($r = .54$, $p = .017$).

**TABLE 4**

Bivariate Correlations for Nondiabetic Subjects

<table>
<thead>
<tr>
<th></th>
<th>FC</th>
<th>HbAl</th>
<th>GLU</th>
<th>CHO</th>
<th>TRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC</td>
<td>.54</td>
<td>.003</td>
<td>-.33</td>
<td>-.51</td>
<td></td>
</tr>
<tr>
<td>HbAl</td>
<td>.02</td>
<td>.99</td>
<td>.15</td>
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</tr>
<tr>
<td>GLU</td>
<td>19</td>
<td>19</td>
<td>20</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>CHO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. Values are $r$, bivariate correlations; $p$, calculated probability; $n$, sample size.

The combined sample (Table 5) demonstrated the same inverse relationship between FC and TRI ($r = -.49$, $p = .0008$) found for diabetics and nondiabetics alone. A significant inverse correlation was also found for FC and HbAl ($r = -.33$, $p = .026$) in the combined sample, a
finding that conflicts with data for diabetics only.

### TABLE 5

Bivariate Correlations for Diabetics and Nondiabetics Combined

<table>
<thead>
<tr>
<th></th>
<th>FC</th>
<th>HbAl</th>
<th>GLU</th>
<th>CHO</th>
<th>TRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC</td>
<td>-.33</td>
<td>-.09</td>
<td>-.18</td>
<td>-.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>.03</td>
<td>.57</td>
<td>.24</td>
<td>.0008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>43</td>
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</tr>
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<td>HbAl</td>
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<td>.54</td>
<td>.27</td>
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<td>.0001</td>
<td>.0002</td>
<td>.07</td>
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<td>44</td>
<td>44</td>
<td>43</td>
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<td>GLU</td>
<td>.28</td>
<td>-.05</td>
<td></td>
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<td></td>
<td>.07</td>
<td>.74</td>
<td></td>
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<td></td>
<td>44</td>
<td>42</td>
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<tr>
<td>CHO</td>
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<td></td>
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<td>.0008</td>
<td></td>
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<tr>
<td></td>
<td>42</td>
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</tr>
</tbody>
</table>

**Note.** Values are $r$, bivariate correlation; $p$, calculated probability; $n$, sample size.

### Regression Analysis

Regression analysis of the stepwise linear regression for diabetic data suggests that the most powerful predictor of FC in insulin-dependent diabetes is the level of serum triglyceride (Table 6). TRI was significant when entered first ($\Delta R^2 = .200$) into the model, and when entered after CHO ($\Delta R^2 = .226$). No other variable was significant when entered after TRI. HbAl
was not found to be significant when entered first ($\Delta R^2 = .021$).

**TABLE 6**

Summary of Stepwise Linear Regression Analysis for Diabetic Data With Fibrinolytic Capacity as the Dependent Variable

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable</th>
<th>$\Delta R^2$</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 1</td>
<td>TRI</td>
<td>.200</td>
<td>.03</td>
</tr>
<tr>
<td>2</td>
<td>HbAl</td>
<td>.001</td>
<td>.84</td>
</tr>
<tr>
<td>3</td>
<td>GLU</td>
<td>.000</td>
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<td>4</td>
<td>CHO</td>
<td>.041</td>
<td>.30</td>
</tr>
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<td>Run 2</td>
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<td></td>
<td></td>
</tr>
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<td>HbAl</td>
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<td>.46</td>
</tr>
<tr>
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<td>GLU</td>
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<td>.03</td>
</tr>
<tr>
<td>3</td>
<td>CHO</td>
<td>.011</td>
<td>.59</td>
</tr>
<tr>
<td>4</td>
<td>TRI</td>
<td>.103</td>
<td>.11</td>
</tr>
<tr>
<td>Run 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>CHO</td>
<td>.001</td>
<td>.90</td>
</tr>
<tr>
<td>2</td>
<td>TRI</td>
<td>.226</td>
<td>.02</td>
</tr>
<tr>
<td>3</td>
<td>HbAl</td>
<td>.024</td>
<td>.43</td>
</tr>
<tr>
<td>4</td>
<td>GLU</td>
<td>.090</td>
<td>.13</td>
</tr>
<tr>
<td>Run 4</td>
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<td>1</td>
<td>GLU</td>
<td>.139</td>
<td>.07</td>
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<td>CHO</td>
<td>.007</td>
<td>.67</td>
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<td>TRI</td>
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<tr>
<td>4</td>
<td>HbAl</td>
<td>.063</td>
<td>.21</td>
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</table>
CHAPTER V
DISCUSSION

Introduction

It has been hypothesized that impaired fibrinolysis may contribute to diabetic vascular complications. Many investigators have examined fibrinolytic activity in diabetes, but conflicting results have been produced because of inconsistent methodologies and inadequate classification of diabetic subjects. To avoid these problems, a standardized measure of fibrinolytic capacity (FC) was used in this study to examine the relationship between metabolic control and FC in subjects meeting the criteria for clinical classification of insulin-dependent diabetes mellitus (IDDM).

