ANALYTICAL EVALUATION OF RED CELL SORBITOL ASSAY IN DIABETIC NIGERIANS

BY

HUSSAINI PETER ANAJA

B Sc (Biochemistry), Sokoto, Nigeria

M Sc (Chemical Pathology), Ibadan, Nigeria

A Dissertation submitted to the postgraduate school, Ahmadu Bello University, Zaria, in partial Fulfillment of the Requirements for the Award of the Degree of DOCTOR Of PHILOSOPHY.

Department of Chemical Pathology

Faculty of Medicine

Ahmadu Bello University

ZARIA, NIGERIA

AUGUST, 1993

DECLARATION
****

I hereby declare that the work reported in this thesis was carried out by me in the Laboratory of the Department of Chemical Pathology, Faculty of Medicine, Ahmadu Bello University, Zaria. The work of other investigators is acknowledged and referred to accordingly. I solemnly declare that no part of this thesis has been submitted elsewhere for a higher degree.

H. P. ANAJA

DATE: 23 August, 1993

This thesis was submitted to the Department of Chemical Pathology, Ahmadu Bello University, Zaria and was accepted as partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

13/4/95

Dr. H.S. Isah (BSc, MSc, PhD, CChem, FRSC)
ASSOCIATE PROFESSOR AND HEAD OF DEPARTMENT

THESIS APPROVAL/CERTIFICATION
****

This thesis entitled "Analytical Evaluation of Red Cell Sorbitol Assay in Diabetic Nigerians"by HUSSAINI PETER ANAJA meets the regulations governing the award of the degree of DOCTOR OF PHILOSOPHY of Ahmadu Bello University, Zaria and is approved for its scholarly contribution to knowledge and literary presentation.

External Examiner
Prof. (Mrs) G. Oladunni Taylor
(BSc, PhD)
Professor and Head,
Department of Chemical Pathology,
College of Medicine,
University of Ibadan,
IBADAN, NIGERIA.

DATE: _____

Internal Examiner
Dr. H.S. Isah (BSc, MSc, PhD,
CChem, FRSC)
Associate Professor and Head,
Department of Chemical Pathology,
Faculty of Medicine,
Ahmadu Bello University,
ZARIA, NIGERIA.

DATE: 13/4/95

Dean, Postgraduate School,
Ahmadu Bello University,
ZARIA, NIGERIA.

Date: _____

Internal Examiner
Dr. I. Abdu-Aguye (MBBS, MSc, FMCP
Associate Professor and Head
Clinical Pharmacology Unit,
Department of Medicine,
Faculty of Medicine,
Ahmadu Bello University,
ZARIA, NIGERIA.

DATE: 18/4/95

DEDICATION
****

This thesis is dedicated to my grand mum, Anyebo Idegwu and my daughter, Glory.

ACKNOWLEDGEMENTS
****

My warmest and deepest gratitude goes to my Supervisors, Dr. H.S. Isah, and Dr. I. Abdu-Aguye. I sincerely register my unalloyed thanks and gratitude to Dr. H.S. Isah, my major Supervisor and Head of Department. I am grateful to him for his invaluable assistance most especially in the areas of financial and moral supports. His constructive criticisms, constant advice and suggestions throughout the period of the study are strongly acknowledged. I am equally thankful to Dr. I. Abdu-Aguye for his assistance in the area of statistical analyses. I thank him also for his constructive criticisms and advice throughout the period of the study. I am grateful to both of them for reading the thesis critically. The generous advice and suggestion of Dr. W.N. Ogala are sincerely appreciated.

I appreciate the assistance and help of all my colleagues in the Department of Chemical Pathology who contributed in no small measure to the success of the study. Special thanks go to Mr. T. Oyeniyi of Immunology Unit for his unqualified support in several ways. I am grateful to Mr. J.I. Attah and other staff of the Blood Transfusion Services, ABU Teaching Hospital, Zaria for their co-operation in obtaining blood specimens and information from donors for this work.

I thank all volunteers who participated in the study. My special regards go to the staff of the Nigerian Railway Corporation, Zaria, Medical Students, Students of School of Medical Laboratory Technology and Alhudahuda College, Zaria for their kind co-operation.

My regards also go to the hospital pharmacy and Simon Mark for the supply of the drugs used in the study.

I appreciate the cooperation of doctors and other staff at the diabetic clinic, ABU Teaching Hospital, Zaria. The assistance and cooperation of Mr. Umoren and other staff at the General Out-patient Laboratory, ABU Teaching Hospital, Zaria is strongly acknowledged.

My thanks and gratitude go to my brethren who joined me in prayers to see to the successful completion of the programme. I sincerely acknowledge the cooperation of my wife and daughter who bore with my long absence from home each day throughout the period of the study. I sincerely register my kind regards to Mr. R.S. Olayanju of the Department of Surgery for his meticulous secretarial assistance. May God richly reward you all for your kind efforts. I acknowledge the assistance of the University Board of Research for the financial support through a research grant. The financial support from the Kogi State Government is sincerely acknowledged.

Finally, and above all, I am grateful to Almighty God who over looked my weaknesses and brought me to the end of the programme successfully.

ABSTRACT

The analytical linearity, detection limit, sensitivity, specificity, precision, accuracy as well as diagnostic sensitivity, specificity and predictive values of red cell sorbitol (RCS) assay were evaluated using the fluorimetric enzymatic technique with the available laboratory facilities in a Nigerian Teaching Hospital. Reference values of RCS in healthy subjects were established and the clinical utility of the assay was explored in diabetic patients.

1. The assay was linear up to 40.0 nmol/ml. The detection limit was 0.55 nmol/ml. The within-assay coefficient of variations (CV) from low to high concentrations were in the range of 2.82 % to 5.48 % while the corresponding values for the between-assay were 3.60 % to 6.05 % respectively. The mean recovery was 90.4 %. High concentrations of some common blood constituents and drugs did not cause significant interference.

2. The 50 % and 95 % central intervals from a probit plot of RCS values for apparently healthy Nigerians were 10.2 nmol/g Hb and 4.1-14.9 nmol/g Hb respectively. These values may be considered as the reference interval for our population. A clinical decision limit of 17.0 nmol/g Hb based on mean+3 SD or greater was thus suggested for diagnostic purposes in our environment. There has been no published data on reference intervals for RCS concentration in subjects from Africa.

3. An evaluation of the diagnostic sensitivity and specificity of RCS in 102 out-patients revealed a sensitivity of 94.4 %, specificity of 98.8 % and efficiency of 98.0 %. The predic-

tive value of a positive test was 94.4 % while that of negative test was 98.8 %.

4. Assessment of the variations of RCS measurements in apparently healthy Nigerians showed that the values were similar in men and women ($P>0.05$). Values of RCS were significantly lower in subjects of lower age group than in adults. The correlation coefficient between RCS and age was 0.30 ($t=4.425$; $P<0.001$).

5. Mean value of RCS concentration for the apparently healthy subjects in the present study significantly differ from values reported in subjects from other parts of the world. This variation could be racial.

6. Thirty two percent of the diabetic patients were obese (Body Mass Index, BMI ≥ 27.0 kg/m^2). The mean RCS value in obese diabetics was significantly higher ($P<0.05$) than in the non-obese patients. A correlation analysis of the data gave a correlation coefficient of 0.294 ($t=3.56$; $P<0.001$).

7. Alcohol consumption seemed to have an effect on the concentration of RCS in the healthy subjects as mean values of sorbitol were significantly higher ($P<0.01$) in drinkers than in non-drinkers.

8. The mean RCS values were significantly higher ($P<0.05$) in hypertensive subjects (non-diabetics and diabetics) than in normotensive (non-diabetics and diabetics).

9. The mean value of RCS was significantly higher (P<0.001) in diabetics (Types I and II) than in healthy controls. The mean value in type I diabetics was significantly higher (P<0.001) than in type II diabetics. The mean values of plasma glucose and fructosamine in diabetics were significantly higher (P<0.001) than in the control as expected. Diabetic patients with RCS $\geq$ 17.0 nmol/g Hb were judged to be in poor control while those with RCS < 15.0 nmol/g Hb were in good control.

10. The mean values of RCS in diabetics were significantly higher (P<0.001) in patients with neuropathy and nephropathy than in control subjects and in those diabetics without such complications. This probably indicates that the affinity of aldose reductase for glucose is higher in patients with neuropathy, and nephropathy than in those without such complications suggesting the implication of sorbitol accumulation in the pathogenesis of these complications.

11. Red cell sorbitol correlated positively and significantly with plasma glucose (r=0.746, P<0.001) in a mixed diabetic population (Types I and II). Similarly, the correlation between plasma fructosamine and RCS in the diabetics (Types I and II) was significant (r=0.478; P<0.001). Red cell sorbitol can thus be used as an index of glycaemic status and probably for monitoring metabolic control in diabetic patients.

12. A two hour post-prandial blood glucose screening conducted on a few diabetics revealed that fasting RCS levels correlated positively and significantly with glucose (r=0.895; P<0.001).

A similar result was obtained on the two hour specimen. Changes of sorbitol in response to changes in blood glucose in diabetic patients appears to be rapid demonstrating that RCS could be an index of coincident glucose level in these patients. As a result of the significantly high level of RCS in diabetic patients in both fasting and after two hours of specimen collection, blood samples for assessing the polyol pathway can be collected at anytime of the day irrespective of time of meal intake.

13. Applying RCS assay as an index in the assessment of metabolic control in diabetic Nigerians using the criteria suggested in the present study, 54.0 % of the diabetic patients were in poor control, 14.8 % were in moderate control and 31.3 % in good control. Serial study of RCS in newly diagnosed diabetics following therapy showed that the mean RCS concentration at presentation was significantly higher ($P<0.001$) than at 8 and 16 weeks. Similar observations were made for glucose and fructosamine.

14. About 70.0 % of the diabetic patients had hyperlipidaemia. Out of these 47.0 % had hypertriglyceridaemia, 4.5 % had hypercholesteraemia and 18.5 % had combined hyperlipidaemia. Mean values of RCS concentration were significantly higher ($P<0.05$) in hyperlipidaemic than normolipidaemic diabetic patients suggesting an inter-relationship between lipid abnormalities and the polyol pathway.

15. The observations in the present study demonstrate that RCS assay could be adopted in our laboratory for clinical applications, namely (i) in the assessment of glycaemic status in diabetic patients (ii) in the study of the pathogenesis of long-term diabetic complications and (iii) in monitoring diabetic patients suffering from any of the late complications who may benefit from aldose reductase inhibitor therapy.

16. Recommendations:

(a) There is a need to undertake analytical evaluation of the assay of red cell sorbitol using any of the available spectrophotometers in the laboratory. This could be done by measuring the absorbance of generated NADH either at 340 nm or 339 nm. A comparative study of the fluorimetric and colorimetric assay of RCS could be carried out. This is because colorimeters are readily available and require limited skill for manipulation. They are more rubust and could easily be adapted for routine use.

(b) The relationship of the polyol pathway to plasma insulin or C-peptide levels should be investigated by measuring red cell sorbitol and insulin and/or C-peptide in non-diabetic and diabetic subjects. This might help to confirm whether or not insulin has any effect on the polyol pathway.

(c) The assessment of the role of the polyol pathway in diabetic patients with cardiovascular diseases should be carried out with the estimation of lipid profiles. This could help to reveal the relationship between the polyol pathway and cardiovascular disease in diabetic patients and possible use of red cell sorbitol assay in the study of the pathogenesis of this complication.

(d) There is a need to undertake a Nigerian multicentre diabetic complication prospective study using a combination of various parameters such as RCS, plasma glucose, fructosamine, and lipid profiles, to select appropriate biochemical indicators of diabetic complication.

(e) The use of RCS in mornitoring therapy with aldose reductase inhibitors in Nigerian diabetic patients needs to be assessed.

TABLE OF CONTENTS

		PAGE
	Title	i
	Declaration	ii
	Certification by Head of Department	iii
	Thesis Approval	iv
	Dedication	v
	Acknowledgements	vi
	Abstact	viii
	Table of Contents	xiv
	Listof Tables	xx
	Listof Figures	xxii
	Abbreviations and Symbols	xxv

CHATER ONE

1.0	INTRODUCTION	1
1.1	GENERAL INTRODUCTION.....	1
1.2	HISTORICAL PERSPECTIVES OF DIABETES MELLITUS.....	3
1.3	INCIDENCE AND PREVALENCE OF DIABETES MELLITUS....	11
1.4	CLASSIFICATION OF DIABETES MELLITUS	15
1.5	ETIOLOGY OF DIABETES MELLITUS	17
1.6	METABOLIC DISORDERS OF DIABETES MELLITUS	19
1.6.1	ormal Metabolism of Glucose and Regulation.....	19
1.6.2	tabolism in insulin Deficiency	24
1.7	DIAGNOSIS OF DIABETES MELLITUS	25
1.8	MANAGEMENT OF DIABETES MELLITUS	28
1.9	LONG-TERM DIABETIC COMPLICATIONS	29

	CHAPTER TWO	PAGE
2.0	LITERATURE REVIEW	33
2.1	THE SORBITOL PATHWAY	33
2.2	THE EFFECT OF SORBITOL ACCUMULATION ON CELL FUNCTION	35
2.3	RELATIONSHIP OF SORBITOL WITH THE LONG-TERM DIABETIC COMPLICATIONS	40
2.3.1	Diabetic Retinopathy (DR)	40
2.3.2	Diabetic Nephropathy (DNP)	43
2.3.3	Diabetic Neuropathy (DNR)	46
2.3.4	Diabetes Mellitus and Vascular Disease	49
2.4	ALDOSE REDUCTASE INHIBITORS (ARIS)	53
2.5	BIOCHEMICAL INDICES OF METABOLIC CONTROL AND COMPLICATIONS IN DIABETICS	56
2.5.1	Urinary and Blood Glucose	57
2.5.2	Glycated Proteins	58
2.5.2.1	Glycated Haemoglobins (HbA_1)	58
2.5.2.2	Glycated Plasma Proteins (Fructosamine)	60
2.5.2.3	Glycated Fibrinogen	61
2.5.3	Red Cell Sorbitol	62
2.5.4	Lipid and Lipoprotein Profiles	65
2.5.5	Urinary and Serum N-acetyl-β-D-Glucosaminidase (NAG)	66
2.6	LOCATION OF STUDY	67
2.7	THE THESIS	67
2.7.1	Statement of Research Problems	67
2.7.2	Aims of the study	68

		PAGE
	CHAPTER THREE	
3.0	MATERIALS AND METHODS	70
3.1	SUBJECTS	70
3.1.1	Apparently Healthy Subjects	70
3.1.2	Subjects from General Medical Out-Patient Department (GMOPD)	71
3.1.3	Diabetic Patients	71
3.1.4	Other Patients	73
3.1.5	Any other Information on the Subjects	73
3.1.6	Ethical Consideration	74
3.2	REAGENTS	74
3.3	EQUIPMENT	74
3.4	METHODS	75
3.4.1	Blood Collection	75
3.4.2	Washing of Red Blood Cells	75
3.4.3	Two-hour Blood Glucose Screening	76
3.4.4	Analytical Methods	76
3.4.4.1	Red Cell Sorbitol	76
3.4.4.2	Red Cell Haemoglobin.....	77
3.5	ASSESSMENT OF RED CELL SORBITOL ASSAY PERFORMANCE CHARACTERISTICS	78
3.5.1	Selection of excitation and Emmission Wavelengths	78
3.5.2	Time Course Studies	78
3.5.3	Calibration Curve	79
3.5.4	Detection Limit	79
3.5.5	Precision Studies	79
3.5.6	Recovery Studies	80
3.5.7	Interference Studies.....	80

		PAGE
3.5.8	Quality Control and Slight Modification	80
3.5.9	Statistical Analyses	81
3.6	OTHER ANALYTES	83
3.6.1	Plasma Fructosamine	83
3.6.2	Plasma Cholesterol	84
3.6.3	Plasma Triglyceride	85
3.6.4	Plasma Glucose	86
3.6.5	Plasma Albumin	87
3.6.6	Plasma Creatinine	88
3.6.7	Urinary Protein	89

CHAPTER FOUR

4.0	RESULTS	90
4.1	RED CELL SORBITOL ASSAY PERFORMANCE CHARACTERISTICS	90
4.1.1	Optimization of Excitation and Emission Wavelengths	90
4.1.2	Time Course Studies	90
4.1.3	Calibration and detection Limit	90
4.1.4	Precision Profile	95
4.1.5	Analytical Recovery and Interference	95
4.1.6	Reference Interval and Clinical Decision Limits	99
4.1.7	Diagnostic Specificity and Sensitivity of Red Cell sorbitol	99
4.2	FACTORS AFFECTING RED CELL SORBITOL CONCENTRATIONS IN APPARENTLY HEALTHY SUBJECTS	103
4.2.1	Sex	103
4.2.2	Age	103
4.2.3	Race	103
4.2.4	Body Mass Index (BMI).....	107

		PAGE
4.2.5	Blood Pressure (BP)	107
4.2.6	Cigarette Smoking	107
4.2.7	Alcohol Intake	111
4.3	RED CELL SORBITOL CONCENTRATION IN NIGERIAN DIABETIC PATIENTS	111
4.4	FACTORS AFFECTING RED CELL SORBITOL CONCENTRATIONS IN DIABETIC PATIENTS	111
4.4.1	Age and Sex	111
4.4.2	Disease Duration	115
4.4.3	Obesity	115
4.4.4	Hypertension	115
4.4.5	Type of Treatment	121
4.4.6	Long-Term Diabetic Complications	121
4.5	RED CELL SORBITOL COMPARED WITH OTHER INDICES OF METABOLIC CONTROL IN DIABETIC PATIENTS	127
4.5.1	Glucose	127
4.5.2	Fructosamine	132
4.5.3	Lipids	132
4.6	RED CELL SORBITOL IN THE ASSESSMENT OF METABOLIC CONTROL IN DM	138

PAGE

CHAPTER FIVE

5.0 DISCUSSION 144

5.1 PERFORMANCE CHARACTERISTICS OF RED CELL SORBITOL ASSAY 144

5.2 REFERENCE INTERVAL AND CLINICAL DECISION LIMITS 146

5.3 VALUES OF RED CELL SORBITOL IN DIABETIC PATIENTS, WITH AND WITHOUT COMPLICATIONS 150

5.4 USE OF RED CELL SORBITOL IN ASSESSMENT OF METABOLIC CONTROL 157

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS 165

REFERENCES 169

APPENDIX 188

LIST OF TABLES

TABLE		PAGE
1.I	Prevalence of Diabetes in African Subjects	13
1.II	Diagnostic blood glucose levels after 75 g oral glucose load in adults as recommended by WHO group in 1985	27
1.III	Long-term Complications of diabetes in University College Hospital, Ibadan, Nigeria	31
3.I	Age and Other details (Mean±SEM) on patients and control subjects studied	72
4.I	Precision profile for red cell sorbitol assay on SFM 25 spectrofluorimeter	96
4.II	Analytical recovery of added sorbitol for red cell sorbitol assay on SFM 25 spectrofluorimeter	97
4.III	Effect of some blood constituents, and drugs on red cell sorbitol assay on SFM 25 spectrofluorimeter	98
4.Iv	Red cell sorbitol and plasma glucose (Mean±SEM) before and after 120 min of 75 g oral glucose	100
4.V	*Diagnostic performance of red cell sorbitol in 102 Nigerians seen at the general out-patients department in Zaria, Nigeria*	102
4.VI	Red Cell sorbitol concentration (Mean±SEM) and selected variables in non-diabetic control subjects	104
4.VII	Red Cell Sorbitol concentration (Mean±SD) in healthy non-diabetic subjects from various parts of the world	106
4.VIII	Influence of Cigarette smoking and alcohol intake on red cell sorbitol and plasma glucose levels (Mean±SEM) in Nigerians	110
4.IX	Red cell sorbitol and other biochemical parameters (Mean±SEM) in diabetic and non-diabetic Nigerians	112

LIST OF TABLES (CONTD.)

TABLE		PAGE
4.X	Effect of duration of diabetes on red cell sorbitol, plasma glucose and plasma fructosamine (Mean±SEM) in diabetic patients	116
4.XI	Serial changes of red cell sorbitol, plasma glucose and fructosamine (Mean±SEM) in 18 newly diagnosed diabetics (Types I and II) following therapy	117
4.XII	Red Cell sorbitol, plasma glucose and fructosamine (Mean±SEM) in obese and non-obese NIDDM and control subjects	118
4.XIII	Red Cell Sorbitol, plasma glucose and fructosamine (Mean±SEM) in diabetics with and without hypertension	120
4.XIV	Red Cell sorbitol, plasma glucose and fructosamine (Mean±SEM) in diabetic patients according to type of treatment	124
4.XV	Red cell sorbitol, plasma glucose and fructosamine (Mean±SEM) in diabetic subjects with and without diabetic complications	125
4.XVI	Lipid profile (Mean±SEM) in diabetic subjects with and without diabetic complications	127
4.XVII	Red cell sorbitol, plasma glucose and fructosamine (Mean±SEM) in hyperlipidaemic and normolipidaemic diabetic patients	136
4.XVIII	Lipid concentrations (Mean±SEM) in diabetic and non-diabetic Nigerians	137
4.XIX	Suggested criteria for evaluation of glucose control in diabetic Nigerians using red cell sorbitol	142
4.XX	Red cell sorbitol in the assessment of metabolic control in Nigerian diabetic patients (Types I and II)	143

LIST OF FIGURES

FIGURES		PAGE
1.1	Schematic representations of proinsulin and insulin	8
1.2	Simplified scheme of the principal pathways of the general metabolism of carbohydrate, protein and fat (triglycerides)	21
2.1	The Sorbitol (Polyol) pathway	34
2.2	The relationship between the polyol pathway, and other pathways of glucose metabolism.	36
2.3	Theoretical implications of intralens sorbitol accumulation.	37
2.4	Proposed self-reinforcing metabolic defect involving polyol (sorbitol) pathway, phosphoinositide metabolism, protein kinase C and Na-K-ATPase.	39
2.5	Scheme of possible biochemical and structural changes in diabetic neuropathy.	48
2.6	Structures of aldose reductase inhibitors under clinical trial.	54
3.1	Quality control charts for between day runs on three red cell pools.	82
4.1	Excitation spectrum of NADH standard, non diabetic red cell, sorbitol standard and diabetic red cell.	91
4.2	Emission spectrum of diabetic red cell, sorbitol standard, NADH standard and non diabetic red cell.	92
4.3	Time course of NADH by diabetic red cell, sorbitol standard and non diabetic red cell.	93
4.4	Standard calibration curve for sorbitol assay on SFM 25 spectrofluorimeter.	94
4.5	A probit plot of red cell sorbitol distribution in a mixed population of 203 apparently healthy Nigerians.	101
4.6	Relationship between age and red cell sorbitol in 203 apparently healthy Nigerians.	105

		PAGE
4.7	Relationship between body mass index (BMI) and red cell sorbitol in 203 apparently healthy Nigerians.	108
4.8	Relationship between diastolic blood pressure and red cell sorbitol in 203 apparently healthy Nigerians.	109
4.9	Red cell sorbitol in apparently healthy subjects and diabetic (Types I and II) Nigerians.	113
4.10	Relationship between age and red cell sorbitol in 182 diabetic Nigerians (Types I and II)	114
4.11	Relationship between body mass index and red cell sorbitol in 182 diabetic Nigerians (Types I and II).	119
4.12	Red cell sorbitol in hypertensive non-diabetics, hypertensive diabetics, and normotensive diabetics.	122
4.13	Correlation between systolic blood pressure and red cell sorbitol in 182 diabetic Nigerians (Types I and II).	123
4.14	Red cell sorbitol in diabetics with an without complications.	126
4.15	Relationship between plasma glucose and red cell sorbitol in 203 apparently healthy Nigerians.	129
4.16	Relationship between plasma glucose and red cell sorbitol in 182 diabetic Nigerians (Types I and II).	130
4.17	Relationship between fasting plasma glucose and fasting red cell sorbitol in 33 newly diagnosed diabetics.	131
4.18	Relationship between plasma fructosamine and red cell sorbitol in 203 apparently healthy non diabetic Nigerians.	133
4.19	Relationship between plasma fructosamine and red cell sorbitol in 182 diabetic Nigerians (Types I and II).	134

		PAGE
4.20	Relationship between plasma glucose and fructosamine in 182 diabetic Nigerians (Types I and II).	135
4.21	Relationship between LDL cholesterol and red cell sorbitol in 156 diabetic Nigerians (Types I and II).	139
4.22	Relationship between Total Cholesterol and red cell sorbitol in 156 diabetic Nigerians (Types I and II).	140
4.23	Correlation between atherogenic index (TC/HDLC) and red cell sorbitol in 156 diabetic Nigerians (Types I and II).	141

hr	hour
HbA	Glycated haemoglobinA
2 HBGS	Two hour blood glucose screening
HLA	Histocompatibility antigens
HDLc	High density lipoprotein cholesterol
HPLC	High performance liquid chromatography
IDDM	*Insulin dependent diabetes mellitus*
IGT	Impaired glucose tolerance
IVGTT	Intravenous glucose tolerance test
Kg	Kilogramme
L	Litre
LDLC	Low density lipoprotein cholesterol
m	metre
M	Molar concentration
mg	milligramme
Min	Minute
ml	millilitre
mmHg	millimetre mercury
mmol	millimole
mM	millimolar
NAD	Nicotinamide adenine dinucleotide
NADP	*Nicotinamide adenine dinucleotide phosphate*
NAG	N-acetyl-β-D-glucosaminidase
NIDDM	non-insulin dependent diabetes mellitus
nmol	nanomole
Ns	Not significant

OGTT	oral glucose tolerance test
OHA	oral hypoglycaemic agent
OHBA	Hydroxybutyric acid
P	Probability
PDPD	protein deficient pancreatic diabetes
PEG	polyethylene glycol
PF	plasma fructosamine
PG	plasma glucose
PO_4	phosphate
PPP	pentose phosphate pathway
Rbc	Red blood cells
RBS	Random blood sugar
RCS	Red cell sorbitol
s	second
SD	Standard deviation
SDH	Sorbitol dehydrogenase
SEM	standard error of the mean
Tc	Total cholesterol
TCA	Tricarboxylic acid cycle
TGS	Triglycerides
Tp	Total protein
UV	ultra violet
VLDL	Very low density lipoprotein
Vs	Versus
WHO	World Health Organisation
yr	year

α -	Alpha
β-	Beta
r	Correlation coefficient
>	Greater than
$\geq$	Greater than or equal to
<	Less than
$\leq$	Less than or equal to
µg	Microgramme
µmol	Micromoles
%	Percentage
±	plus or minus
km	Substrate concentration at ½ Vmax
V	Velocity

CHAPTER ONE

1.0 INTRODUCTION

1.1 GENERAL INTRODUCTION

Intracellular accumulation of sorbitol as a result of increased flux of glucose through the polyol pathway has been implicated in the pathogenesis of some of the long-term complications of diabetes mellitus (Chylack and Kinoshita, 1969; Morrison et al, 1972; Gabbay, 1973). This pathway consists of two enzymes, (i) aldose reductase that converts glucose first to sorbitol and (ii) sorbitol dehydrogenase which then converts sorbitol to the keto sugar, fructose.

Sorbitol, the intermediate in the polyol pathway, does not readily diffuse across cell membranes and has high osmotic effects. Consequently, its accumulation in some tissues such as the lens, neurons and blood vessels has been reported to be one of the factors responsible for cataract formation (Chylack and Kinoshita, 1969; Gabbay, 1973), peripheral neuropathy (Gabbay, 1973) and possibly the vascular complications of diabetes mellitus (Morrison et al, 1972). These reports have been supported by prevention or delay of cataract formation and of impaired nerve conduction velocity by feeding aldose reductase inhibitors to diabetic rats (Yue et al , 1982).

It has been demonstrated that intact red cells are capable of converting glucose to sorbitol because of the presence of aldose reductase (Malone et al, 1980 and Onaya et al , 1990). Animal experiments and clinical trials in human diabetics using specific aldose reductase inhibitors aimed at reducing the level of intracellular sorbitol accumulation have been undertaken (Yue et al , 1982 and Malone et al , 1984). Considerable success has been achieved in this dimension and SORBINIL, a potent aldose reductase

inhibitor, has been suggested as an adjunct in diabetic treatment (Popp-Snijders et al , 1984; Malone et al , 1984; Beyer-Mears and Cruz, 1985).

Recently, Price and coworkers (1987) reported that red cell sorbitol level is not a marker of long-term sorbitol accumulation in other tissues like the retina, neurons and glomeruli. This finding is at variance with those of Malone et al (1980 and 1984) and Vertommen et al (1987). Besides, Malone et al (1980) and Vertommen et al (1987) have proposed that unknown factors other than blood glucose, have some important influence on the polyol pathway activity.

There is a dearth of data with respect to these observations, suggestions and assumptions among diabetic Africans. In addition, an increasing number of diabetic patients in developing countries now live long enough to develop the long-term diabetic complications due to introduction of better facilities for control and management of acute diabetic emergencies. However, because of the adverse economic recession in most developing countries, some patients still die from these acute diabetic emergencies like diabetic ketoacidosis (DKA).

Therefore, an analytical evaluation of red cell sorbitol assay could be of value as a biochemical risk factor in the development of the long-term complications and probably management of diabetes in Nigerians in the nearest future.

1.2 HISTORICAL PERSPECTIVE OF DIABETES MELLITUS

Diabetes mellitus (DM) is a disease which was recognised in antiquity but its history has been characterised by numerous cycles of discovery, neglect and rediscovery. DM is unequivocally characterised by hyperglycaemia and glycosuria. However, the basic defect is an absolute or relative lack of insulin which leads to abnormalities of metabolism not only of carbohydrate but also of protein and lipid (Marble, 1971).

Knowledge of diabetes dates back to the period of the Ebers Papyrus. The term 'diabetes' which is Ionian Greek and means "to run through" or "a siphon", was first used by Aretaeus of Cappadocia in the 2nd Century AD as a generic description for conditions causing increased urine production or output (Polyuria). Galen like Aretaeus, thought diabetes to be a rare disease and apparently encountered only two cases. He employed alternative terms for diabetes including "diarrhoea urinosa" and "dipsakos", the latter emphasising the cardinal symptoms of excessive thirst and drinking.

By the 5th century, Susruta and Charuka from their observations, gave a clinical description which included sugary urine (tasting like honey), sticky to the touch and strongly attracting ants. Obesity and weight loss were also described. During the same dispensation, Chinese and Japanese physicians made similar observations and noted that people with diabetes were prone to develop boils and an affliction which clinically resembles tuberculosis. Avicenna observed and described sugary urine of diabetes, gangrene and impotence (collapse of sexual function)

as two specific complications of the disease.

The diagnostic period in the history of diabetes witnessed the early report of Von Hohenheim (16th Century Swiss physician). He reported that diabetic urine contained an abnormal substance which remained as a white powder after evaporation. He concluded that this substance was salt and that diabetes was due to the deposition of salt in the kidneys, causing 'thirst' of the kidneys and polyuria.

Thomas Willis also observed the sweet taste of diabetic urine and thereby duplicated the observation which had first appeared in Eastern medical writings over one thousand years previously. Besides, distinction had already been made between the urine of diabetes mellitus and that of diabetes insipidus by Johann Peter Frank in 1794.

The concept that diabetes mellitus was a systemic disease was first speculated by Thomas Sydenham. This concept was elaborated a century later by Matthew Dobson of Liverpool. Matthew Dobson in 1776 confirmed that the sweet taste of the urine in diabetes mellitus was due to sugar. He also made the first description of hyperglycaemia by observing the sweet taste of the blood serum in diabetics. This realisation gave rise to therapeutic possibilities and in 1798 John Rollo reported a dietary approach to diabetes involving carbohyrate restriction, a system still in use today.

In 1815 Michel Engine Chevreul, a French chemist gave further characterisation of the sugar in urine and serum of the diabetic as glucose.

The next series of developments related the pathogenesis of diabetes to pancreatic disease. Claud Bernard, the 'father of carbohydrate metabolism' demonstrated that the liver could produce glucose from glycogen, and release it into the blood. He began experiments into the role of the pancreas in diabetes by attempting total pancreatectomy, and also destruction of exocrine tissue by duct injection. These attempts however, proved abortive and it was left for others to pursue the true role of the pancreas.

In 1788, Thomas Cowlay gave a report in which he described a case of diabetes mellitus with numerous calculi in the pancreas (Calcific pancreatitis). Richard Bright in 1831 showed diseased pancreas in some cases of diabetes and Van Reckilinghausen described the presence of pancreatic calculi in diabetes mellitus in 1864. In 1834, Frerich, showed quite convincingly that twenty percent of his cases of diabetes mellitus showed gross change in the pancreas.

Paul Langerhans (German) in 1867 discovered the anatomical site of insulin production. He demonstrated clusters of cells scattered throughout the pancreas and distinctly different from glandular tissue proper. These 'beta cells' were named after him as 'Islets of Langerhans', by Gustave Languesse of Lille in 1893. Laquesse also suggested that the Islets produced and secreted insulin directly into the blood stream.

Joseph Freihew Von Merring and Oscar Minkowski, both Austrian scientists, by inducing the disease in pancreatectomised dogs convincingly demonstrated in 1890 that true diabetes is due to a pancreatic lesion . They also showed that diabetes mellitus was not due to loss of flow of pancreatic juice to bowel and concluded that

the pancreas secreted not only pancreatic juice, but also a substance with a vital role in metabolism. Eugene Lindsay Opie, showed that in many cases of diabetes, the islets had undergone hyaline degeneration. Further clarification of the relationship of the pancreas to diabetes was made around the observation of Eugene Lindsay Opie especially by William McCallum.

The need then arose to find out the active "non-pancreatic juice" component of the pancreas. This search for a 'pancreatic extract' was pioneered by Georg Zuelzer and Nicholas Paulesco in 1921 . Partial success in this regard was made by Ludwig Georg Zuelzer of Berlin who succeeded in obtaining a pancreatic extract, and gave the substance to eight diabetics with good results, though all later developed "toxic" symptoms now thought to be due to hypoglycaemia. Nicholas Paulesco (Rumanian) reported in 1921 similar successful experiments with pancreatic extracts. Some claim that Zuelzer and Paulesco were the real discoverers of insulin. Their work was undoubtedly important, but their extracts were crude and could not be re-produced in large amounts and hence not subjected to proper clinical trials.

The final discovery and isolation of isulin was made by Banting and Best (1921). Frederick Banting, a young Toronto doctor, secured the assistance of Charles Best, a medical student in his believe that he could solve the 'extract' problem. He earlier approached the Scotsman James Mcleod who later brought James Collip into the team after allowing limited facilities because of his doubt. Prior to this discovery, a method of preventing destruction of hormone by trypsin in the pancreas had been described by Soboleff

in 1901. The discovery of insulin gave Banting and Mcleod the Nobel Prize for medicine and physiology in 1923. Insulin (or 'iletin' as it was first called) was synthesised in crystalline form by John-Abel.

The brief history recounted above is from the current review of the history of diabetes mellitus by MacFarlane (1991).

Insulin is synthesized in the endocrine pancreas by the beta calls of the islets of Langerhans as a high molecular weight precursor called pre-proinsulin made up of 108 amino acids (Chan et al, 1976). The pre-proinsulin consists of alpha and beta-chains and two peptide linkages refered to as the signal and connective peptides. Cleavage of the signal peptide by specific peptidase results in the formation of proinsulin with a molecular weight of 9000 (Steiner and Oyer, 1967). Proinsulin has only 5.0 % of the activity of insulin. The proinsulin molecule consists of the alpha and beta-chains connected by disulphide bonds and by a connective peptide called C-peptide. During processing the C-peptide (MW 3000) is removed from the molecule by cleavage catalyzed by specific peptidases. The resulting insulin molecule (MW 6000) consists of alpha and beta-chains connected by two disulphide bonds (Fig. 1.1). The insulin molecule is packed in a vesicle called a beta granule. Cleavage to proinsulin and then to insulin occurs within the granule. Equimolar quantities of C-peptide and insulin are released into the circulation when the granule is dissolved at the plasma membrane of the beta cell following neural, dietary, or hormonal stimuli. Only small quantities of proinsulin are found in circulation.

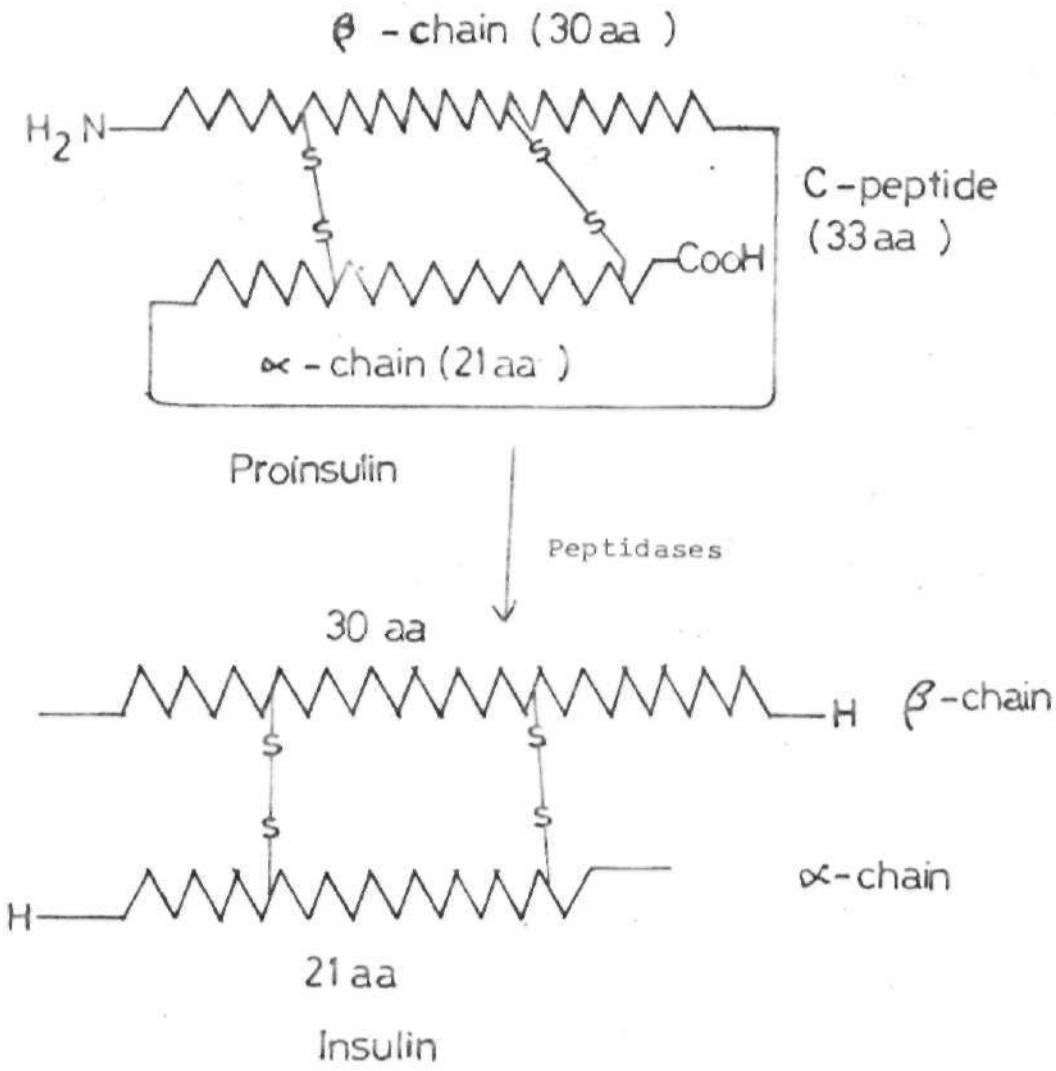


Fig. 1.1 Schematic representations of proinsulin and insulin (modified from palmiter, 1983).

aa = amino acids

The first Radioimmunoassay (RIA), published by Yalow and Berson (1960), was a sensitive and specific technique for measuring insulin levels in plasma. The technique is now widely used in estimating the concentration of many hormones. Yalow and Berson (1960) received the second Nobel prize in biochemistry as a result of this discovery.

Eli Lilly and Company, in cooperation with Genentech, developed the basic recombinant DNA techniques for producing insulin in large quantities in response to the increasing incidence of diabetes worldwide coupled with a desire to reduce the dependency upon animals for this product. Recombinant DNA technology allows manipulation of bacterial host cells by insertion of genes for production of either the alpha chain, the beta-chain of the insulin molecule or the entire proinsulin molecule. Prior to 1986 the alpha and beta chains of the insulin molecule were produced by separate fermentation prucesses (growth of the genetically altered E. coli under controlled conditions). The chains were purified and later combined by chemical techniques to produce insulin.

As of 1986, Humulin began to be produced by a process which involves the enzymatic conversion of human proinsulin. The genetic coding for human proinsulin is inserted into the special E. coli which are then grown in a fermentation process to produce human proinsulin. The fermentation step is stopped by heat sterilization to eliminate any possibility of contamination by E. coli. The connecting peptide is then enzymatically cleaved from the human proinsulin to produce human insulin (Humulin). Sophisticated purification techniques and sensitive analytical procedures, including reverse phase high performance liquid chromatography

(HPLC) and computerized control of various reaction steps, are used to assure the purity of Humulin.

Humulin produced by either of these processes is structurally and chemically identical to pancreatic human insulin. Humulin has been extensively studied in patients new to insulin therapy and in patients transferred from animal-source insulin. Multicentre clinical trials have shown no pharmacokinetic or clinical differences between Humulin made by the two manufacturing processes (Eli Lilly and Company, 1992).

The next significant advancement was made during the Second World War while assessing the usefulness of a sulphonamide drug against endemic typhoid, a compound 2254 RP (a sulphonyl-urea) was demonstrated in Montpellier, France by Lanbatieras and Janhon in 1955 to possess hypoglycaemic properties. This heralded the development of the oral sulphonylureas and later their introduction to the market in 1955. Biguanides were introduced into use shortly after, in 1956, when Pomeranze and associates in New York, USA, showed clinical effectiveness of the first synthetic biguanide-N-Beta-phenethyl formidinylimine urea (DBT) in ten adult diabetics (Pomeranze et al, 1956). The use of biguanides has since been considerably limited by the occasional complication of lactic acidosis.

1.3 INCIDENCE AND PREVALENCE OF DIABETES MELLITUS

The incidence (the number of cases appearing per unit of population within a defined time interval) and prevalence(the proportion of cases occurring during a specified period of time in a defined population) of diabetes mellitus in developing countries is not known (WHO 1985). True incidence and prevalence patterns in Africa are diffcult to come by. In 1901, Albert Cook, a medical missionary in Uganda, reported that 'diabetes is rather uncommon and very fatal in Africa. For the next 50 - 60 years diabetes continued to be regarded as rare in sub-Saharan Africa (Trowell and Burkitt, 1981) although in North Africa it was probably more widely recognised. In other parts of the world, incidence and prevalence patterns are more readily available.

An incidence of 13.2 per 100,000 was reported in Denmark for subjects within the first 29 years of life (Christau et al, 1977) while Taha et al (1983) estimated 22.09 persons per 100,000 population in Kuwait for the same age group. The lowest incidence pattern of diabetes is among the Eskimos. Only 10 cases were seen in a poulation of 12,000 in epidemiological studies conducted between 1962 and 1964. In a field Survey of 4249 Eskimos in Greenland, only one case of unequivocal diabetes was discovered (Sagild et al, 1966). Similarly, Japan and China have low incidence of diabetes, less than one person per 100,000 individuals annually. Kinnear (1963) in a review of 309 diabetics over 5 years (1957-1962) showed an incidence of 1-6 per thousand admission at the University College Hospital (UCH), Ibadan. Osuntokun et al (1971) estimated that about a hundred new diabetics were seen in a year out of 35,000 total new patients

registered at the UCH, Ibadan.

The prevalence of DM is approximately 1.0 to 2.0 % in the western world. A prevalence of 2.0 % was reported for USA as a whole (National Commission on Diabetes, 1975), and age adjusted prevalence rate of 1.0 to 6.0 % in Rochester, USA. While the highest prevalence is among the American Pima Indians with Age and sex adjusted prevalence of 26.5 % (Knowler, 1978). Similar high prevalence rats of 34.4 % has been reported in Central Pacific Islands (Zimmet et al, 1977). The prevalence in Israeli men is about 5.0% (Medalie et al, 1978).

Since the early 1960s, there have been a number of studies to determine the prevalence of diabetes in Africa. Though, studies have involved differing study populations, methodologies, and criteria for the diagnosis of diabetes, most point to a prevalence of 0.0 % to 1.0 % in indigenous African populations. Lester (1983) in Ethiopia reported that diabetes accounted for 2.0 to 3.0 % of admissions to adult medical wards. Studies conducted in some African subjects to determine prevalence of diabetes are listed in Table 1.I. In West Africa, there have been isolated reports of its absence or rarity among rural communities (Teuscher et al, 1987) and of increasing prevalence with urbanisation (Johnson, 1971). A survey in urban Lagos revealed a prevalence of 19.0 % among inmates of an old people's home (Johnson, 1971). Osuntokun et al (1971) found a prevalence rate of 0.45 % of the hospital population. Ohwovorlole et al (1988) reported prevalence of 1.7 % among urban Lagos Community. A prevalence rate of 1.43 % (40/2600) was observed by Erasmus et al (1989) in Ilorin.

