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Glycosaminoglycans of Normal and Diabetic Human Plasma

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GLYCOSAMINOGLYCANS OF NORMAL AND DIABETIC HUMAN PLASMA

by
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GLYCOSAMINOGLYCANS OF NORMAL AND DIABETIC HUMAN PLASMA

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Diabetic complications in nnn-insulin dependent tissues have led researchers to investigate causitive parameters at the biochemical level. The purpose of this project was to measure glycosaminoglycans in diabetic and normal human plasma and to investigate any changes in glycosaminoglycan content for possible roles in diabetic complications.

Glycosaminoglycan content was measured by three methods; the alcian blue assay, protein-bound hexosamine, and total glycosaminoglycan isolate methods. Results show IDDM plasma to have significantly increased levels of glycosaminoglycans as assayed by the alcian blue test and protein-bound hexosamine. The total glycosaminoglycan isolate method did not correlate with either of the two other methods. No difference was demonstrated between normal and NIDDM plasma glycosaminoglycan content.

The protein-bound hexosamine and the alcian blue assays correlate with age and type of diabetes. No differences between the sexes were demonstrated. Of particular interest are the results that indicate a possible correlation between glycosaminoglycans and renal complications.

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Many people have helped me with my studies at Western Michigan University; I wish to thank Dr. Jack Wood for serving as my academic advisor and committee chairman, Dr. Rebecca Norris at The Upjohn Company for her invaluable help in the organization of the protocol for this research project, and Dr. Leonard Ginsberg for his patience and guidence in my research endeavors. I would like to extend a special thanks to Dr. Eric Schreiner for the confidence and encouragement he has given me during my studies at Western. To these people and many more, I remain indebted.

Gloria Badiner

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CHAPTER I

INTRODUCTION

Changes in glycosaminoglycan content in body tissues have been associated with disease. The only confirmed correlation with specific physical finding are the class of disorders called mucopolysaccharidosis. The role of glycosaminoglycans in the pathogenesis of other human disease is currently under investigation. Studies of glycosaminoglycan content of the aorta and kidney have shown changes associated with atherosclerosis and kidney pathology, because of the increased frequency of atherosclerosis and kidney dysfunction among diabetics and the possible role of glycosaminoglycans in the developement of disease, preliminary work was initiated to assay for glycosaminoglycan content in diabetic and normal plasma.

The alcian blue assay is an easy reproducible test for the quantitation of glycosaminoglycans in biological fluids. Our preliminary studies used this procedure to measure the glycosaminoglycan levels in diabetic and normal plasma. Preliminary data showed elevated alcian blue values in diabetic plasma over the normal human plasma. It was further shown this elevation of the alcian blue values was not entirely due to the glycosaminoglycan content alone because the levels of glycosaminoglycan as measured by the alcian blue method were well above the amounts of glycosaminoglycan reported to be present in human plasma.

The purpose of this project was to investigate the correlation between the colorimetric alcian blue assay method and the total glycosaminoglycan fractional isolate method for the determination of plasma glycosaminoglycan content. It is further the intent of this project to investigate the preliminary results which showed an increase in the alcian blue assay absorbance in diabetic plasma. This increase was studied with respect to age, sex, type of diabetes, control of disease and duration of diabetes. Other plasma parameters, protein-bound hexosamine content, total protein, and α_2-macroglobulin levels were also measured.

Type of diabetes was determined by the World Health Organization guidelines. Two types of diabetes were investigated in ths project; insulin dependent diabetes mellitus (IDDM) which is that type of diabetes that is insulin dependent and ketosis prone and non-insulin dependent diabetes mellitus (NIDDM) which is that type of diabetes that is non-insulin dependent and non-ketosis prone.

CHAPTER II

REVIEW OF SELECTED LITERATURE

Structures of Glycosaminoglycans

Glycosaminoglycans are an integral component of the intercellular matrix and are responsible for the visco-elasticity properties of tissures. Glycosaminoglycans (GAG) have been isolated from various human tissues including the aorta, kidney, intestine, brain, liver and plasma. Seven GAG are commonly found in anumal tissues: dermatan sulfate (DS), heparin (HP), heparin sulfate (HS), keratan sulfate (KS), hyaluronic acid (HA), and chondroitin isomers-chondroitin-6-sulfate (C6S) and chondroitin-4-sulfate (C4S). Six of these are structurely related with a carbohydrate backbone of alternating uronic acid and hexosamine residues. All are polyanions that have acidic sulfate and/or carboxyl groups.

Hyaluronic acid is a polymerization products of $[(1\text{-}4)\text{-}\beta\text{-D-glucuronsyl-}(1\text{-}3)\text{-}\beta\text{-D-N-acetylglucosaminyl}]_n$ with no further modification. All other GAG are modified before chain elongation is completed to yeild sulfated polysaccharides, often containing more than one type of disaccharide unit (De Luca, Richmond, and Silvert, 1973).

Chondroitin sulfates are polymerization product of $[(1\text{-}4)\text{-}\beta\text{-D-glucouronosyl-}(1\text{-}3)\text{-}\beta\text{-D-N-acetylgalactosaminyl}]_n$ which is further modified by the addition of sulfate groups on C4 or C6 of the N-acetylgalactosamine residue and usually contains one sulfate group per disaccharide residue.

Dermatan sulfate polymerization is the same as chondroitin except modification can occur at C5 of the glucuronic acid units.

Heparin and heparan sulfate have an intermediate polymerization product of $[(1\text{-}4)\text{-}\beta\text{-D-glucuronosyl-}(1\text{-}4)\text{-}\alpha\text{-D-N-acetylglucosaminyl}]_n$.

Sulfation occurs at the same sites as DS, but deacetylation of N-acetylglucosamines occurs and these sites are then sulfated.

Keratan sulfate is a polymerization product of D-galactose and D-glucosamine. Post polymerization sulfation occurs at the same site as HP.

Minor sugar components do occur giving interchain heterogenity. CS, DS, HS, and HP have been found to contain D-galactose and D-xylose, and KS has been shown to include D-galactosamine, D-mannose, L-fucose, and sialic acid.

Glycosaminoglycans and their binding to macromolecules

Most GAG do not occur as free polysaccharides but as proteoglycans in which several GAG chains are linked to a protein core. HA differs in being a single unbranched polymer attached to protein.

GAG have been found to bind to many macromolecules. All except HA bind to collagen and HP has been found to bind with elastin (Lindahl and Hook, 1978). GAG have also been reported to bind to lippproteins (Nakashima, Di Ferrante, Jackson, and Pownall, 1975), fibrinogen and calcium (Jones and Peterson, 1979), and they have been shown to interact with platelets (Barber, Kaser-Glanzmann, Jakabova, and Luscher, 1972).

In particular, the interaction of GAG with blood elements have shown inconclusive results. Because of the high incidence of vascular disease and the proneness to the developement of atherosclerosis in diabetics, the interaction of GAG and serum components and the GAG associated with the arterial wall will be reviewed.

Low density lipoproteins (LDL) and very low density lipoproteins (VLDL) bind GAG. This binding is dependent on electrostatic forces which increase the charge density of the GAG. Divalent cations are not prerequisite for binding to occur but do stabilize complexes of HP and LDL (Lindahl and Hook, 1978), (Nakashima, De Ferrente, Jackson, and Pownall, 1975).

Camejo demonstrated that mixtures of C6S, DS and HP or HS when bound to a protein core could form insoluble complexes with both purified and crude serum LDL (Camejo, Lalaguna, Lopez, and Starosta, 1980.). This GAG-portein product has been labeled lipoprotein complexing proteoglycan (LCP-3). Specificity of LCP-3 for LDL is influenced by pH, calcium and magnesium ions, and disappears when treated with testicular hyaluronidase or proteolytic enzymes. These researchers suggest LCP-3 may play a role in the arterial intima-media atherogenic changes.

Glycosaminoglycan Changes in Diseased States

Arterial intimia GAG may interact with lipoproteins to promote lipid deposition in the arterial wall which in turn leads to the developement of arterial plaque lesions. At physiological pH; HP, DS, CS, and HA bind to LDL. HP has the greatest affinity for LDL under these conditions and HA has the least affinity for lipoproteins (Inverius, 1972), (Bihari-Varga,

Szlatisz, and Gal, 1981), (Radhakrishnamurthy and Bereson, 1972). Arterial smooth muscle cells synthesize DS and these cells proliferate at the site of atherosclerotic lesions (Ross and Harker, 1976). Cell surface studies of human fibroblasts have shown LDL to bind with high affinity to specific receptor sites (Goldstein, Basu, Brunschede and Brown, 1976). Treatment of cells with HP releases bound LDL from these receptor sites and prevents the release of cholesterol. Abnormal retention of LDL on cell surfaces has been associated with the presence of GAG (Browness, 1977). Donnelly showed HDL and LDL coated red blood cells could agglutinate in the presenee of sulfate rich GAG but did not agglutinate in the presence of low sulfated speices even when high sulfated GAG were added (Donnelly, Di Ferrante, and Jackson, 1978). It was suggested that GAG may play a role in holding LDL to the arterial intimia (Ghiselli and Catapano, 1979).

Kaplan found decreased total content in the human aorta with increased severity of the atherosclerosis. CS and HA content decreased while DS and HS levels increased (Kaplan and Meyer, 1960). Bertelsen and Marker reported a decrease in total GAG concentration (Bertelsen and Marker, 1961) while another study reported and increase in the total GAG concentration in the diseased artery (Schmidt and Dmochowski, 1964).

Fatty streak lesions of the human aorta have also been analyzed for GAG changes. Both Kumar (Kumar, Berenson, Ruiz, Dalferes, and Strong, 1967) and Dalferes (Dalferes,Ruiz, Kumar, Radhakrishnamurthy, and Bereson, 1971) have found fatty streak lesions to contain an increase in the total GAG content with corresponding increases in C4S, C6S and DS. However, these same researchers have found a decrease

in the total GAG content in advanced atherosclerosis with fibrous plaque lesions. This decrease was found to be the result of decreases in the sulfated components, particularly DS. Stevens used the sensitive method of two-dimensional electrophoresis to study GAG changes in the intimia and media of the human aorta in atherosclerosis. This study found normal aortic tissure to contain DS, CS, HA, and HS. With the progression of the disease, there was a decrease in total GAG content with a corresponding decrease in CS and HS while the DS fraction was found to increase. No change occured in the HS fraction (Stevens, Colombo, Hollander, and Schmid, 1976).

Animal studies have shown the bovine aorta to contain C6S, C4S, DS, HP, and HS proteoglycans (Oegema, Hascall, and Eisenstein, 1979). Animals fed atherogenic diets and high doses of ascorbic acid showed a significant increase in the high sulfated GAG content in the aorta (Nambisian and Kurup, 1975). Rabbits fed atherogenic diets with cholesterol developed fatty lesions in the aorta. These lesions were analyzed and found to contain GAG-lipoprotein components. The GAG portion was composed of HS, HA, and C6S (Mawhinney, Augistyn and Fritz, 1978). Since diabetics have increased risks of atherosclerosis and are prone to increased glycosylation of proteins and polysaccharides, there has been much interest in the interaction of GAG in diabetes.

Stender and Astrup have found HP to complex with LDL. This study also showed an increase in the wet weight of the artery with a corresponding decrease in aortic cholesterol in rabbits who were fed high cholesterol and glucosamine diets (Stender and Astrup, 1977). An increase in serum glucosamine levels has been reported in diabetes

(Malathi and Kurup, 1969). Glucosamine is a component of the PAS-positive material. This material is deposited in the basement membrane and may play a role in diabetic microangiopathy which occurs with the thickening of the basement membrane.

Researchers have postulated that changes in the GAG compostion of body tissues may be due to the changes in serum insulin levels. Diabetics often have wide serum insulin level: flucuations. A study by Sirek has linked hyperinsulinemia with GAG alterations in the arterial tree in diabetic dogs. Diabetic dogs subjected to marked insulin flucuations were found to have GAG alterations in many parts of the arterial tree. These alterations involved several GAG fractions. Diabetic dogs subjected to small insulin level flucuations showed alterations in the same branches of the arterial tree, but in only one GAG component. The coronary arteries were particularly sensitive to serum insulin variations and the major GAG consitutent change involved DS (Sirek, Sirek, and Cukerman, 1981).