Fibrinolytic Capacity in Insulin-Dependent Diabetes Mellitus

The data from this research suggest that insulin-dependent diabetics have a reduced capacity for fibrinolysis when compared to healthy controls. This finding concurs with Almer and Nilsson (1975), Almer, Pandolfi, and Nilsson (1975), and Cash and McGill (1969), but is at variance with Tanser (1967) who found normal responses to subcutaneous injection of adrenaline. While there is a possibility that the response to adrenaline injection in diabetes would differ from that produced by venous occlusion or exercise, no differences are seen in healthy subjects (Cash & Allan, 1967; Hedner & Nilsson, 1981).
A more plausible explanation for the conflicting finding of Tanser (1967) is the wide range of diabetic conditions included in his study. A heterogeneous diabetic group may present divergent intervening variables which may affect FC. Previous investigations showing reduced FC in diabetes have chosen to classify subjects on the basis of age at onset (juvenile- vs. maturity-onset). However, The National Diabetes Data Group (1979) has pointed out that age at onset does not necessarily determine insulin-dependency. Existing papers which have investigated fibrinolytic activity in documented IDDM have measured only spontaneous fibrinolysis (Bern et al., 1980; Sharma, 1981). Thus, the present report is unique in that FC has now been examined in IDDM.

Fibrinolytic Capacity and Metabolic Control

Data from the present study do not support a relationship between FC and HbAl for insulin-dependent diabetics. However, it may be of interest to note that a significant negative correlation was observed ($r = -.33$) when normals and diabetics were combined into a single sample. The relationship for the combined sample was remarkably similar to that reported by Gunnarsson, et al (1980), $r = -.35$, who also combined normal and diabetic data for statistical analysis. These data, as well as those which show large group differences in GLU, CHO,
TRI, and HbAl, suggest that it is inappropriate to include normal subjects when attempting to make statements concerning glucose homeostasis among diabetic individuals. It appears that differences between normals and diabetics act as intervening variables to confuse or totally distort the relationship between FC and metabolic control if a combined sample of diabetics and controls is utilized for statistical analysis.

The statistically significant positive relationship between FC and HbAl for normals is interpreted as a chance occurrence and probably has no clinical or physiological significance. This interpretation is based on the assumption that the slope of the relationship has no theoretical basis and also on the assumption that the homogeneous grouping of HbAl for the normal sample could contribute to the unexpected statistical outcome.

**Intervening Variables Affecting Fibrinolytic Capacity**

A significant proportion of variance in FC for insulin-dependent diabetics in the present investigation is explained by levels of serum triglyceride. For many years, lipids have been recognized as having an inhibitory effect on fibrinolysis (Merskey & Markus, 1963), but precise mechanisms have not been identified. It is evident that there is no simple relationship between lipids and fibrinolysis, as divergent results are produced by several different lipid abnormalities (Brakman,
Hyperlipidemia is a common finding in diabetes, but there appears to be a complex interaction between the diabetic syndrome, the genetic background of the individual, and the environment (Goldberg, 1981). In IDDM, several investigations have shown that lipid abnormalities can be related to metabolic control (Glasgow, August, & Hung, 1981; Pietri, Dunn, Grundy, & Raskin, 1983; Sosenko, Breslow, Miettinen, & Gabbay, 1980). Results from the present study lend partial support to these findings in that a significant correlation was found between HbA1 and CHO ($r = .59$), but not between HbA1 and TRI ($r = .24$). Factors other than HbA1, which may have affected TRI levels, include uncontrolled dietary factors, genetic hyperlipidemia, or the presence of diabetic glomerulosclerosis (Tattersall, 1982).

Aimer and Nilsson (1975) found impaired FC to be closely associated with the presence of microvascular disease. The present investigation, however, was unable to place any statistical significance on documented vascular complications. This should not rule out the possibility that latent diabetic angiopathy could have an effect on FC.

**Summary and Conclusions**

1. Insulin-dependent diabetics were shown to have a reduced FC when compared to normal subjects matched for
2. FC appears to be unrelated to HbAl for insulin-dependent diabetics. In addition, the inclusion of data for normal subjects creates spurious results by apparently capitalizing on inter-group differences.

3. Serum triglyceride levels explain a significant proportion of variance in FC for insulin-dependent diabetics. However, a large proportion of variance in FC remains unexplained and requires further elucidation.
REFERENCES
References


Ambrus, C. M., & Markus, G. Plasmin-antiplasmin complex


Bennett, P. H. The epidemiology of diabetes mellitus.