Table 1.I PREVALENCE OF DIABETES IN AFRICAN SUBJECTS

Country	Location	Study population	Number of subjects	Method of detection	Prevalence (%)	Reference
Ethiopia	Urban/rural	Schools/factories	2381	Urine	0.3	Peters (1983)
Ghana	Urban	Outpatients	4000	Urine	0.4	Dodu (1958)
Ghana	Urban	Male community members> 15 yr	5537	Urine	0.2	Dodu and de Hear (1964)
Cote de Vorie	Urban/rural	Community members	5000	Blood	5.7	Zmirou (1979)
Lesotho	Rural	Outpatients	3000	Urine	0.2	Politzer et al (1960)
Malawi	Urban/rural	Outpatients	4725	Urine	0.1	Davidson (1963)
Mali	Urban/rural	Urban workers/ farmers/pastoralists	297	Blood	1.4	Imperato et al (1976)
Mali	Rural	Community members	7472	Blood	0.9	Fish et al (1987)
Nigeria	Urban	Community members by invitation	1627	Urine/Blood	1.7	Ohwovoriole et al (1988)
Nigeria	Urban/rural	Police/farmers/students	2800	Blood	1.4	Erasmus et al (1989)
South Africa	Urban	Outpatients	2122	Urine	1.3	Seftel and Abrams (1960)
South Africa	Urban	Community members	2015	Blood	2.9	Goldberg et al (1969)

Table 1.1 (Contd.)

Country	Location	Study population	Number of subjects	Method of detection	Prevalcence (%)	Reference
Tanzania	Urban/rural	Community members	3145	Blood	0.7	Ahern and Corvigan (1984)
Tanzania	Rural	Community members	6097	Blood	0.9	McLarty et al (1989)
Togo	Rural	Community members	1381	Blood	0.0	Teuscher et al (1987)
Tunisia	Urban	Community members	3826	Blood	3.8	Papoz et al (1988)
Uganda	Urban/rural	Outpatients	7164	Urine	0.2	Tullock (1964)
Zambia	Rural	Community members	369	Blood	1.1	Davidson et al (1969)
Zimbabwe	Urban	Community members	1007	Urine	0.1	Carr and Gelfand (1961)
Zimbabwe	Rural	Outpatients	5456	Urine	0.0	Guidotti and Gelfand (1976)

1.4 CLASSIFICATION OF DIABETES MELLITUS

Diabetes mellitus is usually characterised by hyperglycaemia and glycosuria. The WHO Expert Committee on Diabetes (1985) identified the following classes of diabetes mellitus.

(A) Primary Diabetes: This is not associated with other conditions but based on insulin secretion and action. There are two major types:

(1) Type 1: Insulin dependent diabetes mellitus (IDDM). This is prevalent in childhood and young adults, especially below the age of 40 years and was formerlly called 'juvenile - onset'. Type 1 has an acute onset and often associated with ketosis and coma. Patients in this category are usually non-obese (lean) consequent to unusual weight loss. Serum insulin is low or absent and family history is uncommon, but there is a strong association with certain histo-compatibility antigens (HLA), especially HLA-DR3 and DR4.

Type 2: Non-Insulin dependent diabetes mellitus (NIDDM) (formerly maturity onset or adult onset). This is predominantly prevalent in the middle age and elderly, usually above 40 years and formerly referred to as 'maturity-onset'. The onset is gradual and usually not associated with ketosis. Patients in this category may be obese or non-obese but often obese (about 40.0 %). Weight loss is uncommon. Serum insulin is often

normal or elevated with delayed response to hyperglycaemia. There may be a familial incidence but there is no HLA association.

In 1985, the World Health Organization (WHO) added a third type.

Type 3: Malnutrition-Related Diabetes Mellitus (MRDM).

This is further sub-divided into:

(i) Fibrocalculous panereatic diabetes (FCPD) and

(ii) Protein deficient pancreatic diabetes (PDPD).

Fibrocalculous pancreatic diabetes is characterised by calculus formation in the main pancreatic duct and its branches, together with extensive fibrosis of the pancrease. It is more prevalent in developing countries especially in areas where consumption of cassava is common. It is common in subjects below age of 30 years, mainly during childhood. It is associated with recurrent attacks of abdominal pain, severe weight loss, and other stigma of past or present malnutrition. The key metabolic features are moderate to severe hyperglycaemia that requires insulin for control, sometimes in high doses, and the absence of ketosis.

The key features of PDPD include resistance to the development of ketosis, partial resistance to the action of insulin, extreme degrees of wasting and emanciation, and onset of symptoms before the age of 35 years, commonly between 15 and 35 years of age. Pancreatic calcification and fibrosis are absent.

(b) Secondary Diabetes:

Diabetes can occur secondary to other diseases, for example, chronic pancreatitis, following pancreatic surgery (damage or destruction) and in conditions where there is increased secretion of hormones antagonistic

to insulin, as in Cushing's Syndrome, phaeochromocytoma, thyrotoxicosis and acromegally. It can also be associated with liver disease, obesity, hyperlipidaemia, drugs, chromosomal aberrations and other genetic syndromes.

(C) Gestational Diabete which occurs during pregnancy.

(D) Impared Glucose Tolerance (IGT) (Intermediate category).

IGT is a glycaemic response to a standard (75g) glucose challenge, intermediate between normal and diabetic, and can therefore be determined only by an oral GTT. Patients in this category may be obese, non-obese, or associated with the various syndromes listed in (B) above.

1.5 AETIOLOGY OF DIABETES MELLITUS (DM)

Diabetes mellitus is not a single entity but a syndrome with different underlying aetiological factors. An increasing number of diseases are being recognised as having an association with disturbances of carbohydrate metabolism. Type I (IDDM) is primarily a disease of the islet cells while Type II (NIDDM) is primarily a disease of insulin receptors. Some of the aetiological factors include genetic susceptibility, environmental agents and immunological causes. In case of genetic susceptibility, the major factor is HLA linked gene(s) (HLA B8, BW 15 and B 18) for the Type I while in the Type II it could be polygenic ('dominant') but the mechanism is unknown.

Environmental factor implicated in the case of Type I include viral infections where the mechanism of gene action is to permit interaction with virus resulting in acute or intermittent Beta cell destruction such as mumps'. For the Type II, excess food intake (obesity) and

indolence result in chronic hyper-insulinism, insulin resistance and ultimately hyperinsulinaemia (chronic over-stimulation of beta cells). This may give rise to beta cell failure resulting in relative insulin insufficiency.

This immunological factor is seen more commonly in Type I. Other causes of diabetes mellitus include the followings:

. Beta cytotoxic drugs: streptozotocin, alloxan, certain thiazindes, and haemochromatosis.

. Precipitating Factors:

Pregnancy, cassava (Manioc or tapioca consumption when combined with protein malnutrition) drugs like diphenylhydantoin, colchicine, lithium carbonate, l-asparaginase, cirrhosis and potassium deficiency.

. Factors suspected by some scientists but doubted by others

Dietary fat, dietary sugar, refined carbohydrates, insufficient dietary fibres, some drugs with mild hyperglycaemic effects (phenothiazine, hydrochlorothizides, caffeine, marijuana), contraceptive steroids, male sex in some societies, female sex in others, deficiency states (chromium, iron, zinc, pyridoxine), psychological stress, stress of prolonged or severe illness, specific racial susceptibility (Jews, Indians, American Indiana, polynesians).

1.6 METABOLIC DISORDERS OF DIABETES MELLITUS

Though DM is characterised by hyperglycaemia and glycosuria, the basic defect is an absolute or relative lack of insulin which leads to abnormalities of metabolism not only of carbohydrate but also of proteins and lipids.

1.6.1 Normal Metabolism of Glucose and Regulation

Glucose provides a convenient starting place to discuss the regulation of metabolism in the non-diabetic. Glucose is synthesised mainly in the liver or received in the blood from the gut (John and Alberti, 1976). It then serves as a fuel throughout the body. Certain tissues such as the brain (in the fed man) and red blood cells have an obligatory requirement for glucose. Other tissues such as muscle and adipose tissue are relatively impermeable to glucose, and insulin is required to facilitate glucose transport into these tissues. The liver allows free entry of glucose but may have restrictions on glucose utilization through rate-limiting enzymes such as glucokinase. Blood glucose concentration therefore is the net results of a variable imput and variable utilization.

Once glucose is within cells it is phosphorylated to glucose-6-phosphate and is either converted into glycogen (in muscle and liver for example) or degraded through the glycolytic pathway to pyruvate. The entry of pyruvate into the tricaboxylic acid cycle will be limited by the activity of the enzyme, pyruvate dehydrogenase, and excess pyruvate will equilibrate with lactate (with the equilibrium 10 to 1 in favour of the latter) and these will diffuse into the circulation. Lactate and pyruvate will either be used as oxidative

fuels by tissues such as heart or taken up by the liver to form glucose again through the gluconeogenic pathway (the cori cycle). In normal circumstances lactate provides about 50.0 % of the total gluconeogenic precursor requirement (Searle et al, 1974).

Onto this simple carbohydrate pathway can be superimposed protein metabolism. In extrahepatic tissues, proteins are continually being synthesised from, and broken down to, their constitutent amino acids. These amino acids are not released from the tissues in their original form. It has been shown that branched chain amino acids are transaminated or deaminated with the amino groups transferred to pyruvate, forming alanine or to glutamate, forming glutamine. The carbon skeleton from the amino acids either enter the tricarboxylic acid cycle as intermediates of the cycle or via formation of pyruvate. In animal experiments Blackshear et al (1975) were able to account for almost 80.0 % of the alanine and glutamine formed in peripheral tissues by this mechanism. Much of the amino groups leaving the muscle do so in the form of alanine, and this acts as a major glucose precursor in liver, thus forming the basis of the glucose-alanine cycle (Felig, 1973). Because pyruvate, lactate and alanine are freely interchangeable, it is sometimes referred to as the 'glucose-alanine-lactate cycle (Fig. 1.2). The relative proportions of pyruvate and alanine are influenced by ketone bodies, which either directly inhibit protein breakdown or effectively do so by competing for the coenzyme A required for activation of the branched chain amino acid carbon skeletons.

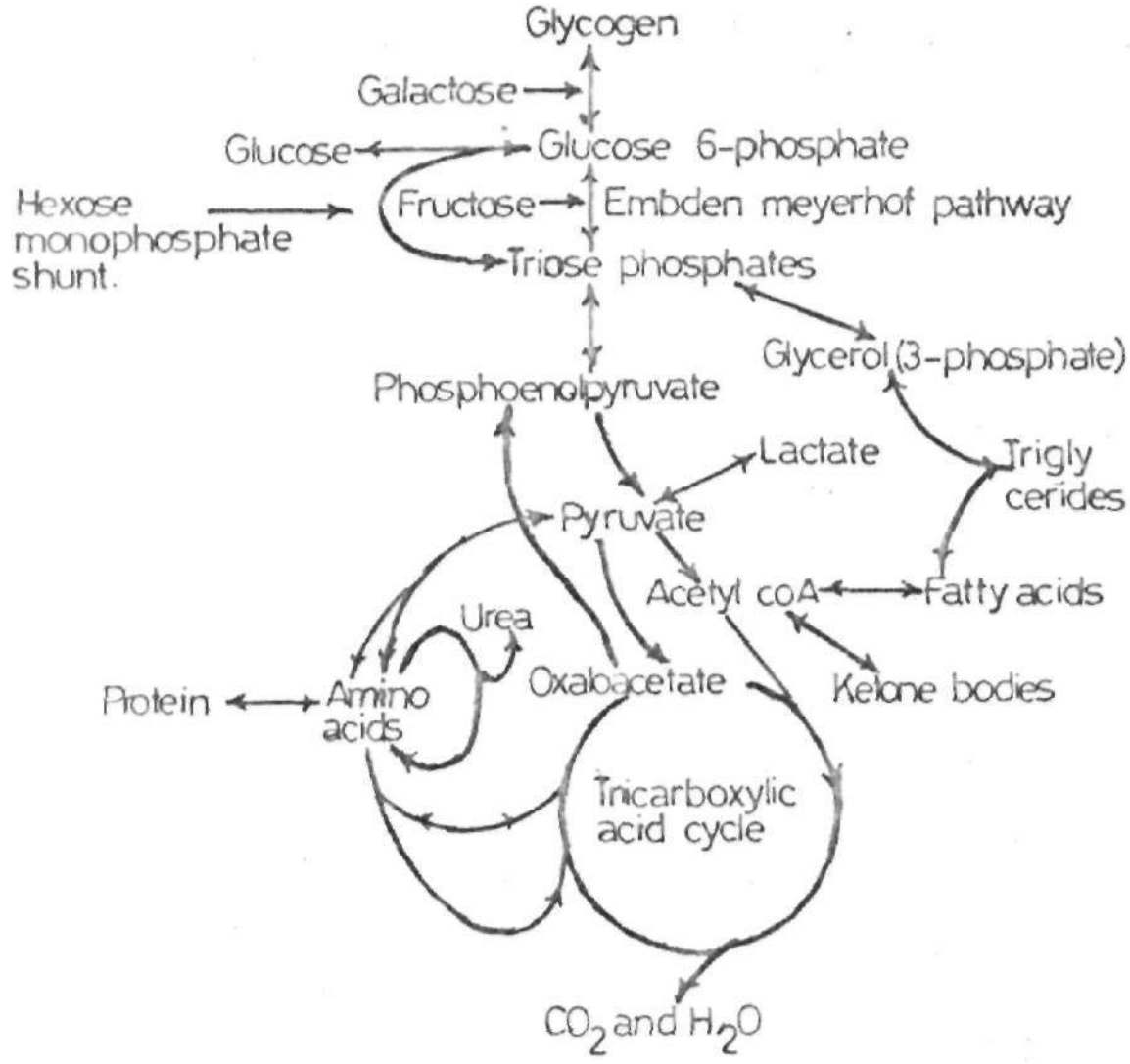


Fig. 1.2 Simplified scheme of the principal pathways of the general metabolism of cabohydrate, protein and fat (triglycerides).

←——→ reversible

Lactate and alanine concentrations in the blood will depend on the rate of breakdown of glucose and protein in peripheral tissues, the activity of pyruvate dehydrogenase, and the activity of the uptake mechanisms for gluconeogenic pathways in the liver. The other gluconeogenic precursor of any note is glycerol which is released from adipose tissue and it provides about 3.5 % of the glucose precursor pool.

Adipose tissue metabolism is basically regulated by glucose entry into the cell. Normally triglyceride degradation and fatty acid re-esterification are finely balanced. The glycerol released from triglycerides cannot be used by the adipocyte due to the absence of the enzyme glycerylkinase. For re-esterification to take place, alpha-glycerophosphate must be provided from glucose. If glucose is either unavailable or cannot enter the cell, alpha-glycerophosphate cannot be synthesized and re-esterification cannot occur. Fatty acids will then leave the cell in increased amounts, though there may be no change in the rate of lipolysis. Normally a small quantity of fatty acid does leave the cell. This is either used by tissues such as muscle for fuel or enters the liver where it is re-converted to triglyceride or broken down in the mitochondrion to acetyl CoA and hence ketone bodies.

The processes involved in carbohydrate metabolism are all subject to hormonal regulation. There are two major groups of hormones (i) the anabolic hormones, mainly insulin and to some extent growth hormone (GH) and (ii) the catabolic hormones, glucagon, cortisol, catecholamines and again to some extent GH. Insulin is unique in that no other hormone can fulfil its role whereas many

of the functions of the catabolic hormones are subserved by two or more hormones. Insulin is anabolic with respect to all phases of metabolism. It stimulates glucose entry into muscle and adipose tissues, activates Pyruvate dehydrogenase, and inhibits gluconeogenesis. Stimulation of glycolysis may at first be considered a catabolic function, but at the same time fatty acid synthesis from acetyl COA is enhanced (particularly in liver), from glucose. Fatty acid metabolism is also influenced by insulin in adipose tissue and liver through stimulation of triglyceride formation and suppression of lipolysis. Protein metabolism is affected by the stimulatory effect of insulin on amino acid transport into cells, and on protein synthesis. Reserves in the form of glycogen, triglyceride and protein are all accumulated under the influence of insulin.

The catabolic hormones oppose these actions. Each has some importance in its own right, but they are better considered as a group (Alberti et al, 1975). Cortisol stimulates breakdown of protein and the release of gluconeogenic precursors from extrahepatic tissues as well as inducing key gluconeogenic enzymes in the liver. It also inhibits glucose-uptake by tissues and has a weak lipolytic action. Glucagon enhances glycogen breakdown in the liver, aids in extraction of gluconeogenic precursors from blood by the liver and stimulates gluconeogenesis. It also has a weak lipolytic action but has more potent effects on ketogenesis. Catecholamines enhance breakdown of glycogen in both liver and muscles, stimulate lipolysis and may enhance gluconeogenesis. Glucagon and catecholamines act rapidly, primarily through stimulation of adenyl cyclase and hence cyclic 3',5'-adenosine

monophosphate function: Cortisol acts more slowly, mainly through enzyme induction.

Under normal circumstances, there is a fine balance between these hormones with insulin acting during and after feeding to lay down reserves and the other hormones mobilising these reserves at times of fasting. The end result is a remarkably stable blood glucose concentration (Newsholme, 1976) with slightly greater fluctuations in the concentrations of the other fuels.

1.6.2 Metabolism in Insulin Deficiency

Removal of a key member of the normal hormonal melieu results in obvious metabolic consequences, hence insulin deficiency will leave the actions of the other hormones unopposed, so that a net catabolic state will result. In addition, concentrations of glucagon and cortisol may rise in insulin deficiency so that catabolism will be further enhanced. This does not necessarily imply a special role for glucagon as has been suggested by others (Unger and Orci, 1976), but that it is simply one member of a group of now unopposed hormones.

Blood glucose concentration will rise for two reasons: First, the normal restraints on gluconeogenesis is removed with increased supplies of gluconeogenic precursors available from the periphery. At the same time, glycogenolysis is increased. Secondly, glucose uptake and utilization in extrahepatic tissues is impaired. Increased concentrations of lipid metabolites will also appear in the blood (Betteridge, 1989). Glucose will not enter adipose tissue so that re-esterification of fatty acids will not take place, and

lipolysis will also be enhanced. Non-esterified fatty acid (NEFA) concentrations will rise in the presence of a relative glucagon excess. The increased delivery of NEFA to the liver will result in enhanced ketogenesis with release of increased amounts of ketone bodies into the blood. A further effect of the increased NEFA and ketone body concentrations in the impairment of glucose utilization by muscle, further exacerbating the hyperglycaemia (the so-called glucose-fatty acid cycle, Randle et al 1963; or glucose-ketone body-fatty acid cycle; Newsholme, 1976). Hyperglycaemia additionally induces excessive non-enzymatic glycation of proteins (Brownlee et al, 1984; Kennedy and Baynes, 1984) and changes in intracellular metabolites, particularly in the polyol pathway (Gabbay, 1973; Malone et al, 1980; Vertommen et al, 1987; Onaya et al, 1990; Small and Collier 1991).

1.7 DIAGNOSIS OF DIABETES MELLITUS

Most cases of DM are diagnosed from the typical clinical features of polyuria, polydipsia, polyphagia, weight loss and glycosuria. This may be supplemented by a single measurement of the blood glucose level. In cases with classical symptoms and signs, a significantly elevated fasting blood glucose level above 7.8 mmol/L or a post absorptive level in excess of 11.1 mmol/L should unequivocally establish or confirm the diagnosis. Fasting blood glucose values of less than 6 mmol/L or random values less than 8 mmol/L virtually excludes the diagnosis. When blood glucose values are borderline or lie between these values, some specialised diagnostic procedures may need to be undertaken.

1. Two-hr blood glucose screening (2HBGS): a single blood glucose estimation made 2 hours after a 75 g oral glucose load, performed fasting or post absorptive, at any time of the day. The values are interpreted as follows:
   i) If the 2-hr value is 6 mmol/L or less, the patient is clearly not diabetic;
   ii) If the 2-hr value is 11.1 mmol/L or more, the patient is clearly diabetic.
2. Oral glucose tolerance test (OGTT) is usually indicated:
   a) When 2-hr BGS values fall between the above stated values besides previous equivocal fasting and/or random blood glucose concentrations.
   b) Unexplained glycosuria, particularly in pregnancy.
   c) Clinical features of DM or its complications with normal blood glucose concentrations.
   d) Diagnosis of acromegaly.

The WHO Expert Committee on Diabetes Mellitus has agreed that an oral glucose load of 75 g in 250-350 ml of water is recommended in adults and 1.75 g/kg body weight (to a maximum of 75 g) for children (WHO, 1985). A subject whose blood glucose concentration is raised, but not significant enough to meet the diagnostic criteria in Table I.II is classified as having impaired glucose tolerance (IGT). Such subjects should be reviewed annually as it has been observed that some will eventually become overtly diabetic and some may not (Jamil et al, 1992).

Diagnostic values are as indicated in Table I.II.

Table 1.II DIAGNOSTIC BLOOD GLUCOSE LEVELS AFTER A 75 g ORAL GLUCOSE LOAD IN ADULTS AS RECOMMENDED BY WHO GROUP IN 1985

	WHOLE BLOOD		PLASMA*	
	VENOUS	CAPILLARY	VENOUS	CAPILLARY
DIABETES MELLITUS				
Fasting	≥6.7	≥6.7	≥7.8	≥7.8
2 hr post glucose load	>10.0	>11.1	>11.1	>12.2
IMPAIRED GLUCOSE TOLERANCE				
Fasting	<6.7	<6.7	<7.8	<7.8
2 hr post glucose load	6.7-10.0	7.8-11.1	7.8-11.1	8.9-12.2

Glucose Concentration, mmol/L

*Used for present study

3. Intravenous Glucose Tolerance Test (IVGTT).

In this procedure, blood glucose levels are measured at intervals after intravenous glucose load. This test is considered useful when glucose absorption is variable in conditions such as thyrotoxicosis and post gastrectomy which favours rapid absorption. It is also useful in hypothyroidism and malabsorption syndromes in which absorption is unpredictably slowed. Other tolerance tests which are used presently for research purposes include:

a. Tolbutamide Response Test: In this test, the response of patients to a glucose load after priming with a sulphonylurea is assessed. Since sulphonylurea stimulates insulin release, lowering of blood glucose is prompt in the non-diabetic or NIDDM with islet reserve. However, patients with IDDM show minimal or no response.

b. Steroid - primed oral GTT in which cortisone or other glucocorticoid, can be used as a 'stress' factor to detect states of impaired glucose tolerance.

1.8 MANAGEMENT OF DIABETES MELLITUS

The services available for adequate management of diabetic petients in terms of health education, diagnosis, therapeutic facilities and follow-up care in Nigeria, like most African countries, are rather sparse. They may not be accessible to many; and where they are, cost is a hurdle. The care of diabetics at the moment is found mainly in the consultant hospitals (teaching hospitals), regional, district and non-governmental hospitals.

Diabetic patients are managed on therapy with:

1. Insulin alone (IDDM)
2. Diet and insulin
3. Diet alone (NIDDM)
4. Oral hypoglycaemia agents (OHA) and diet (NIDDM)
5. Oral hypoglycaemic agents and insulin without diet. (secondary failure of oral agents).

These are aimed at achieving euglycaemia or normoglycaemia. Some of the oral hypoglycaemic agents commonly administered are: the sulphonylureas which include tolbutamide, chlorpropamide, talazamide, acetohexamide and carbutamide. Others are glibenclamide, glipizide, gliclazide, gliquidone and glibernuride. The biguanides include phenformin, metformin and buformin. The biguanides increase insulin receptor binding, potentiate the post-receptor sites of insulin action thus act as synergists to sulphonylureas.

Laboratory monitoring include measurement of blood glucose (instant), urine glucose (several hours) fructosamine (2-3 weeks), glycated haemoglobin (HbA1c) (6-8 weeks) and lipids and lipoprotein profiles. Some of these parameters and red cell sorbitol assay have been suggested to be used in the study of the pathogenesis of chronic complications of diabetes mellitus.(Malone et al, 1980: Onaya et al, 1990).

1.9 LONG-TERM DIABETIC COMPLICATIONS

Diabetes mellitus is a disease with acute and long-term complications. The main acute complications include hypoglycaemia, diabetic ketoacidosis, and hyperosmolar non-ketotic coma. Long-term diabetic complications involve several tissues, the commonest of which is the neuron, resulting in diabetic neuropathy. Others

are the retina of the eye culminating in diabetic retinopathy which may ultimately end up in blindness, the glomeruli of the kidney, resulting in nephropathy, and the vascular tissues resulting in cardiovascular disorders.

Owing to the introduction of insulin therapy about 70 years ago and the availability of potent antibiotics, diabetic keto-acidosis (DKA) and infections are no longer serious problems in the management of diabetic patients especially in deloveped countries. Currently, the diabetic patients face the development of cataract, retinopathy, nephropathy, neuropathy and accelerated generalised atherosclerosis. However, the acute complications of DM still pose serious problems in developing countries.

Despite the lack of precise information on the epidemiology of DM and its long-term complications, the various complications of diabetes have been documented in Nigerians (Kinnear, 1963, Greenwood and Taylor, 1968; Osuntokun et al, 1971; Akanji et al 1989 and Dagogo-Jack, 1991) (Table 1.III). Despite intensive research, the factors that influence the development of diabetic complications remain poorly understood. Although microvascular disease will not occur in the absence of hyperglycaemia, there is wide individual variation in the time of appearance and severity of microvascular complications. Barbosa and Saner (1984) reported that approximately one third of patients with insulin dependent diabetes will develop severe complications whereas in the other two thirds the disease runs a more benign course. Genetic factors clearly play a role: in insulin-dependent diabetes, as there is a familial clustering with respect to diabetic nephropathy (Seaquist et al, 1989) while in the non-insulin dependent, identical twins

Table 1.1II LONG-TERM COMPLICATIONS OF DIABETES MELLITUS IN UNIVERSITY COLLEGE HOSPITAL, IBADAN, NIGERIA

Complication	Kinnear 1963	Greenwood and Taylor 1968	Osuntokun et al 1971	Adetuyibi 1976	Bella et al 1985	Akanji et al 1989
Neuropathy	33.3 %	58.3 %	48.0 %	69.6 %	-	68.0 %
Nephropathy	7.1 %	9.2 %	19.0 %	25.6 %	-	20.0 %
Retinopathy	8.1 %	7.5 %	4.6 %	13.8 %	12.2 %	62.0 %
Cataract	22.0 %	12.5 %				
Gangrene and foot ulceration	1.0 %	-	3.0 %	3.8 %	-	54.0 % (foot Ischaemia)
Hypertension	19.0 %	12.5 %	-	-	-	42.0 %

demonstrate a similar concordance for the severity of nephropathy and retinopathy (Barnett and Pyke, 1986). In addition, a genetic susceptibility to hypertension may influence the development of nephropathy (Tolins and Raji, 1989) and this is an area of current interest and research.

Although, cigarette smoking, alcohol consumption and hormonal factors may also be relevant, a widely held clinical view which is supported by previous studies (Pivart, 1977; Tschobroutsky, 1978) is that the prevalence of diabetic complications is greater in those patients with long-standing poor glycaemic control. Within the next 5-10 years, ongoing prospective multicentre studies such as the Diabetes Control and Complications Trial in the USA and the UK Prospective Diabetes Study, should clarify the importance of glycaemic control in the development of diabetic complications.

There appear to be two possible pathophysiological processes by which hyperglycaemia leads to diabetic complications. A major consequence of hyperglycaemia is excessive non-enzymatic glycation of proteins (Brownlee et al, 1984; Kennedy and Baynes, 1984). Another important consequence of hyperglycaemia is the alteration of steady-state levels of intracellular metabolites particularly in relation to sorbitol, fructose and myoinositol (Gabbay, 1973; Malone et al 1980, 1984; Yue et al 1982; Onaya, et al 1990).

CHAPTER TWO

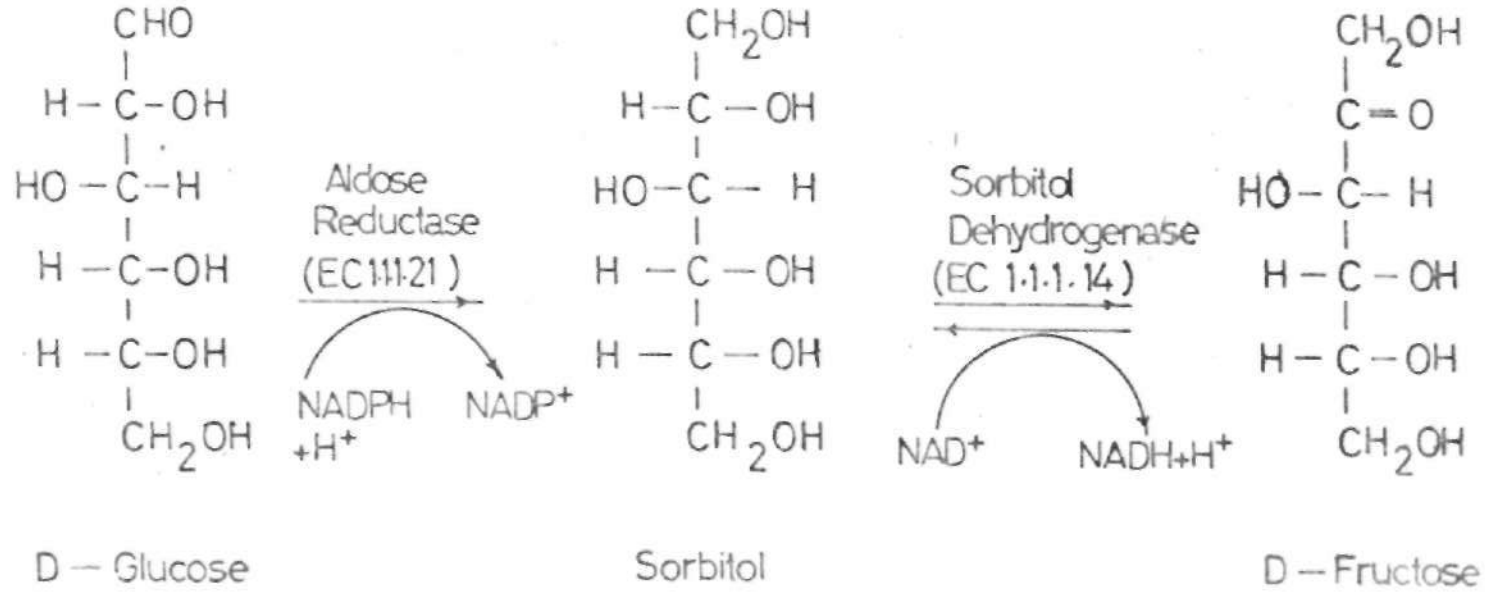
2.0 LITERATURE REVIEW

2.1 THE SORBITOL PATHWAY

The Sorbitol pathway consists of two enzymes that catalyse the conversion of free glucose to its sugar alcohol, sorbitol, and the further conversion of sorbitol to fructose (Fig 2.1). Polyol or sugar alcohol is the reduction product of a carbohydrate. In the sorbitol pathway the rate limiting enzyme, aldose reductase (EC.1.1.1.21) (AR) alditol:NADP oxidoreductase/ which uses reduced nicotinamide adenine dinucleotide phosphate ($NADPH + H^+$) as a cofactor, catalyses the reduction of glucose to sorbitol. This step is irreversible. Aldose reductase possesses broad specificities for many aldoses and is characterized by low affinity (km 50-100mM) for glucose and galactose. During hyperglycaemia and galactosaemia, the availability of a large pool of free intracellular aldoses cause appreciable formation of the sugar alcohols, sorbitol and galactitol respectively. Aldose reductase was first described in sheep seminal vesicles by Hers (1956).

It is hypothesised that in the hyperglycaemia of diabetes mellitus, hexokinase becomes saturated in some tissues and the excess glucose is metabolised to fructose by the polyol pathway (Small and Collier, 1991).

Sorbitol is oxidised by the second enzyme of the pathway, sorbitol dehydrogenase (SDH) (L-iditol:NAD oxidoreductase, EC 1.1.1.14) to form its keto sugar, fructose, using oxidised nicotinamide adenine dinucleotide (NAD^+) as cofactor. This reaction is reversible, and NADPH can be utilized in the reverse action as well as NADH. The



D – Glucose Sorbitol D – Fructose

Figure 2.1 The Sorbitol (polyol) pathway.
(Gabbay, 1973)

forward reaction utilizes NAD^+ only. Sorbitol dehydrogenase has broad substrate specificities for many sugar alcohols and hence capable of coverting xylitol to D-xylulose, and ribitol to D-ribulose (Gabbay, 1973). It has limited ability, however, to metabolise galactitol further, leading to enhanced accumulation in galactosaemia. Fructose can undergo glycolysis following phosphorylation by fructokinase (Fig 2.2).

The sorbitol pathway is operative in tissues such as the lens, the intramural pericytes of retinal capillaries, the glomerulus (kidney), Schwann cells, blood vessels and islet cells (Gabbay 1976).

An aspect crucial to the understanding of the effect of sugar alcohol accumulation in tissues is that these sugar alcohols penetrate cell membranes poorly (LeFvre and Davis, 1951; Wick and Drury 1951). Once formed, they are trapped intracellularly, the only disposition being either conversion to the respective keto sugars or slow leakage from the cell.

2.2 THE EFFECT OF SORBITOL ACCUMULATION ON CELL FUNCTION

Intracellular accumulation of sorbitol may lead to alteration of cell function by three mechanisms.

a) Accumulation of osmotically active sorbitol and fructose.

b) Decreased myoinositol concentration.

c) Altered cellular redox potential.

Intracellular accumulation of sorbitol leads to osmotic swelling which is associated with alteration in membrane permeability resulting in leakage of several substances such as amino acids, glutathione, myoinositol, adenine triphosphate and potassium ions and an influx of sodium and chloride ions (Fig 2.3). Accumulation of sorbitol under non physiological condition as in diabetes is due to its rapid production

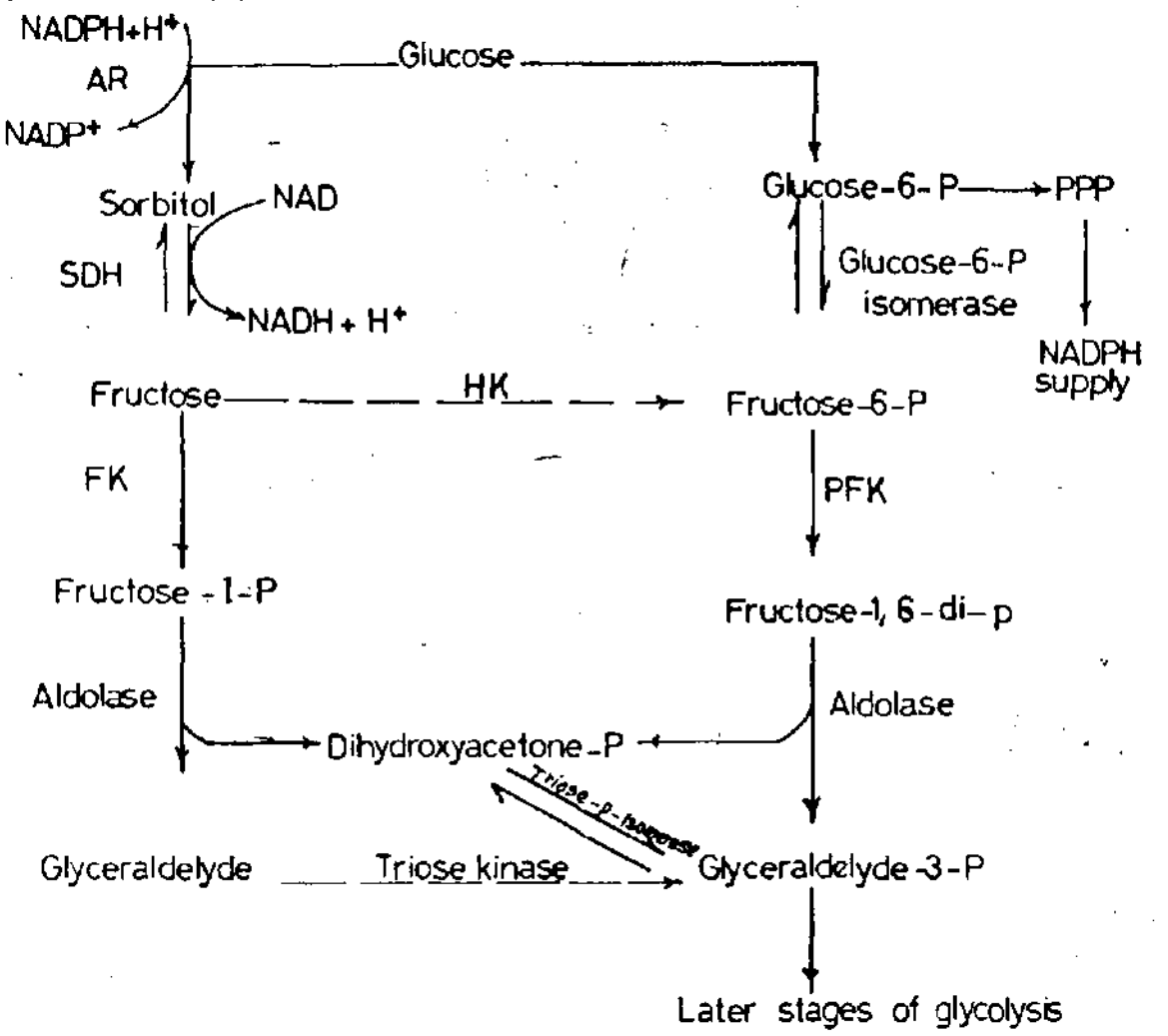


Fig. 2.2 The relationship between the polyol pathway and other pathways of glucose metabolism (Harrison etal 1988).

AR: Aldose Reductase; SDH: Sorbitol Dehydrogenase; P: Phosphate; HK: Hexokinase; PFK: Phosphofructokinase; FK: Fructokinase and PPP: Pentosephosphate pathway.

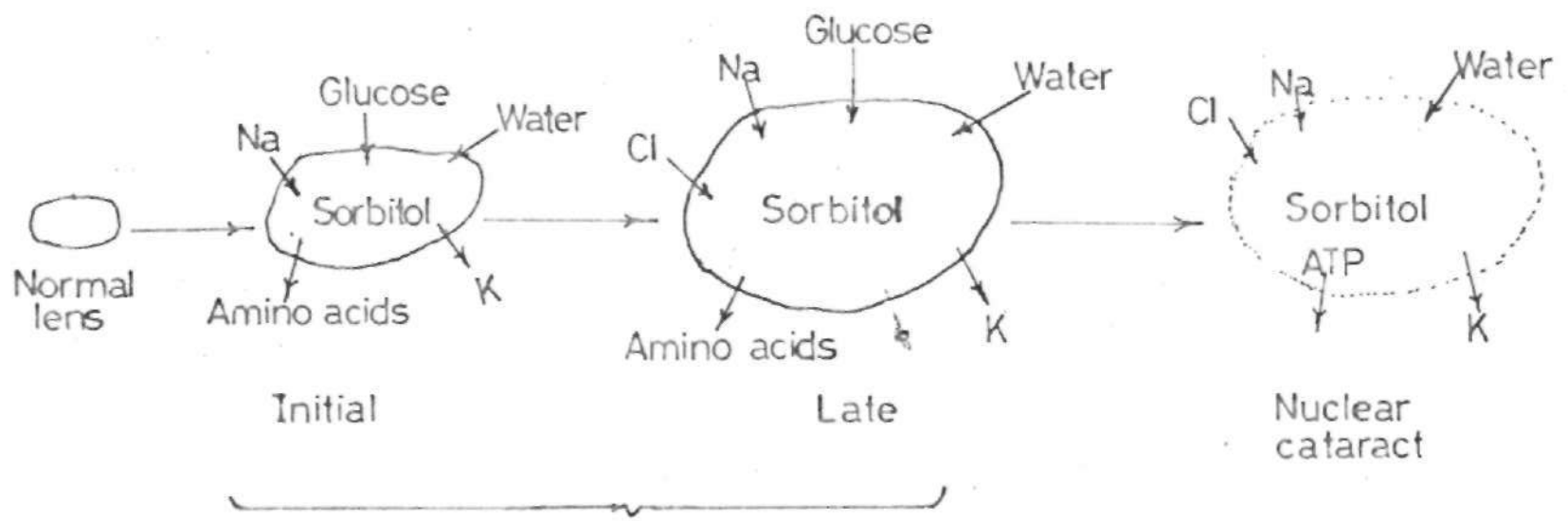


Fig. 2·3 Theoretical implications of intra-lens sorbitol accumulation. (Gabby, 1973).

than conversion to fructose. Increases in tissue sorbitol are accompanied by increases in fructose. Increased fructose concentration which could contribute to tissue damage in diabetes relates to the phosphorylation of fructose to fructose-6-phosphate and fructose-1-phosphate. Metabolism of fructose-1-phosphate avoids the tight control of glycolysis by phospho-fructokinase and could contribute to elevated tissue lactate. Poulsom et al (1983) have shown that lactate concentration is increased in the nerve and retina of diabetic animals, and can be reduced by treatment with an aldose reductase inhibitor. Elevated intracellular lactate concentration could be damaging. Imre (1964) has shown that intraocular injection of lactate in kittens causes neovascularization which is a feature of proliferative retinopathy.

Myoinositol is a six-carbon polyol which is structurally similar to glucose. It is transported in the phospholipid component of cell membranes where it plays an important role in the normal functioning of the membrane-bound Na-k-ATPase pump . Hyperglycaemia leads to myoinositol depletion, although the exact mechanism is not clear (Yue et al, 1982; Popp-Snijders et al, 1984; Greene et al 1987). In experimental animal studies, aldose reductase inhibitors completely prevented depression in myoinositol levels (Yue et al 1982). The fall in myoinositol levels in the cell membrane alters the functioning of the Na-k-ATPase pump and *the integrity of the cell membrane is disrupted* (Baxter, 1991) (Fig. 2.4).

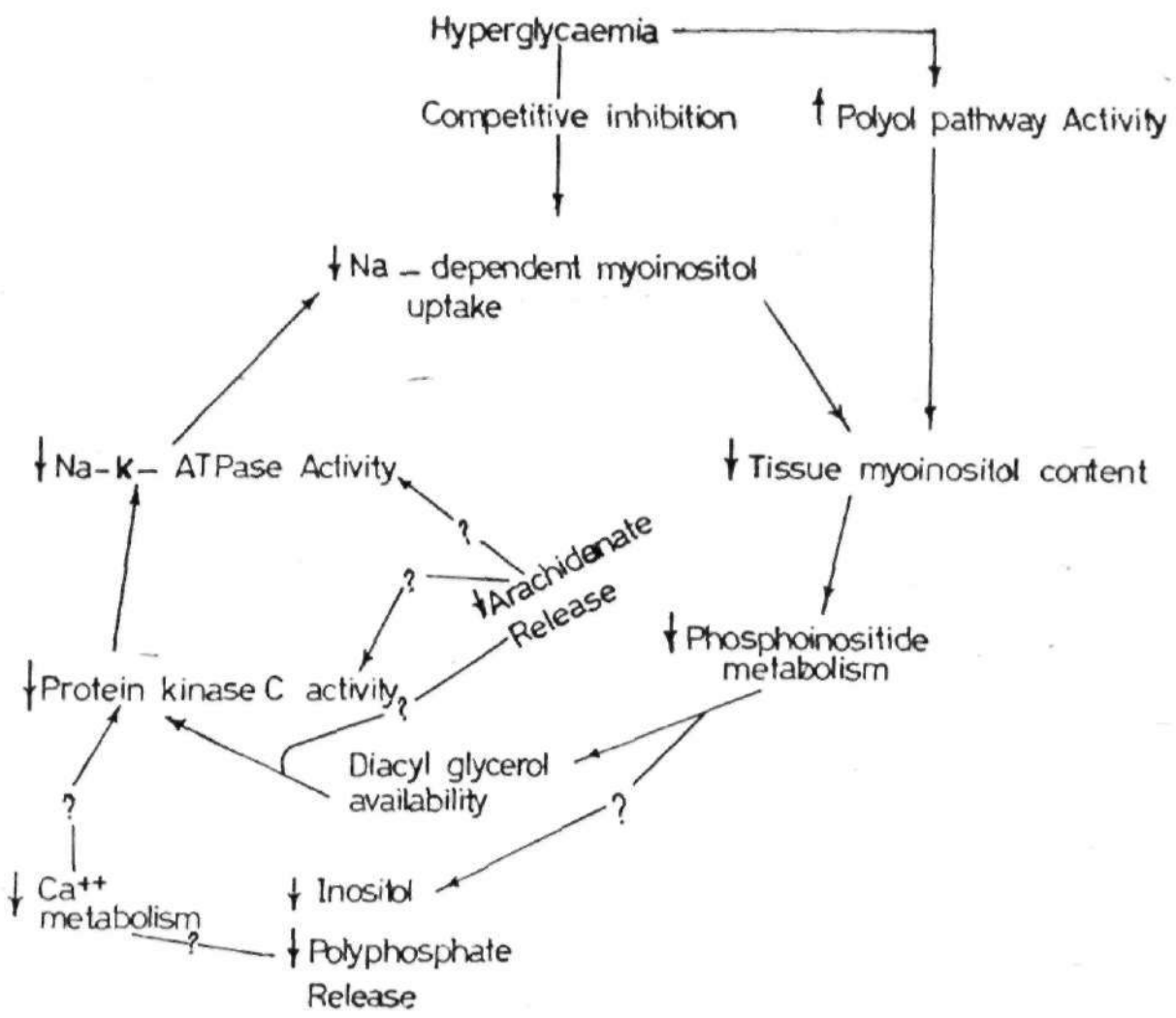


Fig. 2.4 Proposed self–Reinforcing metabolic defect involving Polyol (sorbitol) pathway, Phospholnositide metabolism, Protein kinase C and Na-k – ATPase (Greene etal 1987)

↑ Enhanced / Increased

↓ Depressed / Reduced

Increased polyol pathway activity alters the NADPH: $NADP^+$ ratio in several tissues as a result of the requirements of NADPH + H^+ for the reduction of glucose to sorbitol (Fig 2.1). In addition, oxidation of sorbitol depletes NAD^+ at the expense of elevated NADH + H^+ production. The alterations in the cytoplasmic redox state lead to a reduction in glutathione and possibly other antioxidants such as vitamins C and E and this may lead to reduced protection against oxidative damage at the cellular level (Small and Collier, 1991).