Alterations of metabolism as seen in diabetes plays a role in tissue GAG compostion of many tissues. Like the artery, the kidney has also been the subject of study for many researchers. There is controversy in the literature as to definitive changes in GAG content of diseased tissue. Of particular importance are the studies on the glomerular basement membrane. Beisswenger demonstrated an increase in the basement membrane protein in diabetes of long duration (Beisswenger and Spiro, 1973). This is consistant with electron microscopic studies of basement membrane thickening in diabetic nephropathy. Glomerular permeability to serum proteins is influenced by

the presence of anions in the capillary wall. Proteoglycans have such negative charges and are a fixed component of the glomerular capillary wall. Alterations in the amount and type of GAG could conceivably contribute to glomerulosclerosis as seen in diabetic renal disease (Bennet, Glassock, Chang, Deen, Robertson, and Brenner, 1976). Analysis of renal cortex shows a major GAG component to be HS with a lesser and variable amount of HA. Diabetic kidneys show a slight increase in HS and a variable HA concentration (Brownlee and Cerami, 1981), (Gundersen, Osterby, and Lundbaek, 1978).

α_2-macroglobulin Studies of Diabetic Plasma

Other complex glycoproteins are suspected to have some involvement in the complications of diabetes. Studies of α_2-macroglobulin levels in normal and diabetic plasma have given conflicting results. A study by Di Cesare found no significant increase of α_2-macroglobulin concentrations in diabetic plasma (Di Cesare, Quartarone, Cucinotta, Coglitore, and Squadrito, 1979). This study did not take into account the tupe of diabetes or the treatment of the disease. Of the plasma analyzed, 70% were non-insulin dependent diabetes mellitus and the modes of treatment were variable as were the degrees of retinopathy.

James measured plasma α_2-macroglobulin levels of diabetic and normal plasma. Controls were age and sex matched to diabetics. In addition, the diabetics were grouped with respect to duration of disease, treatment of disease, and degree of retinopathy or nephropathy. Results from this analysis showed an increase in plasma α_2-macroglobulin levels in controls which correlated with the age of the subjects. Regression analysis found age, sex, control of

disease and retinopathy to be contributing variables (James, 1980). The increase in α_2-macroglobulin with increase in age was also reported by Ganrot and Schersten. This study found the increase to be gradual and to level off at thirty years of age (Ganrot and Schersten, 1967).

Brownlee hypothesized that the increase in α_2-macroglobulin levels in human plasma as seen in diabetes may cause thickening of the basement membrane of capillaries. Two pathogenic modes of action were proposed. First, increased plasma insulin or growth hormone levels cause an increase in the basement membrane synthesis that in turn leads to a thickening of the basement membrane. Secondly, an increase in plasma α_2-macroglobulin levels cause a decrease in the polymorphonuclear neutral proteases which in turn decreases basement membrane degradation thereby causing an increase in the basement membrane thickness. The increase in basement membrane material could be a contributing factor in the pathogenesis of diabetic nephropathy and could serve as model for pathogenesis of microvascular disease (Brownlee, 1976).

Isolation of Plasma GAG

Badin isolated hexuronic acid and hexosamine in equimolar amounts form human plasma in 1955 (Badin, Schubert, and Vouras, 1955), Bassiouni extracted components of human blood that matched paper electrophoretograms for chondroitin sulfate and found an unidentified component that displayed lesser motility in the same system (Bassiouni, 1955). Bollet used two-dimensional paper chromatography to isolate two fractions from human plasma. One fraction was indentified as chondroitin

remained unidentified (Bollet, Scraydarian, and Simpson). Kerby found protein-bound GAG in Cohn fractions III and IV-I (Kerby, 1966). Schiller first identified the condroitin component as chondroitn-4-sulfate (Schiller, 1958) and Friman identified the second component as hyaluronic acid by using zone electrophoresis (Firman and Brunish, 1966). Calatroni used anion exchange column chromatgraphy to separate plasma GAG and identified the components as C4S, KS, and HS (Calatroni, Donnelly, and Di Ferrante, 1969). Later work by Singh demonstrated the presence of two C4S components in plasma (Singh, Di Ferrante, Cyorkey, and Wilson 1977). Work by Murata found the major GAG components of human plasma to be under-sulfated C4S isomers followed by a lesser amount of HA with small amounts of HS and over-sulfated C6S. The undersulfated chondroitin was found to contain a small portion of C6S (Murata and Horiuchi, 1977). Breen developed a method to isolate and identify microquantities of tissue GAG in a mono-dimensional electrophoresis system (Breen, Knipper, Weinstein, Blacik, Lewandowski, and Baltrul, 1981). Gold developed a simple spectrophotometric procedure for the quantitation of glycosaminoglycans in human plasma (Gold, 1979).

CHAPTER III

MATERIALS AND METHODS

Experimental Subjects

Human diabetic subjects were selected to achieve a balance between the sexes and duration of disease. Normal subjects were age and sex matched to the diabetics. Exclusion criteria required the normal individuals to be free from acute or chronic disease, to have no history of kidney, heart, or gastrointestinal disease in the last five years, and no diabetic siblings, aunts, uncles, parents or grandparents.

Blood was drawn from 12 hour fasted individuals at Bronson Clinical Investigation Unit and collected in two 5 ml EDTA treated vacuum tubes. Each sample was labeled by Bronson's personnel at the time of collection, date and donor code number. The samples were put on ice and transferred to our laboratory at Western Michigan University. We were given only the sample number so all experiments could remain blind. The samples were centrifuged at 9,000 g in a Sorvall centrifuge at 4°C for 10 minutes after which the plasma protion was carefully drawn off leaving a thin layer of plasma over the white blood cells to avoid contamination of the extracted plasma.

Male Chinese hamsters (Cricetulus griseus) of lines M (normal), XA and AC (diabetic) were donated by The Upjohn Company. The hamsters were fasted for 18 hours and then injected intrapertioneally with radioactively labelled glucosamine and sulfuric acid. After 8 hours blood was withdrawn from the orbital sinus with a heparinized capillary

tube and collected in a 500 ul microfuge tubes to which 5 ul of EDTA had been added. The plasma fraction was extracted as before.

Alcian Blue Assay

The alcian blue test was performed on fresh untreated plasma according to the method of Gold (1979) with some modification Twenty-five ul of plasma were pipetted into clean 5 ml test tubes, the volume was brought to 200 ul with .15 M NaCl, then 2.5 ml of the alcian blue dye mixture as added. The dye mixture was prepared by dissolving alcian blue (Eastman, Lot C7B) in .5M sodium acetate to produce a final dye concentration of 1.4 mg/ml. The samples were vortexed and allowed to react for ten minutes. The reaction mixture was read at 480 nm on a Hitachi dual beam spectrophotometer.

Blanks were prepared by adding 200 ul of .15 M NaCl and 2.5 ml of the alcian blue dye mixture. Standard curves were prepared with various concentrations of chondroitin sulfate (5 to 75 ug/200 ul). The alcian blue dye mixture was prepared fresh each day by dissolving in .5 M sodium acetate. Care was taken to avoid warming of the dye mixture as this caused the dye to aggregate and precipitate. The cuvettes were carefully cleaned with sulfuric acid after each use to prevent color build up on the glass from the dye mixture.

Separation of Glycosaminoglycans from Human Plasma

The method of Breen was modified and used to extract GAG from plasma (Breen, Knipper, Weinstein, Blacik, Lewandowski, and Baltrus, 1981). Two ml of fresh plasma were heated in a boiling water bath for 5 minutes to destroy endogenous hydrolases. The sample was cooled to

room temperature, then 10 mg of protease (Sigma, TypeVI) were added and incubated overnight. The next day the sample was vortexed and again treated with 10 mg protease and incubated for 24 hours at 37°C.

To precipitate any undigested proteins or nucleic acids, 100 ul of 50% trichloroacetic acid (Mallinckroat, AR grade) were added and the sample was refrigerated overnight. The sample was subsequently centrifuged at 15,000 g for 30 minutes at 4°C. The supernatant was carefully drawn off; the precipitant was discarded; and the supernatant was again treated with 100 ul of 50% trichloroacetic acid for 18 hours at 5°C. The supernatant from this treatment was transferred to dialysis membranes (Spectropor, molecular weight cut off 3,500) and dialized against 4 changes of distilled water at 5°C. The samples were concentrated by lyophilization and stored at -70°C until used for electrophoresis.

Separation of Glycosaminoglycans from Hamster Plasma

The proceeding techniques applied to the treatment of hamster plasma with the following modifications. The sample size was reduced to 250 ul with corresponding reductions for all other treatments; and the fresh plasma was diluted and dialyzed prior to boiling.

Acetate Strip Electrophoresis

Cellulose polyacetate electrophoresis strips (Sephaphore-III) were carefully floated on the surface of pyridine-formic acid buffer. The strips were handled with forceps at all times and care was taken to insure the proper side was kept upright. The buffer was prepared by diluting 10 ml of formic acid and 3.8 ml of pyridine to 500 ml with distilled deionized water.

Dry human plasma glycosaminoglycans were dissolved in 30 ul of the pyridine-formic acid buffer and vortexed. Three ul of this sample were applied to the strips and electrophoresis preformed at 180 V for 80 minutes. Following electrophoresis, the strips were suspended in a 1% alcian blue dye mixture for 20 minutes, rinsed in distilled water for 5 minutes, destained in acetic acid for 10 minutes. The strips were cleared in 40% aqueous N-methyl pyrrolidone v/v (Sepra Clear II, Gelman) for 5 minutes and dried onto artist wet media acetate sheets to facillitate photographing and scanning. The strips were photographed with high contrast black and white film (Polaroid Type 55).

The next day the strips were scanned with a light densitometer with a 600 nm filter, 10 nm reading, scan length of 10, and a total material setting of 100. The curves were recorded linear direct/linear inverse and the areas under the curves were measured with a metric polar planometer. Ratios of sulfated GAG (C4S and C6S) to non-sulfated GAG (HA) were determined.

Hamster plasma glycosaminoglycans were dissolved in 25 ul of the pyridine-formic acid buffer and electrophoresis performed

as before. Instead of staining, the strips were dried overnight at 60°C, sandwiched between glass plates and autoradiographic film (LKB Ultrofilm ^{3}H), and exposed for 2 weeks at -70°C.

Hexosamine Assay

The method of Gatt and Berman (Gatt and Berman, 1965) was modified and used for hexosamine determination. Twenty-five ul of plasma were pipetted into 5 ml pyrex screw-top test tubes, the tops of which were wrapped with Teflon tape, and distilled water was added to bring the volume to 400 ul. A tube with 400 ul of distilled water served as the blank. The standard was prepared by using 100 ul of glucosamine-HCl (100 ug/ml water) plus 300 ul distilled water.

To each tube, 200 ul of 6 N HCl was added, stoppered tightly with Teflon caps, contents mixed, and hydrolyzed at 100°C for 4 hours. Upon completion of hydrolysis, the tubes were cooled to room temperature, directly neutralized with 400 ul of 2 M Na_2CO_3 and gently shaken, followed by addition of 500 ul of 2% acetyl-acetone. The tubes were stoppered, mixed, and heated in a boiling water bath for 20 minutes.

After cooling, 1 ml of aboslute ethanol and 500 ul of Ehrlich's reagent were added. The tubes were first shaken gently by hand, then vigorously on a vortex mixer to expell excess CO_2 and allowed to stand overnight at room temperature . This allowed for fading of color due to free glucose (Cessi and Piliego, 1960), (Schloss, 1951). The next day the reaction mixture was read at 530 nm in glass cuvettes.

Reaction solutions were prepred as follows:

1.	2.0 M Na_2CO_3	10.6 g/ml
2.	2% acetylacetone	2 ml 2,4 pentanedione made up to 100 ml with 1.5 M Na_2CO_3
3.	1.5 M Na_2CO_3	15.9 g/100 ml
4.	Absolute alcohol	
5.	Ehrlich's reagent	1.0 g p-dimethylaminobenzaldehyde in a mixture of 15 ml absolute alcohol and 15 ml of concentrated HCl

Protein Determination

Serum protein levels were determined by the method of Lowry (Lowry, Rosebrough, Farr, and Randall, 1951) using bovine serum as the standard.