Bennett, N. B., Ogston, C. M., McAndrew, G. M. & Ogston,


Billimoria, J. D., Pozner, H., Metselaar, B., Best, F. W., & James, D. C. O. Effects of cigarette smoking on lipids, blood coagulation, fibrinolysis, and cel-


Clinical Pathology, 1958, 11, 403-405.


Cash, J. D., & Allan, A. G. E. The fibrinolytic response to moderate exercise and intravenous adrenaline in the same subject. British Journal of Haematology, 1967,


Christensen, L. R., & MacLeod, C. M. A proteolytic enzyme of serum: Characterization, activation, and reaction with inhibitors. *Journal of General Physiology*, 1945, 28, 559-583.


Crutchley, D. J., Conanan, L. B., & Maynard, J. R. Human


Duguid, J. B. Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. *Journal of Patho-


Ferguson, J. E., Mackay, N., Phillip, J. A. D., & Sumner, D. J. Determination of platelet and fibrinogen half-life with $^{75}\text{Se}$ selenomethionine: Studies in normal and in diabetic subjects. Clinical Science and Molecular Medicine, 1975, 49, 115-120.

Fletcher, A. P., Biederman, O., & Moore, D. Abnormal


Gensini, G. F., Abbate, R., Favilla, S., & Serneri, G. G.


Gordon-Smith, I. C., Hickman, J. A., & Le Quesne, L. P. Postoperative fibrinolytic activity and deep vein throm-

Gunnarsson, R., Nyman, D., Walinder, O., & Ostman, J.


McDonald, M. J., Shapiro, R., Bleichman, M., Solway, J., & Bunn, H. F. Glycosylated minor components of human adult hemoglobin. *Journal of Biological Chemistry,*
1978, 253, 2327-2332.


31-34.


Ratnoff, O. D. The surface-mediated initiation of blood coagulation and related phenomena. In D. Ogston, &


Robertson, B. R. Effect of nicotinic acid on fibrinolytic activity in health, in thrombotic disease, and in liver cirrhosis. Acta Chirurgica Scandinavica, 1971, 137,


Skyler, J. S., Skyler, D. L., Seigler, D. E., & O'Sullivan,


Tchobroutsky, G. Relationship of diabetic control to
development of microvascular complications. *Diabetes-
ologia*, 1978, **15**, 143.

Todd, A. S. The histological localization of fibrino-
lysin activator. *Journal of Pathology and Bacteriology*,
1959, **78**, 281-283.

on Diabetes to the Congress of the United States*, 1976,
 Vol. 3, part 4, DHEW publication (NIH) 76-1024.

Tsapogas, M. J., Peabody, R. A., Kwang-Tzen, W., Devaraj,
K. T., & Eckert, C. Depression endogenous fibrinolytic
activity in essential hypertension. *Journal of Cardio-

Walker, I. D., Davidson, J. R., & Hutton, I. Disordered
"fibrinolytic potential" in coronary heart disease.
*Thrombosis Research*, 1976, **10**, 509-520.

Wardle, E. N., Piercy, D. A., & Anderson, J. Some chemical
indices of diabetic vascular disease. *Postgraduate

Weiner, M., de Crines, K., Redisch, W., & Steele, M. J.
Influence of some vasoactive drugs on fibrinolytic

Wilkins, H. J., & Back, N. Fibrinolysis and risk factors
of atherosclerotic disease, with special emphasis on
diabetes mellitus. *Circulatory Risk*, 1978, **6**, 125-
143.
APPENDICES
Appendix A

Clinical Data Form

Name: _______________ Age: __yrs Sex: M F Race: B W O

Height: ____", ____cm Weight: _____lbs, _____kg

Date of Onset: _______ Duration: ___yrs DKA: Yes No

Type of Therapy (check one):

- Conventional (one injection/day)  - Multiple Preprandial Injections
- Split Dose (two injections/day)  - Portable Infusion Pump

Insulin Dose: _____U/24 hr _____U/kg/24 hr

Type of Insulin [check appropriate type(s)]:

- R - NPH - L - U - Actrarapid - Monotard - Ultratard

Other Medication: ____________________________________________

Fasted: Yes No Exercise: Yes No Cigarettes: Yes No

Blood Pressure: Systolic: __mmHg Diastolic: __mmHg

Cholesterol: ___mg% Triglyceride: ___mg% Glucose: ___mg%

Complications (explain):

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

Preocclusion ELT: ___min ___min x ___min

Postocclusion ELT: ___min ___min x ___min

AELT%: ___%

G-Hb%: ___% ___% x = ___%
Appendix B

Subject Consent Form

I, __________________, volunteer to participate in the experiment entitled, "The Relationship Between Fibrinolytic Capacity and Metabolic Control in Insulin-Dependent Diabetes Mellitus," to be conducted at Earl K. Long Memorial Hospital, during the period July 1, 1982 to August 31, 1982, with Guyton Hornsby as Principal investigator. The procedures to be followed and their purposes have been explained to me and I understand them. I further understand that I can withdraw from the experiment at any time, that the data I will provide will be anonymous and my identity will not be revealed without my permission, and that my performance in this experiment may be used for additional approved projects. Finally, I shall be given the opportunity to ask questions prior to the start of the experiment and after my participation is complete.