2.3 RELATIONSHIP OF SORBITOL WITH THE LONG-TERM DIABETIC COMPLICATIONS

2.3.1 Diabetic Retinopathy (DR)

Diabetic Retinopathy (DR) is a microvascular complication of diabetes mellitus. Despite advances in the use of photocoagulation and vitrectomy, diabetic retinopathy has been the leading cause of blindness among diabetics between the ages of 30 and 64 years in Great Britain, North America, Europe and Scandinavia since 1974 (Kohner and Bary, 1984). It is estimated that 5,000 patients with diabetes in the United States and 30,000 to 40,000 worldwide become blind each year from retinopathy.

Many reports have been published on the frequency of diabetic retinopathy in African patients. In Tanzania, Mhnando and Yudkin (1980) reported a prevalence of 24.6 %. Lester (1983) reported a prevalence of 57.1 % in Ethiopian diabetics. Gill and Huddle (1984) on the otherhand, reported a prevalence of 2.9 % in South Africa.

The Nigerian situation is as indicated in Table 2.1. Recently, Akanji et al (1989) reported the highest prevalence of 62.0 % in Ibadan.

Diabetic retinopathy can be subdivided into four categories of background retinopathy, maculopathy, proliferative retinopathy and advanced diabetic eye disease:

i. Background retinopathy is characterized by microaneurysms and haemorrhages, usually most marked at the posterior pole, starting just lateral to the macula.

ii. Diabetic maculopathy with macular oedema is probably the commonest cause of visual loss in diabetics. The visual loss is due to accumulation of oedema fluid or hard exudate plaques at the fovea. The cause of oedema is abnormal permeability of the retinal microvasculature at the posterior pole. No medical treatment has proved effective in reducing macular oedema. In particular, good or even excellent diabetic control and drugs, such as diuretics have not resulted in any sustained improvement. The only effective treatment to date has been photocoagulation as previously reported by Irvine and Norton (1971). Surgical techniques too can be employed, especially in treatment of complications of proliferative diabetic retinopathy, a procedure known as vitrectomy (Machemer and Blankenship, 1981).

iii. Proliferative diabetic retinopathy does not often cause visual loss. However, its complications, such as vitreous haemorrhage, retinal detachment, and retinal distortion are major causes of profound visual loss, resulting in severe blindness that renders the patient totally dependent.

iv. Advanced diabetic eye disease results from fibrovascular proliferation which has a poor visual outcome.

The followings have been suggested as risk factors involved in the development of Diabetic Retinopathy: use or abuse of insulin, longer duration of diabetics, younger age at diagnosis, higher glycated haemoglobin level, excessive sorbitol production and higher systolic blood pressure (Knowler, 1980; Peterson 1982; Rand et al, 1985; Onaya et al, 1990; Brinchmann et al, 1992).

An enhanced activity of the polyol (Sorbitol) pathway has been implicated in the pathogenesis of DR (Robinson et al, 1983). Swelling of the lens fibres due to sorbitol accumulation leads to rupture and lens opacification (Kinoshita et al, 1965, Gabbay, 1973, Kinoshita et al, 1979).

Retinal capillary pericytes are thought to control blood flow through the retinal vasculature and may also contribute to the stability of the vessel wass. Loss of retinal capillary pericytes is the first structural abnormality found in diabetic retinopathy. A postulate that retinopathy is a result of excessive polyol pathway activity is consistent with evidence that AR or AR-like activity occurs in retinal vessels, extravascular cells, endothelial cells and pericytes cultured form retinal vessels (Greene et al 1987, Tilton et al 1988).

The involvement of lipid and lipoprotein abnormalities in DR has been suggested. Abnormal levels of plasma lipids and lipoproteins are found in many insulin dependent diabetic patients with poor glycaemic control (Keiding et al, 1952; Schrade et al, 1963). Higher levels of lipids have been found in patients with retinopathy than in those without (Keiding et al, 1952; Lannaccone

and Kornerup 1954; Esmann et al, 1963, Osuntokun et al, 1971 and Hanna, 1982) suggesting that they may play a part in its development. However, other workers have been unable to confirm this relationship (Duncan et al, 1968; West et al, 1980; Dornan et al, 1982). These conflicting results may have been due to inadequate matching of patients for age and treatment type and failure to take into account the effect of renal impairement on plasma lipids.

2.3.2 Diabetic Nephropathy (DNP)

Diabetic nephropathy (DNP) occurs in up to half of patients with insulin-dependent diabetes mellitus. It is the most common cause of end-stage renal disease in many countries, including the United States (Krolewski et al, 1985; Eggers, 1988).

Many diabetic patients have increased glomerular filtration and renal enlargement early in the course of their illness (Mogensen and Andersen, 1973; Mongensen 1976; Christiansen et al, 1981). The increase in glomerular filtration has been considered a precursor of diabetic nephropathy (Mogensen and Christensen, 1984; Zatz et al, 1986), and suggestions are that high protein diets raise the glomerular filtration rate and renal protein filtration. It has also been shown that high protein diets exacerbate glomerular hypertension and injury in animal models with diabetes and renal disease (Brennar et al, 1982; Zatz et al, 1985).

Renal enlargement, in particular glomerular enlargement, has been implicated as a cause of renal injury in diabetes (Schwieger and Fine, 1990). Hypertrophy and hyperfiltration are associated with

each other (Ellis et al, 1986), but a reduction in kidney size after improved glycaemic control has not been demonstrated consistently in patients with diabetes (Wiseman et al 1985; Feldt-Rasmussen et al, 1986). However, Tuttle et al (1991) showed that both abnormal renal function and renal enlargement can regress after a period of strict glycaemic control in patients with established diabetes.

Many studies in diabetic animals and humans have demonstrated a regression of renal enlargement and improvement in histologic lesions after the normalization of the metabolic milieu by the transplantation of kidneys from diabetic to non-diabetic hosts (Abouna et al 1983), continuous subcutaneous insulin infusion (Petersen et al, 1988), pancreatic transplantation (Bilous et al, 1989) and multiple insulin infusions (Stackhouse et al 1990).

Heavy proteinuria and progressive loss of glomerular filtration are late manifestations of the intercapillary glomerulosclerosis (the Kimmelsteil Wilson Syndrome). The onset of proteinuria indicates grave prognosis of end-stage renal failure which develops in 30.0 to 50.0 % of insulin dependent and 5.0 to 10.0 % of non-insulin dependent diabetics (Watkins 1985). Andersen and coworkers (1983) found that the main cause of death among patients with DNP was uraemia (66.0 %) and ischaemic heart disease (10.0 %). Factors implicated in the development of DNP include sex, calender year, place of domicile, cigarette smoking (Christiansen, 1978), insulin dose/kg body weight (Andersen et al, 1983), increased non enzymatic protein glycation (Hostetter, 1985), enhanced polyol pathway and diminished myoinositol level (Gabbay, 1973; Greene et al, 1987).

Aldose reductase is present in both the glomerulus and the renal tubule (Beyer-Mears et al, 1984). Beyer-Mears and coworkers (1984) reported that glomeruli contain aldose reductase activity and provide the first demonstration that glomerular polyol content increases while myoinositol content decreases in diabetes and that oral sorbinil prevents these changes despite persistent hyperglycaemia. Similarly, these biochemical changes associated with increased sorbitol production have been demonstrated in animal models (Beyer-Mears, 1986). The result obtained by Beyer-Mears et al (1984) is in agreement with that reported by Finegold et al (1983) for the sciatic nerve.

On the other hand, the use of aldose reductase inhibitors (ARIS) has been shown to reduce sorbitol levels and prevent albuminuria (McCaleb et al, 1988). A small scale study in diabetic patients with microalbuminuria was able to demonstrate a reduction in albuminuria while patients were taking an ARI, with a rebound on withdrawal of therapy (Sato et al, 1988).

Increased sorbitol and resultant osmotic swelling in renal papilae have been involved as contributory to the tubular nephropathy that may be associated with uncontrolled diabetes (Gabbay, 1973). This entity does not equate either in anatomic site or pathologic manifestation with diabetic glomerulosclerosis.

Using specific antibodies to AR purified from rat seminal vesicles, Ludvigson and Sorenson (1980) localized AR immunohistochemically in several areas of the kidney. The most intense staining was seen in the inner medulla, while outer medulla and cortex stained faintly and consistently without the cortex. However, AR staining was found in

the convoluted portions of the distal tubule and in glomerular epithelial cells (Podocytes). The ability of sorbinil to prevent polyol accumulation indicates that the agent enters epithelial cells and effectively inhibits AR activity despite persistent hyperglycaemia.

2.3.3. Diabetic Neuropathy (DNR)

The peripheral neuropathy seen in DM is more likely to be due to metabolic changes in the Schwann cell or axon than to vascular damage (Clements, 1979). Although, DNR has generally been considered a late complication of diabetes, diabetic patients with neuropathy in the early course of the disease have been described (Ellenberg, 1958).

Symptomatic polyneuropathy is characterised by presence of bilateral pains and parasthesias in the lower limbs. Studies by Lehtinem et al (1989) have shown that clinical diabetic neuropathy is not common at diagnosis of NIDDM, but disturbances in peripheral and autonomic nerve function, detected by electrophysiological and cardiovascular reflex methods, are often present at that stage of the disease. They also reported that the presence of neuropathy is highly dependent on the criteria used, and that the degree of peripheral nerve dysfunction was associated with the degree of hyperglycaemia but association between glycaemic control and autonomic dysfunction could not be demonstrated.

There is some indication of the possibility of prevention or reversal of early autonomic nerve damage by meticulous glycaemic control and sorbinil therapy (Fagius et al 1985).

Aldose reductase (AR) has been shown to be present in peripheral nerves and it has been demonstrated that activation of the polyol pathway caused a wide range of biochemical, functional and structural abnormalities (Greene et al 1987) in the nerves of diabetic animal models. It has been suggested that the increased concentration of sorbitol in diabetic nerve causes osmotic damage to Schwann cells (Gabbay 1973). However, contrary observations were made by Jacobsen (1978) and Clements (1979). In experimental diabetes, there is a reduced concentration of free myoinositol in sciatic nerve (Stewart et al, 1967; Clements and stockard, 1980). This may be important in the development of neuropathy since inositol lipid metabolism is involved in nerve impulse conduction (Hawthorne and Pickard, 1979). The deficiency of free inositol has been reported in nerves from diabetic patients (Mayhew et al, 1983), though the concentration of inositol was significantly reduced in cerebrospinal fluid from diabetic patients with neuropathy.

Greene et al (1983) studied the effect of polyol pathway blockade with sorbinil, a specific inhibitor of AR, on nerve myoinositol content in streptozotocin induced diabetic rats. They observed that sorbinil administration completely prevented the fall in nerve myoinositol, thereby implicating increased polyol pathway activity as a likely factor in the fall in nerve myoinositol content in experimental diabetes. The scheme of possible biochemical and structural changes in diabetic neuropathy is shown in Fig 2.5. Winegrad et al (1972) noted that during recovery from galactose exposure, lenticular hydration remains increased well after galactitol levels fell and until the depleted tissue myoinositol levels normalize, suggesting a possible interrelationship between polyol

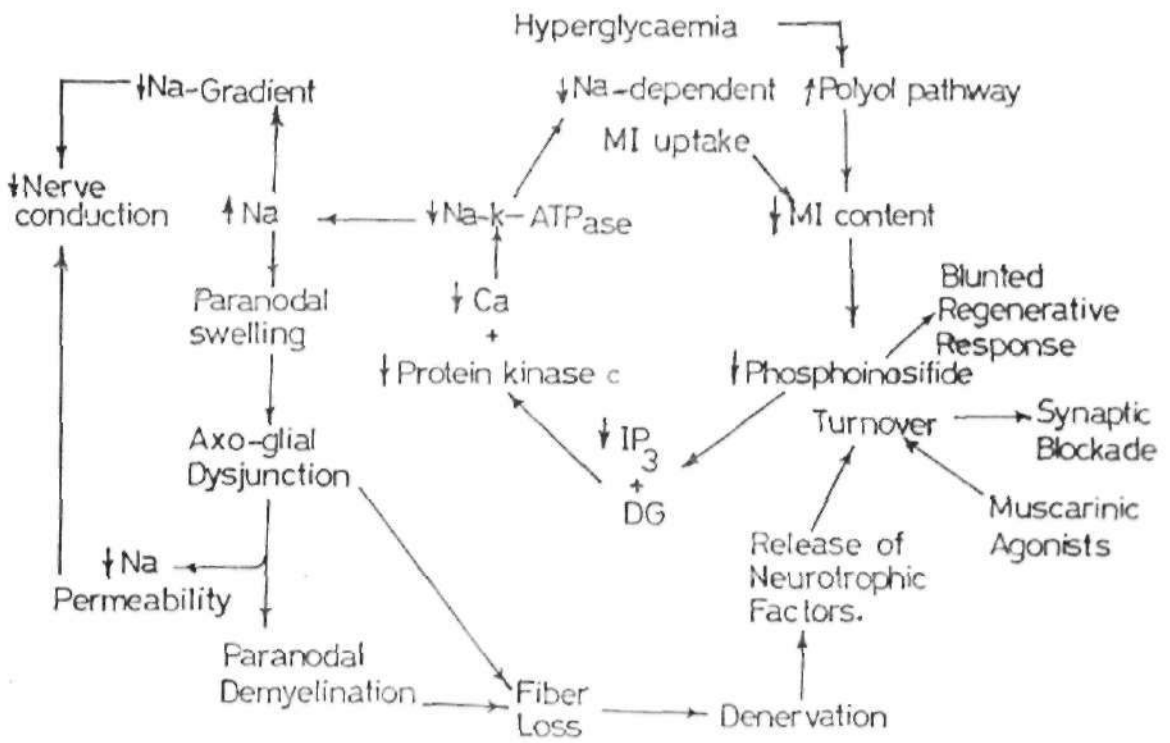


Fig. 2·5 Scheme of possible biochemical and structural changes in diabetic neuropathy (Greene et al 1987)
IP_3 = Inositol triphosphate
DG = Diacyl glycerol; MI = Myoinositol.

pathway and myoinositol metabolism in the lens. However, Dyck et al (1988) demonstrated that myoinositol levels in nerves are neither decreased nor associated with nerve abnormalities or blood glucose levels.

Direct extrapolation of the "osmotic hypothesis" from lens to diabetic peripheral nerve is beset by two conceptual obstacles. First, diabetic peripheral nerve accumulates only micromolar rather than millimolar concentrations of sorbitol which are unlikely to be osmotically significant unless highly localized anatomically (Wingrad et al, 1972). Second, myoinositol administration which corrects the characteristically reduced tissue myoinositol content without influencing nerve sorbitol concentration, normalizes diabetic nerve function (Greene et al, 1983). Oedema does occur in DNR (Griffey et al, 1988). Several studies have confirmed that diabetic patients have supranormal levels of glucose, fructose and sorbitol in their nerves (Hale et al, 1987, Griffey et al, 1988). If sorbitol accumulation leads to nerve oedema and, as a result nerve ischaemic, then a single hypothesis might unify the metabolic and vascular theories of the pathogenesis of DNR (Ananymous, 1989).

2.3.4 Diabetes Mellitus and vascular Disease

The early development of cardiovascular disease in DM is among the most devastating of its long-term complications. Though Cardiovasular diseases represent a major cause of morbidity and mortality in the diabetic patient (West, 1978), there is a lack of information regarding the mechanisms by which diabetes exerts these adverse effects. Atherosclerotic vascular disease

generally occurs at a younger age, progresses more rapidly in diabetic than in non-diabetic subjects and frequently culminates in such fatal or disabling complications as myocardial infarction, stroke and gangrene. Qualitative and quantitative disturbances in lipoprotein metabolism are believed to contribute to the acceleration of atherogenesis in diabetic syndrome (Colwell et al,1981; Howard, 1987). Atherogenesis occurs in all types of diabetes and with all degrees of severity of the diseases. In addition to the hyperglycaemia that is generally regarded to be a cardiovascular risk factor in DM the diabetic patient also often demonstrates other associated risk factors such as hyperlipoproteinaemia, Hypertension, obesity, cigarette smloking and hypercoagulability. Furthermore, diabetics may develop a cardiomyopathy which may be independent of coronary atheroslerosis (Batteridege and Kathryn 1991).

Other factors being speculated in cardiovascular disease in DM include excessive polyol pathway activity (Gabbay 1973 b) and non-enzymatic protein glycation (Kennedy and Baynes, 1984).

Intracellular accumulation of sorbitol has been reported as one of the factors responsible for the vascular complications of diabetes mellitus (Morrison et al, 1972; Gabbay, 1973b). The effects of sorbinil on the polyol pathway activity in the aortic smooth muscle, red blood cell (Rbc) deformability and vascular permeability have been investigated (Morrison and Tampa, 1985; Roby et al 1985: Chang et al, 1985).

Morrison and Tampa (1985) have concluded that increased polyol pathway activity induced an alteration in myoinositol metabolism that contributes to metabolic abnormalities in aortic smooth muscle at elevated glucose concentrations in vitro. Decreased erythrocyte/Rbc deformability, observed in diabetes and implicated in diabetic vascular complications, has been attributed to changes in the cell membrane. Sorbinil prevents changes in membrane bound enzyme activity (Na-K-ATPase), in the nerve, glomeruli and red cells of dibaetic rats. Robbey et al (1985) have demonstrated that sorbinil prevents decreased red cell/ deformability in human diabetes despite persistent hyperglycaemia and suggested that the drug influences membrane fluidity either directly or as consequence of altered cells polyol and/or myoinositol levels. Chang and Coworkers (1985) have indicated that diabetes-induced increase in vascular permeability are aldose reductase-linked phenomena. However, observations on the association of high lipid levels with vascular disease are more convincing.

Patients with DM have more coronary atherosclerosis (Root et al, 1939; Robertson and Strong, 1968) and a higher incidence of clinical coronary heart disease than does the non-diabetic population of similar age (Kessler, 1971; Gracia et al, 1974; Elford et al 1989). The increased risk of developing ischaemic heart disease is evidently related to the duration and severity of diabetes but is less clear as to whether the risk is similar for patients with type I and for type II diabetes. Rolfe et al 1993) have recently observed low prevalence of large-vessel

disease in African patients with diabetes mellitus.

The possibility that elevated serum lipid levels could be responsible was raised in the early reports of Root et al (1939), New et al (1963) and Chase and Glasgow (1976) and it is further supported by more recent data indicating increased prevalence of hyperlipidaemia in diabetic patients (Agbedana et el, 1988; Laakso and Pyrala, 1988).

There is considerable evidence from both cross-sectional and prospective epidemiological studies that plasma cholesterol has similar predictive value for large vessel disease in the diabetic patient as the non-diabetic subjects (Pyrala et al, 1987). Data on the predictive value of plasma triglyceride in diabetics are scanty. However, plasma triglyceride does predict vascular disease in the Paris prospective study and WHO multinational study (Fontbonne et al, 1989; Stevens et al, 1990). In the cross-sectional studies, plasma triglycerides are often more strongly related to the presence of large vessel disease than cholesterol.

There may be a need to perform both primary and secondary intervention trials of cholesterol reduction in the diabetic population in Nigeria and other parts of sub-saharan Africa .

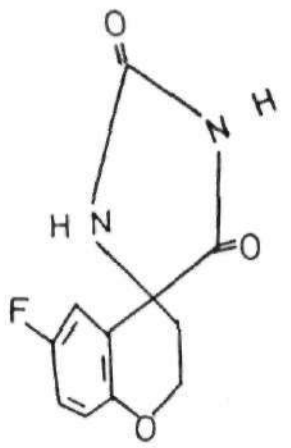
2.4 ALDOSE REDUCTASE INHIBITORS (ARIs)

Aldose reductase inhibitors (ARIs) are drugs used to block the polyol pathway. They specifically inhibit the activity of the enzyme, aldose reductase. The two major structural classes of aldose reductase inhibitors are carboxylic acids and spirohydantoins (Kador et al, 1985).

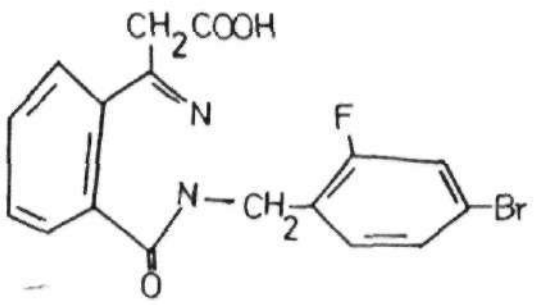
Although, aldose reductase inhibitors appear to be diverse, certain similarities can be seen among them. Both Kinetic and competitive studies (Kador et al, 1985) with purified enzyme reveal that the inhibitors interact with aldose reductase at a common site independent of either the substrate or the nucleotide cofactor binding sites. Most aldose reductase inhibitors display non or uncompetitive inhibition profiles.

The initial interst in aldose reductase inhibitors was stimulated by the wish to define the role of the polyol pathway in the development of cataracts (Kinoshita, 1974). Four potent inhibitors, sorbinil (CP 45, 634) a spirohydantoin, tolrestat (Alredase), Ponalrestat (Statil) and epalrestat (ONO 2235), have undergone extensive evaluation in animal models with diabetic complications and also in numerous clinical studies. Through their use, evidence for the involvement of aldose reductase in other serious diabetic complications has evolved (Kador and Kinoshita, 1984) (Fig. 2.6).

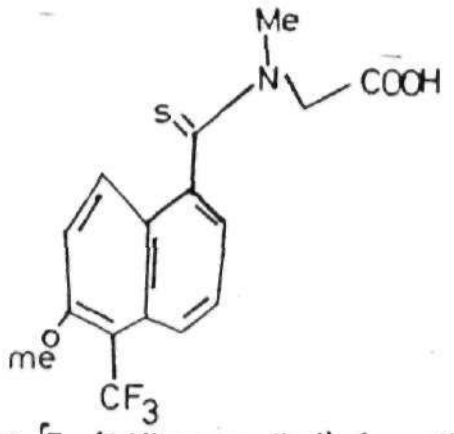
Aldose reductase inhibitors have been reported to improve the transmission abnormalities of peripheral nerves in animal experiments (Dvornik et al, 1973; Peterson et al, 1979; Mayer and Tomlinson, 1983) and in human clinical trials (Handelsman and Tuttle 1981; Judzwitsch



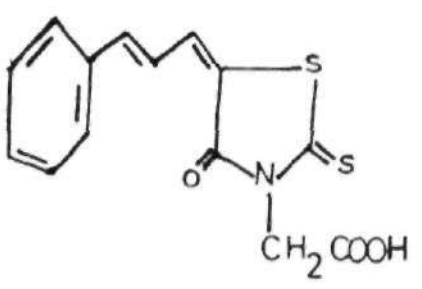
6-Fluoro spirochroman -4-4'-imidazolidine -2', 5'-dione
(Sorbinil, cp 45, 634)



3-(4-bromo-2-Fluorobenzyl-4-OXO -3H-phthalazine -1-ylacetic acid
(Ponalrestat, ICI 128, 436)



N-[5-(trifluoromethyl)-6-methoxy -1-naphthalenyl thioxomethyl] -N-methylglycine
(Tolrestat, AY22 773)



(E)-5[(E)-2-methyl-3-phenyl-propenylidene] rhodamine-3-acetic acid
(Epalrestat, ONO 2235)

Fig. 2.6 Structures of Aldose Reductase Inhibitors under clinical trial.
Small and Collier (1991)

et al, 1981; Jaspan et al, 1983). Dvornik et al (1973) and Handelsman and Tuttle (1981) reported the use of alrestatin while Peterson et al (1979) and Judzwitsch et al (1981) reported the use of sorbinil. On the other hand, Kikkawa et al (1983) reported the use of a new ARI, ONO-2235, which is structurally different from the previous reported inhibitors. ONO-2235 has a potency similar to sorbinil but higher than alrestatin.

In another study, Lewin et al (1984) demonstrated that no beneficial effect of sorbinil on painful diabetic neurophathy was detected in diabetic patients. In contrast, Yue et al (1982) and Judzwitsch et al (1983) demonstrated that ARI has a beneficial effect on nerve conduction in rats with spontaneous galactosaemia and with streptozotocin-induced diabetes. Similar results were also reported by Jaspan et al (1983). Several studies indicate that treatment with an aldose reductase inhibitor reduces the increased nerve levels of sorbitol towards normal (Sherman and Dyck, 1983; Dyck et al, 1988; Sima et al,1988).

Diabetic cataract can be ameliorated by ARIS (Kinoshita et al, 1979; Beyer-Mears and Cruz, 1985). In normal subjects, sorbinil is eliminated slowly from the circulation with a half-time of disappearance of 24-25 hr. A dose of 200 mg/day (Lewin et al, 1984) have been tried. No adverse reactions of ARI administration on human diabetic subjects have been reported except in one study by Lewin et al (1984). One out of 13 patients they studied developed febrile illness with rash and oral ulceration 21 days after starting sorbinil, which resolved on withdrawal. A requirement for future

studies of the efficacy of aldose reductase inhibitors must be the careful measurement of erythrocyte and, where possible, tissue levels of polyols in the experimental and control groups. This will help to assess the degree of aldose reductase inhibition.

2.5 BIOCHEMICAL INDICES OF METABOLIC CONTROL AND COMPLICATIONS IN DIABETICS

The main goal of clinicians involved in diabetic management is the attainment and maintenance of normoglycaemia in their patients. Measurements of urinary and blood glucose have been employed as indicators of glycaemic status in diabetic patients. Alberti et al (1975) have previously shown poor correlation between glucose and other metabolic parameters in insulin requiring diabetics. Consequently, several other biochemical parameters have been suggested as measures of glycaemic status for short, intermediate and long-term complications of diabetes mellitus. Besides, a biochemical marker reflecting early renal damage or retinopathic change would be a useful adjunct to recognising the diabetic patient at risk. Significant progress has been made in these respects in advanced countries while clinical diabetologists in developing countries like Nigeria and other African countries are faced with alot of setbacks. It has been suggested that measurement of several biochemical metabolites other than glucose are necessary. This is because "control" in relation to glucose remains an elusive term (Alberti, 1992).

2.5.1 Urinary and Blood Glucose

Urinary and blood glucose estimations (fasting or random) still stand out as the only means of biochemically establishing the diagnosis of diabetes mellitus. Blood glucose gives an indication of control only at a single moment and urine glucose reflects blood glucose concentation over at most only a few hours. Home urine and blood glucose monitoring are used for day-to-day adjustment of insulin dose. In this respect these measurements remain unchallenged especially that of blood glucose. This is because frequent blood glucose sampling remains the most valuable method of assessing diabetic control.

Home monitoring is however, difficult in most of the third world countries as most of the patients are illiterates. Areas where these measures are available in clinical laboratories involved in providing diabetic service, cost is problem. Moreover, urinary and blood glucose measurements are subject to a lot of limitations in terms of application and clinical utility. Homeostasis of glucose in both the controlled and uncontrolled state of diabetes mellitus involves a large number of interrelated mechanisms whose overall effect have been difficult to quantify (Skylar, 1980). The most important limitation is the lack of correlation of blood glucose to urine glucose in diabetic patients (Walford et al, 1980). Although, estimation of blood glucose level is considered more reliable than urine glucose, in practice, blood glucose concentrations are known to fluctuate greatly during the day, even with the best form of therapy. These parameters do not therefore accurately reflect the degree of

control of diabetes mellitus in relation to other biochemical metabolites.

2.5.2 Glycated Proteins

2.5.2.1 Glycated Haemoglobins (HbA_1)

Huisman and Dozy reported as early as 1962 the presence of elevated levels of glycated haemoglobin in patients with diabetes mellitus but did not recognise this as a significant finding.

There are several fractions of glycated haemoglobin HbA_{1a}, HbA_{1b} and HbA_{1c} but HbA_{1c} is the major component. This is formed by the reaction between glucose and N-terminal valine of the haemoglobin beta-chains (Garlick et al, 1983). As the red cell membrane is freely permeable to glucose, the measurement of glycated haemoglobin provides an integrated measure of plasma glucose concentration during the preceeding 4-8 weeks since the half-life of haemoglobin is about 120 days (Gabbay et al 1977, Peterson 1982).

After the finding of a 'diabetic' haemoglobin component by Rahbar (1968), Trivelli et al (1971) published the first set of figures in diabetic patients. Trivelli and co-workers (1971) found a reference range for HbA of 5.0-8.0 % and values of up 15 % in adult diabetics. Paulsen (1973) found HbA_{1c} levels ranging from 10 to 20 % in insulin-dependent diabetic children. High levels of HbA_{1c} in poorly controlled diabetic patients decreased after effective therapy: 6.8-12.1 % before therapy; 4.2-7.6 % after three months intensive treatment (Koening et al, 1976). These results initiated several investigations regarding the clinical application of the measurement of glycated haemoglobin in the management of diabetes mellitus.

It has been established that the measurement of HbA_1 provides additional information to that obtained by the measurement of blood glucose concentration, glycosuria and physical examination. Gabbay et al (1977) found a good correlation between the actual HbA_1 level and the urinary glucose measure two months earlier. Subsequent several studies could not confirm good correlation between HbA_1 and urinary glucose (Casparie and Miedema, 1977; Ross and Gibson, 1979; Vagues et al, 1979). On the other hand a good correlation with the mean blood glucose concentration was uniformly confirmed (Vagues et al, 1979; Dunn et al, 1979; Tschobroughtsky et al, 1980). Vertommen et al (1987) reported that there is no correlation between HbAl values and fasting plasma glucose.

However, it is clear that HbA_{1c} cannot be used for day-to-day adjustments of insulin dose: in this respect, home blood glucose monitoring remains the only choice. Glycated haemoglobin (HbA_{1c}) measurements cannot also be used as an assessment of impaired glucose tolerance. The measurement of HbA_{1c} provides an objective assessment of post glycaemic control, measure of effectiveness of therapy and patient reliability (Miedema and Casparie, 1984).

Efforts in developing countries in establishing reliable method for assay of HbA_{1c} have not been successful despite the fact that a number of assays are now available. In Nigeria and other African countries, the situation is worse. Previous attempts in this hospital to standardise a method for glycated haemoglobin assay was met with difficulties (Onyemelukwe et al, 1982). This was due to requirements for specialised skill, expensive reagents and instrumentation.

Besides, red cell genetic disorders including haemoglobinopathies, thalassaemic and erythrocyte enzymopathies are associated with decreased red cell stability and hence an alteration in the glycated haemoglobin level (Onyemelukwe et al, 1983; El-Hazmi et al, 1991).

Glycated haemoglobin (HbA_{1c}) assay as a rick factor for long-term diabetic complication has also been investigated in a prospective study by Reichard et al (1990) who reported that mean HbA_{1c} significantly correlated positively with the progression of all the complications evaluated. Further investigations are required in this respect to clarify this relationship.

2.5.3.2 Glycated Plasma Proteins (Fructosamine)

Fluctuating levels of hyperglycaemia may not be reflected in the levels of glycated haemoglobin due to its longer half-life. Glycated haemoglobin does not represent the current quality of diabetic control. This problem led to the introduction of glycated albumin and fructosamine in the clinical management of diabetes (Kennedy and Baynes, 1984).

Fructosamine is a general term for total glycated proteins in plasma. The predominant protein is albumin (Johnson et al, 1982; 1987). Fructosamine has been promoted as an alternative test to HbA_{1c}. The half-life of albumin is about 14-20 days, hence serum or plasma fructosamine indicates the mean glycaemia over 2-3 weeks (Lim and Stalely, 1985). The clinical utility of serum fructosamine assays in diabetic Nigerians has been evaluated in Zaria (Isah et al, 1990). The observations that serum fructosamine responds rapidly to

changes in glycaemia than HbA_{1c} (Baker et al, 1984; Lim and Stalely, 1985) have been confirmed in one study in Zaria (Isah and Abubakar, 1991). In another study in Lagos, a comparative study of the glycated plasma proteins in diabetic Nigerians was undertaken (Ohwovriole et al, 1991). In both studies (Isah et al 1990 and Ohwovoriole et al, 1991), serum fructosamine and glycated plasma proteins clearly differentiated between diabetics and non-diabetics as well as between patients in moderate, poor and good control respectively. These reports agree with the view of Dominiczak (1991) who opined that fructosamine measurement is a good reflection of control in diabetic patients who are in moderate to poor glycaemic control. Data in this respect are still scanty in Nigeria and other African countries.

Fructosamine concentration reflects primarily the concentration of glycated albumin. For that reason, the results must be interpreted with caution in grossly hypoalbuminaemic patients and in patients with hypergammaglobulinaemia. Wolffenbuttel et al (1991) observed that serum fructosamine shows considerable variations, and has no clinical advantage over HbA_{1c} measurements.

2.5.2.3. Glycated Fibrinogen

Fibrinogen is a plasma protein with a half-life of four days and therefore, may be valuable as an index of short-term diabetic control. It has been previously observed that fibrinogen levels are elevated in diabetics irrespective of the duration of the disease (Lutjen et al, 1985).

Hammer et al (1989) have established a new method for measuring glycated fibrinogen and this has been validated clinically. Hammer and coworkers (1989) reported that the differences in the percentage of glycated fibrinogen between normal subjects, well controlled diabetics and poorly controlled diabetics were highly significant. They also observed a significant correlation between glycated fibrinogen and glycated haemoglobin for all the subjects. However, in newly diagnosed diabetics following treatment, glycated fibrinogen fell more rapidly than glycated haemoglobin. Conversely, in deteriorating control, the glycated fibrinogen levels rose much more rapidly, thus alerting clinicians to the alteration in control earlier than could be the case with glycated haemoglobin or fructosamine. It has thus been suggested that glycated fibrinogen provides a simple integrated index of control which responds quickly to altered control and should provide a valuable adjunct to blood glucose estimations in the management of unstable diabetes. In addition, glycated fibrinogen is preferable to glycated haemoglobin when control is monitored over short periods such as in clinical trials and, most notably, in pregnancy (Hammer et al, 1989). However, estimation of this parameter as a measure of diabetic control in this environment has not been evaluated.

2.5.3. Red Cell Sorbitol (RCS)

It has been demonstrated that intact red cells are capable of converting glucose to sorbitol because of the presence of aldose reductase (Malone et al, 1980 and Onaya et al, 1990).

The red cells, as well as certain tissues sensitive to sorbitol damage, such as the lens, neurons, sciatic nerves and glomeruli are freely permeable to glucose (Travix et al, 1971). Malone and his coworkers (1980) suggested that the amount of intracellular sorbitol may be a reflection of the surrounding glucose concentration and/or flux through the polyol pathway.

Malone et al (1984), Kikkawa et al (1984) and Popp-Snigder et al (1984) demonstrated that RCS concentration paralleled the sorbitol content of both lens and peripheral nerves over a wide range in hyperglycaemia, indicating that red blood cells provide a convenient model for assaying the activity of the polyol pathway in these less accessible tissues. Estimation of RCS, therefore, can be useful in studying the pathogenesis of diabetes associated complications.

The utility of red cell sorbitol (RCS) measurements as an index of metabolic control in diabetics have been investigated in several studies with conflicting results (Malone et al, 1984; Vertommen et al, 1987; Price et al, 1987, Wambua et al, 1988; Onaya et al, 1990). In most of these studies it has been confirmed that RCS is significantly elevated in diabetic patients compared with non-diabetic subjects. Red cell sorbitol positively correlated with prevailing blood glucose (Malone et al, 1980; Malone et al, 1984; Lapolla et al, 1985; Vertommen et al, 1987; Price et al, 1987; Wambua et al, 1988; Onaya et al, 1990). Onaya et al (1990) also reported that RCS was significantly elevated in type 2 diabetics with complication compared to those without, contrary to that of blood glucose. Lapolla et al (1985), Vertommen et al (1987) and Onaya et al, (1990) reported that RCS level correlated positively and

significantly with the HbAIc level. However, Onaya et al (1990) used the sorbitol/glucose ratio in line with the suggestion by Malone et al (1984). Price et al (1987) concluded that RCS cannot be used as a measure of long-term sorbitol accumulation in other tissues contrary to earlier report by Malone et al (1984) because Price and coworkers (1987) observed that RCS rapidly returned to normal during normoglycaemia and also found no correlation between RCS and HbA_{1c}.

Assay of RCS in clinical studies may prove valuable in elucidating the role of the polyol pathway in the evolution of diabetic complications as well as in monitoing the success of therapy aimed at blocking the activity of this pathway using aldose reductase inhibitors. Therefore, RCS levels and possibly sorbitol/glucose ratio or sorbitol/haemoglobin ratio may provide information helpful for selecting patients at greater risk for the late complications of DM. The conflicting reports on the results of the red cell sorbitol content in diabetic population may be related to the well-recognised range of patient susceptibility to diabetic complications and probably to the method employed in the study. Yet unknown factors may play significant role. There is need to identify these factors, evaluate and define a reference range of RCS for the non-diabetic control population and agree on a convenient unit of measurement to be employed. Data are accumulating in some of these aspects in advanced nations contrary to the prevailing situation in developing countries (Malone et al 1984; Vertommen et al 1987; Onaya et al 1990, Tawata et al 1992).

2.5.4. Lipid and Lipoprotein Profiles

Lipid and lipoprotein profiles have been suggested as measures for metabolic control in diabetes mellitus (Paterson et al, 1991; Haffner et al 1992). Some data are available on the relationship between lipid profiles and varying degrees of diabetic control in the ambulant Nigerian diabetic (Akanji et al 1989).

In type I diabetic subjects in good glycaemic control, lipid levels are similar to normal controls whereas those with poor control usually have elevated levels (Betteridege and Kathryn, 1991). Hence, well controlled type I diabetics have normal levels of triglyceride, cholesterol, LDLC and VLDL.

Levels of HDL, while low in untreated or poorly controlled type II diabetic patients, are similar to appropriate non-diabetic control subjects or even raised in patients established on insulin therapy (Betteridege and Kathryn, 1991). That HDL levels, estimated as HDL-c, are usually normal or even raised in patients with IDDM has been confusing. This is because epidemiological studies suggest that elevated levels of HDL protect against cardiovascular disease (Miller and Miller, 1975; Nikkila, 1981).

Levels of LDL, are generally raised in poorly controlled patients but return to levels similar to those of control subjects when normoglycaemia is achieved. Levels of LDL, are more likely to be elevated in type II diabetic patients (Betteridege, 1989).

Plasma cholesterol and triglyceride have been suggested as risk factors for vascular disease in diabetes mellitus (Pyrala et al 1987; Fonthonne et al 1989; Stevens et al 1990). Paterson et al (1991)

have reported that HbA_1 was significantly associated only with cholesterol concentrations in type II diabetics. In addition, attention has also been drawn to the low HDL often seen in type II patients. Attempts have been made in Nigeria to assess lipid and lipoprotein status in relation to diabetic control and complications (Agbedana et al, 1988; Akanji et al, 1989). Elevated levels of lipids have been reported in diabetic retinopathy compared with those without(Osuntokun et al, 1971 Hanna, 1982).

Measurement of lipids and lipoproteins can thus be used for evaluation of metabolic control and complications in diabetic patients. Hypercholesteraemia has been observed to be a significant problem in diabetic patients even in those patients with good or acceptable glycaemic control (Paterson et al, 1991). It has thus suggested that lipid-lowering therapy should be instituted in a significant number of patients, especially those having good glycaemic control.

2.5.5. Urinary and Serum N-Acetyl-β-D-Glucosaminadase (NAG)

The lysosomal hydrolase, N-acetyl-β-D-glucosaminidase (NAG) from the proximal tubular cells, is elevated in diabetic patients with poor glycaemic control (Ellis et al, 1983; Brouchard, 1984; Shimojo et al, 1987; Watts et al, 1988). Urinary NAG activity is also high in diabetic patients with severe small vessel disease (Price and Foster, 1979; Whiting et al, 1979; Shimojo et al, 1987; Watts et al, 1988), but general agreement has not been reached about the relationship of this activity to the degree of microangiopathy.

N-Acetyl-β-D-glucosaminidase has also been shown to be elevated in diabetics at diagnosis (Price and Foster 1979) and has been suggested as a good indicator of diabetic retinopathy (Shrka et al, 1987), although Poon et al (1979), and Watts et al (1988) were unable to confirm this observation.

Watts and coworkers (19 8) demonstrated that raised urinary NAG is related to long-term glycaemic control as measured by HbA1. The relationship of raised urinary NAG to microalbuminuria and poor long-term glycaemic control raises the possibility that this urinary enzyme might fulfil a role in studying patients at risk of renal microangiopathy. Agardh et al (1987) reported contrary observation from a small prospective study. Further investigation is required on the clinical usefulness of the measurement of this enzyme in diabetic care.

2.6 LOCATION OF STUDY

The study was carried out at the Ahmadu Bello University Teaching Hospital, Zaria. Zaria is a town located on the Northern part of Kaduna State of Northern Nigeria.

2.7 THE THESIS

2.7.1 Statement of Research Problems

1) Can red cell sorbitol content be estimated reliably in a third world laboratory like ours?
2) Has this assay any clinical utility in the management of long-term diabetic complications in Nigerians or serve as

a specific and reliable biochemical risk factor for long-term diabetic complications?

3) What are the factors that influence red cell sorbitol level in diabetics and non-diabetics?

4) What are the advantages and disadvantages of the use of red cell sorbitol measurement in the assessment of metabolic control in diabetics in relation to other available parameters?

2.7.2 Aims of the Study

There has been no detailed report on analytical performance and clinical validation of red cell sorbitol assay in Nigeria or in other African countries.

Standardization of measurements and the interpretation of data are essential parts of the improvement of the care of diabetic patients in our environment.

The interpretation of sorbitol assay results has been very difficult due to observations by several authors that other factors other than hyperglycaemia interfere with its levels. These factors are yet to be fully characterised. It is believed that results from this investigation will serve as a baseline for a prospective study in Zaria.

The specific objectives of this study are:

1. To standardise an analytical method for the measurement of red cell sorbitol and establish a reference range for the population.

2. To study the effect of sex, age, blood pressure, body mass index (BMI) and disease duration on red cell sorbitol levels.
3. To define the usefulness of red cell sorbitol assay as a measure of metabolic control in diabetics by evaluating the relationship (if any) between red cell sorbitol and fructosamine, red cell sorbitol and lipid concentrations and red cell sorbitol and glucose.
4. To define the status of red cell sorbitol in diabetics with or without complications.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SUBJECTS

A total of 558 subjects (310 males, 248 females) were studied. These consisted of the followings:

3.1.1 Apparently Healthy Subjects

A total of 203 subjects (118 males, 85 females) were recruited for the study. These were divided into two groups. The first group consisted of 101 healthy volunteer blood donors aged 17-55 years (mean age 33.1 years) who presented at the Blood Transfusion Service of the Ahmadu Bello University Teaching Hospital, (ABUTH), Zaria. The majority were engaged in peasant farming and a few were small-scale traders. The second group consisted of 102 subjects aged 12-70 years (mean age 31.6 years). They were made up of 35 laboratory and other hospital staff, 10 medical students, 19 students of the school of Medical Laboratory Technology, all of Ahmadu Bello University Teaching Hospital, Zaria. Others were 10 students of Alhudahuda College, Zaria, and 10 staff of the Nigeria Railway Corporation, Zaria. The rest were persons referred to the laboratory for general medical check-up and others who volunteered to participate in the study. The details on these control subjects are given in table 3.1. None of the subjects in the two groups had personal or family history of diabetes mellitus. Fifty out of the 203 apparently healthy subjects admitted positive smoking habit (ten or more sticks of cigarette per day) while another thirty confirmed to alcohol of five or more bottles of beer per day. Results obtained from the above subjects were used to establish

reference values for red cell sorbitol for the local population and assess the relationship of several variables with RCS.

Twenty two other apparently healthy subjects (11 males; 11 females) aged 22-58 years (mean age 39.1 years) volunteered for a two hour blood glucose screening (2HBGS).