α_2-macroglobulin Assay

This assay measures the amount of trypsin which is protected from anti-trypsin by α_2-macroglobulin. It is not necessarily as quantitative as radioimmunoassay procedures for α_2-macroglobulin because the values are a function of α_2-macroglobulins ability to protect trypsin.

Reaction solutions were prepared as follows:

1. Buffer- 0.1 M Tris-HCl pH 7.65 with 0.1% NaN_3
2. Sample- fresh non-frozen plasma gives consistant results, 5 ul human plasma is sufficient.

Incubate 2 ml buffer with sample, add 5 ul trypsin and let stand for 10 minutes at 37°C. Add 10 ul soybean anti-trypsin and incubate another 10 minutes at 37°C. Add 1 ml BAPNA and incubate 2 hours. Read the samples at 410 nm.

Statistical Analysis

Prepared statistical packages of SAS and SPSS were used to analyze data. Pearson correlations, ANOVA, oneway analysis, scattergrams, histograms, Bartlett-box, LSD procedure, Cochran's test for homogeneity of varience and regression analysis were used. For the unbalanced case the calculated means are harmonic. Fixed and random effects models are given for all significant data

CHAPTER IV

RESULTS

Qualification of Plasma Glycosaminoglycans by Acetate Strip Electrophoresis

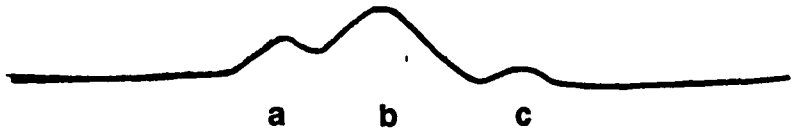
Normal and diabetic human plasma was extracted for GAG content. The major GAG component of human plasma was found to be undersulfated C4S with a lesser amount of HA. The undersulfated C4S fraction was found to contain a small amount of undersulfated C6S. A small amount of oversulfated C6S was also detected (Figure 1), (Table 1).

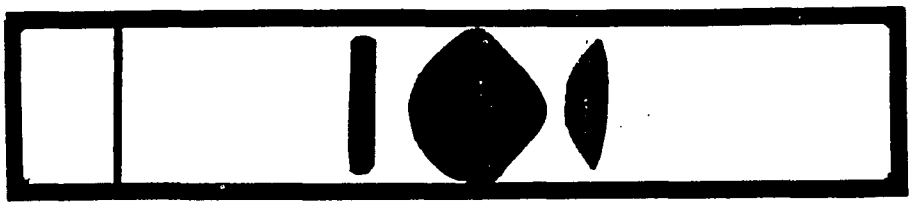
Hamster plasma was analyzed for GAG components and was found to contain the same fractions as human plasma except a HP fraction was also shown to exist.

Relative Quantitation of Plasma GAG

Comparisons of the total areas under the respective curves from the densitometric tracings showed no significant differences in the amounts of gigen GAG fraction between diabetic and normal human plasma determined electrophoretically. Ratios of HA to sulfated (C4S and C6S) plasma GAG fractions increased with increasing age of the individual in normal plasma samples. No difference in the amount of GAG present was detected between the sexes in age matched nomal samples. The results of quantitation by electrophoretic methods did not correlate with those of the spectrophotometric method-the alcian blue assay or those of the protein-bound hexosamines.

DENSITOMETRIC TRACING OF AN ACETATE ELECTROPHORESIS STRIP





o—origin a—HA
b—undersulfated C4S & C6S
c—oversulfated C6S

Figure 1. Relative positions of plasma GAG

TABLE I: MEAN PLASMA PARAMETER VALUES

GROUP	ALCIAN BLUE	LOWRY	ALPHA-2-MAC	HEXOSAMINE	TOTAL GAG	RATIO C4S+C6S / HA
IDDM	.3313 OD	62.0mg/ml	275.6ug/ml	1.56mg/ml	108*	5.689
control 1	.2700	67.0	219.9	1.23	102	6.384
NIDDM	.3241	54.2	205.0	1.81	105	8.639
control 2	.3221	59.5	246.9	1.66	99	13.981

*100=20ug GAG/ml plasma

Insulin dependent diabetes mellitus (IDDM) plasma protein bound hexosamine levels were significantly elevated from the age matched control samples (p=.00018). Non-insulin dependent diabetes mellitus (NIDDM) plasma showed no significant differences from the age matched controls. Plasma protein bound hexosamine levels did correlate with alcian blue positive staining material levels (p=.00013, R=.54), (Figure II).

α_2-macroglobulin Concentration in Human Plasma

IDDM plasma showed significantly elevated levels of α_2-macroglobulin from the matched controls in both sexes and in all age catergories (Figure III). NIDDM showed no significant variations from the age and sex matched controls (Figure IV).

The Alcian Blue Assay

Preliminary data showed an increase in the alcian blue positive staining material in diabetic human and hamster plasma over the control plasma samples (Figure V). Further investigation of human normal and diabetic plasma shows this increase to be a function of the age of the individual and the type of diabetes. No significant differences were demonstrated between the sexes at a given age group. With strict controls for age, the IDDM differed significantly from the age matched controls (P=.02).

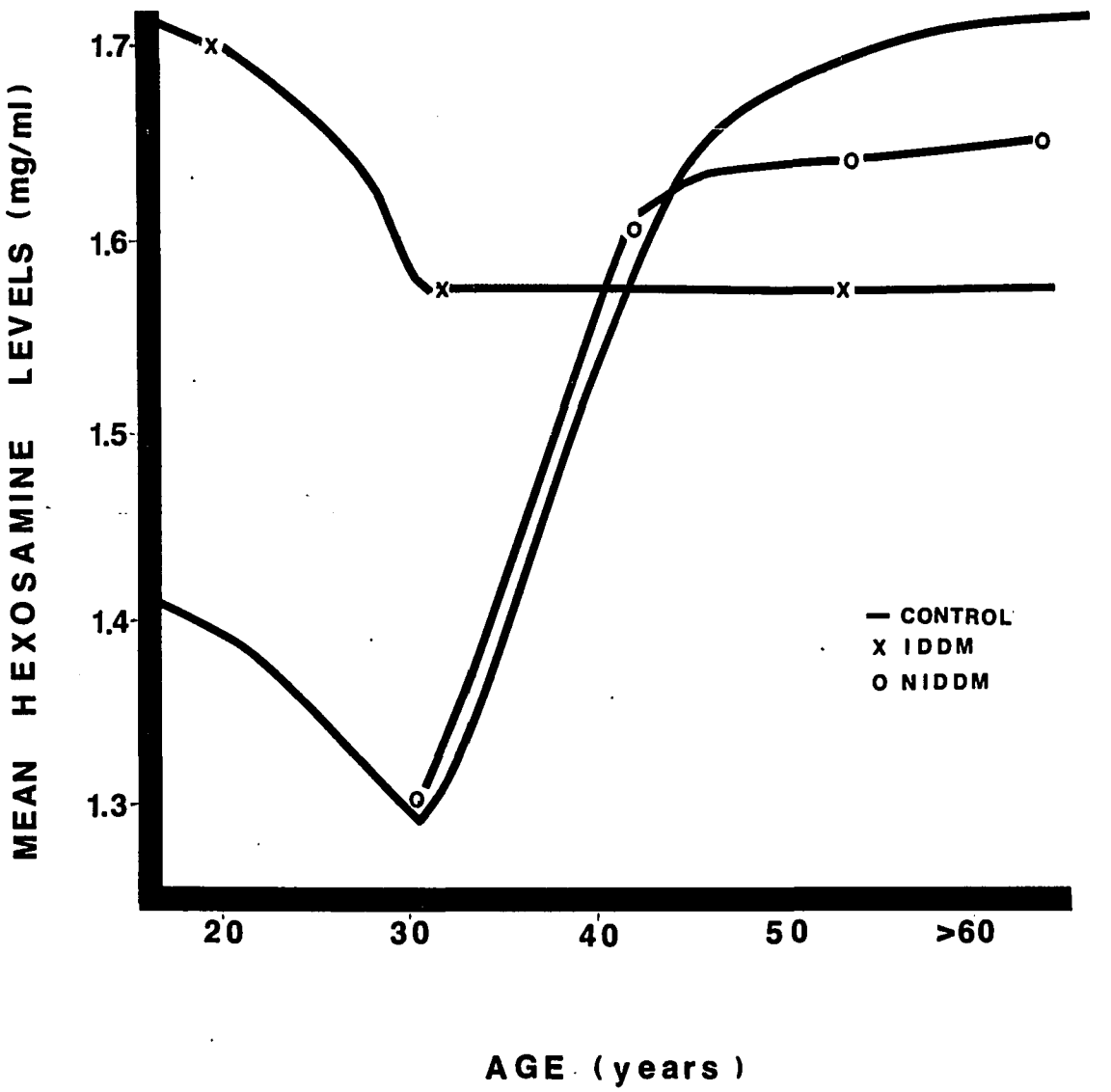


Figure 2. plasma protein-bound hexosamine levels

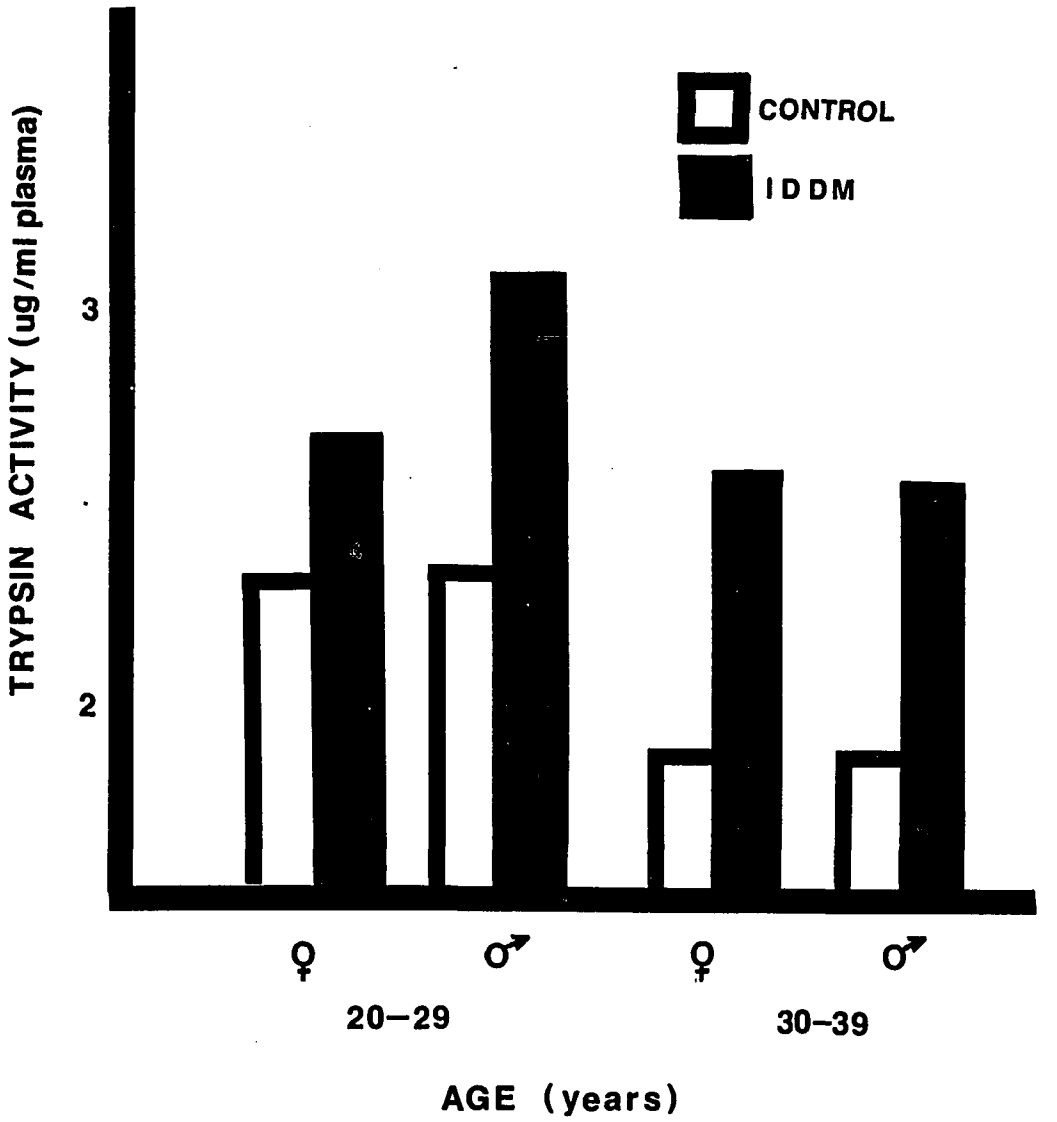


Figure 3. α_2-macroglobulin levels (IDDM)