Subject's signature
Appendix C
Fibrinolytic Capacity, Glycosylated Hemoglobin, and Serum Glucose,
Cholesterol, and Triglyceride for Diabetic Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>PC (%)</th>
<th>HbAl (%)</th>
<th>GLU (mg%)</th>
<th>CHO (mg%)</th>
<th>TRI (mg%)</th>
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<td>94</td>
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<td>193</td>
<td>38</td>
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<td>288</td>
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M  46.6  10.3  209  215  78
± SD  19.3  2.1  102  56  60
n  26  26  26  25  24

* Missing Data
Appendix D

Fibrinolytic Capacity, Glycosylated Hemoglobin, and Serum Glucose, Cholesterol, and Triglyceride for Nondiabetic Subjects

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<th>HbA1</th>
<th>GLU</th>
<th>CHO</th>
<th>TRI</th>
<th>Subject</th>
<th>FC</th>
<th>HbA1</th>
<th>GLU</th>
<th>CHO</th>
<th>TRI</th>
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<td>171</td>
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<td>105</td>
<td>183</td>
<td>53</td>
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<tr>
<td>C2</td>
<td>79.8</td>
<td>8.1</td>
<td>93</td>
<td>215</td>
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<td>C12</td>
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<td>90</td>
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<tr>
<td>C3</td>
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<td>91</td>
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<td>C4</td>
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<td>97</td>
<td>227</td>
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<td>30</td>
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\[
\begin{align*}
\bar{M} & = 64.2 \quad 6.5 \quad 95 \quad 186 \quad 56 \\
\pm \text{SD} & = 12.1 \quad .9 \quad 7 \quad 25 \quad 24 \\
n & = 20 \quad 19 \quad 19 \quad 20 \quad 19
\end{align*}
\]

* Missing Data
## Appendix E

### Clinical Data for Diabetic Subjects

<table>
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<th>Race</th>
<th>Duration of Diabetes (yrs)</th>
<th>Insulin Dose (U/24 hrs)</th>
<th>Type of Therapy</th>
<th>Complications</th>
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<th>Diastolic B.P. (mmHg)</th>
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Clinical Data for Diabetic Subjects

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± SD 6.8 8.0 11.8 4.3 21.9 10.5 7.1

Sex: F - Female, M - Male
Race: B - Black, W - White
Type of Therapy: Conventional - One injection per day, Split - Two injections per day, Multiple - Three or more injections per day, Pump - Continuous subcutaneous injection by portable infusion pump
**Appendix F**

Clinical Data for Nondiabetic Subjects

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Vita

William Guyton Hornsby, Jr., was born in Reynolds, Georgia, on February 14, 1952. Soon after, his family moved to Cayce, South Carolina, where he grew up and graduated from Brookland-Cayce High School in June of 1970.

He attended the University of South Carolina on a scholarship in track and field and graduated magna cum laude with a Bachelor of Science degree in Health Education in May of 1974. After graduation, he accepted a teaching-coaching position at Brookland-Cayce High School, which he held until June of 1977.

He then returned to the University of South Carolina to receive his Master of Arts in Teaching degree in Physical Education in May of 1979. He entered Louisiana State University to work on the Ph.D. degree in Physical Education in August of 1979. His major emphasis of study was in exercise physiology with a minor in zoology.

He married the former Gay Ann Labat of Metairie, Louisiana, on November 29, 1980. He received a Student Research Fellowship from the Louisiana Affiliate, American Diabetes Association to complete work on the study entitled "The Relationship Between Fibrinolytic Capacity and Metabolic Control in Insulin-Dependent Diabetes Mellitus" on June 1, 1982.

He accepted a position as assistant professor in the Division of Health, Physical Education, and Recreation at Delta State University in August of 1982 and was graduated at Louisiana State University in August of 1983.
Candidate: William Guyton Hornsby, Jr.

Major Field: Physical Education

Title of Thesis: The Relationship Between Fibrinolytic Capacity and Metabolic Control in Insulin-Dependent Diabetes Mellitus

Approved:

[Signature]

Major Professor and Chairman

[Signature]

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signature]

Emelyn A. Hall

[Signature]

Scott R. Bren

[Signature]

Ronald Ford

Date of Examination:

June 27, 1983