3.1.2 Subjects from General Medical Out Patient Department (GMOPD)

This group consisted of 102 subjects (49 males, 53 females) aged 6-83 years (mean age 40.8 years) referred from the GMOPD to the Laboratory. Fasting or random plasma/serum glucose estimation was requested on these patients in order to support the diagnosis of diabetes mellitus (DM). The patients presented with one or more of the classical signs and symptoms of DM such as polyuria, polydipsia, nocturia, unexplained weight loss, recurrent boils and some with hypertension. Results from these subjects were used to assess the diagnostic specificity and sensitivity of red cell sorbitol assay.

3.1.3 Diabetic Patients

One hundred and eighty two known Nigerian diabetics were investigated. These were made up of 37 patients aged 17-50 years (mean age 37.2 years; 21 males, 16 females) with insulin dependent diabetes mellitus (IDDM, Type I) and 145 patients aged 33-75 years (Mean age 49.1 years; 83 males, 62 females) with non-insulin dependent diabetes mellitus (NIDDM, Type II; Table 3.1). All the patients were attending the diabetic clinic of the ABU Teaching Hospital, Zaria for assessment of glycaemic control and management. Twenty four out of the 182 diabetic patients

Table 3.1 AGE AND OTHER DETAILS (MEAN±SEM) ON PATIENTS AND CONTROL SUBJECTS STUDIED

Patients	n	Age (years)	Disease Duration (years)	Body Mass Index (kg/m^2)	Blood Pressure S/D (mm Hg)
IDDM (Type 1)	37				134±4*
M/F	21/16	37.2±1.44*	4.7±0.82	23.0±0.55	82±2
NIDDM (Type II)	145				138±2*
M/F	83/62	49.1±0.89*	5.4±0.49	26.7±0.38+	83±1
Controls	203				117±1
M/F	118/85	31.7±0.90	-	22.05±0.48	75±1

n = no of subjects
S = Systolic
D = Diastolic
IDDM = Insulin dependent diabetes mellitus
NIDDM = Non-insulin dependent diabetes mellitus
*P<0.001 = Diabetic Vs non diabetic control
+P<0.001 NIDDM Vs type I and non diabetic controls

presented with neuropathy (painful paraesthesae, loss of tendon reflexes or loss of vibratory sense and impotence), 24 with nephropathy (reagent strip positive for persistent proteinuria and plasma creatinine $\geq$ 160 µmol/L) and 14 with retinopathy (microaneurysms, haemorrhage and exudate). Sixty one were hypertensive (BP $\geq$ 160/95 mm Hg) while 58 were obese (BMI $\geq$ 27kg/m^2). These clinical data were obtained with the help of clinicians at the out-patient diabetic clinic.

Two hr post-prandial blood glucose screening was conducted in 33 newly diagnosed patients aged 30-65 years (mean age 37.5 years, 21 males, 12 females). They were part of the numerous cases referred from the GMOPD and other units within the ABU Teaching Hospital, Zaria to the Laboratory for oral glucose tolerance test (OGTT) and who were subsequently confirmed to have DM.

3.1.4 Other Patients

This group consisted of hypertensive non-diabetic patients attending the Cardiac Clinic at the ABU Teaching Hospital, Zaria. They were made up of 16 patients, aged 24-60 years (Mean age 50.4 years, 7 males; 9 females). Patients who had blood pressure (BP) readings of 160/95 mm Hg or greater on three separate occasions or were on treatment for hypertension, were confirmed to have the disease.

3.1.5 Any Other Information on the Subjects

Data such as age, height, weight, BP, tribe, occupation (Appendix I) were recorded for each subject. In addition, disease duration, type of treatment and presence of complication(s) were

also recorded for the diabetics. Body mass index (BMI) was calculated from weight and height as weight in Kg per height in m^2. Obesity was defined as BMI $\geq$ 27.0 kg/m^2.

3.1.6 Ethical Consideration

The study was approved by the Ethical Committee of the Faculty of Medicine, Ahmadu Bello University, Zaria in accordance with the Declaration of Helsinki.

3.2 REAGENTS

Nicotinamide adenine dinucleotide (β-NAD), sorbitol dehydrogenase (SDH), sorbitol standard, human haemoglobin and retinol were obtained from Sigma (Poole, Dorset, England), ethylacetate and isopropyl alcohol from May and Baker (Dagenham, Essex, England), sodium methoxide from Aldrich Chemical Company, (Gillingham, Dorset, England), and others were procured from BDH (Poole, Dorset, England). All the chemicals were of analytical grade or higher. The drugs used were supplied by the ABU Hospital Pharmacy as well as a private pharmacy Store in Zaria.

3.3 EQUIPMENT

An SFM 25 Spectrofluorimeter (Kontron Instruments, Tegimenta, Switzerland) was used for red cell sorbitol assay. Fructosamine was measured on an Sp 1800 UV Spectrophotometer attached to Sp 1805 programme controller (Pye unicam, Cambridge, England). Plasma glucose, albumin, and creatinine were estimated manually on Corning 253 colorimeter (Corning Medical and Scientific Beds, England). Measurements of erythrocyte haemoglobin, total cholesterol (TC) and high density lipoprotein cholesterol (HDLC) were carried

out on SP6-300 spectrophotometer. Triglyceride was assayed on UV visible ultrospec 4054 plus spectrophotometer (Pharmacia LKB Biochrom, Cambridge, England).

3.4 METHODS

3.4.1 Blood Collection

Venous blood specimens (ante-cubital) were collected into lithium heparin plastic tubes with minimum stasis using dispossable hypodermic syringe and needle. Fasting blood samples were obtained after about 12-14 hr (over night) fast. In some subjects non-fasting specimens (random) were obtained after about 2-3 hr of breakfast. The blood samples were centrifuged on MSE Mistral 4L centrifuge (MSE, England) at 1000 x g for 20 min within one hr of collection and the plasma harvested into plain clean bijou bottles.

Plasma glucose, albumin, red cell sorbitol and haemoglobin were analysed within 24 hr of sample collection. Alliquots of plasma were stored immediately at-20^{o}C until the estimation of other biochemical parameters.

3.4.2 Washing of Red Blood Cells

About 5 ml of red blood cells were suspended in equal volume of cold isotonic saline (Nacl 0.155 mmol/L). This was mixed thoroughly and centrifuged at 1000 x g for 20 min at 4^{o}C. The supernatant and topmost layer of cells were removed and discarded. This procedure was repeated three times on each occasion .

3.4.3 Two-hour Blood Glucose Screening

The test was conducted on all the subjects in accordance with the guidelines recommended by the World Health Organisation (WHO, 1985). After an overnight fast, venous blood was collected from each subject. A drink containing 75 g glucose (dextrose monohydrate) dissolved in about 300 ml of water was given and taken within 5 min. A further blood sample was collected 2 hr after the glucose load. The blood specimens were collected into lithium heparin plastic tubes, and plasma separated as discussed earlier (3.4.1).

3.4.4 Analytical Methods

3.4.4.1 Red Cell Sorbitol

Principle

Red cell sorbitol (RCS) was determined by fluorimetric enzymatic procedure based on the method of Clemens as reported by Malone et al (1980). The equation for the reaction is as shown below.

$$\text{Sorbitol} + NAD^{+} \underset{\longleftarrow}{\overset{SDH}{\longrightarrow}} \text{Fructose} + NADH$$

Sorbitol in the presence of oxidized nicotinamide adenine dinucleotide (NAD^{+}) as coenzyme, is oxidized by sorbitol dehydrogenase (SDH) to form its keto sugar, fructose. In the process, reduced NAD (NADH) is generated. The reaction is reversible but the forward reaction utilizes NAD^{+} only. The interaction of radiant energy with molecules of NADH results in fluorescence. Fluorescence occurs when the NADH molecule, as a fluorophor absorbs light at a shorter wavelength (excitation) and re-emits the light at a longer

wavelength (emission). The relative fluorescence (RF) due to generation of NADH is proportional to the concentration of sorbitol. The sorbitol concentration so obtained is used as an index of the activity of aldose reductase.

Analytical procedure

To 1.0 ml of the washed cells was added 3.0 ml of 1.0 mol/L cold perchloric acid and mixed thoroughly. This was centrifuged at 4°C for 15 min to precipitate the protein. Two millilitres of the clear supernatant was neutralised with 70 µl of 3.0 mol/L potassium hydroxide at 4°C (pH 9-10). To a reaction mixture which consisted of 0.1 mmol/L NAD and 1.0 mg SDH in 1.0 ml of 0.05 M glycine buffer (pH 9,5) was added 0.5 ml of the clear neutralised supernate. Blank (glycine buffer and distilled water) was run simultaneously. Each sample was analysed in duplicate against the blank. Sorbitol standard of 39.6 nmol/ml was used for instrument calibration. The relative fluorescence (RF) due to the production of NADH was measured on an SFM 25 spectrofluorimeter after incubation for 1 hr at room temperature. Concentrations of red cell sorbitol were obtained from a calibration curve.

3.4.4.2 Red Cell Haemoglobin

Red cell haemoglobin was measured by the universally adopted method using ferricyanide and potassium cyanide (KCN) as Drabkin's solution. In this method the Fe (II) of haemoglobin is oxidised to Fe (III) by ferricyanide and then converted into stable cyanmethamoglobin by addition of KCN.

To 5 ml of Drabkin's solution was added 0.02 ml of red blood cell, and mixed thoroughly. This was read at 540 nm after 25 min of incubation at room temperature. Values were obtained by calculation using human haemoglobin as standard.

3.5 ASSESSMENT OF RED CELL SORBITOL ASSAY PERFORMANCE CHARACTERISTICS

3.5.1 Selection of Excitation and Emission Wavelengths

Relative fluorescence of NADH standard were scanned at 360-380 nm for excitation and 450-480 nm for emission respectively. From 28.6 nmol/ml sorbitol standard, 0.5 ml was added to 1.0 ml of the glycine buffer and incubated for 1 hr at room temperature. Relative fluorescence were scanned for the NADH produced at 360-380 nm (excitation) and 450-480 nm (emission). Non-diabetic and diabetic samples were similarly treated.

3.5.2 Time Course Studies

From sorbitol standard (28.6 nmol/ml), 0.5 ml was taken and mixed with 1.0 ml of the glycine buffer and incubated at room temperature. Relative fluorescence due to the NADH produced was recorded serially at excitation wavelength of 368 nm and emission wavelength of 456 nm at 30 min intervals up to a maximum of 90 min. Non-diabetic and diabetic red cells, were similarly assessed.

3.5.3 Calibration Curve

A series of sorbitol standards (1.1-50.5 nmol/ml) were prepared from the stock (0.055 mmol/L) by dilution with distilled water. To 1.0 ml of the glycine buffer.(pH 9.5) was added 0.5 ml of each of the diluted sorbitol standard and mixed. This was then incubated at room temperature for 1 hr from the start of the reaction. Relative fluorescence due to the NADH produced was measured in duplicate at excitation and emission wavelengths of 368 nm and 456 nm. The calculated mean relative fluorescence were used to obtain the calibration curve.

3.5.4 Detection Limit

A measure of the limit of detection of the assay was obtained by measuring replicates of the reagent blank (glycine buffer and distilled water). At three standard deviations (3 SD) above the mean, the relevant figure from the calibration curve was used as a measure of limit of detection.

3.5.5 Precision Studies

Three pools of red blood cells consisting of low, medium and high contents of sorbitol were each assayed 20 times in a single analytical run to assess the within-run precision. Between run precision profiles were assessed using these pools containing low, medium and high concentrations of sorbitol. These were divided into 1500 µl aliquots after precipitation with perchloric acid and stored frozen at - 20^{o}C in plastic containers. An aliquot was assayed for sorbitol daily in duplicate for several days.

3.5.6 Recovery Studies

Aqueous sorbitol standards of 10.0, 20.0, 30.0, 40.0 and 50.0 nmol/ml were prepared. To 1.8 ml of red cell pools from non-diabetics was added 0.2 ml of the above standards. This was assayed in duplicate for five times. Similarly, 1.8 ml of red cell with 0.2 ml of diluent was assayed for sorbitol in duplicate for five times. Recovery was estimated by expressing recovered sorbitol as a percentage of added sorbitol.

3.5.7 Interference Studies

The effects of several blood constituents including some drugs and commonly used oral hypoglycaemic agents on the assay of red cell sorbitol were evaluated. Each analyte or drug was dissolved in water or appropriate diluent. To 1.0 ml of pooled red cell from non-diabetics, 0.5 ml of the analyte or drug was added and assayed in duplicate for five times. The mean was calculated ($\bar{X}_1$). To 1.0 ml of the same pool of red cell, was added 0.5 ml of the appropriate diluent alone. Sorbitol was assayed in duplicate for five times and mean also calculated ($\bar{X}_2$). The magnitude of the interference ($\bar{X}_1 - \bar{X}_2$) was estimated for each analyte or drug. Percentage interference (P I) was calculated as $\frac{\bar{X}_1 - \bar{X}_2}{\bar{X}_1} \times 100$.

3.5.8 Quality Control and Slight Modification

Internal quality control material (red blood cell pools) preserved with perchloric acid was utilized as earlier prepared and used for the precision studies (3.5.5). An liquot

was thawed on each day of analysis and estimated simultaneously with the test samples. Values within the $\bar{X} \pm 2$ SD were accepted as good control (Fig 3.1).

The slight modifications introduced in the technique for the red cell sorbitol assay for possible adaptation were (i) neutralization of protein free supernate with 3.0 mmol/L potassium hydroxide (KOH) instead of potassium carbonate (K_2CO_3) as suggested by Henry and Szustkiewicz (1974) and used by Vertommen et al (1987) (ii) use of 0.1 mmol/L NAD^+ instead of 0.2 mmol/L and (iii) the use of excitation and emission wavelengths of 368 nm and 456 nm respectively. These modifications have the advantages of reduced formation of potassium perchlorate ($KClO_4$) precipitates, reduced costs and increased specificity on the SFM 25 spectrofluorimeter as observed from the wavelength optimization studies.

3.5.9 Statistical Analyses

Standard statistical tests such as Pearson's correlation coefficient and Student's t-test for paired and unpaired data were used. Parametric ANOVA, (analysis of variance) and linear regression analyses were employed. The reference values of red cell sorbitol concentration in the apparently healthy subjects were obtained by probit transformation (Gowans et al 1989). Most of the statistical analyses were performed with a customized programme on an AMSTRAD PCW 8256 computer and CASIO FX-80 Scientific calculator.

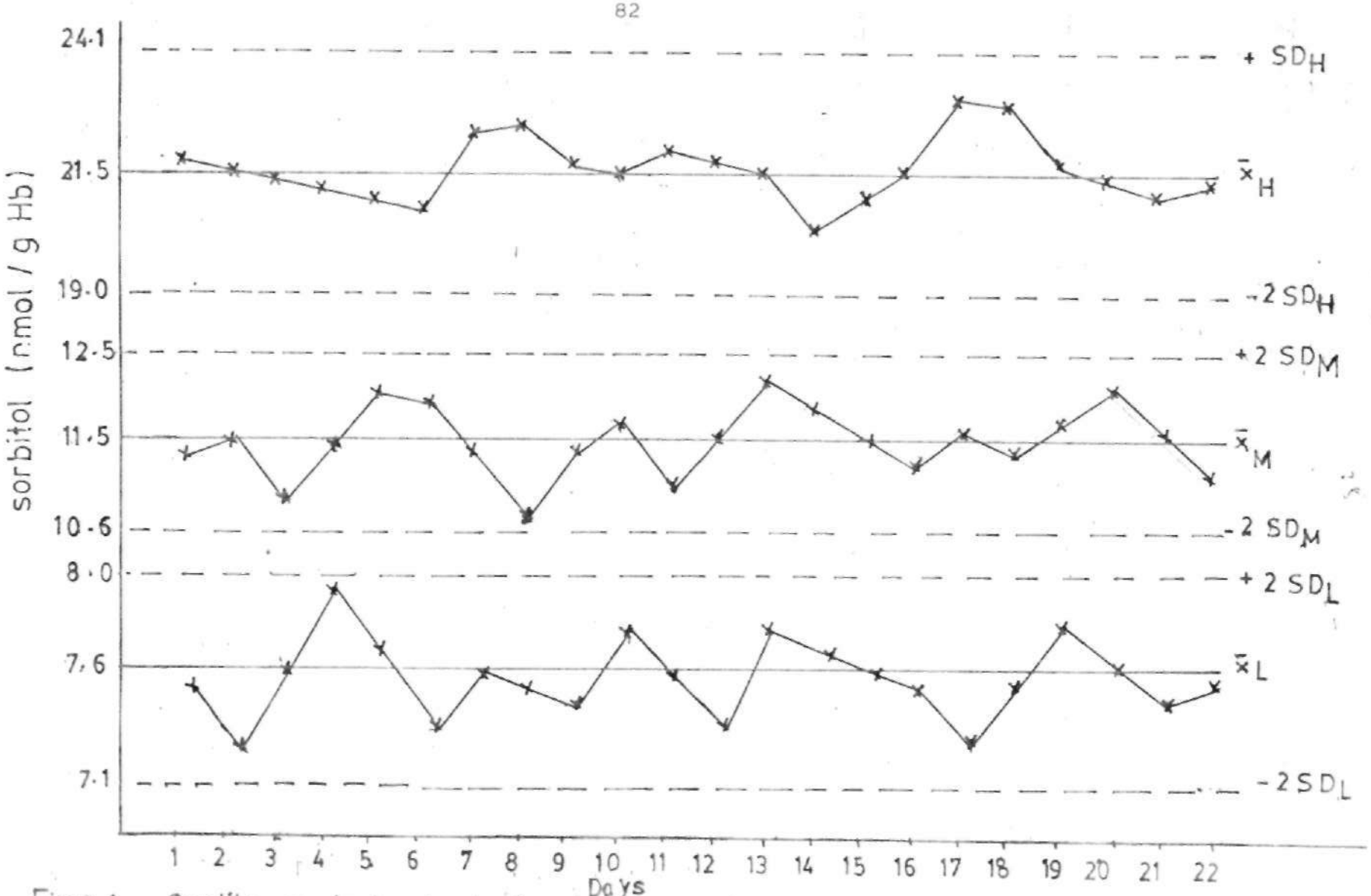


Fig 3·1 Quality control charts for between day runs on three red cell pools (L = low concentration M= medium concentration; H= high concentration)

3.6 OTHER ANALYTES

3.6.1 Plasma Fructosamine

Plasma fructosamine (PF) was estimated by the method of Johnson et al (1982), using nitroblue tetrazolium (NBT) semimanually as recently evaluated in our laboratory by Isah (1990).

3.6.1.1 Principle

Fructosamine (Ketoamine), in an alkaline medium reduces certain tetrazolium salts such as NBT to produce a reddish brown colour which can be read colorimetrically at 540 nm. The intensity of the colour produced is directly proportional to the concentration of fructosamine in the sample.

3.6.1.2 Analytical procedure

To 2.0 ml of carbonate buffer (0.1 mol/L, pH 10.35) containing 0.25 mmol NBT/L, was added 0.2 ml of plasma. This was mixed thoroughly and incubated at 37°C. The absorbance at 540 nm was measured colorimetrically semi-manually at 10 min and 15 min from start of incubation. A calibration curve using standards (0-5 mmol/L) of 1-deoxy 1-morpholino-fructose (DMF) containing albumin (40 g/L) treated in identical fashion as the test was obtained. Distilled water was used as blank. Fructosamine concentrations were obtained from the calibration curve.

3.6.2 Plasma Cholesterol

Total cholesterol and HDLC were estimated by the ferric perchlorate method of Levin and Zac (1964).

3.6.2.1 Principle

Cholesterol reacts with strong and concentrated acids as a typical alcohol. The strong acid removes a molecule of water, then oxidizes the intermediate to produce 3,5-cholestadiene or its cation. Acetic acid and acetic anhydride are used as solvents and dehydrating reagents respectively. Sulphuric acid which is usually used as a dehyrating and oxidizing reagent is converted to sulphur dioxide. The cholestadiene reacts further to form cholestapolyene carbonium ions. In the Liebermann-Burchard reaction, the polyenes are the main chromophores (green colour). In the presence of more concentrated sulphuric acid and ferric ion, the polyene cations are the main chromophores (red colour). The absorbance of the red or green colour is read colorimetrically at specified wavelengths. The intensity of the colour is proportional to the concentration of cholesterol in the sample. The absorbance in the presence of ferric ion is said to be at least 5 times greater than the absorbance of the product that forms in the absence of iron.

3.6.2.2 Analytical Procedure

Total cholesterol was estimated by ferric-per-chlorate method. Five millilitres of cholesterol reagent which consists of ferric perchlorate in 600 ml of ethyl acetate and 400 ml of concentrated sulphuric acid was pipetted into

each tube of test, standard and blank. To the test, standard and blank tubes were added 0.05 ml of plasma, standard and distilled water respectively. The tubes were mixed for 10 s and incubated in heating block at 100°C for exactly 1.5 min and then immersed in cold water for 10 min. The tubes were removed and the exterior dried. The content was mixed by inversion and the test and standard were read at 610 nm using the blank to set the instrument to zero. Values were obtained by calculation using a cholesterol standard of 5.17 mmol/L dissolved in glacial acetic acid.

HDL cholesterol was estimated in plasma after separation of other lipoprotein fractions. Into Khan tubes, 0.5 ml of polyethyleneglycol (PEG) 6000 in distilled water was pipetted and 0.5 ml plasma added. This was mixed for about 30 s and left standing at room temperature for 15 min. It was then centrifuged for 10 min at 1000 x g. The supernatant was decanted into fresh tube and cholesterol content estimated as described above.

3.6.3 Plasma Triglyceride

Plasma triglyceride was measured by the method of Levy (1972).

3.6.3.1 Principle

Fatty acids are first removed by transesterification. The liberated glycerol is converted to formaldehyde by periodate oxidation. The formaldehyde produced is then quantitated by the Hantzsch reaction. In the presence of ammonium acetate, acetyl acetone reacts with formaldehyde to form a yellow coloured

complex, 3,5-di-acetyl-1,4-dihydrolutidine. The coloured complex is read at 412 nm colorimetrically. The intensity of the colour is proportional to the concentration of triglyceride in the sample.

3.6.3.2 Analytical Procedure

The plasma TG was harvested into a heptane layer after precipitation with isopropyl alcohol (13.0 mol/L) and dilute sulphuric acid (0.04 mmol/L H_2SO_4). Glycerol was released from the isolated TG by transesterification with sodium methoxide (9.3 mmol/L). The glycerol was then oxidised to formaldehyde by sodium metaperiodate. The product was treated with acetylacetone and ammonium acetate to produce a fluorescent condensation product (Hantzsch condensation reaction), which was quantitated colorimetrically at 412 nm. Values of TG were obtained by calculation using triolein as standard (2.3 mmol/L in isopropylalcohol).

Low density lipoprotein cholesterol (LDLC) was obtained from the Friedewald formular (Friedewald et al 1972).

$$LDLC = \text{Total cholesterol} - HDLC - \frac{TG\ level}{2.2}$$

measurements are in mmol/L. The analysis of lipid profile was conducted on 156 of the diabetic patients and 50 control subjects.

3.6.4 Plasma Glucose

Plasma glucose (PG) was estimated by the glucose oxidase method using 4-aminophenazone as oxygen acceptor (Trinder 1969).

3.6.4.1 Principle

The aldehyde group of glucose is oxidised by glucose oxidase to give gluconic acid with equivalent amount of peroxide. The peroxide formed is broken down to water and atomic oxygen by a peroxidase. The atomic oxygen produced is transfered to a suitable acceptor such as 4-amino phenazone through phenol with the production of a red coloured complex which is measured colorimetrically. The intensity of the colour formed is proportional to the concentration of glucose in the sample.

3.6.4.2 Analytical procedure

Four millilitres of phenol reagent (21.25 mmol/L) was pipetted into a test tube. To this was added 0.05ml of plasma and mixed. Then 1.0ml of colour reagent(glucose oxidase, peroxidase and 1.48mmol 4-amino-phenazone) was added and mixed thoroughly. The tube was then incubated at 37°C for 20 min. Glucose (18.0mmol/L) and distilled water were used as standard and blank respectively and were treated as the test. The absorbances were read at 520 nm colorimetrically,zeroing with the blank. The glucose values were obtained by calculation using the standard.

3.6.5 Plasma Albumin

Plasma albumin was estimated by the modified method of Batholomew and Dalaney as modified by Doumas et al (1971).

3.6.5.1 Principle

Albumin reacts with the dye, bromcresol green (BCG) which has specific affinity for it in acidic solutions to produce a coloured complex which is measured colorimetrically. The intensity of the colour produced is proportional to the concentration of albumin in the sample.

3.6.5.2 Analytical Procedure

To 4.0 ml of BCG was added 0.02 ml of plasma and mixed properly. Similarly, 0.02 ml of standard (30.0 % bovine albumin) was added to 4.0 ml of BCG. The test and standard test tubes were incubated at 37^{o}C for 10 min. The absorbance readings were taken at 628 nm using the BCG as blank. Albumin concentrations were obtained using the bovine albumin standard.

3.6.6 Plasma Creatinine

3.6.6.1 Principle

Creatinine gives a red colour with alkaline solutions of picric acid (Jaffe's reaction). The red colour is measured in the presence of a high background (yellow colour), and the solution appears orange at low creatinine concentrations.

3.6.6.2 Analytical Procedures

To 3.0 ml of distilled water was added 1.0 ml of plasma. One millilitre of 10.0 % sodium tungstate was added and 1.0 ml of sulphuric acid (1.33 mol/L) to precipate the protein. The tube was mixed well and centrifuged for 10 min. One millilitre of sodium hydroxide (0.75 mol/L) and 1.0 ml of picric acid

(saturated aqueous solution) were added to 3.0 ml of the supernatant. This was mixed and left to stand at room temperature for 15 min and then read colorimetrically at 520 nm. Standard (88.5 mmol/L) and blank (distilled water) were treated simultaneously with the test. Values of creatinine were obtained by calculation using the standard.

3.6.7. Urinary Protein

Urine protein (predominantly albumin) was estimated using the Albustix (Ames).

3.6.7.1 Principle

Albustix reagent strip is impregnated with an indicator, tetrabromophenol blue, buffered to pH 3 with citrate. It is present predominantly in the protonated, yelow form in the absence of protein. In the presence of protein, the affinity of the anionic form of the indicator dye for protein causes a shift of the equilibrium between anionic and protonated forms of the indicator towards formation of the blue anionic species. The intensity of blue colour produced is proportional to the concentration of protein in the urine sample.

3.6.7.2 Analytical Procedure

The reagent end of the strip was dipped in fresh urine and removed immediately. Excess urine was removed by running the edge of the strip against the rim of the urine container. The reagent side of test area was compared with corresponding colour chart immediately. Values were obtained semi-quantitatively as indicated on the chart.

CHAPTER FOUR

4.0 RESULTS

4.1 RED CELL SORBITOL ASSAY PERFORMANCE CHARACTERISTICS

4.1.1 Optimization of Excitation and Emission Wavelength

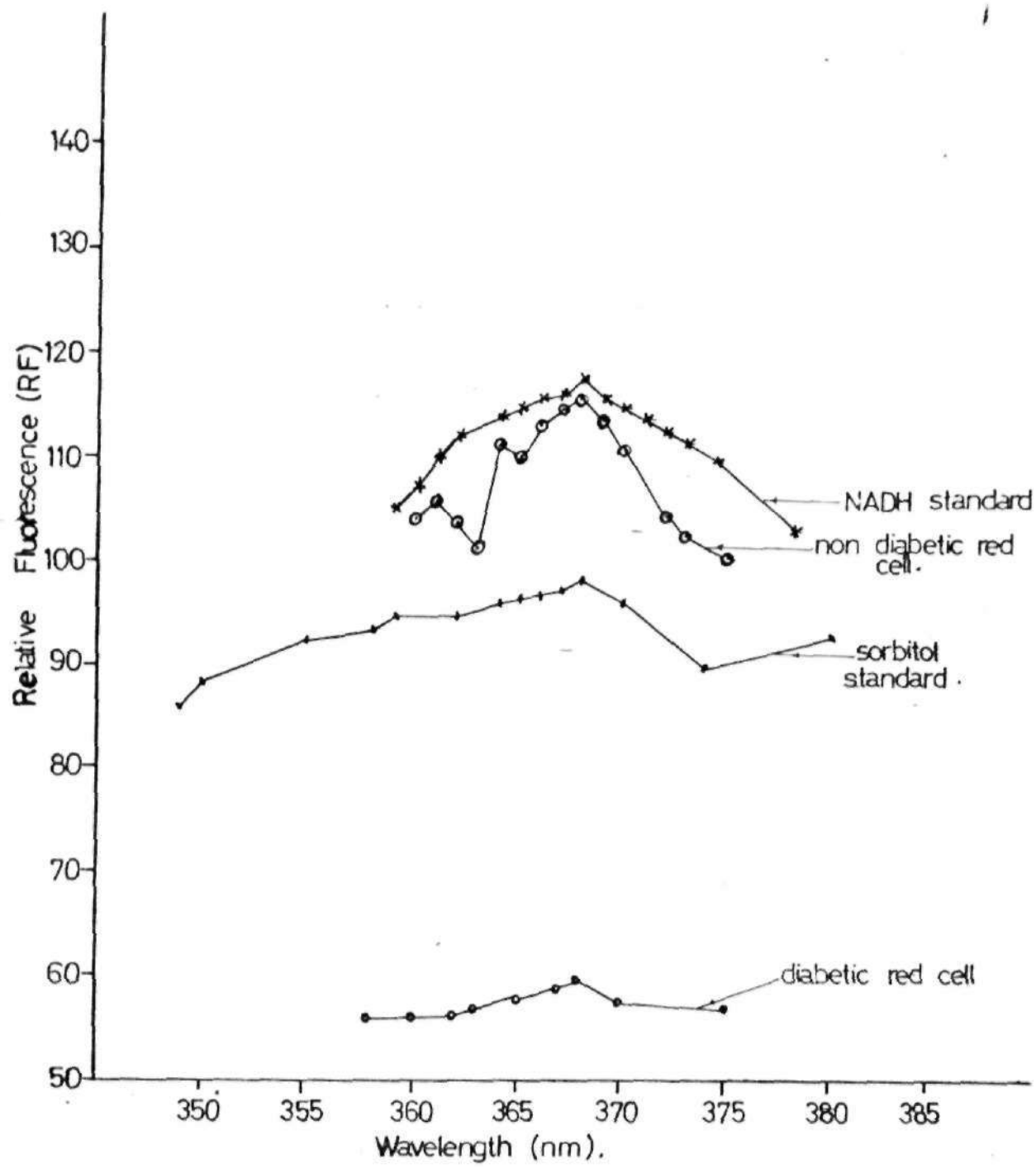
The excitation and emission spectra of NADH standard, sorbitol standard, non-diabetic and diabetic red cells are presented in figures 4.1 and 4.2. The relative fluorescence due to emission of NADH generated from diabetic red cell and sorbitol standard were higher than for that of non-diabetic sample. Differences between the relative fluorescence recorded at emission wavelengths of 454 nm - 460 nm were not significant for the diabetic sample and sorbitol standard respectively. Similarly differences between the relative fluorescence readings at excitation wavelengths of 365 nm - 370 nm were not significant for the same samples.

4.1.2 Time Course Studies

The time course for NADH generated from sorbitol standard, diabetic and non-diabetic samples are illustrated in figure 4.3. There was no significant difference between the relative fluorescence at 60 min and 90 min for all the samples tested.

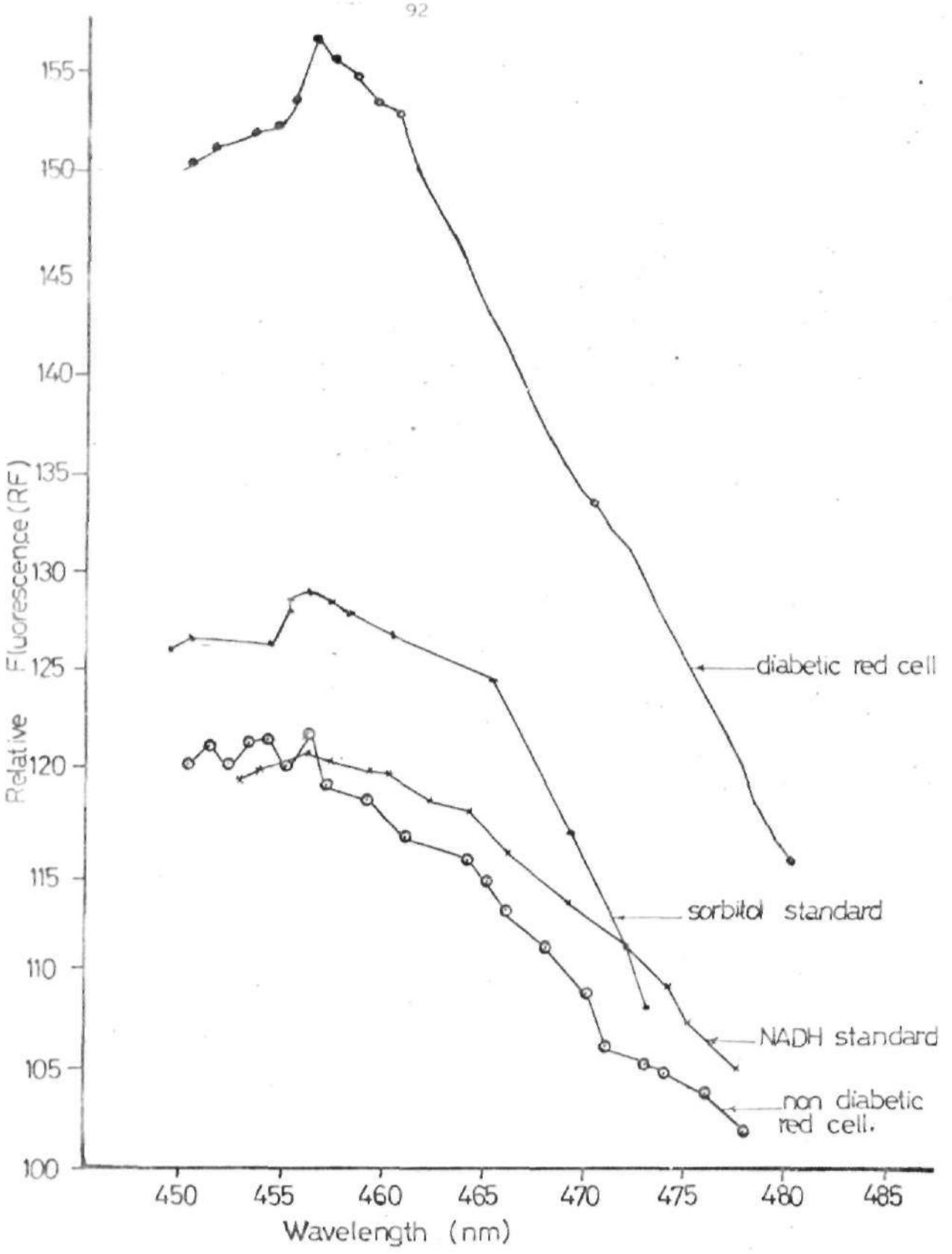
4.1.3 Calibration and Detection Limit

A plot of mean relative fluorescence against known concentrations of sorbitol standard (1.10 - 50.50 nmol/ ml) measured in duplicate is shown in figure 4.4. The linear correlation was positively significant (r=0.998). A limit of detection of 0.55 nmol/ml was extrapolated from the calibration curve, using



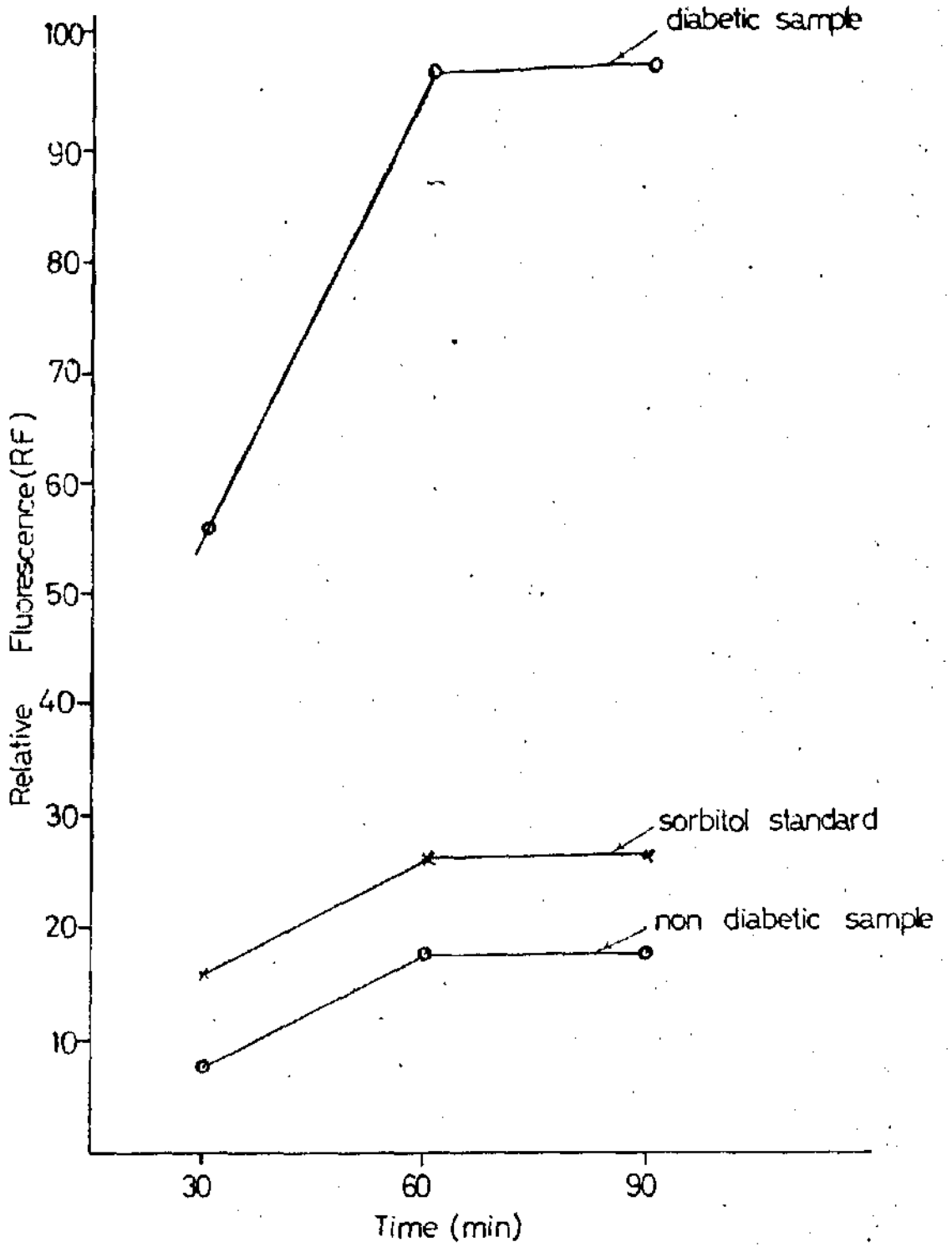
Fig; 4.1 Excitation spectrum of NADH standard, non diabetic red cell, sorbitol standard and diabetic red cell.

—*—*— NADH standard
—o—o— non diabetic red cell
—•—•— Sorbitol standard
—o—o— diabetic red cell.



Fig; 4.2 Emission spectrum of diabetic red cell, sorbitol standard, NADH standard and non diabetic red cell.

NADH standard
non diabetic red cell
Sorbitol standard
diabetic red cell.



Fig; 4.3 Time course of NADH by diabetic red cell, sorbitol standard and non diabetic red cell.

o—— diabetic sample
*—— sorbitol standard
o—— non diabetic sample

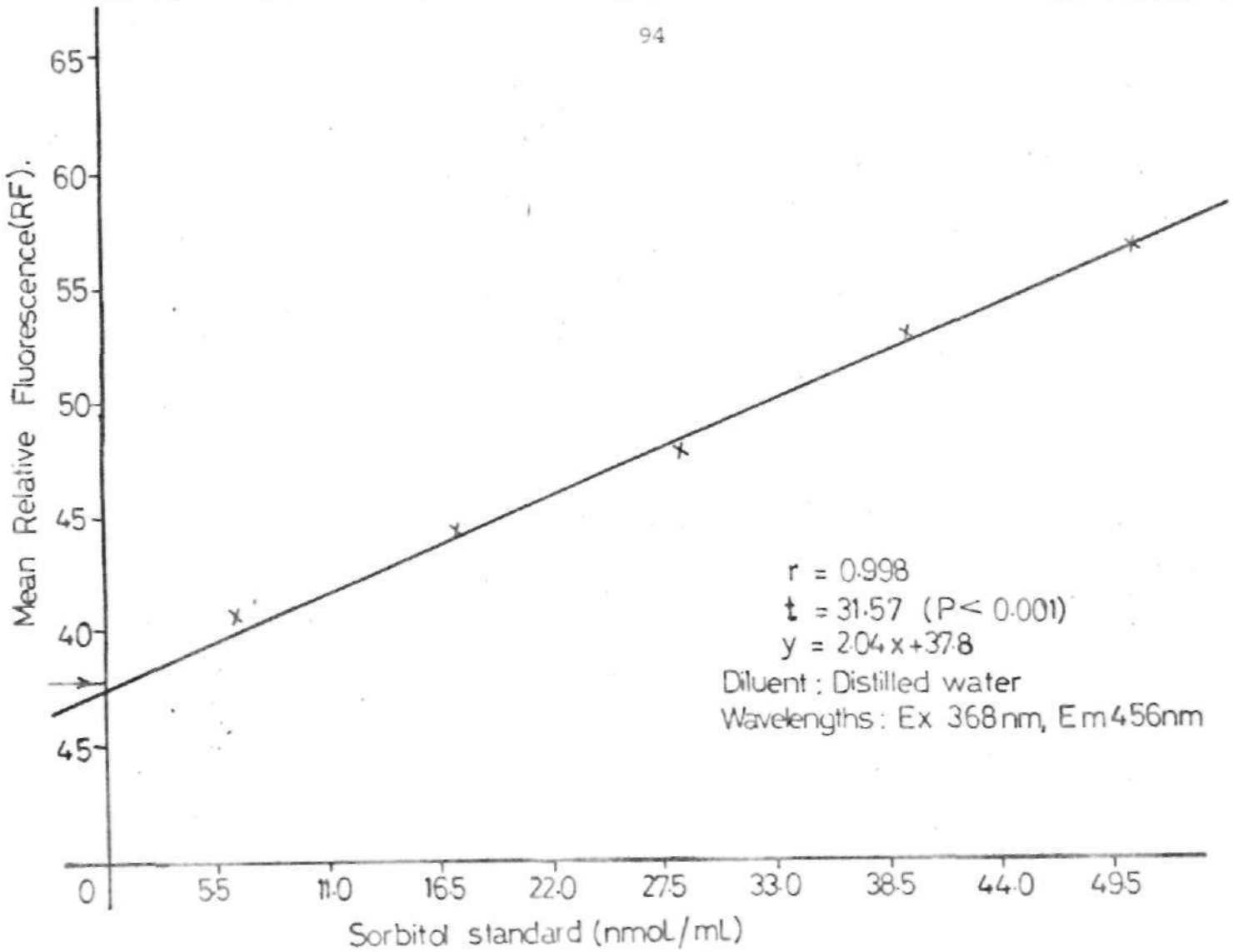


Figure 4.4 Standard calibration curve for sorbitol assay on SFM 25 Spectrofluorimeter.

mean plus 3 SD of the blank.

4.1.4 Precision Profile

The results of the within-assay and between - assay variations are presented in Table 4.1. The within-assay coefficient of variations from low to high concentrations were in the range of 2.82 % to 5.48 % while the corresponding values for the between assay were 3.60 % to 6.05 % respectively.

4.1.5 Analytical Recovery and Interference

Graded concentrations of sorbitol standard added to pool red cell yielded a mean recovery of 90.4 % with a range of 86.0 - 95.0 %. This is shown in Table 4.II.

The data on interference studies are presented in Table 4.III. There was no significant interference on red cell sorbitol assay by any of the analytes or drugs using values of percentage interference of equal to 5.0% or greater as significant.

The unit used for red cell sorbitol concentration in the present study (nmol/g Hb) was suggested by Malone et al (1980), and Lapolla et al (1985) and has been used by others (Vertommen et al, 1987; Wambua et al 1988 and Onaya et al 1990). This unit (nmol/g Hb) is preferable because measurement of red cell haemoglobin is simple, rapid, cheap and reproducible. The earlier unit of nmol/ml packed cells is not used in the present study because it is difficult to reproduce or standardise the packing of red cells. The sorbitol-glucose ratio was not considered due to great variations in glucose values in diabetics and the high cost of procuring the glucose reagents.