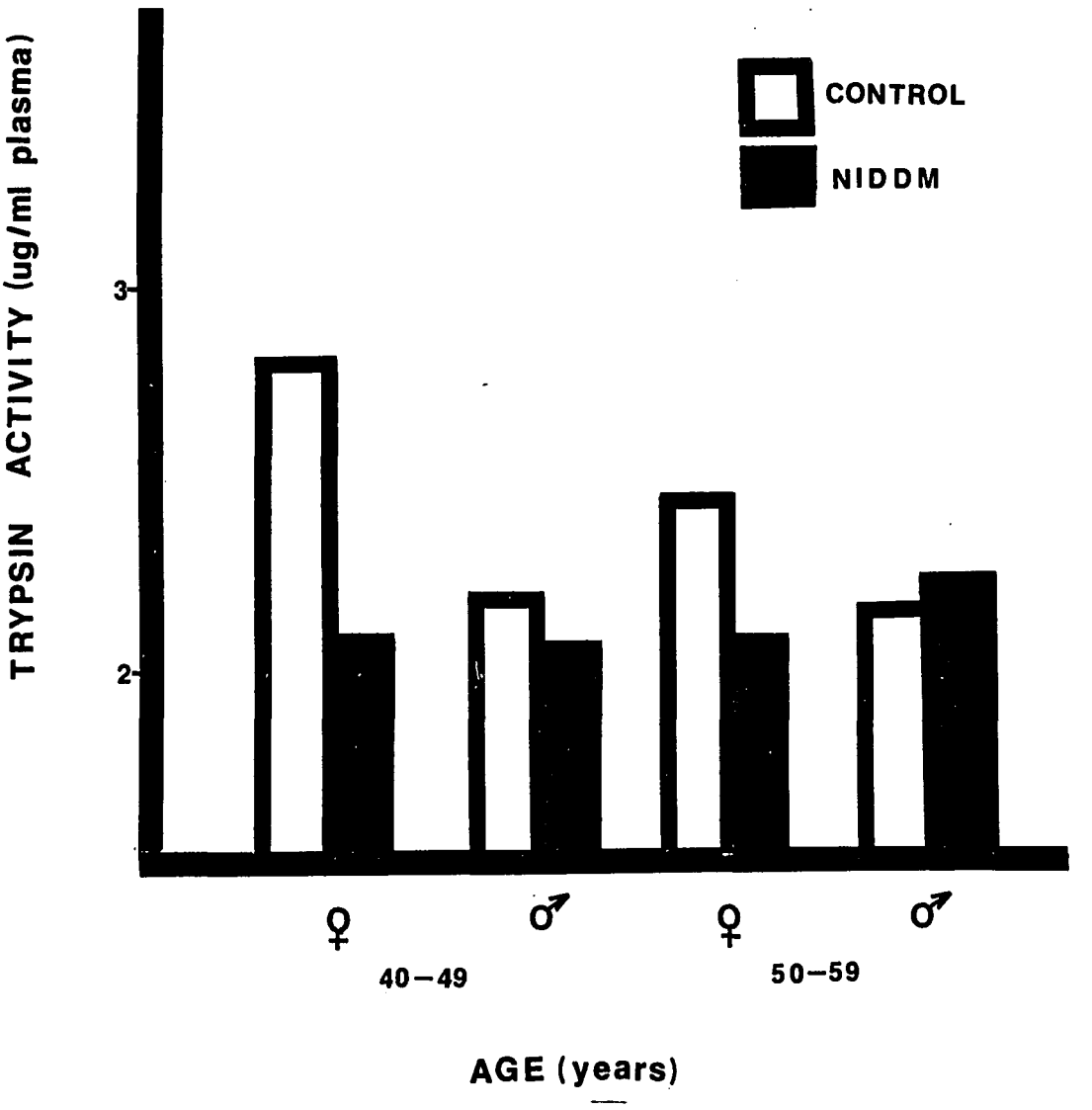


Figure 4. α_2-macroglobulin levels (NIDDM)

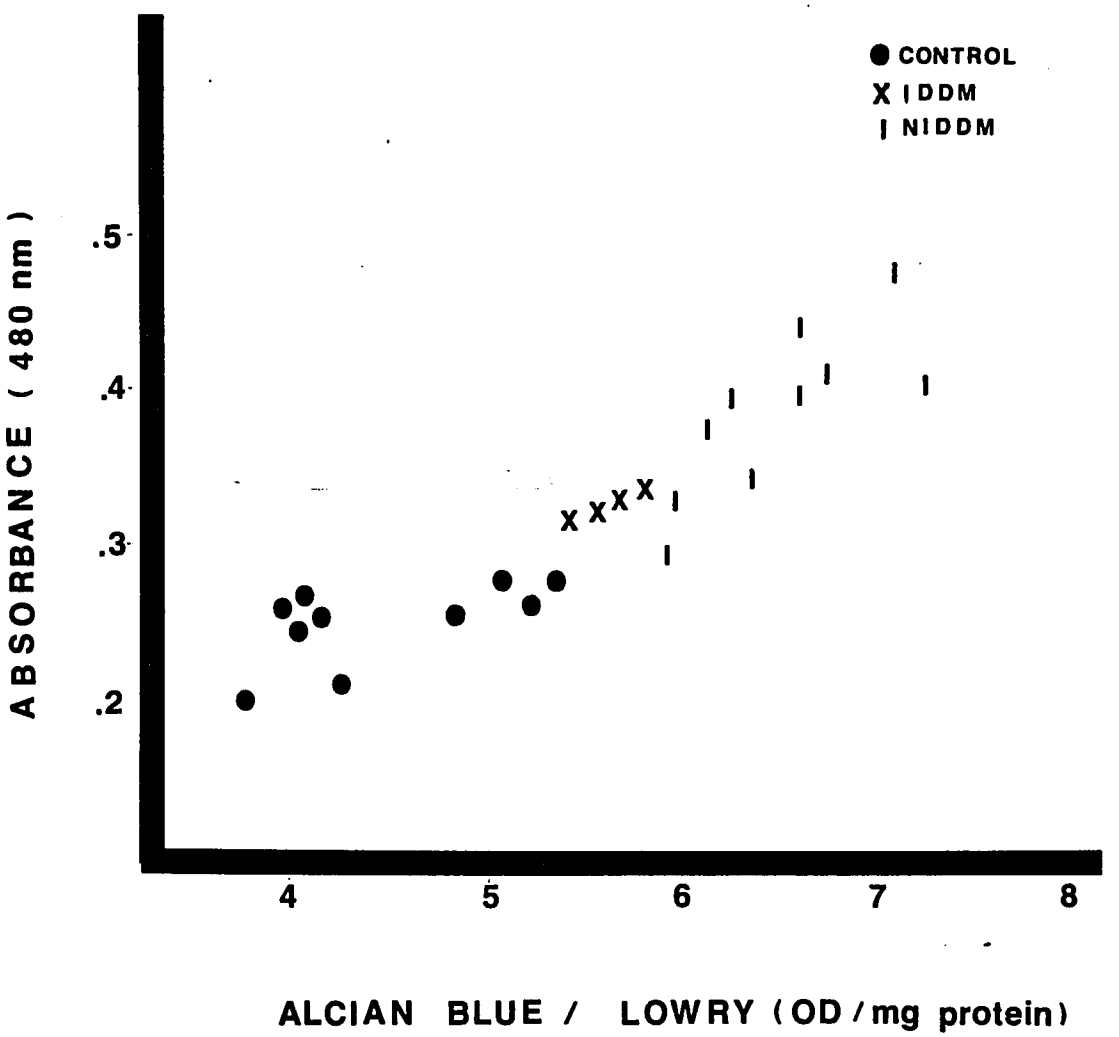


Figure 5. Alcian blue result

NIDDM plasma did not differ significantly from the age matched control plasma in the amount of positive staining alcian blue material (Figure VI).

The alcian blue positive material did not correlate with duration of disease, short term control of disease as measured by fasting blood glucose levels, or long term control of the disease as measured by glycosylated hemoglobulin studies. Serum triglycerides, cholesterol, HDL, LDL, and VLDL levels also showed no direct linear correlation with the alcian blue positive staining material (Appendix B).

One individual who was in the control group had an elevated alcian blue test. Clinically, this individual was normal for all assayed parameters except total urinary protein. Other individuals with excessively elevated alcian blue tests tended to have increased urinary protein levels. However, the sample size of these individuals was low and this could not be shown to be statistically significant.

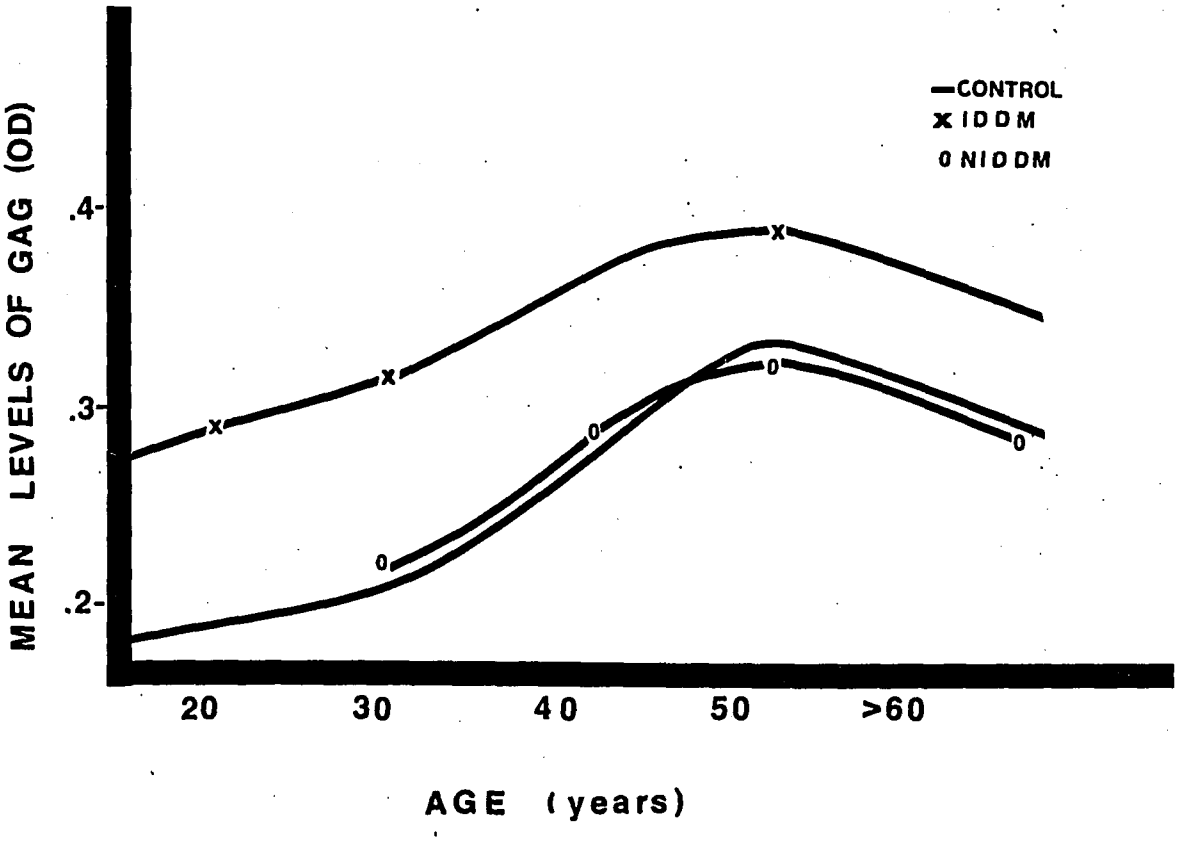


Figure 6. Mean plasma alcian blue assay results

CHAPTER V
DISCUSSION

The study of diabetes is complicated by the heterogenety of the syndrome. Prior to 1979, no systematic standards of nomemclature existed. This complicated the literature and made comparisons of independent studies almost impossible. In 1979 the World Health Organization established a National Data Group to set standards for the classification of diabetes and other glucose intolerant syndromes into uniform and mutually exclusive categories. Recent efforts by researchers to control for type of diabetes in the design of experiments has helped to clarify some of the discrepancies of interest in diabetes research.