Table 4.1 PRECISION PROFILE FOR RED CELL SORBITOL ASSAY ON SFM. 25 SPECTROFLUORIMETER

Sorbitol	Within-assay				Between-assay*			
	$\bar{X}$ (nmol/g Hb)	SD (nmol/g Hb)	CV (%)	n	$\bar{X}$ (nmol/g Hb)	SD (nmol/g Hb)	CV (%)	n
Low	7.5	0.24	3.20	20	7.6	0.27	3.60	10
Medium	11.7	0.33	2.82	20	11.5	0.43	3.74	7
High	21.9	1.20	5.48	20	21.5	1.30	6.05	7

* Based on duplicate determinations over 7-10 days

$\bar{X}$ = mean

SD = Standard deviation

CV = Coefficient of variation

TABLE 4. II ANALYTICAL RECOVERY OF ADDED SORBITOL FOR RED CELL SORBITOL ASSAY ON SFM 25 SPECTROFLUORIMETER

SORBITOL ADDED(nmol/ml)	SORBITOL MEASURED* (nmol/ml)	SORBITOL RECOVERED (nmol/ml)	RECOVERY (%)
0+	126.5[a]	-	-
10.0	135.1	8.6	86.0
0+	126.5[a]	-	-
20.0	144.2	17.7	88.5
0+	126.5[a]	-	-
30.0	153.5	27.0	90.0
0+	212.0[b]	-	-
40.0	249.0	37.0	92.5
0+	212.0[b]	-	-
50.0	259.5	47.5	95.0

a and b =two separate pools of red cell
0+ =No sorbitol added(red cell with only diluent)
* Mean of 5 duplicate measurements
Range = 86.0 - 95.0
Mean recovery = 90.4%

TABLE 4.III EFFECT OF SOME BLOOD CONSTITUENTS AND DRUGS ON RED CELL SORBITOL ASSAY ON SFM 25 SPECTROFLUORIMETER

Analyte added	Unit	C	$\bar{X}_1$ (nmol/g Hb)	$\bar{X}_2$ (nmol/g Hb)	$\bar{X}_1-\bar{X}_2$ (Interference)	PI (%)
Acetylsalicylate	mg/L	300.0	10.10	10.30	-0.20	1.98
Ascorbate	nmol/L	0.60	10.10	10.40	-0.10	0.99
Chlorpropamide	mg/L	300.0	9.40	9.45	-0.05	0.53
Chloroquine	mg/L	100.0	10.50	10.60	-0.10	0.90
Cholesterol	mmol/L	10.0	12.55	12.57	-0.02	0.10
Galactitol	mmol/L	50.0	13.10	13.00	+0.10	0.70
Metformin	mg/L	910.0	12.36	12.44	-0.08	0.60
Paracetamol	mg/L	300.0	9.30	9.20	+0.10	1.07
Retinol	iu/L	20,000	11.67	11.71	-0.04	0.30

C = Final concentration of analyte or drug

$\bar{X}_1$ = Mean of 5 duplicate analyses of red cell with drug or analyte

$\bar{X}_2$ = Mean of 5 duplicate analyses of red cell with equal volume of appropriate diluent

PI = Percentage interference $\frac{\bar{X}_1 - \bar{X}_2}{\bar{X}_1} \times 100$

PI $\geq$ 5% significant

4.1.6 Reference Interval And Clinical Decision Limits

Red cell sorbitol levels (mean ± 2 SD) calculated for random or post-prandial specimens are 6.8 - 15.3 nmol/g Hb while those for fasting are 6.3 - 15.1 nmol/g Hb. These values are not significantly different (P>0.05). This observation was later confirmed from the results of a 2 hr blood glucose screening (2 HBGS) conducted in a few apparently healthy volunteers (Table 4.IV). The differences between the fasting and after two hours were not significant (t = 1.94; P>0,05). The reference range obtained for RCS is 4.1 - 14.9 nmol/g Hb from a probit plot (Fig. 4.5), and a clinical decision limit of 17.0 nmol/g Hb or greater is suggested based on mean + 3 SD.

4.1.7 Diagnostic Specificity And Sensitivity of Red Cell Sorbitol

An evaluation of the diagnostic specificity and sensitivity of red cell sorbitol assay in 102 out-patients revealed a sensitivity of 94.4 %, specificity of 98.8 % and efficiency of 98.0 % (Table 4.V). The concentrations (mean ± SEM) of plasma glucose and red cell sorbitol in the out-patients were 7.2 ± 0.59 mmol/L and 12.5 ± 0.44 nmol/g Hb respectively. These values were significantly higher (P<0.05) than the equivalent values of 4.5 ± 0.08 mmol/L and 10.6 ± 0.21 nmol/g Hb in control subjects respectively. Further blood glucose estimation and the presence of one or more of the classical symptoms of DM confirmed the diagnosis of the disease in the 17 patients with true positive red cell sorbitol.

Table 4.IV RED CELL SORBITOL AND PLASMA GLUCOSE (MEAN±SEM) BEFORE AND AFTER 120 MIN OF 75 g ORAL GLUCOSE

	Fasting	After 2 h	P (Paired t-test)
Non-diabetic subjects (n = 22)			
Glucose (mmol/L)	5.1 ± 0.26	7.2 ± 0.31	(P<0.001)
Sorbitol (nmol/g Hb)	9.6 ± 0.65	10.6 ± 0.56	(P>0.05)
Sorbitol-glucose ratio	43.9 ± 5.14	31.2 ± 2.09	(P>0.05)
Diabetic Patients (n = 33)			
Glucose (mmol/L)	14.0 ± 2.1	18.9 ± 2.6	(P<0.001)
Sorbitol (nmol/g Hb)	20.3 ± 1.2	23.3 ± 1.4	(P<0.001)
Sorbitol-glucose ratio	29.4 ± 2.2	25.1 ± 2.5	(P<0.001)

● · 446877

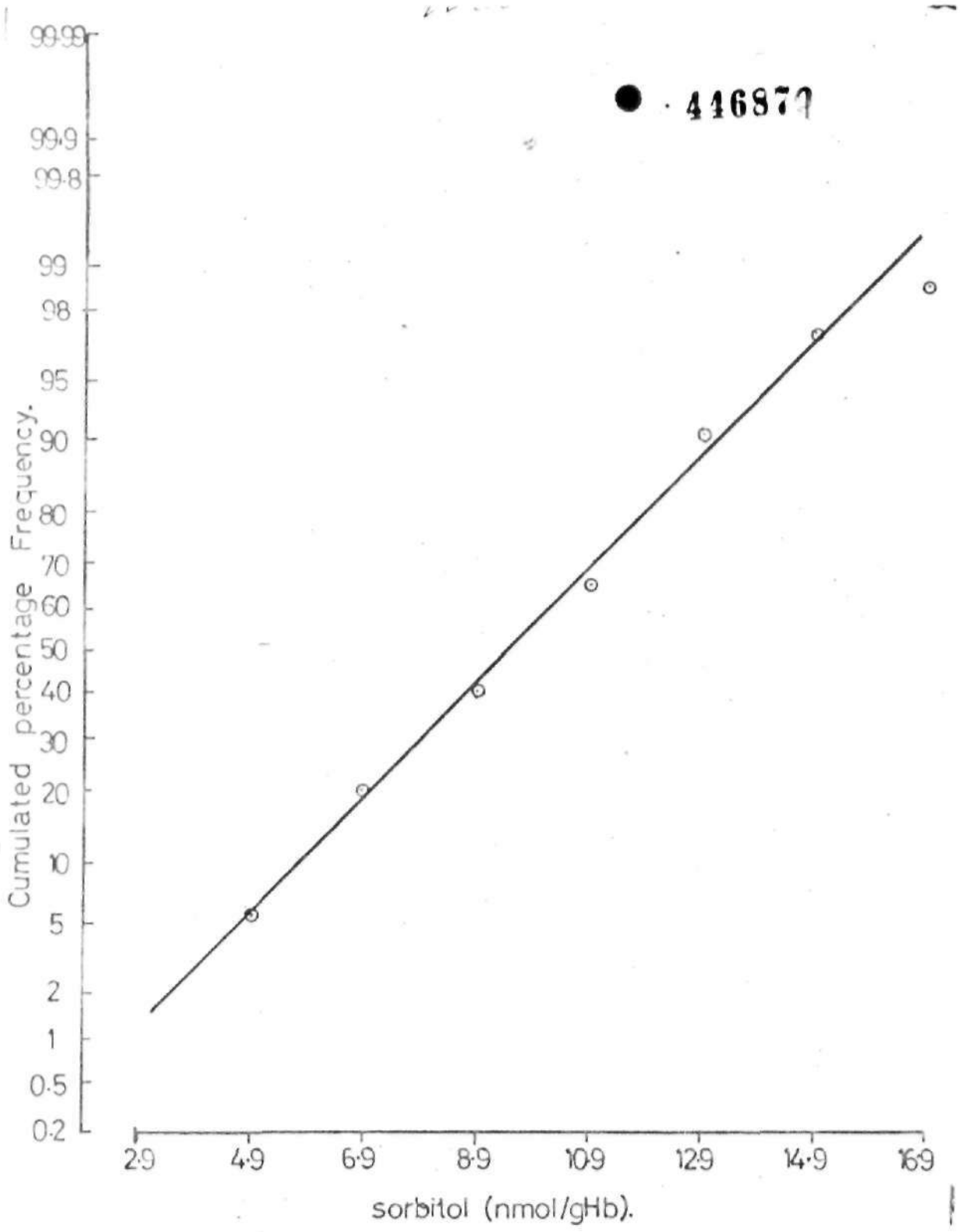


Fig. 4.5 A probit plot of Red cell sorbitol distribution in a mixed population of 203 apparently healthy Nigerians.

Table 4.V DIAGNOSTIC PERFORMANCE OF RED CELL SORBITOL IN 102 NIGERIANS SEEN AT THE GENERAL OUT-PATIENT DEPARTMENT IN ZARIA, NIGERIA

	Sorbitol		
	Positive*	Negative	Total
Diabetic**	17	1	18
Non-diabetic	1	83	84
Total	18	84	102
Sensitivity (%)			94.4
Specificity (%)			98.8
Predictive value of positive test (%)			94.4
Predictive value of negative test (%)			98.8
Efficiency (%)			98.0

*Positive red cell sorbitol (RCS) = RCS $\geq$ 17.0 nmol/g Hb)

Negative red cell sorbitol = RCS $<$ 17.0 nmol/g Hb

**Diabetes was diagnosed according to WHO Criteria (1985)

Sensitivity (%) = $\frac{TP}{TP+FN}$ X100

Predictive value(+) = $\frac{TP}{TP+FP}$X100%

Specificity (%) = $\frac{TN}{TN+FP}$X100

Predictive (-)

Efficiency (%) = $\frac{TP+TN}{TP+FP+TN+FN}$X100

= $\frac{TN}{TN+FN}$ X100%

TP = True positive FP = False positive

TN = True Negative FN = False Negative

4.2 FACTORS AFFECTING RED CELL SORBITOL CONCENTRATIONS IN APPARENTLY HEALTHY SUBJECTS

4.2.1 Sex

The red cell sorbitol levels in the 203 apparently healthy subjects (mean ± SEM) were 10.7 ± 0.13 nmol/g Hb in men and 10.5 ± 0.28 nmol/g Hb in women. The difference between men and women was not significant (P>0.05).

4.2.2 Age

The results on the influence of age, BMI and Bp are presented in Table 4.VI. The difference in the mean values of red cell sorbitol between the various age groups was significant as assessed by one way analysis of variance (ANOVA) (F = 9.4, P<0.001). Additional comparisons using the student t-test revealed that differences for ages 22 to 51 years and above were not statistically significant (P>0.05). The mean red cell sorbitol for ages 12-21 years was significantly lower (P<0.001) than in the other groups. However, the level of red cell sorbitol fell for age range of 32 - 41 years even though the decrease was not statistically significant. A correlation analysis (Fig 4.6) gave Pearsons correlation coefficient, r = 0.30 (t = 4.425; P<0.001).

4.2.3 Race

Results of mean red cell sorbitol values in healthy non-diabetic individuals from various parts of the world are presented in Table 4.VII. These results, to some extent, reveal the effect of race on red cell sorbitol levels in man. The mean values for Blacks as obtained in the present study and that reported by Wambua et al (1988) were significantly lower than those reported

TABLE 4. VI RED CELL SORBITOL CONCENTRATION (MEAN + SEM) AND SELECTED VARIABLES IN NON-DIABETIC CONTROL SUBJECTS

VARIABLE		n	SORBITOL (nmol/g Hb)	F-VALUES BY ANOVA	P
AGE(YEARS)	GROUP				
12 - 21	I	58	9.0 + 0.35		
22 - 31	II	57	11.4 + 0.30		
32 - 41	III	48	10.4 + 0.38	9.4	< 0.001
42 - 51	Iv	19	11.9 + 0.49		
> 51	v	21	12.2 + 0.58		
Body mass index (kg/m^2)					
17.0	I	2	10.8 + 1.84		
17.0 - 27.0	II	177	10.3 + 0.20	10.2	< 0.001
≥ 27.0	III	24	13.4 + 0.70		
Blood pressure (Systolic) (mm Hg)					
< 100	I	7	11.6 + 1.30		
100 - 134	II	190	11.0 + 0.48	2.13	> 0.05
≥ 134	III	6	14.4 + 0.99		

n = Number of subjects

ANOVA = Analysis of Variance.

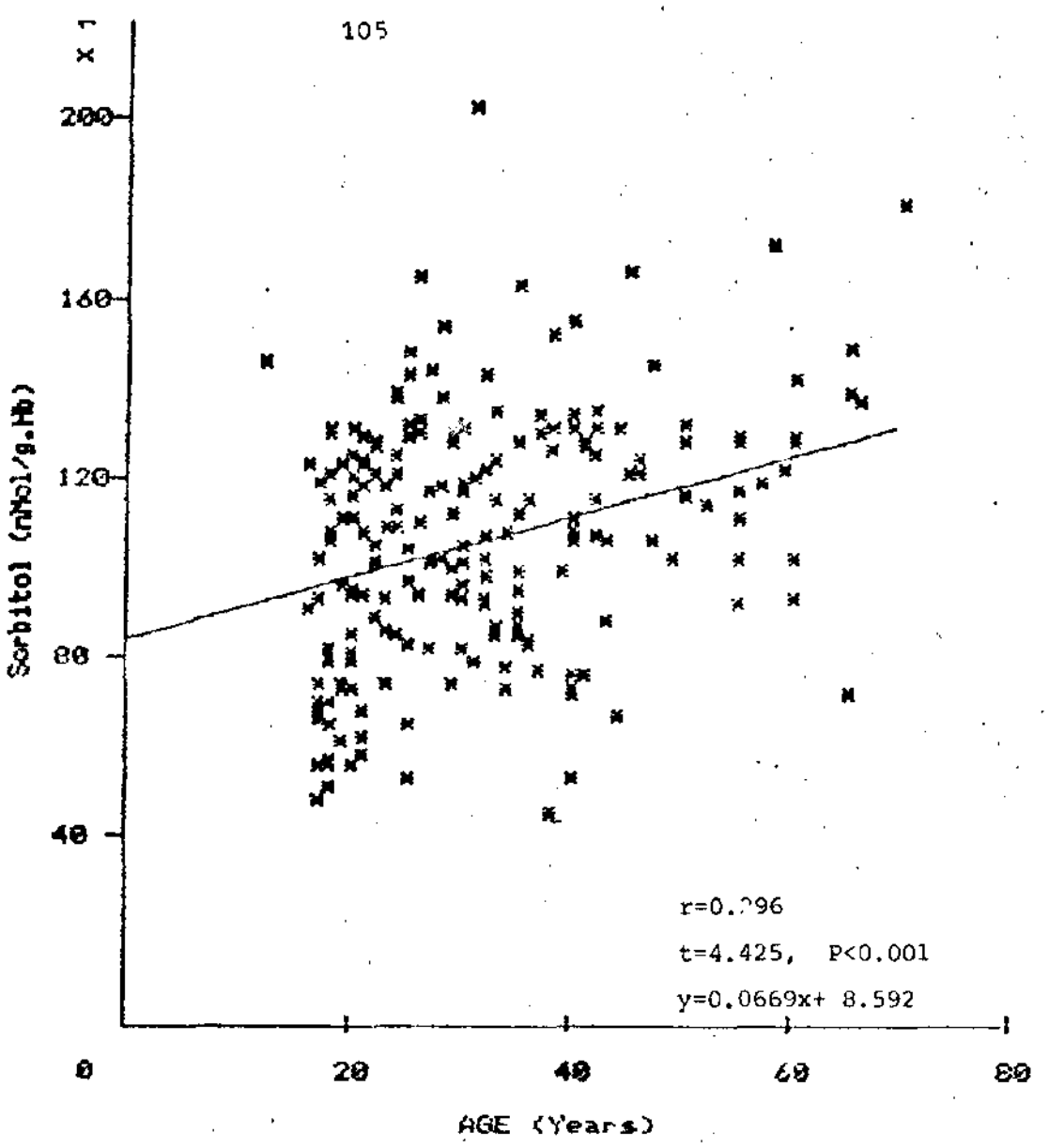


Fig. 4.6

Relationship between age and red cell sorbitol in 203 apparently healthy Nigerians.

Table 4.VII RED CELL SORBITOL CONCENTRATION (MEAN ± SD) IN HEALTHY NON DIABETIC SUBJECTS FROM VARIOUS PARTS OF THE WORLD

S/No.	Location	n	Sorbitol	Reference
1.	Florida (USA)	30	10.1 ± 7.2*	Malone et al (1980)
2.	Amsterdam (Netherlands)	30	5.2 ± 1.6[a]	Popp-Snijders et al (1984)
3.	Leeds (UK)	16	6.7 ± 2.7[b]	Price et al (1987)
4.	Antwerp (Belgium)	54	42.8 ± 16.5*	Vertommen et al (1987)
5.	Nairobi (Kenya)	12	10.6 ± 8.2[c]	Wambua et al (1988)
6.	Yamanashi (Japan)	100	31.7 ± 10.0*	Onaya et al (1990)
7.	Zaria (Nigeria)	203	10.6 ± 3.0*	Present study

a and b: Capillary gas chromatographic method (Unit = nmol/ml packed red cells)

*Similar method and unit(Enzymatic fluorimetric; nmol/g Hb)

c: Enzymatic colorimetric method (unit: nmol/g Hb)

n = No. of subjects.

for whites by Vertommen et al (1987) and Onaya et al (1990).

4.2.4 Body Mass Index

The difference in mean RCS concentrations in the different BMI groups was significant (Table 4.VI, $F = 10.2$, $P<0.05$). Similar result was obtained for the correlation analysis ($t = 2.12$, $P<0.05$, Fig. 4.7). Further statistical test, using the student's t-test for unpaired data, showed that the difference was significant for groups I and III, groups II and III ($P<0.05$). The difference between groups I and II was not significant ($P>0.05$).

4.2.5 Blood Pressure

The interaction of systolic Bp with red cell sorbitol did not demonstrate any significant effect ($F = 2.13$, $P>0.05$). These results are shown in Table 4.VI. The relationship of the diastolic Bp with RCS is presented in Figure 4.8 ($r = 0.221$ $P<0.01$). Red cell sorbitol values (mean $\pm$ SEM) in subjects with diastolic BP $\geq$ 90 mm Hg (13.4 $\pm$ 0.55 nmol/g Hb) was significantly higher ($t = 4.69$, $P<0.001$) than similar value (10.6 $\pm$ 0.20 nmol/g Hb) in those with diastolic Bp < 90 mm Hg.

4.2.6 Cigarette Smoking

The mean red cell sorbitol concentration in the smokers (11.2 $\pm$ 0.08 nmol/g Hb) was not statistically higher ($P>0.05$) than the value in control non smokers 9.9 $\pm$ 0.33 nmol/g Hb. This result is shown in Table 4.VIII.

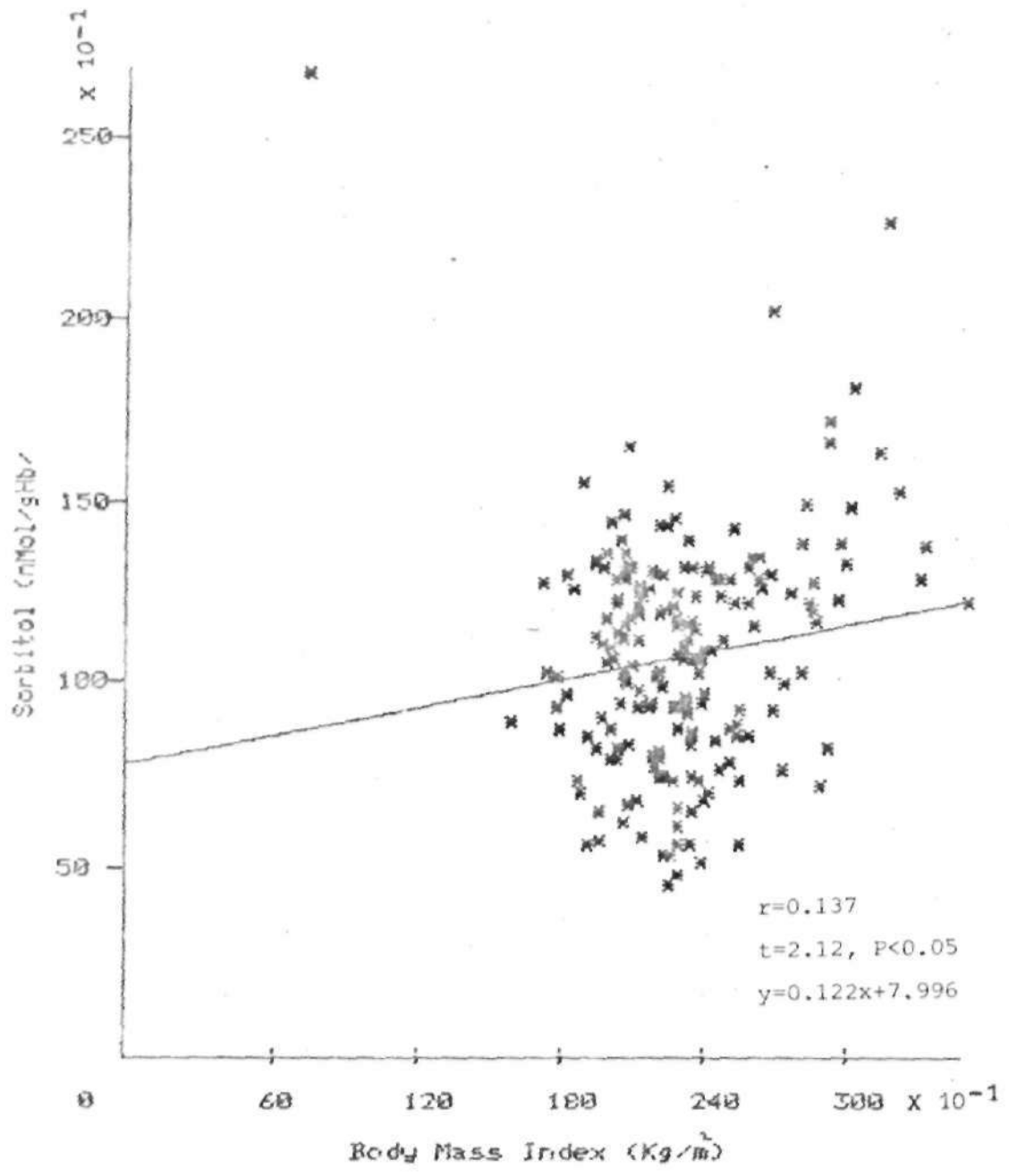


Fig. 4.7

Relationship between body mass index (BMI) and red cell sorbitol in 203 apparently healthy Nigerians.

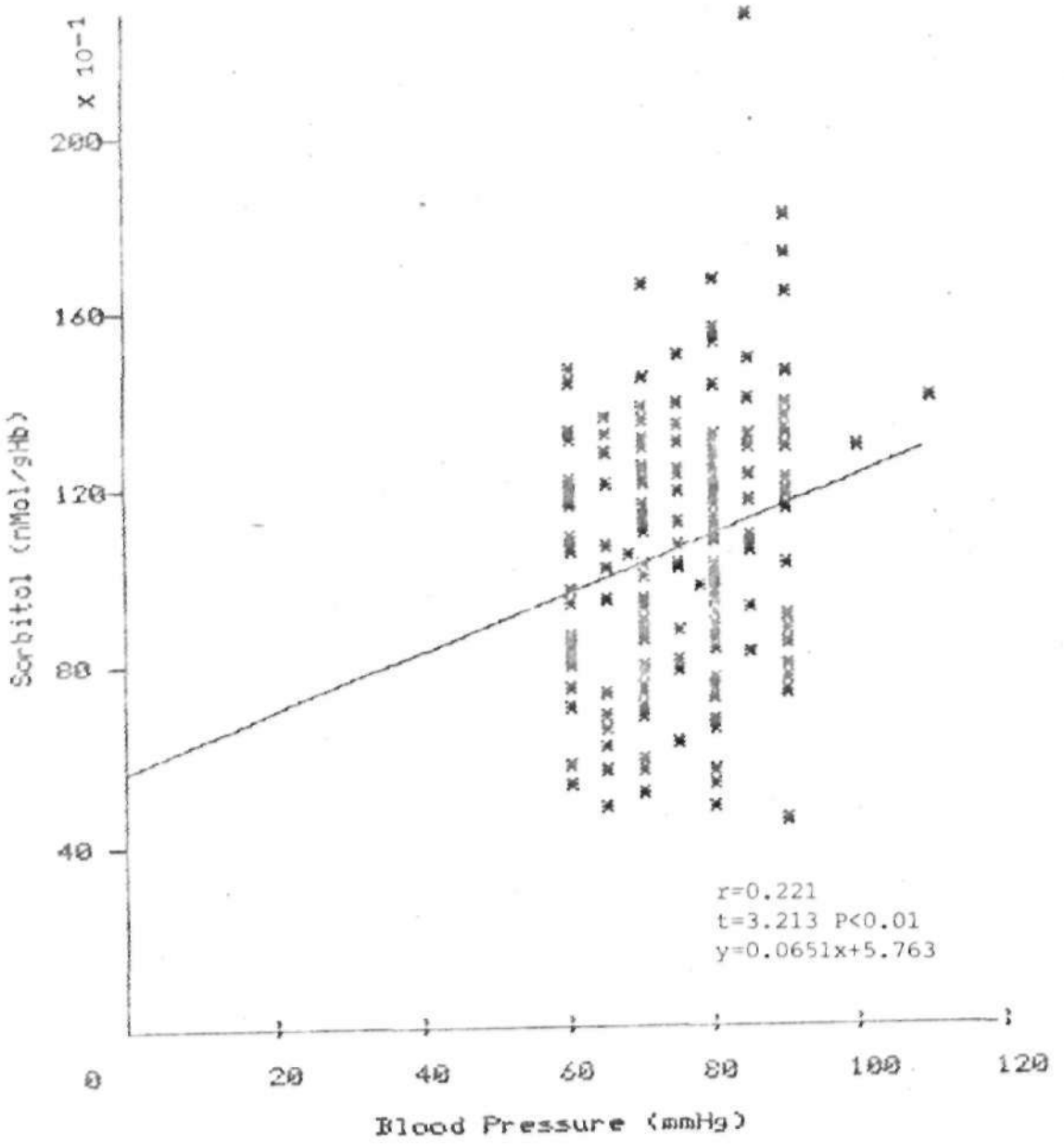


Fig. 4.8

Relationship between diastolic blood pressure and red cell sorbitol in 203 apparently healthy Nigerians.

Table 4.VIII INFLUENCE OF CIGARETTE SMOKING AND ALCOHOL INTAKE ON RED CELL SORBITOL AND PLASMA GLUCOSE LEVELS (MEAN ± SEM) IN NIGERIANS

	n	Sorbitol (nmol/g Hb)	Glucose (mmol/L)
Smokers	50	11.2 ± 0.08	5.0 ± 0.56
Drinkers	30	12.1 ± 0.51*	4.6 ± 0.36
Controls	130	9.9 ± 0.33	4.2 ± 0.21

*P<0.01 drinkers compared with controls (i.e. Negative smoking and drinking habits)

Smokers ≥ 10 sticks of cigarette/day

Drinkers ≥ 5 bottles of bear/day

4.2.7 Alcohol Intake

The result of the influence of alcohol intake on red cell sorbitol level is presented in Table 4.VIII. The mean red cell sorbitol values in the drinkers (12.1 ± 0.51 nmol/g Hb) was significantly higher ($P<0.01$) than that obtained for non-drinkers (9.9 ± 0.38 nmol/g Hb).

4.3 RED CELL SORBITOL CONCENTRATION IN NIGERIAN DIABETIC PATIENTS

The mean red cell sorbitol values and other parameters in diabetic and control subjects are presented in Table 4.IX. The mean red cell sorbitol values were significantly higher ($P<0.001$) in diabetics (Types I and II) than in the healthy controls (Fig. 4.9). The mean value in the type I was significantly higher ($P<0.001$) than the type II. Similarly, the values of plasma glucose and fructosamine were significantly higher ($P<0.001$) in diabetics than in the control as expected.

4.4 FACTORS AFFECTING RED CELL SORBITOL CONCENTRATIONS IN DIABETIC PATIENTS

4.4.1 Age and Sex

The relationship between age and red cell sorbitol in diabetic patients is presented in Fig. 4.10. The correlation was not significant ($r = -0.02$, $P>0.05$). The mean red cell sorbitol in male (22.2 ± 0.85 nmol/g Hb) was similar to that of female (21.9 ± 0.82 nmol/g Hg).

Table 4.IX RED CELL SORBITOL AND OTHER BIOCHEMICAL PARAMETERS (MEAN±SEM) IN DIABETIC AND NON DIABETIC NIGERIANS

	n	Sorbitol (nmol/g Hb)	Glucose (mmol/L)	Fructosamine (mmol/L)	Creatinine (µmol/L)	Albumin (g/L)
IDDM (Type I)	37	25.1 ± 1.83*	13.6 ± 1.49*	2.73 ± 0.22*	89.4 ± 4.50*	36.0 ± 1.10
NIDDM (Type II)	145	19.6 ± 0.48*	11.3 ± 0.58*	2.13 ± 0.10*	90.7 ± 2.50*	36.0 ± 0.53
Non diabetic controls	203	10.6 ± 0.21	4.5 ± 0.08	1.2 ± 0.04	77.8 ± 1.63	40.0 ± 0.46

*P<0.001 Diabetics Vs Non diabetic controls

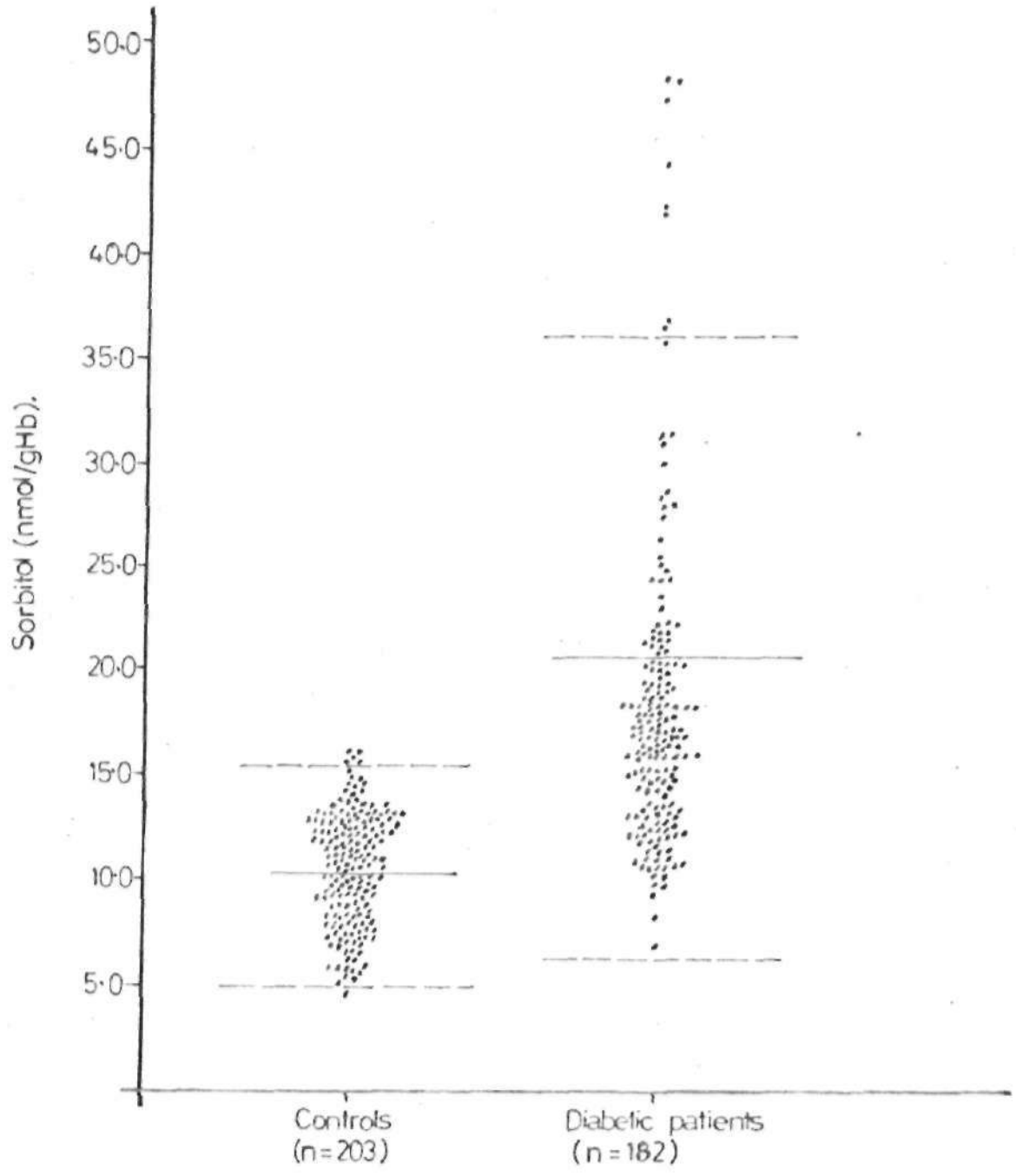


Fig 4.9 Red cell sorbitol (—mean;----2 SD) in apparently healthy subjects and diabetic (Types 1 and 11) Nigerians.

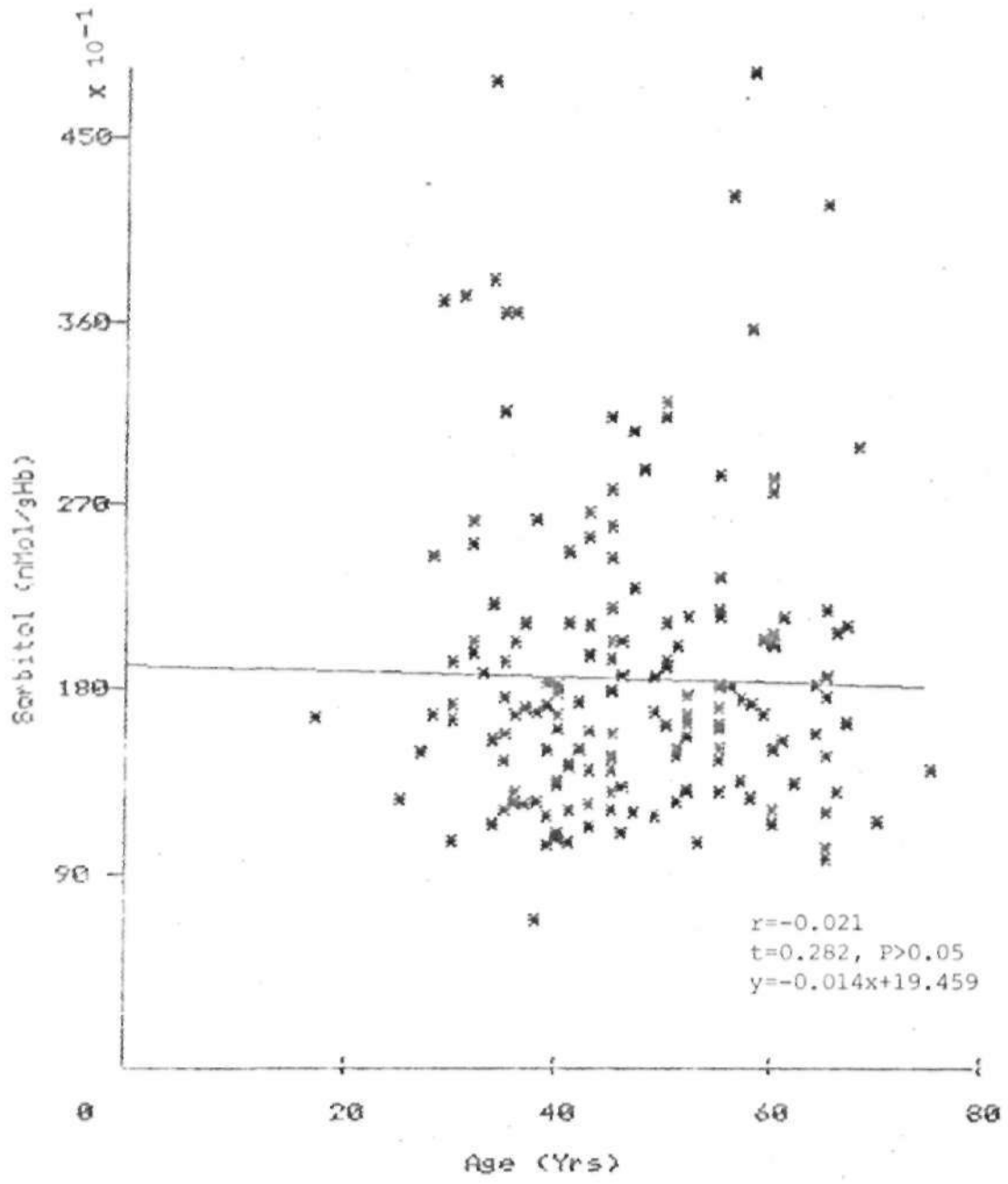


Fig. 4.10

Relationship between age and red cell sorbitol in 182 diabetic Nigerians (Types I and II).

4.4.2 Red Cell Sorbitol and Disease Duration

The impact of disease duration on red cell sorbitol is presented in Table 4.X. Duration of diabetes did not produce any significant effect on red cell sorbitol concentrations ($F=1.48$, $P>0.05$). Similar observation was made for plasma glucose and fructosamine ($F=2.02$, $P>0.05$, and $F=2.07$, $P>0.05$, Table 4.X).

The results of sequential changes in red cell sorbitol, fructosamine and glucose in 18 newly diagnosed diabetics are presented in Table 4.XI. The mean values of glucose, fructosamine and RCS were significantly higher ($P<0.001$) in the diabetics than in controls as expected. The changes observed in the three parameters were similar except for glucose at week 16. The mean plasma glucose concentration was significantly higher ($P<0.05$) at presentation than at weeks 8 and 16.

4.4.3 Obesity

Thirty two percent of the diabetics were obese (BMI$\geq$27.0 Kg/m^2). Red cell sorbitol values in obese and non obese diabetic and control subjects are shown in Table 4.xII. The mean red cell sorbitol in obese diabetics was significantly higher ($P<0.05$) than in non obese diabetics. A correlation analysis of the data gave a correlation coefficient of 0.294 ($t = 3.56$, $P<0.001$). This is presented in Fig. 4.11

4.4.4 Hypertension

Sixty one of the diabetic patients were hypertensive (Bp $\geq$ 160/95 mm Hg). Mean values of red cell sorbitol, plasma glucose and fructosamine are presented in Table 4.XIII and red

Table 4.X EFFECT OF DURATION OF DIABETES ON RED CELL SORBITOL, PLASMA GLUCOSE AND PLASMA FRUCTOSAMINE (MEAN ± SEM) IN DIABETIC PATIENTS

	n	Sorbitol (nmol/g Hb)	Glucose (mmol/L)	Fructosamine (mmol/L)
Newly diagnosed	18	21.6 ± 1.48	15.5 ± 2.03	3.0 ± 0.33
Previously diagnosed				
≤ 1 yr	14	17.8 ± 1.42	9.4 ± 1.32	2.0 ± 0.34
1 - 4 yr	72	16.7 ± 0.64	10.9 ± 0.78	2.2 ± 0.14
5 - 9 yr	44	16.5 ± 0.85	11.2 ± 1.03	2.1 ± 0.17
> 9 yr	32	18.8 ± 0.97	12.8 ± 1.52	2.2 ± 0.17
F Value	-	1.48	2.02	2.07

n = No. of patients

F Value (P>0.05) for all parameters for the previously diagnosed

Table 4.XI. SERIAL CHANGES OF RED CELL SORBITOL, PLASMA GLUCOSE, AND FRUCTOSAMINE (MEAN±SEM) IN 18 NEWLY DIAGNOSED DIABETICS (TYPES I AND II) FOLLOWING THERAPY

	Sorbitol (nmol/g Hb)	Glucose (mmol/L)	Fructosamine (mmol/L)
Diabetics at presentation	21.6 ± 1.48*	15.5 ± 2.03*	3.0 ± 0.33*
8 weeks	17.3 ± 1.04	10.3 ± 1.53	2.6 ± 0.29
16 weeks	17.9 ± 1.37	9.4 ± 1.52	2.8 ± 0.29
Control subjects	10.6 ± 0.21	4.5 ± 0.08	1.2 ± 0.04

*P<0.05 Diabetics at presentation Vs weeks 8 and 16

Table 4.XII RED CELL SORBITOL, PLASMA GLUCOSE AND FRUCTOSAMINE (MEAN±SEM) IN OBESE AND NON OBESE NIDDM AND CONTROL SUBJECTS

NIDDM	n	Sorbitol (nmol/g Hb)	Glucose (mmol/L)	Fructosamine (mmol/L)
Obese	58	19.4 ± 0.85[+]	11.0 ± 6.70	1.9 ± 0.10
Non Obese	85	16.0 ± 0.75	10.4 ± 6.37	2.2 ± 0.12
Non diabetic controls				
Obese	24	13.4 ± 0.70[++]	4.6 ± 1.19	1.1 ± 1.19
Non Obese	177	10.3 ± 0.20	4.5 ± 0.11	1.2 ± 0.09

n = No. of subjects

+P<0.05 Obese NIDDM Vs Non Obese NIDDM

++P< 0.05 Obese controls Vs Non Obese controls

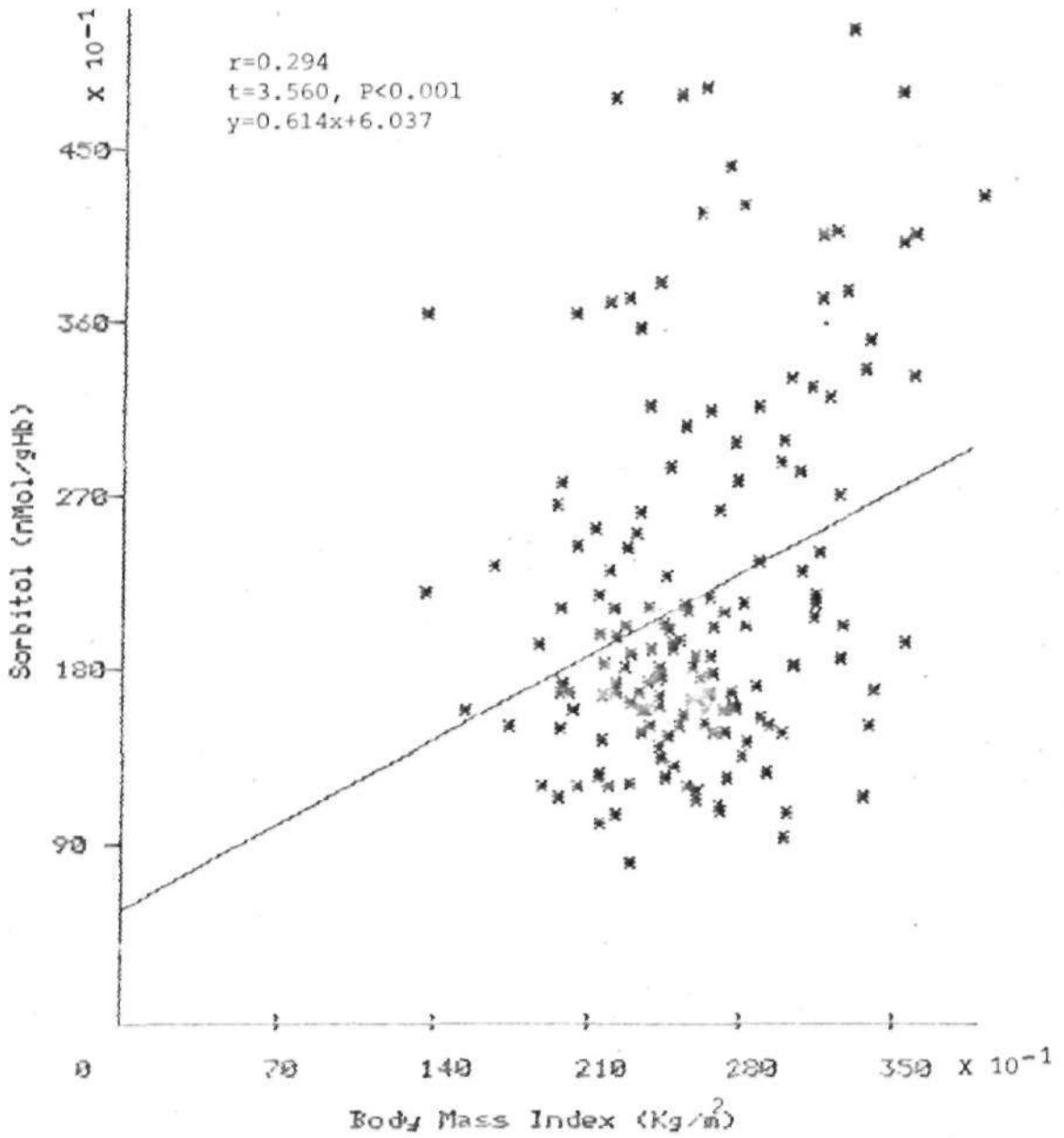


Fig. 4.11

Relationship between body mass index and red cell sorbitol in 182 diabetic Nigerians (Types I and II).