This project controlled for type of diabetes, control of disease as measured by fasting blood glucose and glycosylated hemoglobin, family history, treatment of disease, age and sex. It was found age and type of diabetes must be controlled for in the selection of controls for the analysis of plasma glycosaminoglycans. No difference between the sexes was demonstrated. Analysis of plasma α_2-macroglobulin levels show age, sex, and type of diabetes to be contributing parameters and therefore must be included in the experimental design.

The GAG content of diabetic and normal plasma was of particular interest because of the preliminary results. The alcian blue assay method was reported as an easy, reproducible spectrophotometric method for the assessment of GAG content of biological fluids. Our preliminary results indicated elevated levels of alcian blue positive staining material in diabetic plasma. Further work was needed to

investigate this increase with the proper controls and to determine if this increase was a result of a GAG.

Three methods of GAG analysis were preformed, the alcian blue assay, protein-bound hexosamine, and the total GAG isolate method. Of these three methods the alcian blue assay and the protein-bound hexosamine assay were found to correlate. The total GAG isolate method did not correlate with either the alcian blue assay or the protein-bound hexosamine assay. All three tests are individually consistant and highly repeatable.

The correlation of the alcian blue assay and the protein-bound hexosamine is expected if these assays are measuring plasma GAG. The lack of correlation with the total GAG isolate method can be explained by several hypothesis, the alcian blue assay is not measuring GAG, the GAG isolate technique involves loss of sample, or the alcian blue assay measures proteoglycans and is a function of the configuration of the proteoglycan and not the amount of the proteoglycan.

The alcian blue assay and the protein-bound hexosamine both are performed in a single test tube and loss of any GAG would be minimized. The total GAG isolate method takes 11 days to complete and involves multiple transfers of the sample between vessels. The opportunity for loss does exist, the dialysis step and the treatment with trichloroacetic acid are of particular concern. If any of the GAG remains protein-bound following protease digestion this fraction would be precipitated out with the trichloroacetic acid treatment.

The alcian blue test must be performed on fresh plasma to give consistant results. Freezing significantly alters the absorbance at 480 nm. Results of this assay detect more GAG to be present then is known to exist in plasma. This amplifaction was shown to be due to the presence of protein. Digestion of plasma with protease reduces the absorbance by 75%. Digestion of plasma with ABCase reduces the absorbance by 25%. Neither protease nor ABCase react with the dye mixture to cause a change in absorbance. If plasma is first digested with ABCase and then digested with protease the absorbance approaches zero. These finding are consistant with the fact that most GAG do not occur as free GAG but as protein-bound GAG in the body.

The glycosaminoglycan content of human plasma has been analyzed by different researchers by a variety of methods. Of the many electrophoretic methods tested the method of Hata and Nagai gave best results for the resolution of glycosaminoglycans. This method involves two-dimensional electrophoresis and is time consuming. Good results were also obtained by the method of Breen. This method also removed nucleic acids that contaminate the electrophoresis analysis. The anionic exchange column method gave inconsistant results and variable repeatability and was therefore abandoned. Several buffer systems were tried and best results were obtained with the pyridine-formic acid system, as this gave good resolution between the CS and HA. However, if a DS component is present it aligns with the chondroitin isomers, causes smearing of the bands and difficulty differentiating the GAG species.

Our results of the alcian blue assay show an age and type of diabetes dependency. Normal plasma levels of alcian blue positive staining material increase with age. IDDM plasma levels resemble those of there older diabetic counterparts. This is consistant with the hypothesis that IDDM is a disease that contributes to premature aging. NIDDM plasma does not differ significantly from the age and sex matched controls.

One individual in the control group was found to have a highly elevated alcian blue test. This patient was clinically normal in all parameters except total urinary protein. Other individuals with excessively increased alcian blue tests also demonstrated a tendency toward higher total urinary protein levels, this was not statistically significant. This is suggestive of role for GAG in renal metabolism. The control of proteins in the kidney is determined by anionic charges in the glomerular capsule. GAG have been demonstrated to be a component of this system. Serum levels of GAG may be a reflection of glomerular changes in the kidney. To investigate this possibility an analysis of total urinary protein and plasma GAG levels over time may demontrate if a relationship exists.

In conclusion, the findings of this project show IDDM plasma to contain increased levels of alcian blue positive staining material. This material is most likely a protein-bound GAG as shown by selective digestion experiments and by correlation with the protein-bound hexosamine assay. NIDDM shows no significant difference in the alcian blue positive staining material from the age and sex matched controls.

The experimental design must control for age, sex and type of diabetes, as these parameters contribute significantly to the variables measured. Also a new parameter to investigate is that of kidney function as assayed by total urinary protein.

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APPENDIX A

EXPERIMENTAL DESIGN

Volunteers-eight groups of five volunteers each except group two had six volunteers.

SUBJECT CHARACTERISTICS

Group	Number	Diabetes	Type	Sex	Age
1	5	yes	IDDM	Male	18-35
2	6	yes	IDDM	Female	18-35
3	5	no	-	Male	18-35
4	5	no	-	Female	18-35
5	5	yes	NIDDM	Male	≥ 30
6	5	yes	NIDDM	Female	≥ 30
7	5	no	-	Male	≥ 30
8	5	yes	-	Female	≥ 30

Volunteer selection among diabetics was done to achieve a balance between the sexes with relationship to duration of disease. Volunteer selection among non-diabetics was performed to select for age to match the age of the diabetics.

APPENDIX B

INFORMED CONSENT

Purpose

This study will examine blood and urine from diabetics and non-diabetics for possible differences that may increase scientific knowledge concerning development of atherosclerosis (hardening of the arteries) in diabetics and non-diabetics.

General Information

Diabetics have a greater than average risk of having problems that involve their blood vessels (vascular disease). Certain substances involved in transporting lipids ("fats", such as cholesterol and triglyceride) in the blood play a role in the development of such vascular diseases as atherosclerosis (large blood vessels) and micro-angiopathy (small blood vessels). This study will investigate these substances and may increase our knowledge of diabetes and its vascular complications.

Study Drug

None. You will not be asked to take any medication other than what you normally require. You are specifically required to refrain from any other medications for 7 days before the bood and urine tests.

Study Procedures

You will be asked to come to BCIU for two visits. On the first visit you will undergo brief physical examination, an electrocardiogram, and measurement of blood pressures in the legs. You will also receive instructions and containers for a 24 hour urine collection to be done just prior to the second visit. For the second visit you will be asked

to refrain from any food or beverage other than water from 8:00 P.M. the previous evening. You will be asked to refrain from any alcohol for two days before the visit, to collect your urine for 24 hours and to come to the clinic where blood will be drawn. You will then be free to go. If your blood or urine tests are unsuccessful, you may be asked to return for a second blood and urine collection.

Risk and Benefit

Your risk is that of the venipuncture which may include bleeding or fainting. This entails possible pain and bruising and the needles site. The total blood drawn is expected to be 1-1/2 ozs but could be 3 ozs. if we needed to repeat the tests. There is no personal medical benefit to you as a volunteer. The potential benefit is to the science and medical knowledge of diabetes and its complications.

Volunteer Statement

I acknowledge that I have been given an opportunity to ask questions regarding this research study and that these questions have been answered to my satisfaction.

In giving my consent, I acknowledge that my participation in this research project is voluntary and that I may withdraw at any time. This study is of no benefit to me.

I hereby authorize Bronson Methodist Hospital Clinical Investigational Unit to release the information obtained in this research study to medical science literature. I understand that I will not be identified by name.

In the event of physical injury resulting from the research procedures, BCIU will provide or arrange to provide for all necessary medical care

to help me recover promptly at no expense to me. BCIU does not commit itself to provide any compensation in addition to medical care. Further information may be obtained from R.M. DeHaan, M.D., BCIU Medical Director.

Volunteer	Date
Investigator	Date
Witness	Date

3/12/81

APPENDIX C
TABLE I
GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
THE DESCRIPTIVE STATISTICS OF EACH GROUP
GROUP 1

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE
AGE	5	28.400	5.030	23.000	34.000
DUR	5	15.600	4.393	11.000	22.000
ABLUE	5	0.339	0.047	0.283	0.410
GLUCOSE	5	306.000	91.304	196.000	434.000
HBA1C	5	10.704	1.767	8.240	12.680
TG	5	117.000	23.558	81.000	142.000
CHOL	5	212.000	36.729	165.000	249.000
HDL	5	45.400	7.301	35.000	55.000
LDL	5	139.600	32.485	97.000	180.000
VLDL	5	24.800	5.167	18.000	30.000
NAG	5	97.000	55.745	47.000	186.000
TPR	5	751.600	1233.452	45.000	2919.000
TGLUC	5	84.600	43.793	10.000	118.000
CCC	5	88.600	28.945	42.000	122.000
HEXO	5	1.650	0.086	1.540	1.760
LOWRY	5	67.220	3.745	63.000	71.500
GROUP-2					
AGE	6	31.500	11.640	23.000	54.000
DUR	6	13.667	7.941	3.000	24.000
ABLUE	6	0.325	0.058	0.232	0.405
GLUCOSE	6	216.333	59.752	144.000	306.000
HBA1C	6	11.463	3.425	7.210	17.470
TG	6	95.167	56.130	48.000	203.000
CHOL	6	190.167	20.400	155.000	218.000
HDL	6	55.000	8.556	45.000	66.000
LDL	6	115.000	19.950	92.000	150.000
VLDL	6	18.333	8.756	9.000	33.000
NAG	6	52.833	29.862	15.000	89.000
TPR	6	168.000	225.528	26.000	618.000
TGLUC	6	68.167	95.936	1.000	256.000
CCC	6	92.833	41.701	39.000	139.000
HEXO	6	1.535	0.130	1.440	1.790
LOWRY	6	65.683	6.097	54.000	70.800

TABLE 2 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
THE DESCRIPTIVE STATISTICS FOR EACH GROUP
GROUP 3

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE
AGE	5	28.600	5.983	23.000	36.000
ABIUE	5	0.234	0.031	0.194	0.266
GLUCOSE	5	93.800	9.203	78.000	101.000
HBA1C	5	5.038	0.347	4.660	5.370
TG	5	70.600	21.276	40.000	93.000
CHOL	5	192.000	30.042	154.000	227.000
HDL	5	59.400	4.722	53.000	64.000
LDL	5	114.200	35.280	74.000	157.000
VLDL	5	13.600	6.841	6.000	24.000
NAG	5	20.800	6.261	15.000	31.000
TPR	5	26.600	14.690	12.000	46.000
TGLUC	5	0.740	0.329	0.400	1.100
CCC	5	104.400	18.284	76.000	122.000
HEXO	5	1.342	0.133	1.190	1.540
LOWRY	5	65.900	3.808	61.300	70.200
GROUP 4					
AGE	5	24.800	5.357	20.000	33.000
ABIUE	5	0.300	0.061	00.246	0.396
GLUCOSE	5	90.400	4.615	84.000	97.000
HBA1C	5	4.688	0.225	4.350	4.940
TG	5	48.600	9.072	39.000	62.000
CHOL	5	171.200	14.516	159.000	196.000
HDL	5	54.400	7.537	46.000	63.000
LDL	5	96.400	16.637	81.000	123.000
VLDL	5	12.200	1.483	10.000	14.000
NAG	5	31.400	11.760	21.000	51.000
TPR	5	90.200	116.158	7.000	294.000
TGLUC	5	0.240	0.358	0.000	0.800
CCC	5	106.000	20.952	81.000	137.000
HEXO	5	1.370	0.121	1.230	1.510
LOWRY	5	67.400	1.751	64.800	69.300