Table 4.XIII RED CELL SORBITOL, PLASMA GLUCOSE AND FRUCTOSAMINE (MEAN±SEM) IN DIABETICS WITH AND WITHOUT HYPERTENSION

Subjects	n	Blood Pressure $\frac{S}{D}$ (mm Hg)	Sorbitol (nmol/g Hb)	Glucose (mmol/L)	Fructosamine (mmol/L)
Hypertensive diabetics	61	$\frac{156 \pm 3}{92 \pm 2}$	21.5 ± 1.23*	11.0 ± 0.87+	2.2 ± 0.15+
Hypertensive Non-diabetics	16	$\frac{130 \pm 1}{82 \pm 1}$	13.6 ± 0.99	5.3 ± 0.47	1.1 ± 0.11
Normotensive[a] diabetics	100	$\frac{126 \pm 2}{77 \pm 1}$	16.5 ± 0.54	11.1 ± 0.65+	2.2 ± 0.11+
Controls (Normotensive	203	$\frac{117 \pm 1}{75 \pm 1}$	10.6 ± 0.21	4.5 ± 0.08	1.2 ± 0.04

n = No. of subjects

S = Systolic

D = Diastolic

*P<0.05 Hypertensive diabetics Vs normotensive diabetic and non diabetic subjects

+P<0.001 Diabetics Vs non-diabetics

cell sorbitol in Fig. 4.12. Mean red cell sorbitol in hypertensive diabetics were significantly higher ($P<0.001$) than in normotensive diabetics. Correlation of the systolic Bp with red cell sorbitol was significant ($r = 0.418$, $t = 5.78$, Fig. 4.13). On the other hand, a weak correlation was obtained for diastolic Bp ($r = 0.15$, $t = 1.93$).

4.4.5 Type of Treatment

The effect of type of treatment of diabetics on red cell sorbitol levels was assessed. The results are presented in Table 4.XIV. Values of red cell sorbitol, plasma glucose and fructosamine in patients treated with diet alone or oral hypoglycaemic agents were similar ($P>0.05$; Table 4.XIV). The mean values of red cell sorbitol and plasma glucose were significantly lower ($P<0.001$) in patients treated with diet alone or oral hypoglycaemic agents than corresponding values in insulin treated patients. The mean value of plasma fructosamine was significantly lower ($P<0.001$) in patients treated with oral hypoglycaemic agents than in those treated with insulin, while values for diet and insulin treated patients were similar ($P>0.05$).

4.4.6 Long-term Diabetic Complications

The mean concentrations of red cell sorbitol in patients with diabetic complications are shown in Table 4.XV and in Fig. 4.14. Twenty four diabetic patients had neuropathy, 24 had nephropathy and 14 had retinopathy. The mean values of red cell sorbitol were significantly higher ($P<0.001$) in diabetic patients with neuropathy and nephropathy than in those without such complications (Table 4.XV).

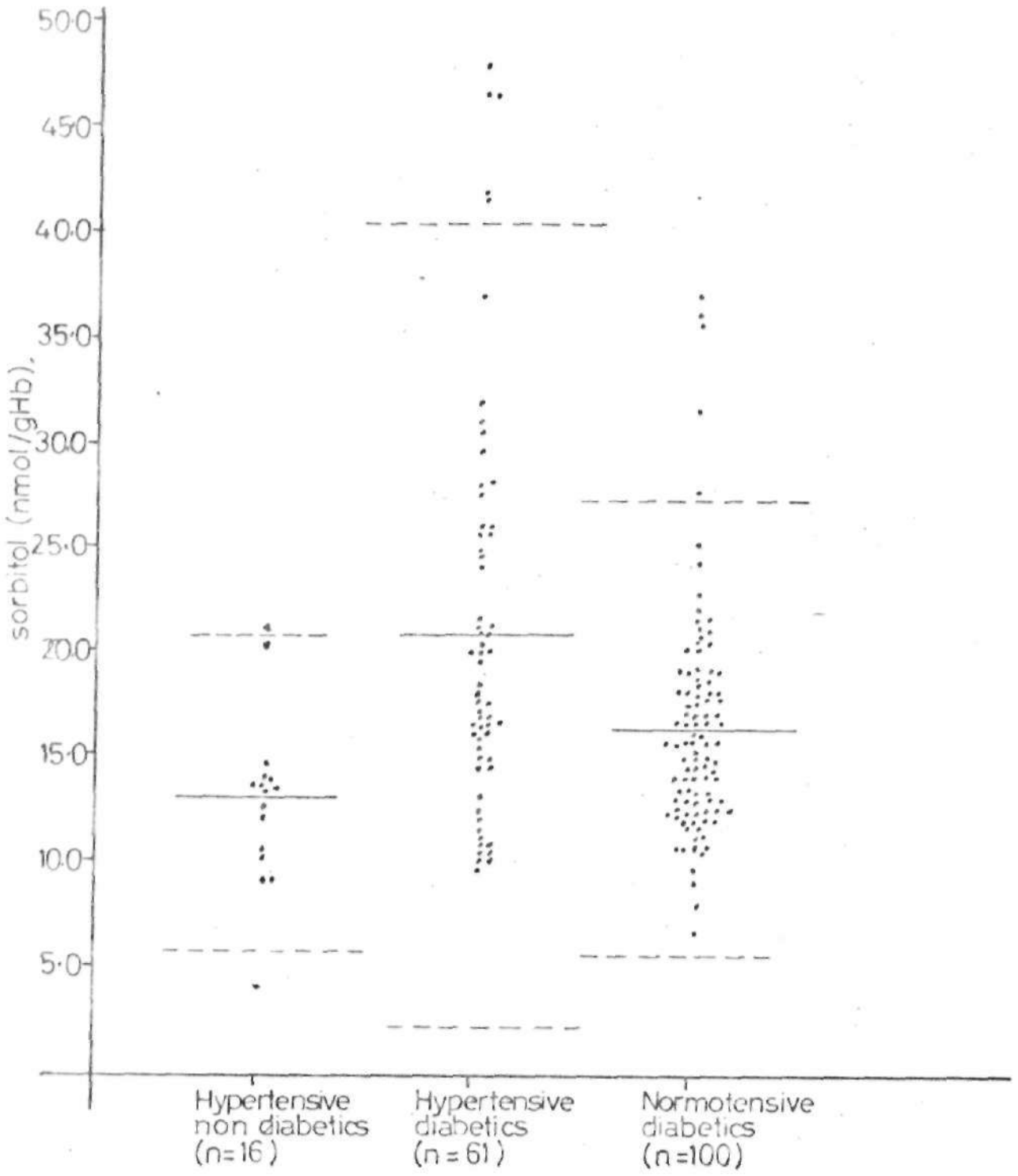


Fig. 4·12 Red cell sorbitol (— mean and -----2 SD) in hypertensive non diabetics, hypertensive diabetics and normotensive diabetics

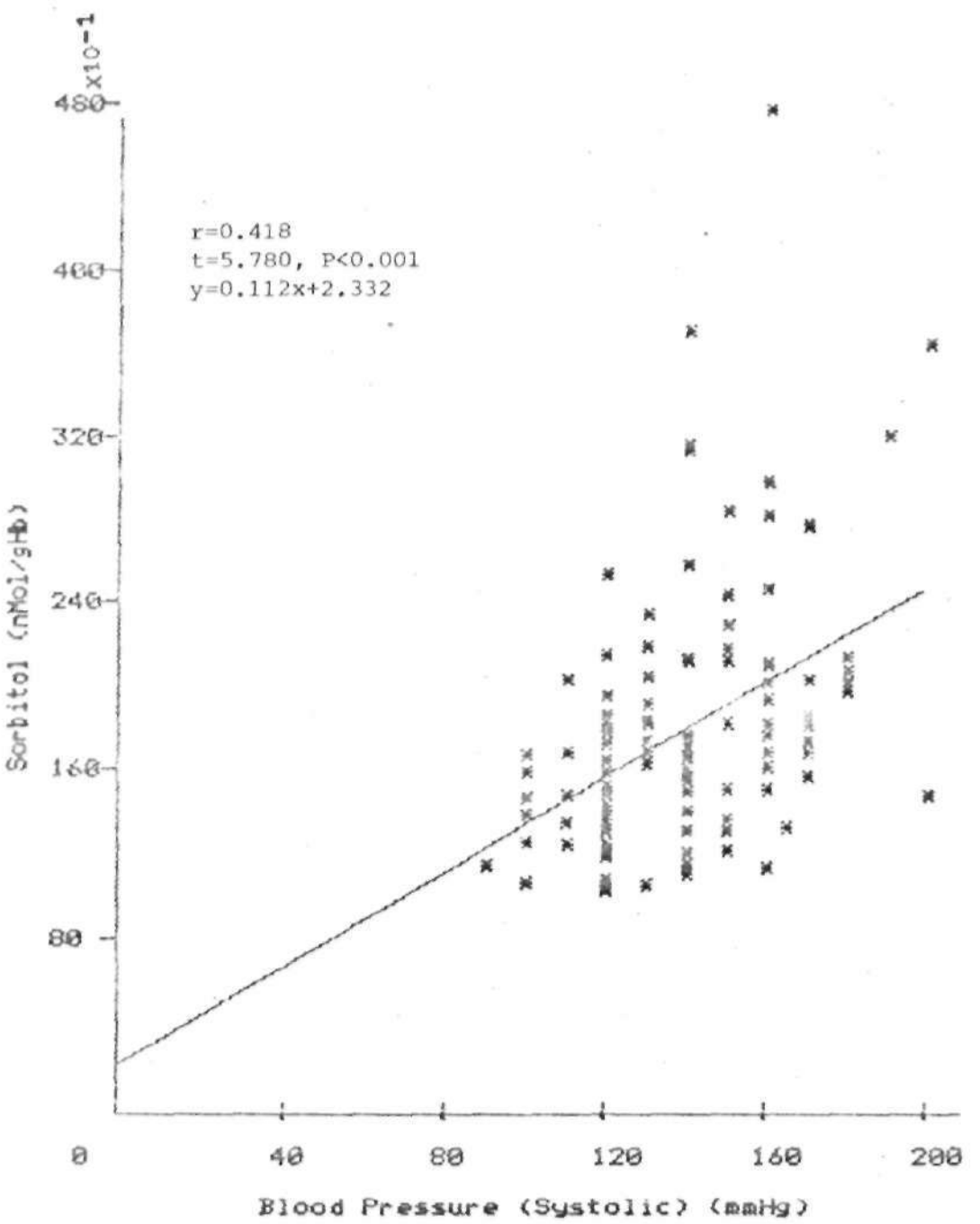


Fig. 4.13

Correlation between systolic blood pressure and red cell sorbitol in 182 diabetic Nigerians (Types I and II).

Table 4.XIV RED CELL SORBITOL, PLASMA GLUCOSE AND FRUCTOSAMINE (MEAN±SEM) IN DIABETIC PATIENTS ACCORDING TO TYPE OF TREATMENT

Treatment	n	Sorbitol (nmol/g Hb)	Glucose (mmol/L)	Fructosamine (mmol/L)
Diet alone	9	17.4 ± 1.10	10.9 ± 2.10*	2.2 ± 0.21
Oral hypoglycaemic agents (OHA)	118	16.8 ± 0.55+	10.6 ± 0.58+	2.0 ± 0.09
Insulin	37	25.1 ± 1.80	13.6 ± 1.50	2.7 ± 0.22

n = No. of subjects

*P<0.001 Diet alone Vs Insulin

+P<0.01 OHA Vs Insulin

Table 4.XV RED CELL SORBITOL,PLASMA GLUCOSE AND FRUCTOSAMINE (MEAN ± SEC) IN DIABETIC SUBJECTS WITH AND WITHOUT DIABETIC COMPLICATIONS

	n	Sorbitol (nmmol/g Hb)	Glucose (mmol/L)	Fructosamine (mmol/L)
Non diabetic controls	203	10.6 ± 0.21	4.5 ± 0.08	1.2 ± 0.04
Diabetics without complications	71	16.0 ± 0.74	11.7 ± 0.91	2.2 ± 0.13
Diabetics with Complications:				
Neuropathy	24	22.0 ± 1.80*	11.5 ± 1.10	2.2 ± 0.20
Nephropathy	24	23.0 ± 2.20*	14.2 ± 2.10	2.6 ± 0.30
Retinopathy	14	16.5 ± 1.18	9.1 ± 1.16	1.6 ± 0.29

n = No. of subjects

*P<0.001 Diabetics with neuropathy and nephropathy Vs diabetics without complications and non diabetic controls.

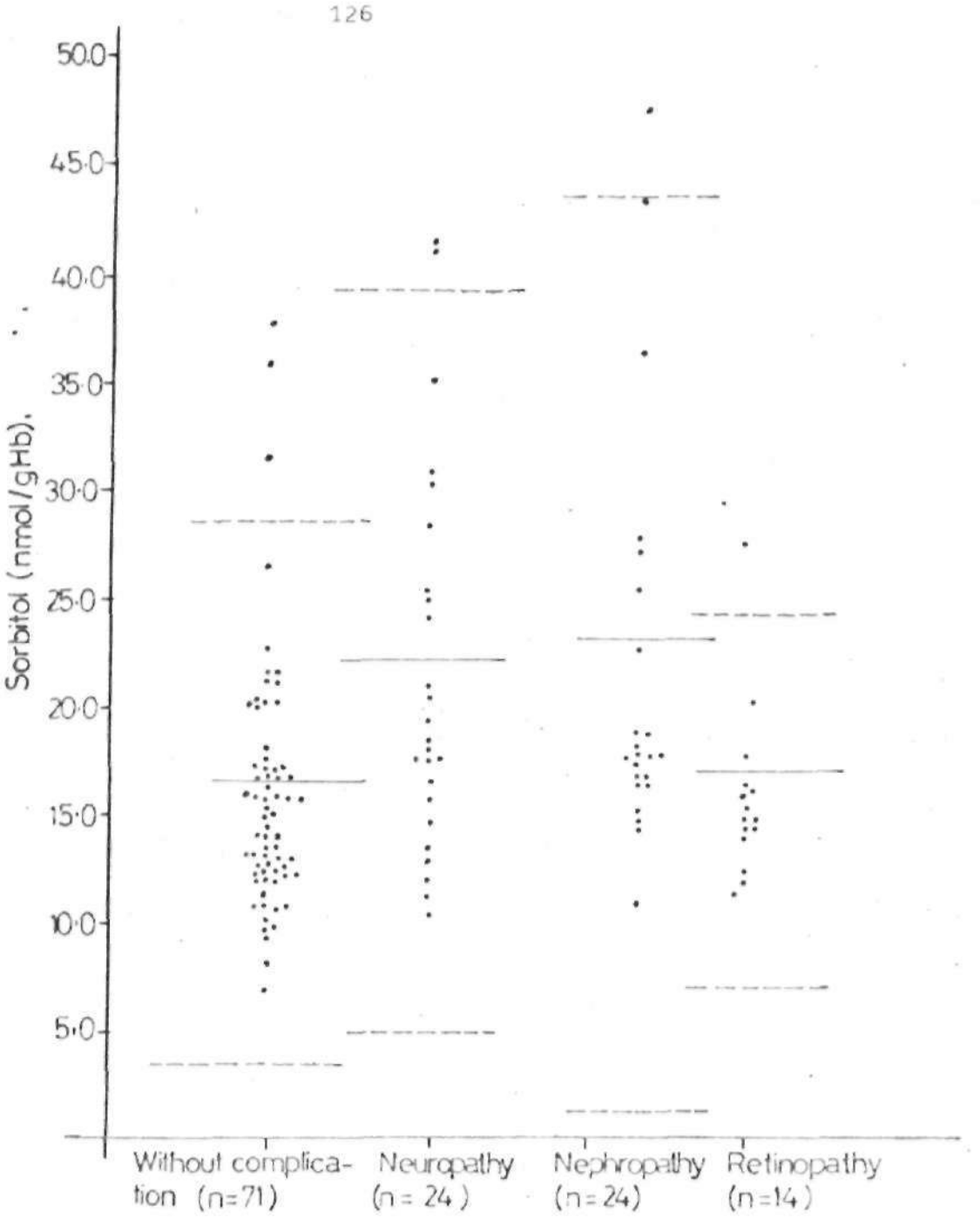


Fig. 4·14 Red cell sorbitol (—, mean and - - - -, 2SD) in diabetics with and without complications.

On the other hand, mean red cell sorbitol values were similar for patients with retinopathy and those without complications. Mean values of plasma glucose, fructosamine, total cholesterol, triglyceride and HDL-C were similar in both patients with and without complications (Table 4.XV and Table 4.XVI). The mean LDL-C was significantly higher ($P<0.05$) in diabetics with nephropathy than in those without and controls (Table 4.XVI).

4.5 RED CELL SORBITOL COMPARES WITH OTHER INDICES OF METABOLIC CONTROL IN DIABETIC PATIENTS

4.5.1 Glucose

A weak but positive correlation was obtained between red cell sorbitol and glucose ($r = 0.08$, $P>0.05$; Fig 4.15) for non diabetic control subjects. Results of two hr post prandial blood glucose in 22 healthy volunteers are shown in Table 4.IV. Correlation between red cell sorbitol and glucose at fasting ($r = 0.01$, $P>0.05$) and after 2 hr ($r = 0.06$, $P>0.05$) were not significant.

Red cell sorbitol correlated positively and significantly with plasma glucose ($r = 0.746$, $P<0.001$; Fig 4.16) in a mixed diabetic population (Types I and II).

The fasting plasma glucose correlated positively, and significantly ($r = 0.895$, $P<0,001$, Fig. 4.17) with fasting red cell sorbitol. Similar significant correlation was obtained after 2 hr of glucose load. These results indicate that red cell sorbitol increase progressively with glucose in diabetics but not in non-diabetics.

Table 4.XVI LIPID PROFILE (MEAN ± SEM) IN DIABETIC SUBJECTS WITH AND WITHOUT DIABETIC COMPLICATIONS

	n	Total cholesterol (mmol/L)	Triglyceride (mmol/L)	LDL-Cholesterol (mmol/L)	HDL-Cholesterol (mmol/L)
Non diabetic controls	50	4.1 ± 0.10	1.4 ± 0.06	2.3 ± 0.11	0.90 ± 0.30
Diabetics without complications	71	5.4 ± 0.32	3.4 ± 0.32	2.4 ± 0.20	0.85 ± 0.06
Diabetics with Complications:					
Neuropathy	24	5.1 ± 0.30	3.7 ± 0.43	2.4 ± 0.30	0.76 ± 0.07
Nephropathy	24	5.8 ± 0.40	3.4 ± 0.26	3.3 ± 0.40*	0.82 ± 0.10
Retinopathy	14	5.2 ± 0.33	3.0 ± 0.36	2.4 ± 0.45	0.91 ± 0.11

n = No. of subjects

*P<0.05 Diabetics with nephropathy Vs controls, diabetics without complications and those with neuropathy and retinopathy.

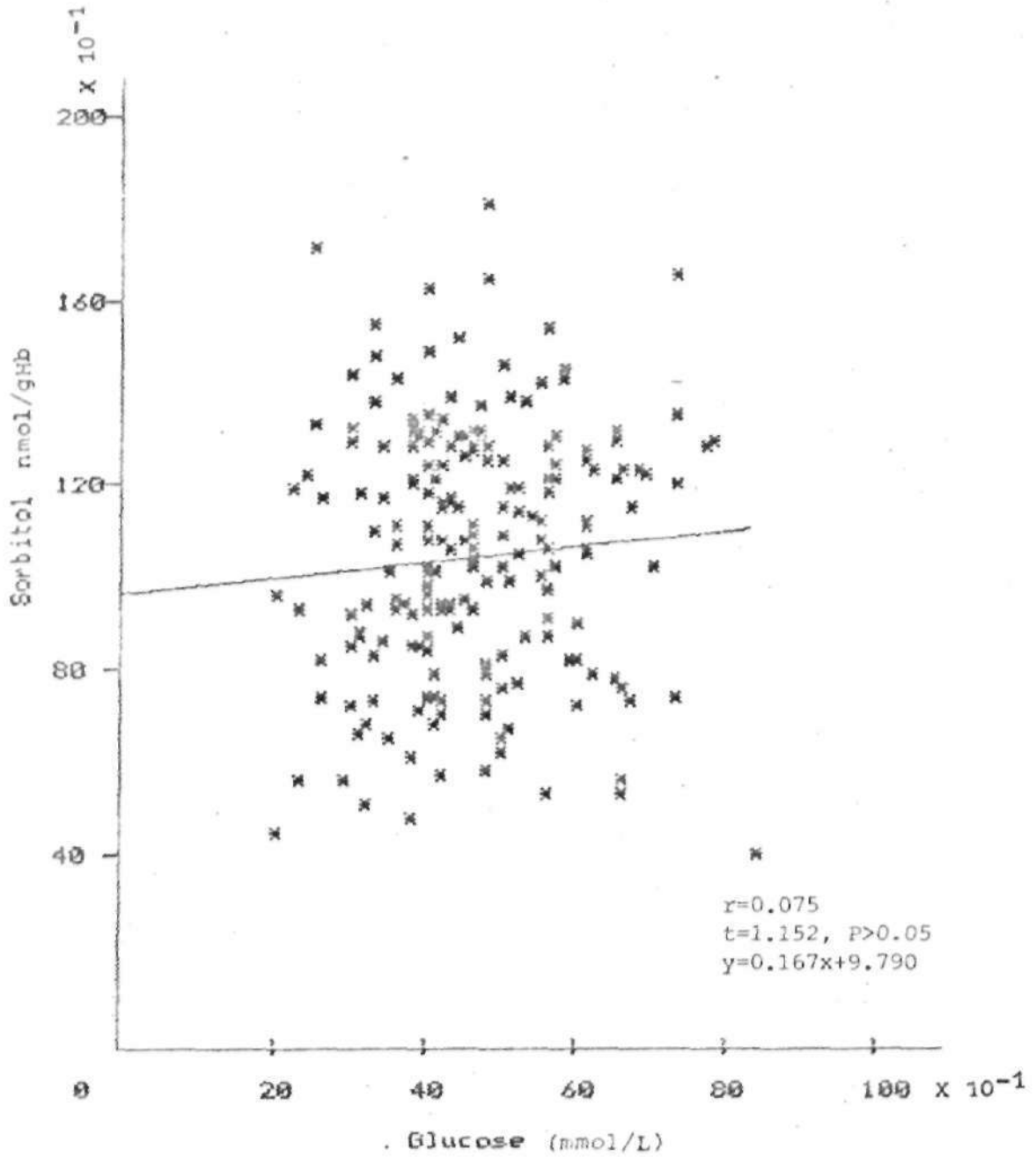


Fig. 4.15

Relationship between plasma glucose and red cell scrbitol in 203 apparently bealthy Nigerians.

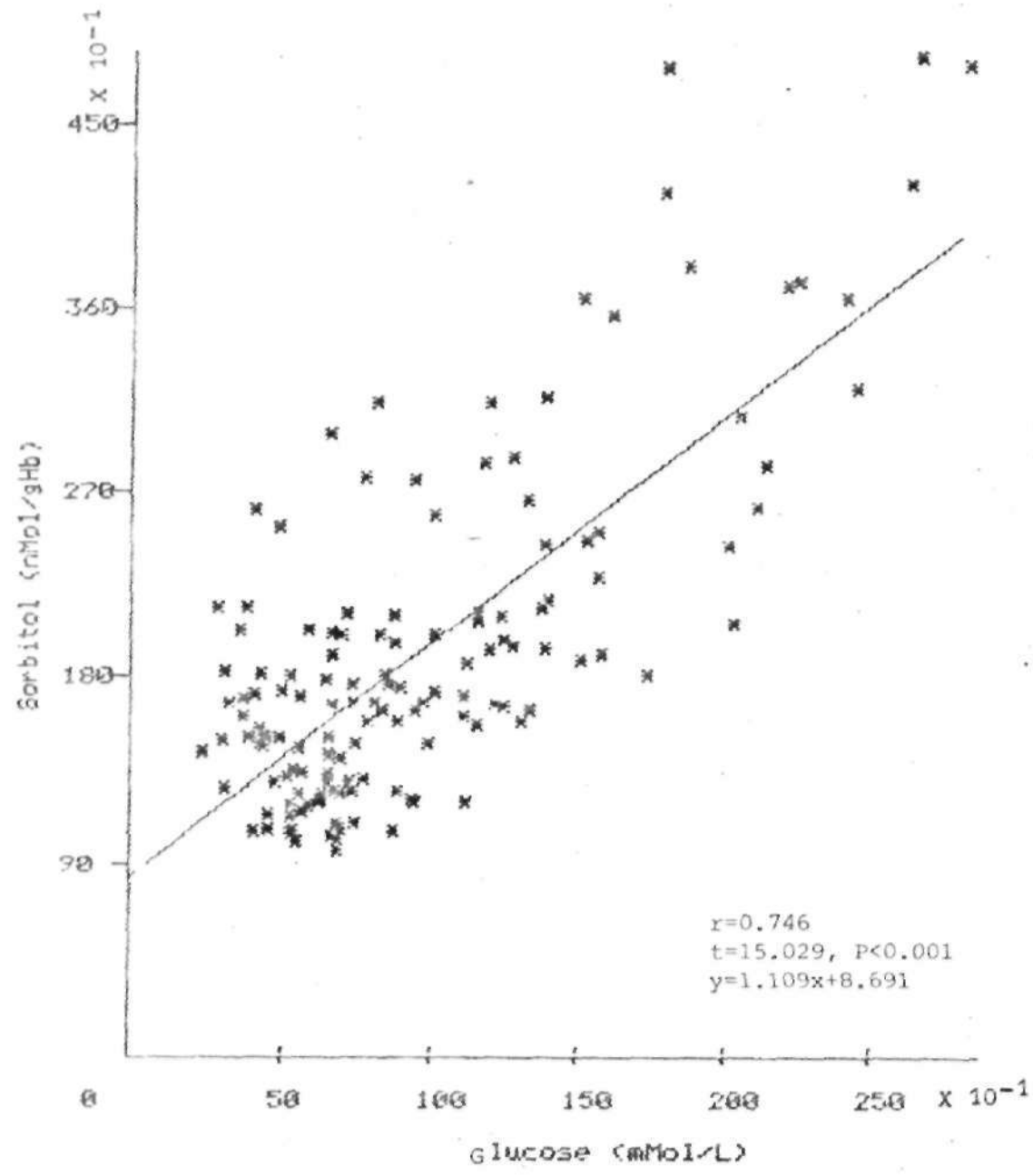


Fig. 4.16

Relationship between plasma glucose and red cell sorbitol in 182 diabetic Nigerians (Types I and II).

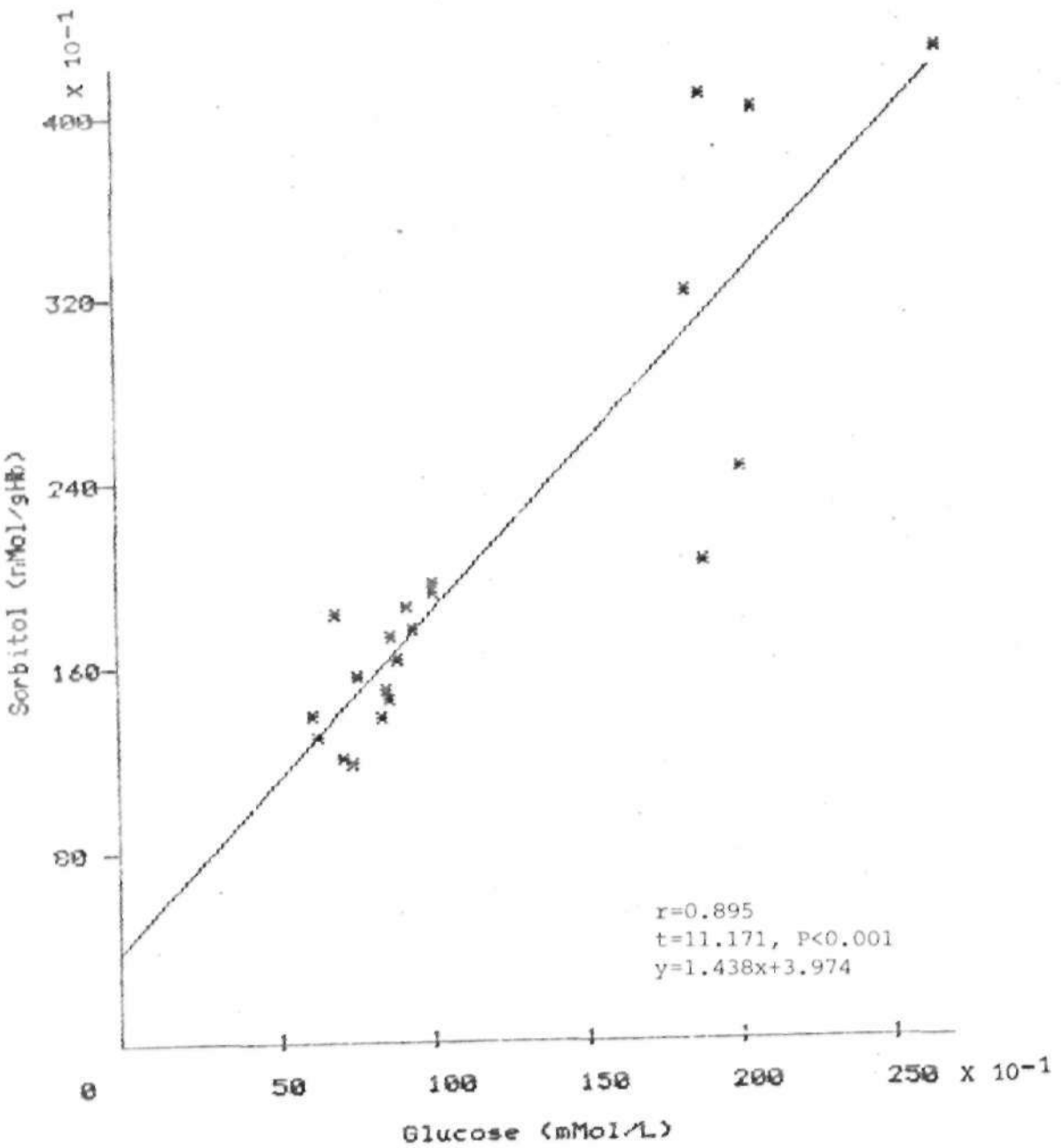


Fig. 4.17

Relationship between fasting plasma glucose and fasting red cell sorbitol in 33 newly diagnosed diabetics.

4.5.2 Fructosamine

The relationship of red cell sorbitol with plasma fructosamine are shown in Figures 4.18 and 4.19 for the non diabetic and diabetic subjects respectively. The correlation between plasma fructosamine and red cell sorbitol in the non diabetics was not significant ($r = 0.146$, $P>0.05$, Fig. 4.18), but was significant for diabetics (Types I and II) ($r = 0.478$, $P<0.001$, Fig. 4.19). The results of the correlation studies between red cell sorbitol and plasma fructosamine were similar to that obtained between glucose and fructosamine ($r = 0.411$, $P<0.001$; Fig. 4.20).

4.5.3 Lipids

Hypercholesteraemia was defined as TC $\geq$ 6.5 mmol/L while hypertriglyceridaemia was defined as TG $\geq$ 2.5 mmol/L and combined hyperlipidaemia as elevation of both cholesterol and triglyceride. By these definitions, 7 patients were hypercholesteraemic, 74 hypertriglyceridaemic and 29 had combined hyperlipidaemia. Values of RCS, glucose and fructosamine in both hyperlipidaemic and normolipidaemic diabetic patients are presented in Table 4.XVII. The results of lipid profiles are presented in Table 4.XVIII.

Mean red cell sorbitol concentration in hypercholesteraemic diabetics was significantly higher ($P<0.05$) than in normolipidaemic patients. Similar results were obtained in hypertriglyceridaemic patients ($P<0.02$) and combined hyperlipidaemia ($P<0.05$). Mean plasma glucose and fructosamine values were similar in the three groups of hyperlipidaemia.

Low density lipoprotein cholesterol (LDL-C) correlated positively and significantly with red cell sorbitol ($r = 0.290$, $P<0.01$, Fig.

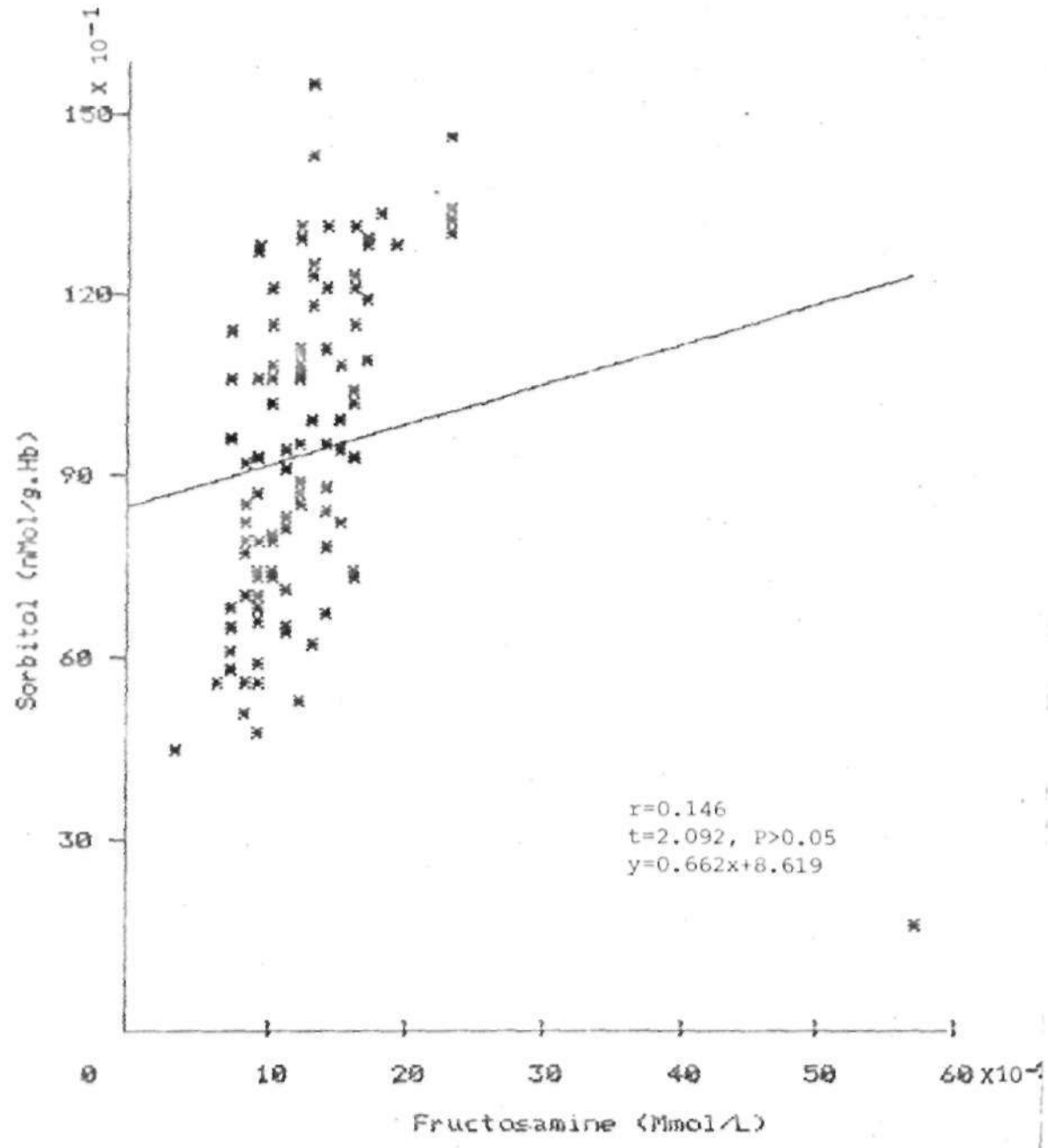


Fig. 4.18

Relationship between plasma fructosamine and red cell sorbitol in 203 apparently healthy non diabetic Nigerians.

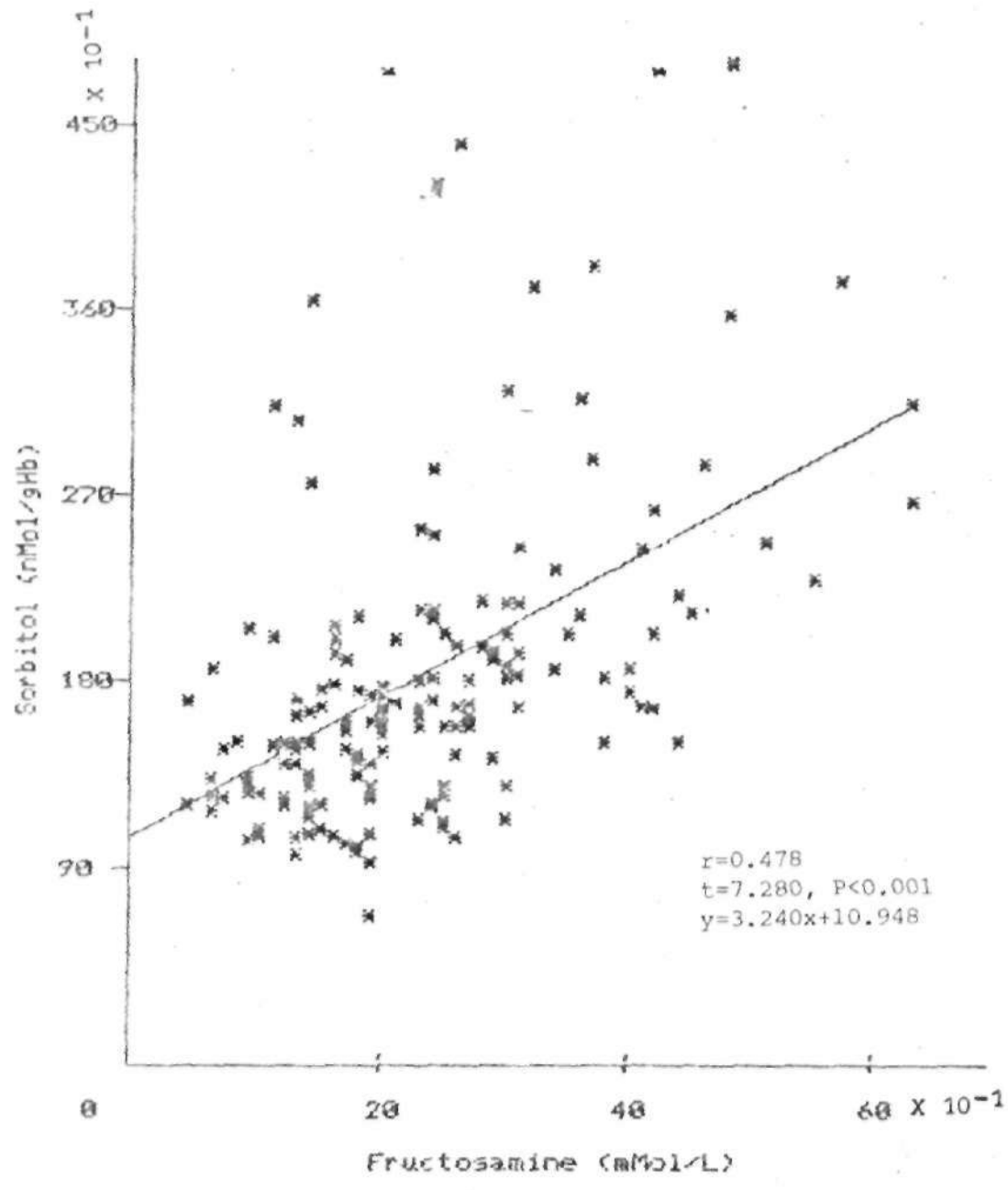


Fig. 4.19

Relationship between plasma fructosamine and red cell sorbitol in 182 diabetic Nigerians (Types I and II).

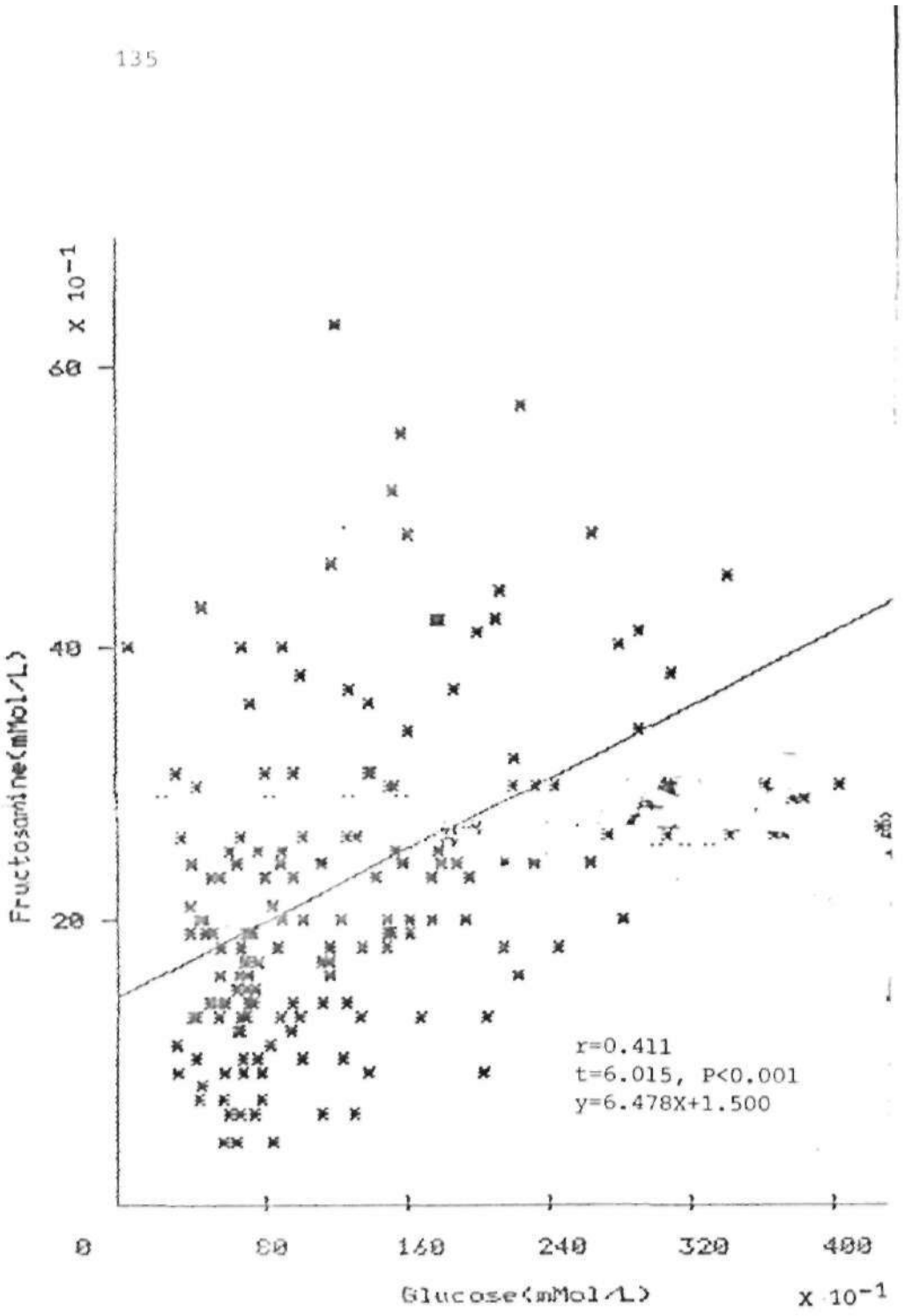


Fig. 4.20

Relationship between plasma glucose and fructosamine in 182 diabetic Nigerians (Types I and II).

Table 4.XVII RED CELL SORBITOL, PLASMA GLUCOSE AND FRUCTOSAMINE (MEAN±SEM) IN HYPERLIPIDAEMIC AND NORMOLIPIDAEMIC DIABETIC PATIENTS

	n	Sorbitol (nmol/g Hb)	Glucose (mmol/L)	Fructosamine (mmol/L)
Hypercholesteraemia alone	7	20.5±1.47*	14.5±1.48	2.6±0.22
Hypertriglyceridaemia alone	74	20.0±0.82+	12.2±0.76	2.3±0.11
Combined hyperlipidaemia	29	20.7±1.67*	14.8±1.62	2.6±0.23
Normolipidaemia	46	16.9±0.91	11.3±1.14	2.1±0.17

n = No. of subjects

*P<0.05 Hypercholesteraemia alone and combined hyperlipidaemia Vs normolipidaemia

+P<0.02 Hypertriglyceridaemia alone Vs normolipidaemia

DEFINITIONS

*Hypercholesteraemia alone = Tc $\geq$ 6.5 mmol/L

*Hypertriglyceridaemia alone = TG $\geq$ 2.5 mmol/L

*Combined hyperlipidaemia = Elevation of both Tc and TG

*Normolipidaemia = TC < 6.5 mmol/L; TG < 2.5 mmol/L

Table 4.XVIII. LIPID CONCENTRATIONS (MEAN±SEM) IN DIABETIC AND NON DIABETIC NIGERIANS

Subjects	n	Total cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-Cholesterol (mmol/L)	LDL-Cholesterol (mmol/L)	TC/HDLC (Atherogenic index)
IDDM	32	4.9 ± 0.31	3.7 ± 0.28*	0.8 ± 0.10	2.3 ± 0.27	5.0 ± 0.06*
NIDDM	34	5.3 ± 0.15‡	3.4 ± 0.16*	0.8 ± 0.05	2.7 ± 0.28	5.2 ± 0.30*
Controls	50	4.1 ± 0.10	1.4 ± 0.06	0.9 ± 0.30	2.3 ± 0.11	4.1 ± 0.14

n = No. of subjects

*P<0.001 Diabetics Vs Controls

‡P<0.01 NIDDM Vs Controls

+P<0.02 NIDDM Vs Controls

4.21). Similarly, triglyceride correlated positively and significantly with red cell sorbitol ($r = 0.520$, $P<0.001$). However, the relationship between red cell sorbitol and plasma total cholesterol was not significant ($r = 0.057$, $P>0.05$, Fig. 4.22). The atherogenic index (TC/HDLC) equally correlated with red cell sorbitol ($r = 0.226$, $P<0.01$, Fig. 4.23). These results demonstrate that polyol pathway and lipid abnormalities in diabetes mellitus may be related.

4.6 RED CELL SORBITOL IN THE ASSESSMENT OF METABOLIC CONTROL IN DM

The suggested criteria for evaluation of glucose control in diabetic Nigerians using red cell sorbitol are presented in Table 4.XIX. The performance of red cell sorbitol in comparison with glucose, fructosamine and their combinations in the assessment of metabolic control in diabetic patients is presented in Table 4.XX. Sorbitol alone indicated 54.0 % in poor control, 14.8 % in moderate control and 31.3 % in good control. Values of RCS(mean $\pm$ SEM) in the 58.2 % of the patients with poor glucose control (21.1 $\pm$ 0.84 nmol/g Hb) was significantly higher ($P<0.001$) than similar values in the 33.0 % of the patients with good glucose control (14.3 $\pm$ 0.64 nmol/g Hb). Patients with moderate glucose control had mean RCS values of 15.4 $\pm$ 0.92 nmol/g Hb.

These results demonstrate that RCS could differentiate between good, moderate and poor controls. Most of the reported complications are common in diabetics with abnormal glucose, fructosamine and RCS values.

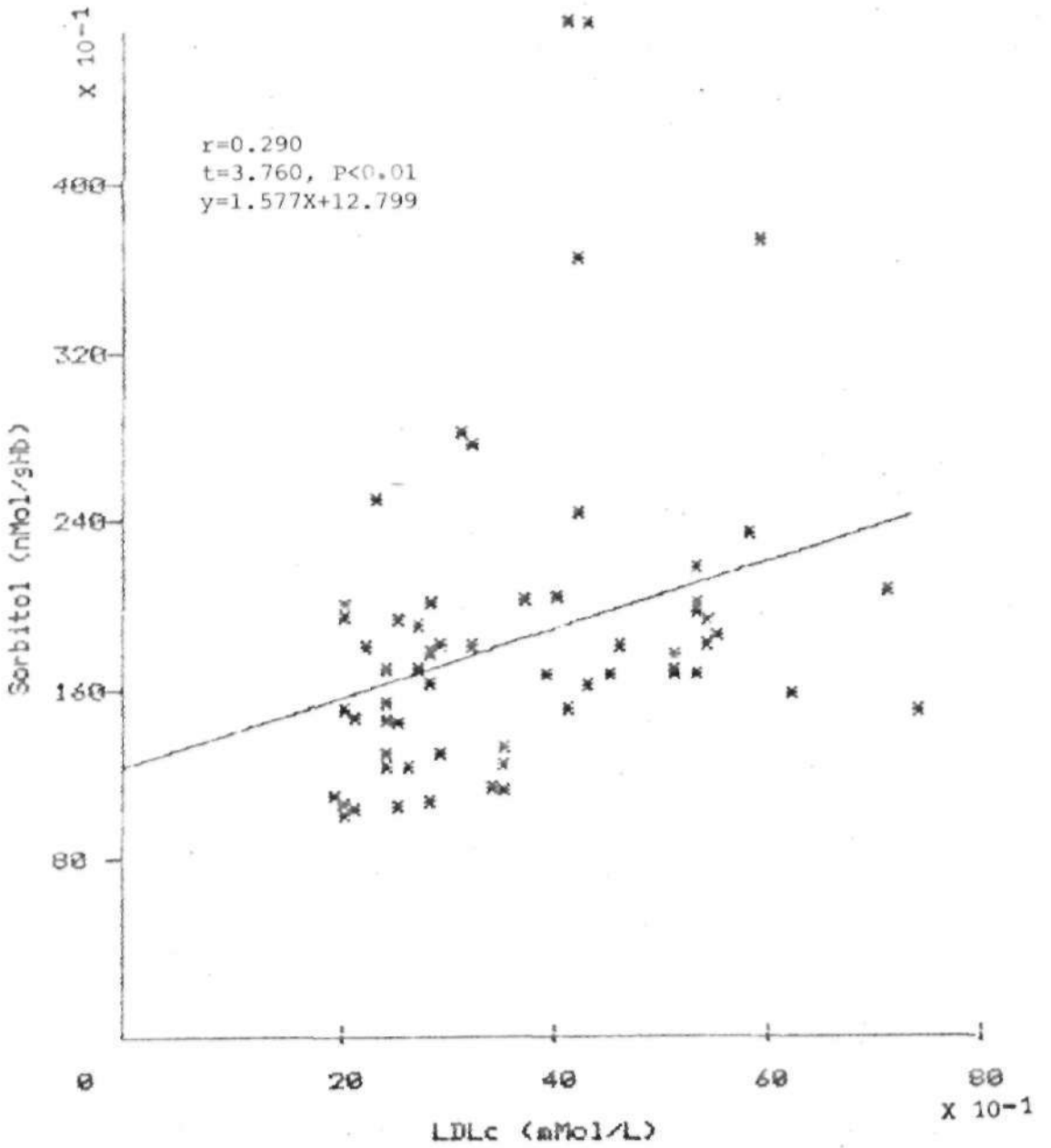


Fig. 4.21

Relationship between LDL cholesterol (LDLC) and red cell sorbitol in
156 diabetic Nigerians (Types I and II).

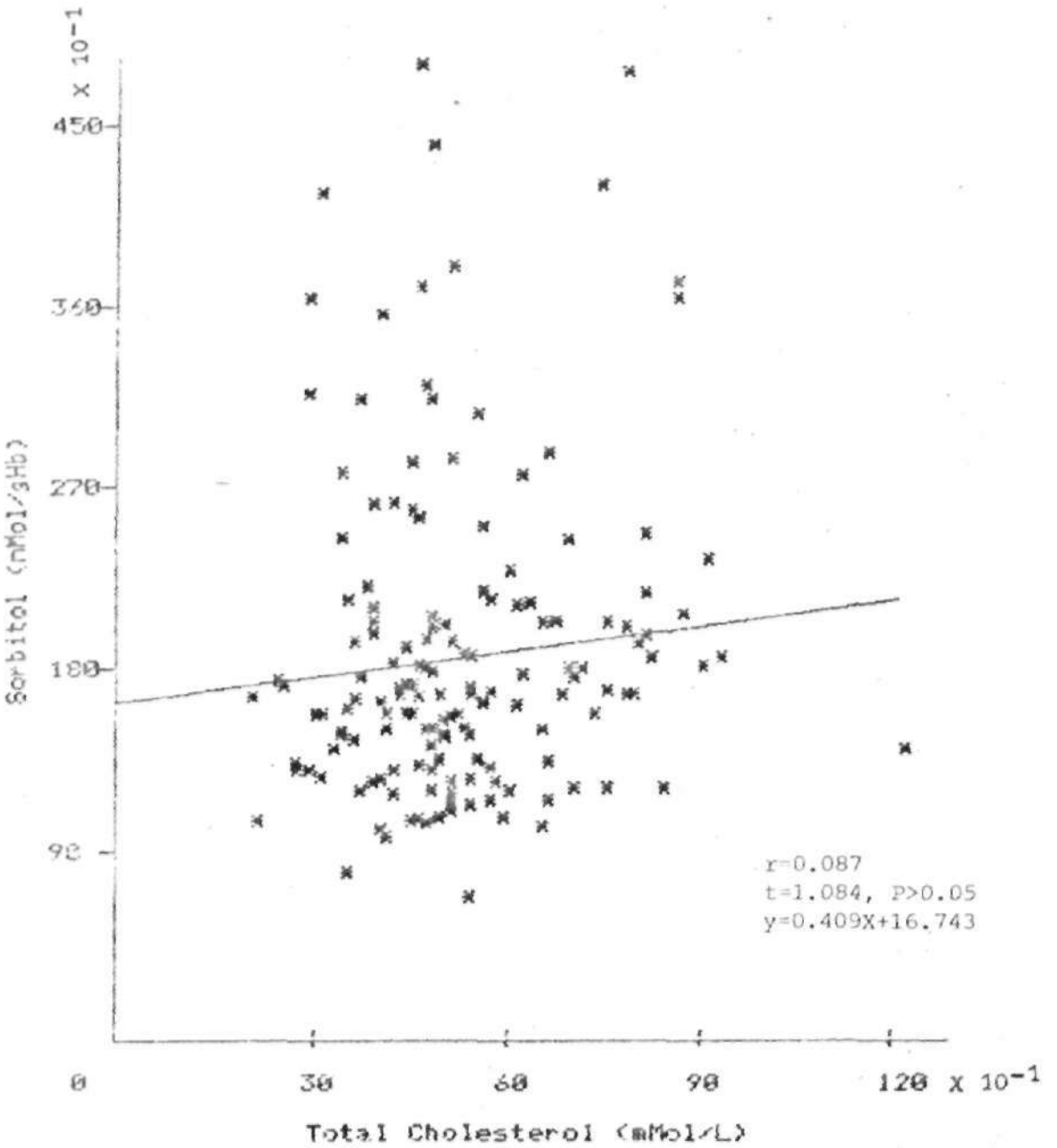


Fig. 4.22

Relationship between Total Cholesterol and red cell sorbitol in 156 diabetic Nigerians (Types I and II).

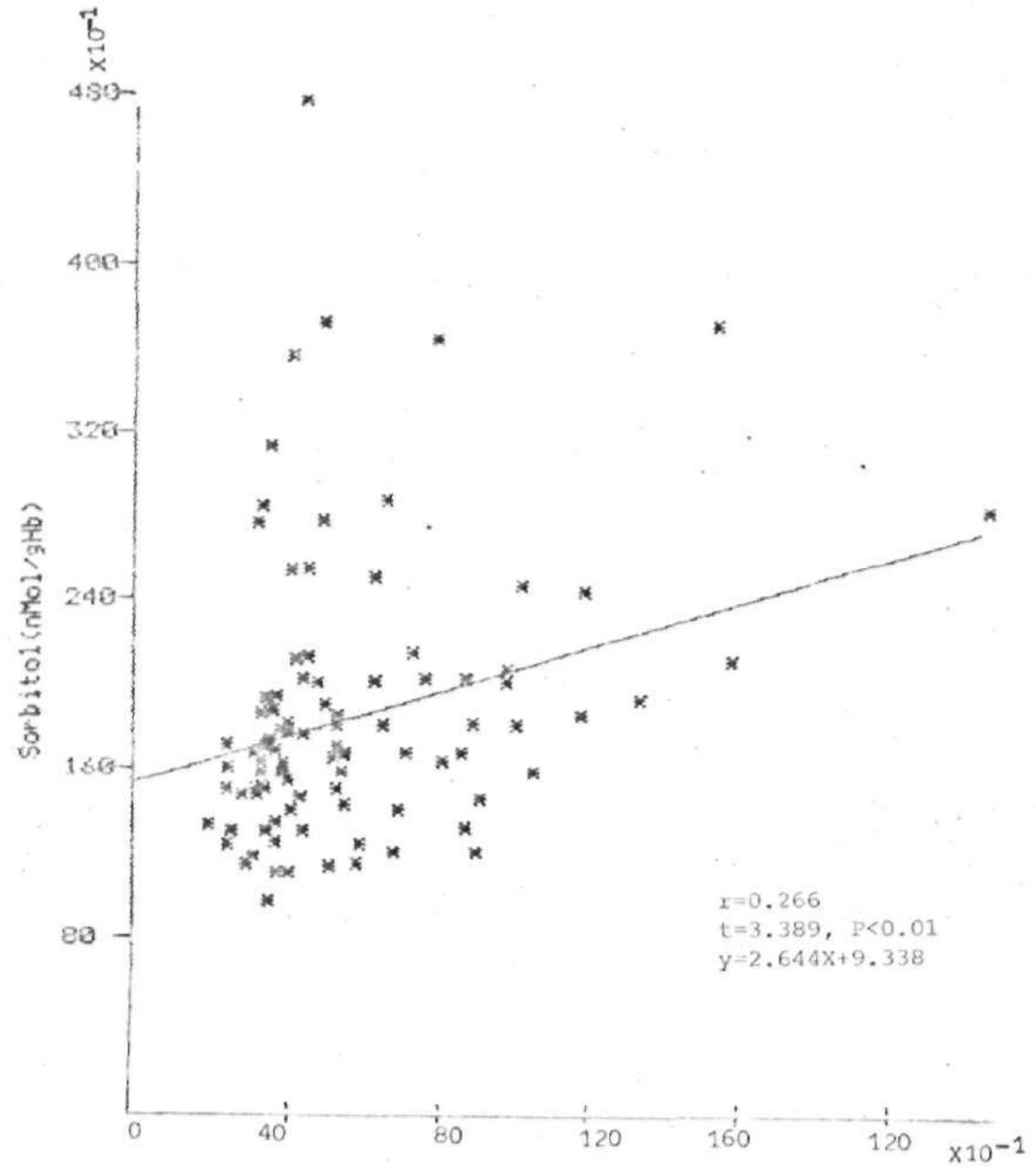


Fig. 4.23

Correlation between atherogenic index (TC/HDLC) and red cell sorbitol in 156 diabetic Nigerians (Types I and II).

Table 4.XIX. SUGGESTED CRITERIA FOR EVALUATION OF GLUCOSE CONTROL IN DIABETIC NIGERIANS USING RED CELL SORBITOL

Assessment	Sorbitol (nmol/g Hb)	Fructosamine (mmol/L)	Glucose (mmol/L)
Poor	$\geq$ 17.0	$\geq$ 2.0	FPG $\geq$ 7.8 RPG $\geq$ 11.1
Moderate/Satisfactory	15.0-17.0	1.8-2.0	FPG 6.5-7.8 RPG 8.0-11.0
Normal/good	< 15.0	< 1.80	FPG 2.5-6.5 RPG 3.0-8.0

FPG = Fasting Plasma Glucose

RPG = Random Plasma Glucose

TABLE 4. XX RED CELL SORBITOL IN THE ASSESSMENT OF METABOLIC CONTROL IN NIGERIAN DIABETIC PATIENTS (TYPES 1 AND II)

INDICES	POOR		MODERATE		GOOD	
	n	%	n	%	n	%
Sorbitol alone	98	54.0	27	14.8	57	31.3
Glucose alone	106	58.2	16	8.8	60	33.0
Fructosamine alone	95	52.2	21	11.5	66	36.3
Glucose and Sorbitol	85	46.7	45	24.7	52	28.6
Fructosamine and Sorbitol	83	45.6	53	29.1	46	25.3
Glucose, Fructosamine and Sorbitol	79	43.4	70	38.5	33	18.1

See Table 4. X1X for criteria

n No. of patients

CHAPTER FIVE

5.0 DISCUSSION

5.1 PERFORMANCE CHARACTERISTICS OF RED CELL SORBITOL ASSAY

Results from the wavelength optimization studies showed that maximum relative fluorescence occurred at 368 nm for excitation and 456 nm for emission for all the samples assessed. Malone et al (1980) reported measurements at excitation and emission wavelengths of 366 nm and 452 nm while Williams and Marks (1988) measured at 365 nm excitation and 457 emission respectively. All subsequent analyses in this study were made at 368 nm excitation and 456 nm emission because the relative fluorescence responses were more sensitive on the SFM 25 spectrofluorimeter. The choice of wavelengths for analysis on a spectrofluorimeter may be dictated by the band width of the monochromators which may differ from one model of the equipment to another.

Results from the time course studies showed no significant difference between the relative fluorescence at 60 min and 90 min for the samples tested. This indicates that the reaction is complete at about 60 min of starting the reaction. This result is in agreement with that reported by Vertommen et al (1987). Malone et al (1980), however, reported a time lapse of 25 min. In the present study, all measurements were made after 60 min of starting the reaction.

The limit of detection is the smallest single result which is distinguishable from a suitable blank (Butter et al, 1976). This is estimated as mean plus 3 SD above measured blank (ACS 1980). The mean plus 3 SD of ten consecutive blank readings yielded a

detection limit of 0.55 nmol/ml from a calibration curve. Vertommen and co-workers (1987)using a similar method, reported limit of detection of 0.69 nmol/ml and Malone et al (1980) reported 0.33 nmol/ml. A limit of detection less or equal to 1.0 nmol/ml seems appropriate for similar analytical technique. This is because low levels of sorbitol are of no clinical significance. A limit of detection of 1.0 nmol/ml is equivalent to sorbitol levels of 8.0 nmol/ml for biological sample and thus demostrate acceptable sensitivity for the assay.

The values of the within-assay and between-assay variations are in close agreement with those reported by Price et al (1987) and Vertommen et al (1987). Price et al (1987) used the capillary gas chromatographic method. Similar findings have been reported by Wambua and colleagues (1988) even though they used the colorimetric method. The results obtained in the present study demonstrate that red cell sorbitol assay has coefficient of variations within acceptable levels.

Data on recovery studies from previous studies are limited. Results from the recovery experiments in the present study demonstrate acceptable recovery and is an indication of the accuracy of the assay.

The results of interference studies are well in agreement with those reported by Malone et al (1980). Malone and his colleagues (1980) did not, however, assess the interfering effect of common oral hypoglycaemic agents. Vertommen et al (1987) did not observe any significant effect of oral hypoglycaemic agents in their studies of diabetic patients. Observations from the present studies and those of Vertommen et al (1987) indicate that specimens for red cell sorbitol assay can be collected from patients on oral hypoglycaemic agents.

It can be concluded from these findings that red cell sorbitol assay in our laboratory is linear within clinically relevant range, sensitive, precise, specific and free from common interferents. The fluorimetric assay technique can be sustained on a regular basis in our department provided the necessary reagents are procured as and when due. In addition, these results demonstrate that red cell sorbitol assay is feasible and a result could be made available within 2½ hr of sample collection. The method as evaluated costs about twenty five naira (₦25.00) per test (equivalent to US $1.14) and could easily be included in the routine laboratory test.

5.2 REFERENCE INTERVAL AND CLINICAL DECISION LIMIT

In order to establish a reference interval for clinical use, the random and fasting results of RCS were pooled and analysed by probit transformation (Gowans et al, 1989). The technique of probit transformation of gaussian and log-gaussian distributions of data into straight lines is rarely used. However, it has many advantages in that it is easy to perform, sophisticated hardware and software are not required, displays of data are easily prepared and the straight line format is easy to interprete. In this approach every step is visualized and there is the possibility of drawing many lines on the same plot and the lines may be easily distinguished. The 95 % central interval from the plot for the apparently healthy population is 4.1-14.9 nmol/g Hb. These values may be considered as the reference interval for our population. A clinical decision limit of red cell sorbitol of 17.0 nmol/g Hb or greater is thus suggested for diagnostic purposes in our

environment. There has been no published data on the reference intervals for red cell sorbitol concentration in Africans. Wambua et al (1988) used only 12 healthy subjects as controls. They observed extreme variation of sorbitol levels in the control subjects which made it impossible to construct a 95 % confidence interval for the population. It was then suggested that a study with larger sample size was required to define appropriate reference interval. The present study represents an important response to this problem.

In none of the apparently healthy subjects in the present study was red cell sorbitol less than 4.5 nmol/g Hb recorded. Therefore, the suggested reference interval seems appropriate for our population and thus could be employed for clinical application.

The data on the diagnostic specificity and sensitivity of red cell sorbitol using the above decision limit suggest acceptable diagnostic performance characteristics for red cell sorbitol in the out-patients. Information on the diagnostic performance of red cell sorbitol assay is lacking. However, despite the suggestion of the influence of other non-glucose factors on the polyol pathway activity (Vertommen et al, 1987), the assay seems to possess a potential for application as a screening test for DM. This awaits additional investigations in the general population for full assessment of this potential.

The variation of sorbitol with sex in the controls showed that red cell sorbitol levels (mean $\pm$ SEM) were 10.7 $\pm$ 0.13 nmol/g Hb in men and 10.5 $\pm$ 0.28 nmol/g Hb in women. These results demonstrate that there is no sex difference, agreeing with the known fact that aldose reductase activity or the polyol (sorbitol)

pathway is similar in both men and women (Onaya et al, 1990). As a result of these findings a single reference interval was defined for both men and women for our local population.

The results of the relationship between RCS and age indicate that mean value of red cell sorbitol was significantly higher ($P<0.05$) in adults than in adolescence, however, value fell for ages between 32 to 41 years. It is important to note that basal insulin concentration is unaffected by age, though insulin secretion in response to glucose load is reduced in the elderly adults (Alberti et al 1975) indicating that in adults, more substrate is presented to the enzyme than in childhood and adolescence. The activity of the polyol pathway depends on the glucose concentration (Malone et al, 1980; Onaya et al, 1990; Tawata et al, 1992). Glucose levels in adults are slightly higher than in younger subjects owing to reduced insulin response and hardening of the vessel walls (Alberti et al 1975). There has been no report on the influence of age on red cell sorbitol concentrations in non-diabetic Nigerians. However, age did not produce any significant effect on the level of red cell sorbitol in diabetic patients, confirming earlier observation by Onaya et al (1990).

The mean values of red cell sorbitol are similar for Blacks as seen in the present study and the report by Wambua et al (1988) for Kenyan Africans. However, results of the present study and that of Wambua et al (1988) were significantly lower when compared with those of Vertommen et al (1987), and Onaya et al (1990). Even

though sorbitol was not estimated in White subjects in the present study, these observations suggest an indication of racial difference. Genetically, the activity of aldose reductase may be higher in certain White populations than Blacks. The risk of the development of any of the late complications of diabetes mellitus may be higher in these populations.

The relationship between body mass index and red cell sorbitol was assessed in the apparently healthy subjects. Body mass index (BMI) equal to 27 kg/m^2 or greater was used as an index of obesity. Obesity is the most important predicator of the development of type II diabetes (Owusu, 1992). However, none of the subjects with BMI greater than 27 kg/m^2 had fasting or random blood glucose higher than the diagnostic cut-off points. The BMI recorded for the majority of the subjects in this study were of low or moderate obesity (BMI $\leq$ 17 kg/m^2/BMI $\geq$ 17 $\leq$ 27 kg/m^2). The results demonstrate weak positive relationship between BMI and RCS in the controls.

The interaction of systolic blood pressure (Bp) with red cell sorbitol did not demonstrate any significant effect while the diastolic Bp seems to have a weak but significant effect as indicated in a correlation analysis ($r = 0.221$, $P<0.01$). Similarly, using the student's t-test for unpaired data, the difference in the mean red cell sorbitol values between the subjects with diastolic Bp $\geq$ 90 mm Hg and those with Bp < 90 mm Hg was significant ($t = 4.69$, $P<0.001$). However, only few subjects had diastolic Bp greater than 90 mm Hg. The importance of this observation is that the interpretation of red cell sorbitol results should be with caution in hypertensive individuals. In addition, the risk of

the development of any of the long-term diabetic complications resulting from the accumulation of sorbitol may be higher in the hypertensive than nomotensive patients.

Cigarette smoking is one of the factors implicated in hypertension (Modan, 1985). This assessment was undertaken to explore the possible influence of cigarette on the polyol pathway. Results from this assessment demonstrate that cigarette does not play any significant role in enhancing or depressing the polyol (sorbitol) pathway. A previous study in our laboratory revealed that Nigerian smokers have significantly elevated level of thiocyanate compared with non-smokers (unpublished data). The mean plasma glucose values were similar in both smokers and non-smokers ($P>0.05$).

Red cell sorbitol in alcohol drinkers revealed that alcohol consumption may be one of the factors influencing aldose reductase activity, though the values of red cell sorbitol were within our reference interval. The alcohol drinkers have higher mean sorbitol value than the non-drinkers. The reason for this observation is obscure. Alcohol may depress cellular up take of glucose, thus making available more substrate for the enzyme aldose reductase. It may be important, therefore, to suggest that alcohol intake should be avoided two to three days before sample collection for sorbitol estimation.

5.3 VALUES OF RED CELL SORBITOL IN DIABETIC PATIENTS WITH AND WITHOUT COMPLICATIONS

Diabetes mellitus is a disease with various hyperglycaemic and long-term complications. The role of hyperglycaemia in the pathogenesis of the long-term complications of diabetes continues to attract active research. Intracellular accumulation of sorbitol

as a result of increased flux of glucose through the polyol pathway has been implicated in the pathogenesis of retinopathy, neuropathy, nephropathy and cardiovascular diseases (Chylack and Kinoshita, 1969; Morrison et al, 1972; Gabbay 1973; Onaya et al, 1990; Tawata et al, 1992). The accumulation of sorbitol in the cell may lead to alteration of cell function. Sorbitol is osmotically active and its accumulation leads to osmotic swelling which is associated with alteration in membrane permeability resulting in leakage of several substances such as amino acids, glutathione, myoinositol, potassium ions and influx of sodium and chloride ions. Sorbitol accumulation may also result in decreased myoinositol concentration. The fall in myoinositol levels in the cell membrane alters the functioning of the Na-k-ATPase pump and the integrity of the cell membrane is disrupted (Baxter, 1991). In addition, accumulation of sorbitol in the cell may alter the cellular redox potential. This alters the NADPH:$NADP^+$ ratio in several tissues as a result of the requirements of $NADPH+H^+$ for the reduction of glucose to sorbitol. Oxidation of sorbitol depletes NAD^+ at the expense of elevated $NADH + H^+$ production.

Red cell sorbitol assay has been suggested to be used in the study of the pathogenesis of diabetic complications (Malone et al, 1980; Onaya et al, 1990). Red cell sorbitol concentrations in diabetic patients with and without complications have been assessed in the present study.

Results obtained in this study closely agree with previous report by Crable et al (1980) for diabetics with retinopathy. Onaya et al (1990), however, reported a significantly higher sorbitol-glucose ratio in diabetics with retinopathy than those

without. The enhanced activity of the polyol (sorbitol) pathway has been implicated in the pathogenesis of diabetic retinopathy (Robinson et al, 1983). This is due to involvement of sorbitol accumulation in retinal capillary basement. Retinal capillary pericytes are thought to control blood flow through the retinal vasculature and may also contribute to the stability of the vessel wall. Loss of retinal capillary pericytes is the first structural abnormality found in diabetic retinopathy. A postulate that retinopathy is as a result of excessive polyol pathway activity is consistent with evidence that aldose reductase (AR) activity occures in retinal vessels. Engerman and Kern (1989) reported that preliminary results on the effect of administration of high doses of an aldose reductase inhibitor (ARI) indicate that marked suppression of polyol production and accumulation may have little influence on retinopathy. Whether the development of retinopathy can be inhibited by inhibition of the polyol pathway has yet to be established. Correlation between sorbitol accumulation in retinal capillary basement membrane and red blood cell needs to be established.

Higher levels of lipids have been found in patients with retinopathy than in those without (Keiding et al, 1952; Iannaccone and Kornemp 1954; Esmann et al 1963 and Hanna 1982) suggesting that they may play a part in its development, but other workers have been unable to confirm this relationship (Duncan et al, 1968, West et al, 1980, Dornan et al, 1982). Our result also failed to confirm higher lipid levels in diabetics within retinopathy than in those without. These conflicting results may have been due to inadequate matching of patient groups for age

and type of treatment. Information on fructosamine values in diabetics with retinopathy is lacking. However, a two to five fold elevation in glycation of myelin and myelin associated proteins have been demonstrated in both the peripheral and central nervous system in diabetes which is comparable to increases measured for plasma proteins (Vlassara et al, 1981; 1983).

The role of sorbitol in diabetes complications has been well characterized using ARI by several workers (Greene et al, 1983; Greene et al 1987; Tilton et al, 1988; McCaleb et al, 1988; and Sato et al, 1988). Previous findings (Onaya et al 1990) and finding in this study more specifically suggest that the affinity of aldose reductase for glucose may be greater in patients with diabetic complications, indicating that the polyol pathway is implicated in the pathogenesis of diabetic complications.

Assay of red cell sorbitol could thus prove valuable as a biochemical risk factor in early detection of patients prone to the development of any of the long-term diabetic complications who may benefit from aldose reductase inhibitor therapy. As seen in this study, some of the diabetic patients, inspite of good metabolic control judged by glucose levels, had elevated red cell sorbitol and plasma fructosamine levels. The question remains whether these patients with high red cell sorbitol values are more at risk in developing diabetic complications.

The observation of significant elevation of RCS levels in diabetics compared with non diabetics Nigerians confirmed earlier reports of several workers (Malone et al 1980, Lapolla et al 1985, Vertommen et al 1987, Price et al 1987, Onaya et al 1990). The rate limiting enzyme in the polyol pathway, aldose reductase

(AR) has a high Km (50-100 mM). Thus its affinity for glucose is low in normoglycaemia. During hyperglycaemia as commonly encountered in uncontrolled DM, a lot of glucose is diverted towards this pathway as the activity of AR is increased. Tawata et al (1992) have observed the induction of AR mRNA expression and its activity by glucose in fetal rat aortic smooth muscle (A10) cells. Besides, other workers (Vertommen et al 1987) have reported similar elevated level of RCS in type I (insulin-dependent) than type II (non-insulin dependent) diabetics as observed in the present study. It has been stated that insulin administration as commonly practised in type I diabetic patients may tend to enhance the activity of AR. The conventional insulin treatment of once or twice daily injections does not mimic physiological insulin secretion, and many patients are subjected to wide fluctuations of blood glucose levels and basal hyperglycaemia. In addition, other metabolic and hormonal abnormalities remain uncontrolled (Ruskin, 1982) and tendency to higher insulin levels especially when given as isophane (NPH) insulin at night. The case for Nigerian diabetic patients may be more compounded as in most third world countries due to lack of insulin and even when available, cost, storage and high rate of illiteracy may worsen the problems. Thus, the significantly higher ($P<0.001$) level of RCS in type I may be due primarily to poor diabetic state consequent to poor management or non-compliance in these patients. There has been no report on red cell sorbitol concentration in diabetic Nigerians. These could serve as a baseline for further investigations on this aspect in Nigeria and other parts of Africa.

The mean of plasma glucose and plasma fructosamine in diabetics were significantly higher than the controls. This confirmed previous reports by Isah et al (1990) for similar diabetic Nigerians.

Changes in red cell sorbitol, plasma glucose and fructosamine were not consistent with the progression in the duration of the disease in the present study.. In the newly diagnosed diabetics, the significantly elevated level of RCS and other parameters may be due to ignorance of the classical signs and symptoms of the disease which may cause delays in reporting for medical care. The observation for patients with more than 9 years duration may be due to religious belief, change to traditional doctors, poor compliance, cost of drugs, lack of adequate monitoring, poor clinic attendance and above all, illiteracy. In the newly diagnosed patients and patients with more than 9 years duration, there is severe hyperglycaemia. During hyperglycaemia, the availability of a large pool of free intracellular glucose cause appreciable formation of sorbitol. This is because the enzyme, aldose reductase has a high Km (50-100 mM) for glucose. Thus in normoglycaemia the activity is low due to reduced affinity for glucose and high during hyperglycaemia. This contributed significantly to the elevated RCS levels observed in these patients.

The mean red cell sorbitol value in obese diabetics was significantly higher ($P<0.05$) than in non-obese diabetics. A correlation analysis of the data gave a correlation coefficient of 0.294 ($t = 3.56$; $P<0.001$).

It has been reported that obesity is correlated with high circulating insulin levels (even after fasting), and this leads to down-regulation of receptor levels (O'Malley and Binbaumer, 1977).

Insulin is one of the drugs said to have a significant effect on the activity of the polyol pathway. Obesity is one of the causative factors for DM, especially type II. It has been observed that results of red cell sorbitol concentration in NIDDM are conflicting (Vertommen et al 1987) suggesting the involvement of non-glucose factors. Obesity does not seems to influence plasma glucose concentration as mean values were similar in the two subject groups. Campbell and Carlson (1993) concluded recently that obesity diminishes the insulin sensitivity of both hepatic and peripheral tissues in patients with NIDDM. The mechanism by which obesity influences the sorbitol pathway is still obscure.

The affinity of aldose reductase for glucose seems to be higher in hypertensive individuals than in normotensitive subjects. This could be due to the presence of underlying renal lesions in the hypertensive subjects. Reports on the relation of hypertension to aldose reductase activity are lacking. It has been established that hypertension is more prevalent in diabetic than non-diabetic populations (Medan, 1985).

The results of RCS on the various types of treatment are in close agreement with previous reports (Onaya et al 1990). It is worth mentioning that the mean values of the three parameters were lower in patients treated with oral hypoglycaemic agents (OHA) than in the other groups even though the values were not within our laboratory reference intervals for controlled patients. This observetion could be as a result of availability of the OHA compared with insulin. However, diet therapy does not seem to be adequate because of poor socio-economic conditions and the high rate of illiteracy among most diabetic Africans.

5.4 USE OF RED CELL SORBITOL IN ASSESSMENT OF METABOLIC CONTROL

Estimation of red cell sorbitol concentration has been suggested as a good indicator of metabolic control in diabetic patients (Malone et al 1980; Lapolla et al, 1985; Vertommen et al 1987) especially in developed nations. The relationship between red cell sorbitol and glucose, fructosamine as well as lipids were examined in this study.

The weak positive correlation obtained between red cell sorbitol and glucose ($r = 0.08$; $P>0.05$) for non-diabetic control subjects confirmed previous observations by Others (Malone et al 1980; Onaya et al 1990). Aldose reductase is an enzyme with high Km and thus low affinity for glucose during normoglycaemia. Changes in glucose concentration from fasting to random seem not to correspond positively with the activity of AR in non-diabetic subjects. A 2 hr blood glucose screening conducted on 22 healthy volunteers confirmed this observation. Correlation analyses between red cell sorbitol and glucose at fasting ($r = 0.01$, $P>0.05$) and 2 hr after a glucose load ($r = 0.06$, $P>0.05$) were not significant. Though, 2 hr blood glucose screening(2 HBGS) is used to detect undiagnosed diabetes mellitus, in the present study, it was used to assess influence of short term glucose changes on red cell sorbitol levels.

Red cell sorbitol correlated positively and significantly with plasma glucose ($r = 0.746$, $P<0.001$) in a mixed diabetic population (Types I and II). Other workers have reported similar results for the diabetic subjects (Malone et al, 1980, Lapolla et al, 1985; Vertommen et al 1987 and Onaya et al 1990). A similar result was obtained for newly diagnosed diabetics who were

subjected to the 2 HBGS. The results of the correlation studies between fasting glucose and RCS show that in diabetics the activity of AR increases linearly as glucose concentration increases. As a result of the significantly high level of red cell sorbitol in DM in both fasting and random specimen, specimens for assessing the polyol pathway can be collected at anytime of the day irrespective of time of meal intake. Malone et al (1980) reported elevated level of fasting red cell sorbitol in diabetics compared with non diabetics. Onaya et al (1990) used specimens collected 2 hr after break fast and still reported similar elevated red cell sorbitol in diabetic patients as obtained in this study. These reports indicate that abnormalities of the polyol pathway occur mainly in DM. The results also show that control of hypergiycaemia to a large extent may play a significant role in decreasing the frequency of the polyol pathway abnormality.

Therefore, it can be concluded from these findings that estimation of red cell sorbitol is a good marker of glycaemic status in DM. Individual differences may still exist and should not be overlooked.

The results of correlation studies between fructosamine and RCS especially in the diabetics demostrate that red cell sorbitol assay could be employed as an index of metabolic control. There has been no report on the relationship between red cell sorbitol and fructosamine in diabetics before now. However, other workers have observed significant correlation between red cell sorbitol and HbA_{1c} (Vertommen et al 1987; Onaya et al 1990). Price and co-workers (1987) could not demonstrate any correlation between red cell sorbitol and HBA_{1C}. A similar correlation

between plasma fructosamine and red cell sorbitol was obtained between plasma glucose and fructosamine in the present study ($r = 0.411$ $P<0.001$). This confirms earlier report by Isah et al (1990) for a similar Nigerian diabetic population. It may then be suggested that red cell sorbitol could be used as a measure of long-term sorbitol accumulation in other tissues since the levels parallel those of tissues like the lens and sciatic nerve (Kikkawa et al, 1983; Malone et al, 1984).

There is no information associating red cell sorbitol levels to lipid profiles in diabetic patients. The results of RCS values in hyperlipidaemic and normolipidaemic patients and the relation of RCS with TG, LDLC and TC/HDLC demonstrate that deranged lipid profiles as commonly encountered in diabetics relate to abnormal polyol pathway. Triglyceride estimation has been suggested as a risk factor for vascular disease in diabetes mellitus (Pyrala et al, 1987; Fontbonne et al,1989; Stevens et al, 1990). In cross-sectional studies, plasma triglycerides are often more strongly related to the presence of large vessel disease than cholesterol (Stevens et al, 1990). There are adequate data to support a causal relationship between serum plasma total cholesterol and low density lipoprotein (LDL) cholesterol and macrovascular disease in the non-diabetic population (consesus conference 1985; study group of the European Atherosclerosis Society, 1987) and it is likely that such a relationship also applies in diabetic subjects. Significant correlations of these parameters and the atherogenic index with red cell sorbitol in the present study suggest that the polyol pathway plays a salient role in vascular diseases in diabetes

mellitus. Diabetes mellitus, hypertension and hyperlipidaemia are considered as serious risk factors in atherosclerotic cardiovascular diseases which are related to red cell sorbitol as demonstrated in this study.

Intracellular accumulation of sorbitol has been reported as one of the factors responsible for the vascular complications of diabetes mellitus (Morrison et al, 1972). The effects of sorbinil, an aldose reductase inhibitor, on the polyol pathway activity in the aortic smooth muscle, RBC deformability and vascular permeability have been investigated (Morrison and Tampa, 1985, Rabey et al, 1985, Chang et al, 1985). Morrison and Tampa (1985) concluded from their study that increased polyol (sorbitol) pathway activity may induce an alteration in myoinositol metabolism that contributes to metabolic abnormalities in aortic smooth muscle at elevated glucose concentration in vitro. A similar conclusion has recently been made by Tawata et al (1992).

The findings in the assessment of metabolic control using RCS demonstrate the effectiveness of red cell sorbitol in assessing glycaemic control in established diabetics. The plasma fructosamine assay, although cheap and simple, is difficult to standardise and represents the total glycated plasma protein and a wide range of plasma proteins of different half-lives will contribute to the final value. In addition, fructosamine assays are based on a simple reducing chemistry and there could be interference from any reducing substances present in the plasma (Johnson et al 1982). The concentration of albumin in the serum/plasma also affects the fructosamine value. Thus it has been suggested that the result must be interpreted with caution in

grossly hypoalbumineamic and hypergammaglobulinaemic patients, in pregnancy and in patients with chronic renal failure. Frequent blood glucose or urine sampling remains the most valuable method of assessing diabetic control. Unfortunately, patients are often reluctant to perform frequent sampling and the results can be imprecise or falsely recorded (Mazze et al 1984). The problem is even more pronounced in most Third World countries due to the fact that most of the patients are illiterate. Although estimation of blood glucose level is considered more reliable than urine glucose, in practice, blood glucose concentrations are known to fluctuate greatly during the day, even with the best form of therapy.

Red cell sorbitol seems to provide a simple integrated index of control and should be a valuable adjunct to plasma fructosamine and blood glucose in the management of unstable diabetes.

Changes in red cell sorbitol seem to be rapid with poor glucose control than fructosamine. This is confirmed from the observations in the present study of the effect of 75 g oral glucose on red cell sorbitol concentrations in diabetic patients. The correlation between glucose and red cell sorbitol both at fasting and 2 hr was significant. However, the use of red cell sorbitol assay for the assessment of long-term metabolic control is still tentative as the half-life of this index in circulation is not yet known.

The results of sequential changes in red cell sorbitol, fructosamine and glucose indicated changes for the three parameters as 20.0 %, 14.0 % and 33.5 % between time of presentation and the 8th week respectively. However, differences in the mean

values for red cell sorbitol, fructosamine and glucose were not significant between weeks 8 and 16 ($P>0.05$).

A gradual fall in plasma glucose level was recorded unlike red cell sorbitol and plasma fructosamine. This discrepancy though not significant, could be as a result of the mode of treatment and patient compliance. At the 16th week, three of the patients were lost to follow-up. The poor control recorded for these patients within the period under investigation may be due to several factors such as poor patient compliance, cost of drugs, religious belief, lack of adequate home monitoring, and illiteracy. These observations support the suggestion of the use and significance of red cell sorbitol assay in the assessment of glycaemic status in Nigerian diabetics and demonstrate the value of red cell sorbitol measurements for serial assessment of glycaemic status of diabetics in our environment. The mean red cell sorbitol concentration decreased to 17.9 nmol/g Hb, while glucose decreased to 9.4 mmol/L from 15.5 mmol/L and fructosamine to 2.76 mmol/L at week 16 in the patients considered. Critical assessment of the patients revealed that by the 8th week, 53.0 % attained normoglycaemia and the same percentage had red cell sorbitol comparable to the normal or well controlled patients. However, by week 16, 60.0 % attained normoglycaemia while 50.0 % had red cell sorbitol levels within the controlled patients. The difference at week 16 showed that the use of glucose alone as an index of metabolic control could be misleading as control in terms of glucose is an elusive term.

Because of the large number of diabetics with poor control owing to several factors as earlier stated, and the occurrence of long-term diabetic complications, attempts should be made to develop more biochemical assays for adequate assessment of

diabetics in this and other developing countries. Red cell sorbitol assay could prove very valuable in this respect in the nearest future as data are accumulating.

An interesting observation in the present study was in a 66 year old Hausa businessman referred from Kofan-Gayan, General Hospital, Zaria to the diabetic clinic, ABU Teaching Hospital, Zaria with paraesthesae in both lower limbs, erectile impotence, BP of 180/80 mm Hg and obesity (BMI of 31.2 kg/m^2). Biochemical results obtained were fasting blood glucose 11.5 mmol/L, red cell sorbitol 21.0 nmol/g Hb, fructosamine 1.6 mmol/L, TC 8.7 mmol/L, LDLC 7.1 mmol/L, and HDLC 0.9 mmol/L. At week 8 the patient attained near normal normoglycaemia with the red cell sorbitol still elevated. This observation illustrates the involvement of sorbitol accumulation in diabetic complications. It is possible that therapy with aldose reductase inhibitor as an adjunct in the management of the patient would have decreased the level of sorbitol and ameliorated the patient's condition. Gingliano (1993) recently concluded that progression of mild diabetic autonomic and peripheral neuropathy may be halted or even reversed by pharmacologic intervention with the aldose reductase inhibitor, tolrestat • Boland et al (1993) could not demonstrate significant effect of ponalrestat on a range of standard cardiovascular autonomic nerve function tests in diabetic patients with autonomic dysfunctions. It is not clear whether the use of an aldose reductase inhibitor reduces the level of red cell sorbitol in diabetics with normoglycaemia but elevated red cell sorbitol concentration. These types of patients may be in danger of habouring risks that are not easily identified. Long-term prospective

studies are required to provide some answers to these questions. It may be important, therefore, to be able to identify diabetics who have abnormal polyol pathway activity early so that appropriate treatment can be provided and the development of complications delayed or prevented.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

The assay of red cell sorbitol using the fluorimetric enzymatic technique has been standardised. It can be concluded from observations in this study that red cell sorbitol assay in our laboratory is feasible, linear within clinically relevant range, sensitive, precise, specific and free from common interferences. With the observed performance characteristics, the assay could be very useful for clinical applications in our enviroment. The technique evaluated is not too expensive as against the use of capillary gas chromatographic and mass spectrometric method.

However, it is recommended that the performance characteristic of the colorimetric enzymatic technique should be assessed.

A reference range of 4.1 - 14.9 nmol/g Hb was defined for our population. A clinical decision limit of red cell sorbitol of 17.0 nmol/g Hb or greater is suggested for diagnostic purposes in our environment. These established reference interval and clinical decision limit for red cell sorbitol concentration seem appropriate for our population and thus could be used for clinical applications especially as adjunct in monitoring metabolic control and complications in diabetic patients.

Studies with a larger sample size is recommended including subjects from other states in the North of Nigeria since ABUTH serves as a reference hospital for most hospitals in the Northern parts of the country.