TABLE 3 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
THE DESCRIPTIVE STATISTICS FOR EACH GROUP
GROUP 5

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE
AGE	5	58.800	9.230	44.000	66.000
DUR	5	10.600	5.771	5.000	17.000
ABLUE	5	0.320	0.037	0.279	0.368
GLUCOSE	5	166.400	53.482	103.000	251.000
HBA1C	5	8.696	2.180	7.100	12.480
TG	5	204.000	192.546	87.000	541.000
CHOL	5	220.000	36.528	183.000	272.000
HDL	5	46.000	9.950	31.000	55.000
LDL	5	139.400	23.565	118.000	179.000
VLDL	5	40.800	29.727	22.000	93.000
NAG	5	60.400	20.635	45.000	83.000
TPR	5	88.000	115.030	23.000	293.000
TGLUC	5	3.200	2.799	0.600	7.300
CCC	5	105.800	28.613	61.000	130.000
HEXO	5	1.616	0.198	1.330	1.840
LOWRY	5	57.860	2.045	54.700	60.000
---GROUP-6---					
AGE	5	48.400	5.771	42.000	55.000
DUR	5	4.600	3.209	2.000	10.000
ABLUE	5	0.339	0.042	0.292	0.405
GLUCOSE	5	178.200	123.210	60.000	317.000
HBA1C	5	7.894	2.939	5.010	11.410
TG	5	173.400	99.158	85.000	329.000
CHOL	5	227.800	51.041	172.000	284.000
HDL	5	59.800	20.981	44.000	96.000
LDL	5	141.400	33.193	98.000	180.000
VLDL	5	33.400	20.981	14.000	68.000
NAG	5	119.200	192.766	14.000	463.000
TPR	5	206.600	201.455	18.000	519.000
TGLUC	5	21.360	35.260	0.800	82.800
CCC	5	156.200	132.538	77.000	392.000
HEXO	5	1.594	0.094	1.460	1.710
LOWRY	5	61.940	4.424	57.400	68.500

TABLE 4 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
THE DESCRIPTIVE STATISTICS FOR EACH GROUP
GROUP 7

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE
AGE	5	58.400	8.562	47.000	66.000
ABLUE	5	0.310	0.047	0.256	0.376
GLUCOSE	5	97.400	14.977	80.000	121.000
HBA1C	5	5.424	0.335	5.020	5.930
TG	5	152.400	78.600	71.000	275.000
CHOL	5	255.400	49.908	174.000	308.000
HDL	5	51.000	5.292	44.000	58.000
LDL	5	176.000	47.429	100.000	228.000
VLDL	5	32.600	8.204	21.000	44.000
NAG	5	34.200	22.532	8.000	69.000
TPR	5	29.000	11.554	11.000	39.000
TGLUC	5	0.240	0.230	0.000	0.600
LOWRY	5	56.980	1.983	54.400	59.600
CCC	5	92.600	4.393	88.000	99.000
HEXO	5	1.706	0.235	1.380	1.980

GROUP 8

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE
AGE	5	48.800	5.263	44.000	55.000
ABLUE	5	0.334	0.055	0.273	0.395
GLUCOSE	5	89.400	6.465	82.000	98.000
HBA1C	5	5.174	0.310	4.770	5.540
TG	5	101.000	24.566	66.000	127.000
CHOL	5	223.600	28.298	196.000	264.000
HDL	5	65.600	9.209	58.000	81.000
LDL	5	145.000	34.329	97.000	180.000
VLDL	5	20.400	4.450	13.000	25.000
NAG	5	44.600	21.801	22.000	72.000
TPR	5	114.600	122.627	41.000	331.000
TGLUC	5	0.660	0.594	0.000	1.500
CCC	5	107.800	28.244	78.000	148.000
HEXO	5	1.616	0.255	1.250	1.900
LOWRY	5	61.920	3.448	58.000	67.200

TABLE 5 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
THE DESCRIPTIVE STATISTICS FOR EACH GROUP
GROUP - ALL DIABETICS

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE
AGE	21	41.286	14.907	23.000	66.000
DUR	21	11.238	6.782	2.000	24.000
ABLUE	21	0.331	0.045	0.232	0.410
GLUCOSE	21	216.714	95.733	60.000	434.000
HBA1C	21	9.774	2.912	5.010	17.470
TG	21	144.905	110.873	48.000	541.000
CHOL	21	211.524	37.210	155.000	284.000
HDL	21	51.714	13.210	31.000	96.000
LDL	21	132.952	27.892	92.000	180.000
VLDL	21	28.810	19.156	9.000	93.000
HMG	21	80.952	95.573	14.000	463.000
TPR	21	297.000	630.309	18.000	2919.000
TGLUC	21	45.467	63.750	0.600	256.000
CCC	21	110.000	70.853	39.000	392.000
HEXO	21	1.596	0.131	1.330	1.840
LOWRY	21	63.295	5.500	54.000	71.500

TABLE 6 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
THE DESCRIPTIVE STATISTICS FOR EACH GROUP
GROUP - MALE DIABETES

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE
AGE	10	43.600	17.488	23.000	66.000
DUR	10	13.100	5.507	5.000	22.000
ABLUE	10	0.330	0.041	0.279	0.410
GLUCOSE	10	236.200	101.930	103.000	434.000
HBA1C	10	9.700	2.150	7.100	12.680
TG	10	160.500	137.210	81.000	541.000
CHOL	10	216.200	34.816	165.000	272.000
HDL	10	45.700	8.233	31.000	55.000
LDL	10	139.500	26.755	97.000	180.000
VLDL	10	32.800	21.811	18.000	93.000
NAG	10	78.700	44.073	45.000	186.000
TPR	10	419.600	896.792	23.000	2919.000
TGLUC	10	43.900	51.927	0.600	118.000
CCC	10	97.200	28.608	42.000	130.000
HEXO	10	1.633	0.145	1.330	1.840
LOWRY	10	62.540	5.695	54.700	71.500

TABLE 7 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
THE DESCRIPTIVE STATISTICS FOR EACH GROUP
GROUP - FEMALE DIABETICS

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE
AGE	11	39.182	12.608	23.000	55.000
DUR	11	9.545	7.621	2.000	24.000
ABLUE	11	0.331	0.049	0.232	0.405
GLUCOSE	11	199.000	90.852	60.000	317.000
HBA1C	11	9.841	3.577	5.010	17.470
TG	11	130.727	84.720	48.000	329.000
CHOL	11	207.273	40.453	155.000	284.000
HDL	11	57.182	14.797	44.000	96.000
LDL	11	127.000	28.806	92.000	180.000
VLDL	11	25.182	16.594	9.000	68.000
NAG	11	83.000	128.494	14.000	463.000
TPR	11	185.545	205.113	18.000	618.000
TGLUC	11	46.891	75.476	0.800	256.000
CCC	11	121.636	94.821	39.000	392.000
HEXO	11	1.562	0.114	1.440	1.790
LOWRY	11	63.982	5.499	54.000	70.800

TABLE 8 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
THE DESCRIPTIVE STATISTICS FOR EACH GROUP
GROUP - NON-DIABETES

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE
AGE	20	40.150	15.469	20.000	66.000
ABLUE	20	0.295	0.059	0.194	0.396
GLUCOSE	20	92.750	9.419	78.000	121.000
HGA1C	20	5.081	0.393	4.350	5.930
TG	20	93.150	55.711	39.000	275.000
CHOL	20	210.550	44.716	154.000	308.000
HDL	20	57.600	8.500	44.000	81.000
LDL	20	132.900	44.855	74.000	228.000
VLDL	20	19.700	9.857	6.000	44.000
NAG	20	32.750	17.885	8.000	72.000
TTR	20	65.100	87.312	7.000	331.000
TGLUC	20	0.470	0.438	0.000	1.500
CCC	20	102.700	19.290	76.000	148.000
HEXO	20	1.508	0.240	1.190	1.980
LOWRY	20	63.050	4.917	54.400	70.200

TABLE 9 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
THE DESCRIPTIVE STATISTICS FOR EACH GROUP
GROUP - MALE NON-DIABETES

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE
AGE	10	43.500	17.180	23.000	66.000
ABLUE	10	0.272	0.055	0.194	0.376
GLUCOSE	10	95.600	11.872	78.000	121.000
HBA1C	10	5.231	0.381	4.660	5.930
TG	10	111.500	68.822	40.000	275.000
CHOL	10	223.700	51.232	154.000	308.000
HDL	10	55.200	6.477	44.000	64.000
LDL	10	145.100	51.126	74.000	228.000
VLDL	10	23.100	12.288	6.000	44.000
NAG	10	27.500	17.116	8.000	69.000
TPR	10	27.800	12.524	11.000	46.000
TGLUC	10	0.490	0.375	0.000	1.100
CCC	10	98.500	13.994	76.000	122.000
HEXO	10	1.524	0.263	1.190	1.980
LOWRY	10	61.440	5.504	54.400	70.200

TABLE 10 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
THE DESCRIPTIVE STATISTICS FOR EACH GROUP
GROUP - FEMALE NON-DIABETES

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE
AGE	10	36.900	13.604	20.000	55.000
ABLUE	10	0.317	0.058	0.246	0.396
GLUCOSE	10	89.900	5.322	82.000	98.000
HBA1C	10	4.931	0.362	4.350	5.540
TG	10	74.800	32.673	39.000	127.000
CHOL	10	197.400	34.818	159.000	264.000
HDL	10	60.000	9.888	46.000	81.000
LDL	10	120.700	36.096	81.000	180.000
VLDL	10	16.300	5.334	10.000	25.000
MAG	10	38.000	17.920	21.000	72.000
TPR	10	102.400	113.337	7.000	331.000
TGLUC	10	0.450	0.513	0.000	1.500
CCC	10	106.900	23.464	78.000	148.000
HEXO	10	1.493	0.513	1.230	1.900
LOWRY	10	64.660	3.872	58.000	69.300

TABLE 11 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP-1

PEARSON CORRELATION COEFFICIENTS / PROB !R! UNDER HO: RHO=0 / N=5

	ABLUE	HEXO	LOWRY
AGE	0.35230 0.5609	-0.01155 0.9853	0.16005 0.7971
DUR	0.11106 0.8589	-0.08600 0.8906	0.68435 0.2025
ABLUE	1.00000 0.0000	0.52911 0.3592	0.78539 0.1154
GLUCOSE	-0.24272 0.6940	0.36827 0.5419	-0.13156 0.4681
HBA1C	0.12295 0.8438	-0.32392 0.5949	-0.46352 0.4317
TG	-0.25192 0.6827	0.20601 0.7984	-0.65282 0.2324
CHOL	0.51438 0.3752	0.15905 0.7984	0.46635 0.4285
HDL	0.60435 0.2805	0.53342 0.3546	0.55736 0.3290
LDL	0.47200 0.4221	0.00448 0.9943	0.53063 0.3576
VLDL	0.09728 0.8763	0.46119 0.4343	-0.40796 0.4954
MAG	0.78931 0.1124	0.45461 0.4418	0.24344 0.6931
[illegible]PR	0.81659 0.0917	0.67277 0.2133	0.35153 0.5618
TGLUC	-0.46645 0.4284	-0.33646 0.5796	-0.82303 0.0870
CCC	-0.79821 0.1055	-0.75203 0.1426	-0.65947 0.2260
HEXO	0.52911 0.3592	1.00000 0.0000	0.44928 0.4478
LOWRY	0.78539 0.1154	0.44928 0.4478	1.00000 0.0000

TABLE 12 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP-2

PEARSON CORRELATION COEFFICIENTS / PROB |R| UNDER HO: RHO=0 / N=6

	ABLUE	HEXO	LOWRY
AGE	0.66435 0.1501	-0.01911 0.9713	-0.89998 0.0145
DUR	0.67749 0.1392	-0.06956 0.8958	-0.62843 0.1814
ABLUE	1.00000 0.0000	-0.63467 0.1758	-0.45302 0.3670
GLUCOSE	-0.45818 0.3608	0.19850 0.7062	0.10192 0.8477
HBA1C	-0.02888 0.9452	-0.08435 0.8738	-0.06301 0.9056
TG	0.03654 0.9452	-0.17345 0.7424	-0.06808 0.8980
CHOL	0.35236 0.4933	-0.16284 0.7579	-0.18763 0.7219
IDL	-0.28324 0.5865	0.73888 0.0934	-0.64262 0.1688
LDL	0.55139 0.2567	-0.21612 0.6809	-0.21294 0.6854
VLDL	0.47996 0.3353	-0.62560 0.1840	0.01773 0.9734
NAG	0.48084 0.3343	0.01259 0.9811	-0.54478 0.2637
TPR	0.12201 0.8179	-0.10933 0.8367	0.29718 0.5673
TGLUC	-0.20783 0.6927	-0.12387 0.8151	0.13983 0.7916
CCC	0.16370 0.7566	-0.37991 0.4576	0.48622 0.3281
HEXO	-0.63467 0.1758	1.00000 0.0000	-0.32705 0.5269
LOWRY	-0.45302 0.3670	-0.32705 0.5269	1.00000 0.0000

TABLE 13 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP-3

PEARSON CORRELATION COEFFICIENTS / PROB !R! UNDER HO: RHO=0 / N=5

	ABLUE	HEXO	LOWRY
AGE	-0.38632 0.5206	-0.78548 0.1154	-0.85697 0.0635
ABLUE	1.00000 0.0000	0.77729 0.1219	-0.08141 0.8965
GLUCOSE	0.87115 0.0544	0.43445 0.4648	-0.50649 0.3839
HBA1C	0.01959 0.9751	-0.60510 0.2796	-0.89169 0.0421
TG	0.82589 0.0849	0.81604 0.0921	0.14447 0.8167
CHOL	0.75445 0.1406	0.25220 0.6823	-0.52515 0.3635
HDL	-0.72643 0.1645	-0.32724 0.5909	0.42265 0.4784
LDL	0.63890 0.2459	0.11206 0.8576	-0.56236 0.3237
VLDL	0.76568 0.1313	0.87286 0.0534	0.21881 0.7236
NAG	-0.52753 0.3609	-0.08627 0.8903	0.51172 0.3781
TPR	0.51136 0.3785	0.74607 0.1476	0.68960 0.1976
TGLUC	-0.73024 0.1612	-0.27620 0.6529	0.40555 0.4982
CCC	0.80416 0.1009	0.83965 0.0752	0.25566 0.6781
HEXO	0.77729 0.1219	1.00000 0.0000	0.52994 0.3583
LOWRY	-0.08141 0.8965	0.52994 0.3583	1.00000 0.0000

TABLE 14 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP-4

PEARSON CORRELATION COEFFICIENTS / PROB !R! UNDER HO:RHO=0 / N=5

	ABLUE	HEXO	LOWRY
AGE	-0.30101 0.6226	-0.20434 0.7416	-0.43981 0.4386
ABLUE	1.00000 0.0000	0.83590 0.0778	0.71019 0.1789
GLUCOSE	-0.79333 0.1092	-0.63998 0.2448	-0.26919 0.6614
HBA1C	-0.00123 0.9984	0.52905 0.3593	0.56706 0.3188
TG	-0.70985 0.1792	-0.46674 0.4281	-0.04879 0.9379
CHOL :	-0.21054 0.7339	-0.17787 0.7747	0.35612 0.5563
HDL	-0.92318 0.0253	-0.81670 0.0916	-0.65748 0.2279
LDL	0.07478 0.9049	0.07573 0.9037	0.57335 0.3122
VLDL	0.32991 0.5877	0.27851 0.6500	0.39473 0.5108
NAG	-0.08454 0.8925	-0.28980 0.6363	0.21978 0.7224
TPR	0.92311 0.0253	0.69331 0.1942	0.69962 0.1885
TGLUC	-0.59641 0.2884	-0.43876 0.4598	0.03193 0.9594
CCC	0.84470 0.0717	0.84187 0.0737	0.55750 0.3289
HEXO	0.83590 0.0778	1.00000 0.0000	0.85417 0.0654
LOWRY	0.71019 0.1789	0.85417 0.0654	1.00000 0.0000

TABLE 15 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP-5

PEARSON CORRELATION COEFFICIENTS / PROB !R! UNDER HO: RHO=0 / N=5

	ABLUE	HEXO	LOWRY
AGE	-0.20551 0.7402	0.15574 0.8025	-0.62029 0.2643
DUR	0.34997 0.5637	0.73506 0.1570	0.06609 0.9159
ABLUE	1.00000 0.0000	0.58232 0.3029	0.43042 0.4694
GLUCOSE	-0.62056 0.2640	-0.45056 0.4464	0.26051 0.6721
HBA1C	-0.50937 0.3807	-0.33648 0.5789	0.17789 0.7747
TG	0.37652 0.5322	0.22897 0.7110	0.60208 0.2826
CHOL	0.30912 0.6128	0.47800 0.4145	0.47812 0.4153
HDL	-0.64589 0.2391	-0.60666 0.2780	-0.62286 0.2617
LDL	-0.05123 0.9348	0.76566 0.1313	0.08289 0.8946
VLDL	0.34771 0.5664	0.16500 0.7909	0.55124 0.3355
NAG	-0.71128 0.1779	-0.15037 0.8093	-0.60493 0.2797
TPR	-0.32098 0.5985	-0.22750 0.7129	0.32219 0.5970
TGLUC	-0.73426 0.1577	-0.63100 0.2341	-0.20525 0.7405
CCC	0.4339 0.4653	-0.02185 0.9722	-0.46625 0.4286
HEXO	0.58232 0.3029	1.00000 0.0000	0.35821 0.3281
LOWRY	0.43042 0.4694	0.55821 0.3281	1.00000 0.0000

TABLE 16 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX

GROUP-6

PEARSON CORRELATION COEFFICIENTS / PROB > !R! UNDER HO: RHO=0 / N=5

	ABLUE	HEXO	LOWRY
AGE	-0.34604 0.5684	0.28677 0.6399	0.83940 0.0754
DUR	0.34497 0.5696	0.77758 0.1216	0.69162 0.1958
ABLUE	1.00000 0.0000	0.72102 0.1693	0.00292 0.9963
GLUCOSE	-0.19354 0.7551	0.37412 0.5350	0.78856 0.1129
HBA1C	-0.42017 0.4812	0.10187 0.8705	0.63901 0.2458
TG	0.02906 0.9630	0.28124 0.6467	0.49809 0.3931
CHOL	-0.20870 0.7362	0.16388 0.7923	0.56622 0.3197
HDL	-0.14282 0.8188	-0.03500 0.9555	0.06367 0.9190
LDL	-0.04955 0.9369	0.30874 0.6132	0.61783 0.2667
VLDL	-0.11684 0.8516	0.11596 0.8527	041299 0.4895
NAG	0.09649 0.8773	0.71211 0.1772	0.86069 0.0621
TPR	0.22932 0.7106	0.10431 0.8674	0.06888 0.9124
TGLUC	0.02451 0.9688	0.67325 0.2129	0.90373 0.0342
CCC	-0.60479 0.2799	-0.83032 0.0817	-0.63631 0.2484
HEXO	0.72102 0.1693	1.00000 0.0000	0.68566 0.2013
LOWRY	0.00292 0.9963	0.68566 0.2013	1.00000 0.0000

TABLE 17 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP-7

PEARSON CORRELATION COEFFICIENTS / PROB !R! UNDER HO:RHO=0 / N = 5

	ABLUE	HEXO	LOWRY
AGE	0.52159 0.3674	-0.05873 0.9253	0.51010 0.3799
ABLUE	1.00000 0.0000	0.63636 0.2484	0.68363 0.2032
GLUCOSE	-0.34775 0.5663	0.40528 0.4985	-0.39448 0.5111
HBA1C	0.94660 0.0147	0.81753 0.0910	0.52222 0.3667
TG	-0.09376 0.8808	0.61819 0.2664	-0.45952 0.4362
CHOL	0.89392 0.0408	0.82426 0.0861	0.33987 0.5757
HDL	-0.39089 0.5153	-0.75088 0.1436	-0.10722 0.8638
LDL	0.89860 0.0382	0.83729 0.0768	0.32909 0.5887
VLDL	0.14740 0.8130	0.75468 0.1404	-0.39097 0.5152
NAG	0.60916 0.2755	0.75329 0.1415	0.32017 0.5994
TPR	-0.00459 0.9942	-0.15857 0.7989	0.63724 0.2475
TGLUC	-0.18891 0.7609	-0.18138 0.7703	-0.20591 0.7397
CCC	0.66156 0.2240	0.43209 0.4675	0.88563 0.0456
HEXO	0.63636 0.2484	1.00000 0.0000	0.11475 0.8542
LOWRY	0.68363 0.2032	0.11475 0.8542	1.00000 0.0000

TABLE 18 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP-8

PEARSON CORRELATION COEFFICIENTS / PROB !R! UNDER HO:RHO=0 / N=5

	ABLUE	HEXO	LOWRY
AGE	0.46009 0.4596	0.84274 0.0731	-0.08099 0.8970
ABLUE	1.00000 0.0000	0.71829 0.1717	-0.01094 0.9861
GLUCOSE	-0.90262 0.0259	-0.76121 0.1349	0.33370 0.5831
HBA1C	0.00316 0.9960	0.08593 0.8907	0.64355 0.2413
TG	0.14226 0.8195	0.48867 0.4035	-0.88118 0.0483
CHOL	0.62478 0.2598	0.79968 0.1043	-0.19306 0.7557
HDL	-0.68058 0.2060	-0.89583 0.0397	0.68208 0.2046
LDL	0.78104 0.1189	0.90207 0.0362	-0.44263 0.4554
VLDL	0.47227 0.4219	0.81002 0.0965	-0.80223 0.1024
NAG	0.53901 0.3486	0.55253 0.3341	-0.41553 0.4866
TER	0.14758 0.8128	0.44339 0.4545	-0.24863 0.6867
TGLUC	0.63203 0.2526	0.32196 0.5973	-0.42169 0.4794
CCC	-0.02982 0.9620	-0.42622 0.4742	-0.25432 0.6797
HEXO	0.71829 0.1717	1.00000 0.0000	-0.44320 0.4348
LOWRY	-0.01094 0.9861	-0.44320 0.4548	1.00000 0.0000

TABLE 19 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETIC AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP - ALL DIABETICS

PEARSON CORRELATION COEFFICIENTS / PROB !R! UNDER HO: RHO=0 / N = 21

	ABLUE	HEXO	LOWRY
AGE	0.08761 0.7057	0.08622 0.7102	-0.72301 0.0002
DUR	0.32804 0.1466	0.22970 0.3165	0.15820 0.4934
ABLUE	1.00000 0.0000	0.14103 0.5420	0.03951 0.8650
GLUCOSE	-0.19079 0.4074	0.14789 0.5223	0.45612 0.0377
HBA1C	-0.15611 0.4992	-0.17109 0.4584	0.31849 0.1594
TG	0.09613 0.6785	0.19554 0.3956	-0.12985 0.5748
CHOL	0.19840 0.3886	0.26049 0.2541	0.03568 0.8780
HDL	-0.09096 0.6950	-0.09066 0.6959	-0.07571 0.7443
LDL	0.27965 0.2196	0.33138 0.1423	0.04836 0.8351
VLDL	0.13020 0.5738	0.11812 0.6101	-0.15451 0.5037
NAG	0.20911 0.3630	0.28004 0.2189	0.26181 0.2516
TPR	0.41286 0.0629	0.24557 0.2833	0.32369 0.1523
TGLUC	-0.13738 0.5526	-0.04217 0.8560	0.40419 0.0692
CCC	-0.19149 0.4057	-0.32269 0.1537	-0.27382 0.2297
HEXO	0.14103 0.5402	1.00000 0.0000	0.06767 0.7707
LOWRY	0.03951 0.8650	0.06767 0.7707	1.00000 0.0000

TABLE 20 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON- DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP-MALE DIABETES

PEARSON CORRELATION COEFFICIENTS / PROB !R! UNDER HO:RHO=0 /N=10

	ABLUE	HEXO	LOWRY
AGE	-0.2114 0.5576	-0.06443 0.8596	-0.83248 0.0028
DUR	0.30635 0.3893	0.50834 0.1335	0.58521 0.0755
ABLUE	1.00000 0.0000	0.50714 0.1346	0.53261 0.1130
GLUCOSE	-0.06762 0.8528	0.03309 0.9277	0.53412 0.1118
HBA1C	-0.03670 0.9198	-0.21640 0.5482	0.34387 0.3306
TG	0.10877 0.7649	0.16287 0.6530	-0.18739 0.6042
CHOL	0.37539 0.2851	0.33275 0.3475	0.11372 0.7544
HDL	-0.04789 0.8955	-0.32451 0.3630	-0.00896 0.9804
LDL	0.31093 0.3810	0.41094 0.2381	0.20339 0.5730
VLDL	0.10822 0.7660	0.11739 0.7467	-0.24325 0.4983
NAG	0.48054 0.1598	0.16333 0.6521	0.42394 0.2221
TPR	0.64914 0.0423	0.27493 0.4420	0.48557 0.1548
TGLUC	-0.01802 0.9606	0.00605 0.9868	0.31106 0.1311
CCC	-0.31407 0.3768	-0.25343 0.4799	-0.54398 0.1041
HEXO	0.50714 0.1346	1.00000 0.0000	0.30687 0.3884
LOWRY	0.53261 0.1130	0.30687 0.3884	1.00000 0.0000

TABLE 21 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP-FEMALE DIABETES

PEARSON CORRELATION COEFFICIENTS / PROB !R! UNDER HO:RHO=0 / N=11

	ABLUE	HEXO	LOWRY
AGE	0.41371 0.2059	0.22255 0.5107	-0.58586 0.0582
DUR	0.36782 0.2657	-0.10148 0.7666	-0.04842 0.8876
ABLUE	1.00000 0.0000	-0.18237 0.5915	-0.34853 0.2935
GLUCOSE	-0.29960 0.3707	0.18281 0.5906	0.45926 0.1553
HBA1C	-0.21363 0.5282	-0.15955 0.6394	0.32077 0.3362
TG	0.09997 0.7699	0.17331 0.6103	-0.00883 0.9794
CHOL	0.08958 0.7934	0.15266 0.6541	0.00475 0.9890
HDL	-0.13875 0.6841	0.27387 0.4151	-0.23715 0.4826
LDL	0.27825 0.4074	0.16121 0.6358	-0.02279 0.9470
VLDL	0.17149 0.6141	-0.02136 0.9712	0.00420 0.9902
NAG	0.15621 0.6465	0.42713 0.1901	0.24947 0.4594
TPR	0.17029 0.6166	-0.00839 0.9805	0.16798 0.6215
TGLUC	-0.20090 0.5536	-0.07390 0.8290	0.34984 0.2916
CCC	-0.19111 0.5735	-0.38414 0.2435	-0.29177 0.3840
HEXO	-0.18237 0.5915	1.00000 0.0000	-0.12159 0.7217
LOWRY	-0.34853 0.2935	-0.12159 0.7217	1.00000 0.0000

TABLE 22 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETIC AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP-NON-DIABETICS

PEARSON CORRELATION COEFFICIENTS / PROB !R! UNDER HO: RHO=0 / N=20

	ABLUE	HEXO	LOWRY
AGE	0.39332 0.0862	0.62192 0.0034	-0.80753 0.0001
ABLUE	1.00000 0.0000	0.69560 0.0007	-0.16491 0.4872
GLUCOSE	-0.27000 0.2496	0.11443 0.6309	-0.28994 0.2150
HBA1C	0.24231 0.3033	0.51645 0.0197	-0.53919 0.0142
TG	0.18503 0.4348	0.69509 0.0007	-0.70524 0.0005
CHOL	0.49227 0.0275	0.77615 0.0001	-0.64631 0.0021
HDL	-0.35949 0.1195	-0.44958 0.0467	0.23800 0.3123
LDL	0.53964 0.0141	0.78844 0.0001	-0.65813 0.0016
VLDL	0.27850 0.0998	0.80550 0.0001	-0.75282 0.0001
NAG	0.52264 0.0181	0.55061 0.0119	-0.18639 0.4314
TPR	0.51190 0.0210	0.26138 0.2656	0.14398 0.5448
TGLUC	-0.15162 0.5234	-0.07947 0.7391	0.07408 0.7563
CCC	0.28921 0.2162	-0.05345 0.8229	0.25441 0.2791
HEXO	0.69560 0.0007	1.00000 0.0000	-0.51357 0.0206
LOWRY	-0.16491 0.4872	-0.51357 0.0206	1.00000 0.0000

TABLE 23 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP-MALE NON-DIABETES

PEARSON CORRELATION COEFFICIENTS / PROB !R! UNDER HO: RHO=0 / N=10

	ABLUE	HEXO	LOWRY
AGE	0.73299 0.0159	0.59356 0.0704	-0.83192 0.0028
ABLUE	1.00000 0.0000	0.84706 0.0020	-0.54288 0.1049
GLUCOSE	0.11629 0.8490	0.39519 0.2583	-0.33693 0.3411
HBA1C	0.71298 0.0206	0.55067 0.0990	-0.63338 0.0493
TG	0.47986 0.1604	0.79015 0.0065	-0.60431 0.0642
CHOL	0.91873 0.0002	0.82746 0.0031	-0.59878 0.0674
HDL	-0.75218 0.0121	-0.79553 0.0059	0.66453 0.0361
LDL	0.89306 0.0005	0.79012 0.0065	-0.61461 0.0587
VLDL	0.73793 0.0148	0.90367 0.0003	-0.70043 0.0241
NAG	0.55996 0.0923	0.68713 0.0281	-0.22740 0.5273
TPR	0.22086 0.5398	0.21277 0.5551	0.25639 0.4742
TGLUC	-0.71483 0.0202	-0.61042 0.0609	0.68836 0.0277
CCC	0.01588 0.9653	-0.02324 0.9492	0.52683 0.1177
HEXO	0.84706 0.0020	1.00000 0.0000	-0.52402 0.1200
LOWRY	-0.54288 0.1049	-0.52402 0.1200	1.00000 0.0000

TABLE 24 GLYCOSAMINOGLYCANS IN HUMAN PLASMA
FROM DIABETICS AND NON-DIABETICS
CORRELATION COEFFICIENTS MATRIX
GROUP-FEMALE NON-DIABETICS

PEARSON CORRELATION COEFFICIENTS / PROB !R! UNDER HO:RHO=0 / N = 10

	ABLUE	HEXO	LOWRY
AGE	0.30810 0.3864	0.67021 0.0340	-0.74085 0.0142
ABLUE	1.00000 0.0000	0.72446 0.0178	-0.08431 0.8169
GLUCOSE	-0.81988 0.0037	-0.64551 0.0438	0.18745 0.6041
HBA1C	0.22047 0.5405	0.51571 0.1271	-0.23915 0.5058
TG	0.21475 0.5513	0.63122 0.0503	-0.89557 0.0005
CHOL	0.42013 0.2267	0.75487 0.0114	-0.62400 0.0538
HDL	-0.41504 0.2330	-0.22171 0.5382	-0.29459 0.4087
LDL	0.55146 0.0984	0.83630 0.0026	-0.64292 0.0450
VLDL	0.46161 0.1793	0.81295 0.0042	-0.84724 0.0020
NAG	0.37252 0.2891	0.50891 0.1330	-0.46076 0.1802
TPR	0.54839 0.1007	0.46955 0.1709	-0.04703 0.8973
TGLUC	0.24873 0.4883.	0.35798 0.3098	-0.51114 0.1311
CCC	0.35246 0.3178	-0.05463 0.8808	-0.05130 0.8881
HEXO	0.72446 0.0178	1.00000 0.0000	-0.52807 0.1167
LOWRY	-0.08431 0.8169	-0.52807 0.1167	1.00000 0.0000

ANALYSIS OF VARIANCE ALCIAN BLUE BY TYPE OF DIABETES: ALL DATA INCLUDED

source	d.f.	sum of squares	mean squares	F-ratio	F-prob
between groups	2	0.0116	0.0058	2.169	0.1283
within groups	38	0.1013	0.0027		
total	40	0.1129			

group	count	mean	std dev	std error	95% C.I. for mean
1	11	0.3313	0.0513	0.0155	0.2968 to 0.3657
2	10	0.3241	0.0295	0.0093	0.3030 to 0.3452
3	20	0.2946	0.0595	0.0133	0.2668 to 0.3225
total	41	0.3117	0.0531	0.0083	0.2949 to 0.3284
fixed effects model			0.0516	0.0081	0.2953 to 0.3280
random effects model				0.0124	0.2582 to 0.3651

random effects model-estimate of between component variance 0.002

TABLE 25

ANALYSIS OF VARIANCE ALCIAN BLUE BY TYPE OF DIABETES: IDDM AND MATCHED CONTROLS

source	d.f.	sum of squares	mean squares	F-ratio	F-prob
between groups	1	0.0215	0.0215	7.310	0.0141
within groups	19	0.0559	0.0029		
total	20	0.0774			

group	count	mean	std dev	std error	95% C.I. formean
1	11	0.3313	0.0513	0.0155	0.2968 to 0.3657
3	10	0.2672	0.0574	0.0181	0.2262 to 0.3082
total	21	0.3008	0.0622	0.0136	0.2724 to 0.3291
fixed model effects			0.0542	0.0118	0.2760 to 0.3255
random model effect				0.0321	-0.1066 to 0.7082

random effects model-estimate of between component variance 0.0018

TABLE 26

ANALYSIS OF VARIANCE: α_2-MACROGLOBULIN BY TYPE OF DIABETES: NIDDM AND AGE MATCHED CONTROLS

source	d.f.	sum of squares	mean squares	F-ratio	F-prob
between groups	1	8778.0456	8778.0457	6.826	.0176
Within groups	18	23147.5501	1285.9750		
total	19	31925.5960			

group	count	mean	std dev	std error	95% C.I. for mean
2	10	204.9500	29.2721	9.2567	184.0100 to 225.8900
3	10	246.8500	41.4137	13.0962	217.2244 to 276.4756
total	20	225.9000	40.9914	9.1660	206.7154 to 245.0846
fixed effects model			35.8605	8.0187	209.0534 to 242.7466
random effects model				20.9500	-40.2948 to 492.0948

random effects model-estimate of between component variance 749.2071

Cochran;s C = 0.6668, P =.316

Bartlett-box F = 1.007, P = .316

Maximum variance/minimum variance = 2.002

TABLE 27

ANALYSIS OF VARIANCE HEXOSAMINE BY AGE: ALL DATA INCLUDED

source	d.f.	sum of squares	mean squares	F-ratio	F-prob
between groups	4	0.3050	0.0762	2.258	0.082
within groups	36	1.2155	0.0338		
total	40	1.5205			

group	count	mean	std dev	std error	95% C.I. for mean
1	14	1.4936	0.1597	0.0427	1.4013 to 1.5858
2	6	1.4267	0.2031	0.0829	1.2135 to 1.6398
3	8	1.5750	0.1801	0.0637	1.4245 to 1.7255
4	7	1.6686	0.1435	0.0542	1.5359 to 1.8012
5	6	1.6550	0.2557	0.1044	1.3866 to 1.9234
total	41	1.5532	0.1950	0.0304	1.4916 to 1.6147
Fixed model effects			0.1837	0.0287	1.4950 to 1.6114
random model effects				0.0451	1.4278 to 1.6785

TABLE 28

STATISTICS HEXOSAMINE WITH AGE: ALL DATA INCLUDED

CORRELATION (R) ---------- -0.54030	R SQUARED---------- 0.29192
STANDARD ERROR OF ESTIMATE---------- -0.04527	MISSING VALUES---------- -0
PLOTTED VALUES---------- -41	ECLUDED VALUES---------- -0
INTERCEPT (A)---------- 0.08300	SLOPE (B)---------- 0.14722
SIGNIFICANCE---------- -0.00013	

MULTIPLE RANGE TEST LSD PROCEDURE: ALL DATA INCLUDED

Ranges of the 0.050 level 2.87

* denotes pairs of groups significantly different at the .05 level

			group 1	group 2	group 3	group 4	group 5
			group	group	group	group	group
			1	2	3	4	5
mean	1.4267	group 1					
mean	1.4936	group 2					
mean	1.5750	group 3					
mean	1.6550	group 4	*				
mean	1.6686	group 5	*	*			

The ranges are table ranges. The value actually calculated compared with mean (J) - mean (I) is:

$$0.1299 \times \text{range} \times \sqrt{1/N(I) + 1/N(J)}$$

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