The diagnostic sensitivity and specificity of red cell sorbitol assay recorded in the present study suggest acceptable performance characteristics. A community based study probably

using glucose and RCS is recommended as results in present study are limited to out-patients. However, cost may be a problem in this endeavour.

The present study revealed that age, BMI, alcohol consumption and BP could affect the concentrations of red cell sorbitol in non diabetic subjects. Thus, these factors should be considered in the interpretation of red cell sorbitol results.

Studies with a large sample size and lower age group (below 12 years) is needed.

Mean red cell sorbitol value was significantly higher in diabetics than in the healthy controls. Red cell sorbitol to a greater extent clearly differentiates diabetics from non diabetics. Similarly, mean values of plasma glucose and fructosamine were significantly higher in diabetics than the controls. The activity of the polyol pathway seems to be influenced by types of diabetic treatment, BMI, and BP. However, the diabetic state plays dominant role.

It can also be concluded that the activity of aldose reductase is higher in diabetics with nephropathy and neuropathy than in those without. Thus RCS assay could be utilised in study of the pathogenesis of these complications. In the light of these observations, studies with the use of aldose reductase inhibitors in the management of the late complications of DM are suggested and RCS results in the diabetics should be interpreted with caution with respect to BMI and BP.

Red cell sorbitol indicates changes in deterioting control or current quality of diabetic control. Attainment of normoglycaemia or good glycaemic control could reduce the frequency

of abnormal polyol pathway in diabetic patients. Red cell sorbitol could serve as an index of long-term sorbitol accumulation in diabetic patients. The assay of RCS could be used as a risk factor for cardiovascular disease in DM. However, further research involving the estimation of red cell sorbitol in a population with high mortality from cardiovascular diseases to confirm this observation is recommended. It is also recommended that the estimation of RCS and lipid profiles be undertaken in diabetics with cardiovascular accidents or strokes. A multicentre study for Nigeria is needed for this investigation.

The observations in the present study show that red cell sorbitol could differentiate diabetic patients who are in poor, moderate and good controls with respect to glucose control. The findings from the serial study of RCS in the newly diagnosed diabetics following therapy demonstrate the effectiveness of red cell sorbitol in assessing glycaemic control in established diabetics.

Serial measurements of RCS and fructosamine are recommended in diabetic in-patients or pregnant diabetics to select appropriate criteria for metabolic control.

Finally, this study has shown that red cell sorbitol assay could be applied as an index of metabolic control in diabetics as an adjunct to glucose, fructosamine and lipid profiles. In addition, the assay, could be used as one of the biochemical risk factors to select patients susceptible to the development of any of the late diabetic complications. The assay could equally be used to monitor therapy with aldose reductase inhibitor drugs in patients who may benefit from this treatment in the foreseable

future.

The results in the present study could form the baseline for further research in Nigeria as there has been no report on red cell sorbitol concentration in Nigerians before now.

REFERENCES

1. Abouna GM, Al-Adanai MS, Kremer GD, Kumar SA, Daddah SK, Kusma G (1983): Reversal of diabetic nephropathy in human cadaveric kidneys after transplantation into non-diabetic recipients, Lancet 2: 1274-6.

2. Adetuyibi A (1976): Diabetes in the Nigerian African. I. Review of long term complications. Trop Geogr Med 28: 155-159.

3. Agardh CD, Arardh E, Hultberg B (1987): Lack of relationship between betahexosaminidase activity and retinopathy in insulin-dependent diabetes. Clin Chim Acta 167: 37-42.

4. Agbedana EO, Akanji AO, Ugbade C (1988): Plasma lipid profiles and Vascular disease in type 2 (non-insulin dependent) Nigerian diabetic patients. Trop Geogr Med 40: 88-92.

5. Ahren B, Corringan CB (1984): Prevalence of diabetes mellitus in North -Western Tanzania. Diabetologia 26: 333-336.

6. Akanji AO, Famuyiwa OO, Adetuyibi A (1989): Factors influencing the outcome of treatment of foot lesions in Nigerian patients with diabetes mellitus. Q J Med 73: 1005-1014.

7. Akanji AO, Agbedana EO, Ugbode C (1989): Plasma lipid profiles in relation to diabetic control in Nigerians. Afr J Med Med Sci 18: 228-234.

8. Alberti KG, Dornhirst A, Rowe AS (1975): Metabolic rhythms in old age. Biochem Soc Trans 3: 132-3.

9. Alberti KGMM (1992): The diagnosis of diabetes: Problems and prospectives. Int Diab Dig 3: 65-66.

10. A C S (1980): American chemical Society, Committee on Environmental Improvement: Guidelines for data acquisition and data quality evaluation in environmental chemistry. Ann Chem 52: 532-539.

11. Anonymous (1989): Diabetic nephropathy Lancet 1: 1113.

12. Andersen AR, Christiansen J, Anderson JK, Kreiner S, Deckert T (1983): Diabetic Nephropathy in type I (insulin-dependent) Diabetes: An Epidemiological study. Diabetologia 25: 496-501.

13. Baker JF, Johnson RM, Scott DJ (1984): Serum fructosamine concentrations in patients with type II (non-insulin-dependent diabetes mellitus during changes in management. Br Med J 288: 1484-1486.

14. Banting FG, Best CH (1922): The internal secretion of the pancreas J Lab Med 7: 251-266.

15. Barbosa J, Saner B (1984): Do genetic factors play a role in the pathogenesis of diabetic microangiopathy? Diabetologia 27: 487-92.

16. Barnett AH, Pyke DA (1986): The genetics of diabetic complications. Baillieres Clin Endocrinol Metab 15: 715-26.

17. Baxter MA (1991): The role of myoinositol in the pathogenesis of diabetic complications. TEM 2: 187-190.

18. Bella AF, Famuyiwa OO, Akintewe T et al (1985): The prevalence of diabetic retinopathy and effect of improved diabetic control in Ibadan. West Afr J Med 4: 27-29.

19. Betteridege DJ (1989): Lipids, diabetes and Vascular disease: The time to act. Diabetic Med 6: 195-218.

20. Betteridege DJ, Kathryn T (1991): Hyperlipidaemia in diabetes mellitus. Prac Diab Dig 2: 94-96.

21. Beyer-Mears A, Ku L, Cohen MP (1984): Glomerular Polyol accumulation in diabetes and its prevention with sorbinil. Diabetes 33: 89A (Suppl.)

22. Beyer-Mears M, Cruz E (1985): Reversal of diabetes Cataract by Sorbinil, an aldose reductase inhibitor. Diabetes 34: 15-21.

23. Beyer-Mears A (1986): The polyol pathway, Sorbinil and renal dysfunction. Metabolism 35 (Suppl. 1): 46-54.

24. Bilous RW, Mauer SM, Sutherland EOR, Najarian JS, Goetz FC, Steffes MW (1989): The effects of pancreas transplantation on the glomerular structure of renal allografts in patients with insulin dependent diabetes. N Engl J Med 321: 80-85.

25. Blackshear PJ, Holloway PAH, Alberti KGMM (1975): Factors regulating amino acid release from extraplancnic tissues in the rat. Interactions of alanine and glutamine. Biochemical Journal 150: 379-87.

26. Boland OM, Blackwell CC, Clarke BF, Ewing DJ (1993): Effects of Ponalrestat, an aldose reductase inhibitor, on neuthrophil killing of Escherichia Coli and autonomic function in patients with diabetes mellitus. Diabetes 42: 336-40.

27. Brenner BM, Meyer TW, Hostetter TH (1982): Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging renal ablation, and intrinsic renal disease. N Engl J Med 307: 652-9.

28. Brinchmann H, Dahl-Jorgensen K, Sandvik K, Hanssen KF (1992): Blood glucose concentrations and progression of diabetic retinopathy: the seven year results of the Oslo study. Br Med J 304: 19-22.

29. Brouchard BH, La Grone L, Travis LB et al (1984): Response of urinary N-acetyl-β-D-glucosaminidase to rapid decrease in blood glucose. Clin Chim Acta 140: 197-202.

30. Brownlee M, Vlassara M, Cerami A (1984): Non-enxymatic glycosylation and the pathogenesis of diabetic complications. Ann Intern Med 101: 527-37.

31. Buttner J, Berth R, Boutwell JW et al (1976): (IFCC Committee on Standards): Provisional recommendation on quality control in clinical chemistry. I. General Principles and terminology. Clin Chem 22: 532-539.

32. Campbell PJ, Carlson MG (1993): Impact of obesity on insulin action in NIDDM. Diabetes 42: 405-410.

33. Carr WR, Gelfand M (1961): The incidence of diabetes mellitus in the African. A survey carried out in Highfield, Salisbury, South Rhodesia. Cent Afr J Med 7: 332-335.

34. Casparie AF, Miedema I (1977): Glycosylated haemoglobin A_{1c} in comparison with other parameters as index of diabetic control. Neth J Med 21: 73-6.

35. Chan SJ, Keim P, Steiner DF (1976): Cell-free synthesis of rat pre-proinsulin: Characterization and partial amino acid sequence determination. Proc Natl Acad Sci, USA 73: 1964.

36. Chang K, Williamson JR, Rowold E et al (1985): Diabetes - Induced increases in vascular permeability are prevented by castration and by sorbinil. Diabetes 34: (Suppl. 1) 108A.

37. Chase HP. Glasgow AM (1976): Juvenile diabetes mellitus and serum lipid and lipoprotein levels. Am J Dis Child 130: 1113-17.

38. Christau B, Kromann H, Anderson OO et al (1977): Incidence, seasonal and geographical patterns of juvenile-on set (insulin-dependent) diabetes mellitus in Denmark. Diabetologia 13: 281-284.

39. Christiansen JS (1978): Cigarette smoking and prevalence of microangiopathy in juvenile-on set (insulin-dependent) diabetes mellitus. Diabetes Care 1: 146-149.

40. Christiansen JS, Gammelgaard J, Frandsen M, Parving HH (1981): Increased kidney size, glomerular filtration rate, and renal plasma flow in short-term insulin-dependent diabetics. Diabetologia 20: 451-6.

41. Chylack LT Jr, Kinoshita JH (1969): A biochemical evaluation of a cataract Ophthalmol. 8: 401-12

42. Clements Jr RS (1979): Diabetes neuropathy, new concepts of its etiology. Diabetes 28: 604-611.

43. Clements Jr RS, Stockard CR (1980): Abnormal Sciatic nerve myoinositol metabolism in the streptozotocin-diabetic rat. Effect of insulin treatment. Diabetes 29: 227-235.

44. Cohen AM, Michaelson IC, Yonko L (1971): Retinopathy in rats with disturbed carbohydrate metabolism following a high sucrose diet. I. Vascular changes. Am J Opthalmol 73: 863-9.

45. Colwell JA, Lopes, Virella M, Halushka PV (1981): Pathogenesis of atherosclerosis in diabetes mellitus. Diabetes Care 4: 121-33.

46. Consensus Conference: Lowering blood cholestereol to prevent heart disease. J Am Med Assoc 1985: 253: 2080-6.

47. Cook AR (1991): Notes on the diseases met within Uganda, Central Africa. J Trop Med 4: 175-178.

48. Corder CN, Braughler JM, Cupl P (1979): Quantitative histochemistry of the sorbitol pathway in glomeruli and small arteries of human diabetic kidney. Folip Histochem Cytochem 17: 137-46.

49. Crable MJC, Pecker CO, Halder AB, Cheng H (1980): Erythrocyte glyceraldehyde-reductase levels in diabetics with retinopathy and cataract. Lancet 2: 1268-70.

50. Dagogo-Jack S (1991): Pattern of diabetic foot ulcer in Port-Harcourt, Nigeria. Prac Diab Dig 2: 74-78.

51. Davidson JC (1963): The incidence of diabetes in Nyasaland. Cent Afr J Med 9: 92-94.

52. Davidson JC, McGlashan ND, Nightingale EA, Upadhay JM (1969): The prevalence of diabetes in the Kalene Hill area of Zambia. Med Proc 15: 426-432.

53. Dodu SRA (1958): The incidence of diabetes mellitus in Accra (Ghana). A study of 4000 patients. W Afr Med J 7: 129-134.

54. Dodu SRA, de Heer N (1964): A diabetes case finding survey in Ho, Ghana. Ghana Med J 3: 75-80.

55. Dominiczak M (1991): Laboratory methods for the assessment of past glycaemic control in diabetics. Prac Diab Dig. 2: 46-50.

56. Dornan TL, Carter RD, Bron AJ, Turjer RC, Mann JI (1982): Low density lipoprotein Cholesterol: An association with the severity of diabetic retinopathy. Diabetologia 22: 167-170.

57. Doumas BT, Watson WA, Briggs HG (1971): Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta 31: 87-96.

58. Duncan LJP, Cullen JF, Ireland JT, Nolan J, Clarke BF, Olive MF (1968): A three-year trial of Atromid therapy in exudative diabetic retinopathy. Diabetes 17: 458-457.

59. Dunn PJ, Cole RA, Soeldner JS et al (1979): Temporal relationship of glycosylated haemoglobin concentrations to glucose control in diabetes. Diabetologia 17: 213-20.

60. Dvornik D, Simard-Duquesne N, Kraml M et al. (1973): Polyol accumulation in galactosaemic and diabetic rats: Control by aldose reductase inhibitor. Science 182: 1146-1148.

61. Dyck PJ, Zimmerman BP, Vilen TH et al (1988): Nerve glucose, fructose, sorbitol, myoinositol, and fibre degeneration and regeneration in diabetic neuropathy. N Engl J Med 319: 452-48.

62. Eggers PW (1988): Effect of transplantation on the medicare end-stage renal disease program. N Engl J Med 318: 223-9.

63. Elford J, Philips AN, Thomson AG, Shaper AG (1989): Migration and geographic variations in ischaemic heart disease in Great Britain. Lancet i: 343-6.

64. Ellenberg M (1958): Diabetic neuropathy presenting as the initial clinical manifestation of diabetes mellitus. Ann Intern Med 49: 620-31.

65. El-Hazmi MAF, Al-Momen AK, Warsy AS (1991): Glycosylated haemoglobin in normal individuals and patients with red cell genetic diseases. Prac Diab Dig 2: 73-75.

66. Eli Lilly and Company (1992): Personal Communications.

67. Ellis EN, Brouhard BH. LaGrone L et al (1983): Urinary excretion of N-acetyl-Beta-D-glucosaminidase in children with type I diabetes mellitus. Diabetes Care 6: 251-255.

68. Ellis EN, Stettes MW, Goetz FG, Sutherland D-E R, Mauer SM (1986): Glomerular filtration surface in type I diabetes mellitus. Kidney Int 29: 889-94.

69. Engerman RL, Kern TS (1989): Determinants of experimental diabetic retinopathy. In Proc Congr Int Diabetes Fed 13th Larkins R G Ed, Amsterdam, Elsevier, p 221-24.

70. Erasmus RT, Fakeye T, Olukoya O et al (1989): Prevelence of diabetes mellitus in a Nigerian Population. Trans R Soc Trop Med Hyg 83: 417:418.

71. Esmann V, Lundback K, Madsen PN (1963): Types of exudates in diabetic retinopathy. Acta Med Scand 174: 375-384.

72. Fagius J, Wallin BG, Sundlef G (1985): Limited benefit of treatment of diabetic polyneuropathy with an aldose reductase inhibitor a 24-week controlled trial. Diabetologia 28: 323-329.

73. Falko JM, Parr JH, Simpson RN, Wynn V (1987): Lipoprotein analyses in varying degrees of glucose tolerance and control populations Am J Med 83: 461-47.

74. Fajans SS, Floyd JC, Knopf RF et al (1972): Current unresolved problems in diabetes managenent. Diabetes 21: Suppl. 2: 678-84.

75. Feldt-Rasmussen B, Mathiesen ER, Hededus L, Deckert T (1986): Kidney function during 12 months of strict metabolic control in insulin-dependent diabetic patients with incipient nephropathy N Engl J Med 314: 665-70.

76. Felig P (1973): Diabetic Ketoacidosis. Metabolism 22: 179.

77. Finegold D, Lattimer SA, Nolle S, Bernstein M, Greene DA (1983): Polyol pathway activity and myoinositol metabolism. A suggested relationship in the pathogenesis of diabetic neuropathy. Diabetes 32: 988-92.

78. Fish A, Pichard E, Prazuck T, Lablanc H, Sidibe Y, Brucker G (1987): Prevalence and risk factors of diabetes mellitus in the rural region of Mali (West Africa): A practical approach. Diabetologia 30: 859-862.

79. Fontbonne A, Eschwege E, Cambien F et al (1989): Hypertriglyceridaemia as a risk factor of coronary heart disease mortality in subjects with impared glucose tolerance or diabetes. Result from the 11 year follow-up of the Paris prospective study. Diabetologia 32: 300-4.

80. Friedewald WT, Levy RI, Fredrickson DS (1972): Estimation of the concentration of low density liporotein cholesterol in plasma without the use of preparative ultracentrifuge. Clin Chem 18: 499.

81. Gabbay KH (1973[a]): Role of Sorbitol pathway in neuropathy. Adv Metab Disord Suppl 2: 417-24.

82. Gabbay KH (1973[b]): The polyol pathway and the complications of diabetes. N Engl J Med 288: 831-36.

83. Gabbay KH, Hasty K, Breslow JI et al (1977): Glycosylated haemoglobins and long-term blood glucose control in diabetes mellitus. J Clin Endocrinol Metab 44: 859-864.

84. Gabbay KH, Spack N, Loo S, Hirsch JJ, Ackil AA (1979): Aldose reductase inhibition; studies with alrestatin. Metabolism 28: 471-76 (Suppl.)

85. Garlick RJ, Mazer JS, Higgins PJ, Bunn HF (1983): Characterization of glycosylated haemoglobins. Relevance of monitoring of diabetic control and analysis of other proteins. J Clin Invest 1062-1072.

86. Gill GV, Huddle KR (1984): Patterns and problems of diabetes in Soweto. Cent Afr J Med 30: 189-195.

87. Goldberg MD, Marine N, Ribeiro F et al (1969): Prevalence of Glycosuria and diabetes among Indians and Bantus. S Afr Med J 43: 733-738.

88. Gingliano D (1993): Tolrestat for mild diabetic neuropathy. Ann Intern Med 118: 7-8.

89. Gowans EMS, Fraser CG, Hytoft Petersen P (1989): A guide to the use of probit transformation of Gaussian distributions. Biochemica Clinica 13: 327-336.

90. Gracia MJ, McNamara PM, Gordeon T et al (1974): Morbidity and mortality in diabetics in the Frammigham population - sixteen year follow-up study. Diabetes 23: 106-11.

91. Greene DA, Lattimer SA, Sima AAF (1983): Impaired rat sciatic nerve Na-k adenosine triphosphate in acute streptozotocin diabetes and its correction by dietary myo-inositol supplementation. J Clin Invest 72: 1058-63.

92. Greene DA, Lattimer SA, Sima AAF (1987): Sorbitol, phosohoinositides, and Na-k-ATPase in the pathogenesis of Diabetic complications. N Engl J Med 316: 599-606.

93, Greenwood BM, Taylor JR (1968): The complications of diabetes in Nigerians. Trop Geogr Med 20: 1-8.

94. Griffey RH, Eaton P, Sibbitt RR, Sibbitt WC, Dicknell JM (1988): Diabetic neuropathy: Structural analysis of nerve hydration by magnetic resonance spectroscopy. J Am Med Assoc 260: 2872-78.

95. Guidotti L, Gelifand M (1976): Frequency of diabetes mellitus in Mtoko. Cent Afr J Med 22: 28-29.

96. Haffner SM, Morales MA, Stern MP, Gruber MK (1992): Lp(a) concentration in NIDDM. Diabetes 41: 1267-1272.

97. Hale TJ, Nattrass M, Silvermann SH et al (1987): Peripheral nerve concentrations of glucose, fructose, sorbitol and myoinositol in diabetic and non-diabetic patients. Diabetologia 30: 464-67.

98. Hammer MR, John PN, Flymn MD, Bellingham AJ, Leslie EDG (1989): Glycated fibrinogen: A new index of short-term diabetic control. Ann Clin Biochem 26: 58-62.

99. Handelsman DJ, Tuttle JR (1981): Clinical trial of an aldose reductase inhibitor in diabetic neuropathy. Diabetes 30: 459-64.

100. Hanna Ak (1982): Identified significant association between proliferative diabetic retinopathy and poorer diabetic control, increased diastolic BP, impairement of renal function, higher triglyceride levels and impaired motor and sensory nerve conduction. In diabetic complications: Report of the tenth Canadian workshop on Diabetes by Sirek OV. Diabetologia 23: 76-77.

101. Harrison EE, Stribling D, Armstrong PM, Parkins AM (1988): Aldose reductase in the etiology of Diabetes complications: 1. Introduction. J Diabetic Complications 2: 6-11.

102. Hawthorne JN, Pickard MR (1979): Phospholipids in Synaptic function. J Neurochem 32: 2-14.

103. Henry RJ, Szustkiewicz CP (1974): The preparation of protein-free filtrates in clinical chemistry: in principles and technics 2nd Ed. Harper and Row, Maryland pp 389-396.

104. Hers HG (1956): Le mecanisme de la transformation de glucose en fructose lar les vesicules seminales. Biochim Biophys Acta 22: 202-203.

105. Hostetter TH (1985): Diabetic Nephropathy. N Engl J Med 312: 642-644.

106. Howard BV (1987): Lipoprotein Metabolism in diabetes Mellitus. J Lipid Res 28: 613-28.

107. Iannaccone A, Kornerup P (1954): Plasma lipids and diabetic retinopathy. Acta Med Scand 148: 411-416.

108. Imperato PJ, Handelsman MB, Fofana B, Sow O (1976): The prevalence of diabetes mellitus in three population groups in the Republic of Mali. Trans R Soc Trop Med Hyg 70: 155-158.

109. Imre G (1964): Studies on the mechanism of retinal neovascularisation. Role of lactic acid. Br J Ophthalmol 48: 75-82.

110. Irvine AR, Norton EWD (1971): Photocoagulation for diabetic retinopathy. Am J Ophthalmol 71: 437-445.

111. Isah HS (1990): Performance Characteristics of serum fructosamine assay with a semi-manual procedure. Biochimica Clinica 14: 1565-1572.

112. Isah HS, Ogunkeye OO, Abdu-Aguye I (1991): Serum fructsamine in the assessment of glycaemic status in diabetic Nigerians. Biochemica Clinica 14: 1555-1560.

113. Isah HS, Abubakar HB (1991): Monitoring Metabolic Control in Diabetes: experience with the fructosamine test in Zaria, Nigeria. Prac Diab Dig 2: 1555-157.

114. Jamil Z, Gazzaz A, Home PD, Alberti KGMM (1992): The reliability of the oral glucose tolerance test in subjects with varying degree of glucose intolerance in Saudi Arabia Int Diab Dig 3: 71-75.

115. Jaspan J, Masselli R, Herold K, Bartkus C (1983): Treatment of severely painful diabetic neuropathy with an aldose reductase inhibitor: Relief of pain and improved somatic and autonomic nerve functions. Lancet ii: 258-62.

116. Jacobsen J (1978): Peripheral nerves in early experimental diabetes: Expansion of endoneural space as a cause of increased water content. Diabetologia 14: 113-119.

117, Johnston DG, Alberti KGMM (1976): Carbohydrate metabolism in Liver Diseases. Clinics in Endocrinol and Metab 5: 675.

118, Johnson TO (1971): Comparative study of screening methods for diabetes mellitus in the elderly Nigerian Subject. W Afr Med J 20: 243-246.

119. Johnson RN, Metcalf PA, Baker JR (1982): Fructosamine: A new approach to the estimation of serum glycosylprotein. An index of diabetic control. Clin Chim Acta 127: 87-95.

120. Johnson RN, Metcalf PA, Baker JR (1987): Relationship between albumin and fructosamine in diabetic and non-diabetic sera. Clin Chim Acta 164: 151-162.

121. Judzewitsch RG, Pfeifer MA, Best JD, Beard JC, Balter B, Porte D Jr. (1981): Chronic Chlorpropamide therapy of non-insulin dependent diabetes augments basal and stimulated insulin secretion by increasing islet sensitivity to glucose. J Clin Endocrinol Metab 55: 321-8.

122. Kador PE, Kinoshita JH (1984): Diabetic galactosaemic cataracts. Ciba Found Symp Human Cataract Formation 106: 110-23.

123. Kador PF, Robison WGJ, Kinoshita JD (1985): The pharmacology of aldose reductase inhibitors. Ann Rev Pharmacol 25: 691-714.

124. Keiding NR, Namm GV, Root HF, Lawry EY, Marble A (1952): Serum lipoproteins and cholesterol levels in normal subjects and in young patients with diabetes in relation to vascular complications. Diabetes 1: 434-440.

125. Kennedy L, Baynes JW (1984): Non-enzymatic glycosylation and the chronic complications of diabetes: an overview. Diabetologia 26: 92-98.

126. Kessler II (1971): Mortality experience of diabetic patients. A twenty-six year follow-up study. Am J Med 51: 715-24.

127. Kikkawa R, Hatawaka I, Yasuda H et al (1983): Effect of a new aldose reductase inhibitor. (E)-3-Carboxymethyl-5 (2E)-methyl-3-phenyl-properylidine Rhodamine (ONO-2235) on peripheral nerve disorders in streptozotocin-diabetic rats. Diabetologia 24: 290-292.

128. Kikkawa R, Hatanaka I, Yasuda H, Kobayashi N, Shigeta Y (1984): Prevention of peripheral nerve dysfunction by an aldose reductase inhibitor in streptozotocin diabetic rats. Metabolism 33: 212-214.

129. Kinnear TWG (1963): The pattern of diabetes mellitus in a Nigerian Teaching Hospital. East Afr Med J 40: 288-294.

130. Kinoshita JH, Hayman S, Merola LO (1965): Osmotic effects on the amino acid concentrating mechanism in the rabit lens. J Biol Chem 240: 310-15.

131. Kinoshita JH, Pukushi S, Kador P, Merla LO (1979): Aldose reductase in diabetic complications of the eye. Metabolism 28: 462-69.

132. Kinoshita JH (1974): Mechanisms initiating cataract formation. Invest Ophthalmol Vis Sci 13: 713-724.

133. Knowler WC, Bennett PH, Ballintine EJ (1980): Increased incidence of retinopathy in diabetics with elevated blood pressure. N Engl J Med 302: 645-650.

134. Knowler WV, Bennison LJ, Drobny E et al (1978): Diabetes incidence and prevalence in Pima Indians: A 19-fold greater incidence than in Rochester, Minnessota. Am J Epidemiol 198: 497-505.

135. Koening RJ, Peterson CM, Jones RL et al (1976): Correlation of glucose regulation and HBA_{1c} in diabetes mellitus. N Engl J Med 295: 417-20.

136. Kohner EM, Bary PJ (1984): Prevention of blindness in diabetic retinopathy. Diabetologia 26: 173-179.

137. Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR (1985): The changing natural history of nephropathy on type I diabetes. Am J Med 78: 785-94.

138. Laakso M, Pyrala K (1988): Lipid and lipoprotein abnormalities in diabetic patients with peripheral vascular disease. Atherosclerosis 74: 55-64.

139. Lapolla A, Poli T, Valeria A, Fedele D (1985): Is red cell sorbitol content a good marker of glycaemic control in diabetic patients? Diabetes Res 216: 283-286.

140. LeFvre PG, Davis RI (1951): Active transport into the human erythrocyte: Evidence from comparative Kinetics and competition among monosaccharides. J Gen Physiol 34: 515-524.

141. Lehtinem JM, Uusitupa M, Siitonen O, Pyorala K (1989): Prevalence of neuropathy in newly diagnosed NIDDM and non-diabetic control subjects. Diabetes 38: 1370-13.

142. Lester FT (1983): Long standing diabetes Mellitus in Ethiopia: A survey of 105 patients. Diabetologia 25: 222-225.

143. Levine JB, Zak B (1964): Automated determination of serum total cholesterol. Clin Chim Acta 10: 381-4.

144. Levy AL (1972): Triglycerides by nonane extraction and colorimetry, manual and automated. Ann Clin Lab Sci 6: 474.

145. Levin IG, O'Brian IAD, Morgan MH, Corral RJM (1984): Clinical and Neurophysiological studies with the aldose reductase inhibitor, in symptomatic diabetic neuropathy. Diabetologia 26: 445-48.

146. Lim YS, Stalely MJ (1985): Measurement of plasma fructosamine evaluated for monitoring diabetes. Clin Chem 31: 731-733.

147. LudVigson MA, Sovenson RL (1980): Immunohistochemical distribution of aldose reductase II. Rat eye and Kidney. Diabetes 29: 450-459.

148. Lutjens A, te velde AA, Veen EA vd, Meer J vd (1985): Glycosylation of human fibrinogen in vivo. Diabetologia 28: 87-9.

149. MacFarlane IA (1991): Mathew Dobson of Liverpool (1735-1784) and the history of diabetes. Prac Diab Dig 2: 54-58.

150. Machemer R, Blankenship GW (1981): Vitrectomy for Proliferative diabetic retinopathy associated with vitreous haemorrhage. Ophthalmology 88: 643-648.

151. Malone JI, Knox G, Benford S, Tedesco TA (1980): Red cell sorbitol: An indicator of diabetic control. Diabetes Care 29: 861-864.

152. Malone JI, Knox G, Harvey C (1984): Sorbitol accumulation is alrered in type I (insulin-dependent) diabetes mellitus. Diabetologia 27: 509-13.

153. Marble A (1971): In Joslin's Diabetes Mellitus (Ed.) Lea and Febiger, Philadelphia P 1.

154. Mayer JH, Tomlinson DR (1983): Prevention of defects of axonal transport and nerve conduction velocity by oral administration of myoinositol or an aldose reductase inhibitor in streptozotocin-diabetic rats. Diabetologia 25: 433-38.

155. Mayhew JA, Gillon KRW, Hawthorne JN (1983): Free and lipid inositol, sorbitol and sugars and sciatic nerve obtained post-mortem from dabetic patients and control subjects. Diabetologia 24: 13-15.

156. Mazze RS, Shamoon H, Pasmantier E et al (1984): Reliability of blood glucose monitoring by patients with diabetes mellitus. Am J Med 77: 211-217.

157. McCaleb ML, Sredy J, Millen J, Ackerman DM, Dvornik D (1988): Prevention of urinary albumin excretion in 6 months steptozotocin diabetic rats with the aldose reductase inhibitor, tolrestat. J Diabetic Complications 2: 16-18.

158. McLarty DG, Swai AMB, Kitange HM et al (1889): Prevalence of diabetes and impaired glucose tolerance in rural Tanzania. Lancet i: 871-875.

159. Medalie JH, Goldbourt U, Neufeld HN, Oron D (1978): Variations in incidence of diabetes among 10,000 adult Israel males and the factors related to their development. Adv Metab Disord 9: 93-110.

160. Mhnando PA, Yudkin JS (1980): The pattern of diabetic complications in African patients in Dares Salaam. Trop Geogr Med 32: 317-323.

161. Miedema K, Casparie T (1984): Glycosylated haemoglobins: Biochemical evaluation and clinical utility. Ann Clin Biochem 21: 2-15.

162. Miller GJ, Miller NE (1975): Plasma high density lipoprotein concentrations and development of ischaemic heart disease. Lancet 1: 16-19.

163. Modan EJ (1985): Hyperinsulinaemia, a link between hypertension, obesity and glucose intolerance. J Clin Invest 75: 809-17.

164. Mogensen CE, Anderson MJF (1973): Increased kidney size and glomerular filteration rate in early juvenile diabetes. Diabetes 22: 706-12.

165. Mogensen CE (1976): Renal function changes in diabetes. Diabetes 25: 872-9.

166. Mogensen CE, Christiansen CK (1984): Predicting diabetic nephropathy in insulin dependent patients. N Engl J Med 311: 89-93.

167. Monnier VM, Cerami A (1983): Non enzymatic glycosylation and browning of proteins in vivo. In: Walter GR, Feather MS eds. The maillard reaction in foods and nutrition - Symposium series No. 215. Washington DC. American Chemical Society 431-9.

168. Morrison AD, Clements RS Jr., Winegrad AI (1972): Effects of elevated glucose concentration on the metabolism of the aortic wall. J Clin Invest 51: 3114-23.

169. Morrison AD, Tampa FL (1985): Linkage of Polyol pathway activity and myoinositol in aortic smooth muscle. Diabetes 34 (Suppl.): 12A.

170. Mulhauser I (1990): Smoking and Diabetes. Diabetic Med 7: 10-15.

171. National Commission on Diabetes (1975): Report to the Congress of the US, Vol III Part I, US DHEN. Publication No. (NIH) 76-1021.

172. New MI, Roberts TN, Bierman EL et al (1963): The significance of blood lipid alterations in diabetes mellitus. Diabetes 12: 208-12.

173. Newsholme EA (1976): Carbohydrate metabolism in vivo: regulation of the blood glucose level. Clinics in Endocrinol and Metab 5: 543.

174. Nikkila EJ (1981): HDLc in diabetes. Diabetes 30 (Suppl. 2): 82-87.

175. Ohwovoriole AE, Kuti JA, Kabiawu SIO (1988): Casual blood glucose levels and prevalence of undiscovered diabetes mellitus in Lagos metropolis, Nigeria. Diabetes Res Clin Pract 4: 153-158.

176. Ohwovoriole AE, Awojobi AO, Okotore RO, Johnson TO (1991): A comparative study of the glycosylated plasma proteins in diabetic Nigerians. West Afr J Med 10: 343-46.

177. O'Malley BW, Birnbaumer L (1977): Receptors and hormone Action, 3 vols. New York: Academic Press.

178. Onaya T, Shindo H, Tawata M, Aida K (1990): Clinical significance of erythrocyte sorbitol-blood glucose ratios in type II diabetes mellitus. Diabetes Care 13: 461-467.

179. Onyemelukwe GC, Isah HS, Mba EC, Ekanem E (1982): Glycosylated haemoglobin in tropical antenatal care. East Afr Med J 59: 469-472.

180. Onyemelukwe GC, Isah HS, Mba EC, Awunor-Renner C, Mohammed I (1983): Glycosylated haemoglobin (HbA_1) for diabetic control in Africans: Preliminary findings with the microcolumn technique. Trop Geogr Med 35: 346-351.

181. Osuntokun BO, Akinkugbe FM, Francis TI, Reddy S, Osuntokun O, Taylor GOL (1971): Diabetes Mellitus in Nigerians: A study of 832 Patients. W Afr Med J 20: 295-312.

182. Owusu SK (1992): Diabetes in the Yemen: A Prelimimary Study. Int Diab Dig 3: 101-107.

183. Palmiter R (1983): Hormone Action. In Zubay GL(Ed) Biochemistry. Addison-Wesley Publishing Company p.1116.

184. Paterson JR, Pettigrew AR, Dominiczak MH, Small M (1991): Screening for hyperlipidaemia in diabetes mellitus. Relationship to glycaemic control. Ann Clin Biochem 28: 354-58.

185. Papoz L, Ben Khalifa F, Eschwege E, Ban Ayed H (1988): Diabetes mellitus in Tunisia; Descriptions in urban and rural populations. Int J Epidemiol 17: 419-422.

186. Paulsen EP (1973): Glycosylated haemoglobin (HbA_{1c}) In childhood diabetes. Metabolism 22: 269-71.

187. Peters WH (1983): A study on the prevalence of diabetes mellitus in Northern Ethiopia (Gondar Survey). De Gesundh-Wesen. 38: 1283-89.

188. Peterson MJ, Sarges R, Aldinger CG, Mac Donald DP (1979): CP-45634: A novel aldose reductase inhibitor that inhibits polyol formation pathway activity in diabetic and galactosaemic rats. Metabolism 28: 456-61.

189. Petersen J, Ross J, Rabkin R (1988): Effect of insulin therapy on established diabetic nephropathy in rats. Diabetes 37: 1346-50.

190. Peterson CM (1982): Symposium on non enzymatic glycosylation and browning reactions. Diabetes 31: (Suppl.): 11-82.

191. Pivart J (1977): Diabetes mellitus and its degerative complications: a prospective study of 4400 patients observed between 1947-1977. Diabete Metab 3: 97-107.

192. Politzer WM, Hardegger B, Schneider T (1960): Incidence of diabetes mellitus in the Bulha-Huthe district of Basutoland. Br Med J i: 615-617.

193. Pomerange J, Fujy H, Mouratoff GI (1956): Clinical report on a new hypoglycaemic agent (23164). Proc Sci Exptl Biol Metab 95: 193.

194. Poon PYW, Dornan TI, Ellis RB, Truber RC (1979): Increased plasma actvities of N-Acetyl-B-D-glycosaminidase isoenzymes in human diabetes mellitus. Clin Endocrinol 97: 191-195.

195. Popp-Snijders C, Lomecky-Janousek MZ, Schouten JA, Vonderveen EA, (1984): Myo-Inositol and sorbitol in erythrocytes from diabetic patients before and after sorbinil treatment. Diabetologia 27: 514-516.

196. Poulson R, Mirrlees DJ, Earl DCN, Health H (1983): The effects of an aldose reductase inhibitor upon the sorbitol pathway, fructose-1-phosphate and lactate in the retina and nerve of streptozotocin-diabetic rats. Exp Eye Res 36: 751-760.

197. Price CP, Foster KJ (1979): Serum and urine glycosidase activities in diabetes mellitus. Clin Biochem 12: 231-233.

198. Price DE, Grant PF, Airey CM, Stickland MH, Kemp J, Wales JK (1987): Red cell sorbitol levels during hyper and hypoglycaemic clamp studies in insulin-dependent diabetic patients. Diabetic Med 4: 229-232.

199. Pyrala K, Lawsko M, Uusitryia M (1987): Diabetes and atherosclerosis: an epidemiologic view. Diabetes/metabolism Reviews 3: 436-524.

200. Rahbar S (1968): An abnormal haemoglobin in red cells of diabetics. Clin Chim Acta 22: 296-8.

201. Rana BS, Botha JL (1990): Should diabetics be advised not to smoke? A review of the epidemiological evidence. Practical Diabetes 7: 61-66.

202. Rand LJ, Prud'homme GJ, Ederer F, Canner PL (1985): The diabetic retinopathy study research group. Factors influencing the development of visual loss in advanced diabetic retinopathy. Invest Ophthalmol Vis Sci 26: 893-897.

203. Randle PJ, Garland PB, Hales CN, Newsholme EA (1963): The glucose fatty-acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet i: 785.

204. Raskin P (1982): Treatment of insulin-dependent diabetes mellitus with portable insulin infusion devices. Med Clin North Am 66: 1269-83.

205. Reichard P, Britz P, Carlsson I et al (1990): Metabolic control and complications over 3 years in patients with insulin dependent diabetes (IDDM): The stockholm Diabetes Intervention study (SDIS) J Intern Med 228: 511-517.

206. Robertson WB, Strong JP (1968): Atherosclerosis in person with hypertension and diabetes mellitus. Lab Invest 18: 538-51.

207. Robey C, Dasmahapatra A, Coben MP, Suarez S (1985): Sorbinil prevents decreased erythrocyte (RBC) deformability in diabetes mellitus. Diabetes 34 (Suppl.): 41A

208. Robinson WG, Kador PF, Kinoshita JH (1983): Retinal Capillaries basement membrane thickening by galactosaemia prevented with aldose reductase inhibitor. Science 277: 1177-79.

209. Rolfe M, Mendelsohn D, Armstrong JRM (1993): Lipid levels in diabetic patients from Central Africa. Int Diab Dig 4: 154-157.

210. Root HF, Bland CF, Gordon WH et al (1939): Coronary atherosclerosis in diabetes mellitus. J Am Med Assoc 113: 27-30.

211. Ross IS, Gibson PF (1979): A semi-automated method for the determination of glycosylated haemoglobin. Clin Chim Acta 98: 53-9.

212. Sagild U, Littaner J, Jesparson CS et al (1966): Epidemiological studies in Greenland. 1962-1964. I. diabetes mellitus in Eskimos, Acta Med Scand 179: 29-39.

213. Sato S, Kador PF, Kinoshita JH (1988): Rat kidney aldehyde reductase: Purification and comparison with rat lens aldose reductase. Sakamoto N, Kinoshita JH, Kador PF, Hot N (eds): Polyol pathway and its role in diabetic complications, Amsterdam. Excerpta Medical Congress series 76: 72-81.

214. Schrade W, Boechle E, Biegler R, Harmuth E (1963): Fatty acid composition of lipid fractions in diabetic serum. Lancet 1: 285-290.

215. Schwieger J, Fine LG (1990): Renal hypertrophy, growth factors and nephropathy in diabetes mellitus. Semin Nephrol 10: 242-53.

216. Seaquist ER, Goetz FC, Rich S, Barbosa J (1989): Familial Clustering of diabetic kidney disease: Evidence for genetic susceptibility to diabetic nephropathy. N Engl J Med 320: 1161-5.

217. Searle GL, Sharmes D, Cavalieri RR et al (1974): Kinetics of lactate turnover and oxidation in man. In dynamic studies with radiosotopes in medicine Vol. 1: IAEA SM 185/47.

218. Seftel HC, Abrams GJ (1960): Diabetes in the Bantu. Br Med J i: 1207-08.

219. Sherman WR, Dyck PJ (1983): Free inositol, sorbitol and fructose levels in sciatic nerve (letter). Diabetologia 25: 452-3.

220. Shimojo N, Kitahashi S, Keiichi N et al (1987): Comparison of N- Acetyl-β-D-glucosaminidase and Alanine aminopeptidase Activities for evaluation of microangiopathy in Diabetes Mellitus. Metablism 36: 277-280.

221. Shrka J, Pernsicora J, Stolba P, Stirbor V, Par J (1982): Comparison of N- acetyl-β-D-glucosaminidase and albuminuria with clinical findings of microangiopathy in type I diabetes mellitus. Clin Chim Acta 166: 135-141.

222. Sima AAF, Bril V, Nathaniel V et al (1985): Regeneration and repair of myelinated fibres in sural-nerve biopsy specimens from patients with diabetic neuropathy treated with sorbinil. N Engl J Med 319: 548-55.

223. Skylar JS (1980): Symposium on home blood glucose monitoring. Diabetes Care 3: 57.

224. Small M, Collier A (1991): The role of the polyol pathway in diabetes mellitus. Br J Hosp Med 45: 38-40.

225. Stackhouse S, Miller PL, Park KW, Meyer TW (1990): Reversal of glomerular hyperfiltration and renal hypertrophy by blood glucose normalization in diabetic rats. Diabetes 39: 989-95.

226. Steiner DF, Oyer PE (1967): The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. Proc Natl Acad Sci,USA 57: 473.

227. Stevens CN, Head JA, Fuller JH (1990): Hypertriglyceridaemia and mortality in diabetic subjects: The WHO Multinational Study. Diabetologia 33: A 57.

228. Stewart AM, Sherman WR, Kuren MM, Moonsamm GI, Wisqehot M (1967): Polyol accumulation in nervous tissue of rats with experimental diabetes and galactosaemia. J Neuro Chem 14: 1057-1066.

229. Study Group of the European Atherosclerosis Society. Strategies for the prevention of Coronary heart disease, a policy statement of the European Atherosclerosis Society. Eur Heart J 1987; 8: 77-88.

230. Taha TH, Mousse MA, Rashid AR, Fenesh FF (1983): Diabetes Mellitus in Kuwait. Incidence in the first 29 years of life. Diabetologia 15: 306-308.

231. Tawata M, Ohtaka M, Hosaka Y, Onaya T (1992): Aldose reductase mRNA expression and its activity are induced by glucose in fetal rat aortic smooth muscle (A10) cells. Life Sci 51: 719-726.

232. Teuscher T, Rosman JB, Baillod P, Tuesher A (1987): Absence of diabetes in a rural West African Population with a high carbohyrate/cassava diet. Lancet 1: 765-768.

233. Tilton RG, Change K, Weigel C, Eades D et al (1988): Increased ocular blood flow and 125 I-albumin permeation in galactose fed rats, inhibition by sorbinil. Invest Ophthalmol 106: 1099-102.

234. Tolins JP, Raji L (1989): Concerns about diabetic nephropathy in the treatment of diabetic hypertensive patients. Am J Med (Supl 6A): 29-33.

235. Travix SF, Morrison AD, Clements RS Jr et al (1971): Metabolic alterations in the human erythrocyte produced by increases in glucose concentration. The role of the polyol pathway. J Clin Invest 50: 2104-12.

236. Trinder P (1969): Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 6: 24-7.

237. Trivelli AL, Ranney HM, Lai HT (1971): Haemoglobin Components in patients with diabetes mellitus. N Engl J Med 284: 353-7.

238. Trovell HC, Burkitt DP eds. (1981): Western Diseases: Their emergence and prevention. Cambridge, Massachusetts: Harvard Univ Press 22-26.

239. Tschobroutsky G (1978): Relation of diabetic control to development of microvascular complications. Diabetologia 15: 143-152.

240. Tschobroutsky G, Charitamki D, Bluquit Y et al (1980): Diabetic control in 102 insulin-treated out-patients. Diabetologia 18: 447-52.

241. Tullock JA (1964): The incidence of glucosuria and diabetes mellitus among hospital out-patients in Uganda. East Afr Med J 41: 572-580.

242. Tuttle KR, Bruton JL, Perusek MC, Lancaster JL, Kopp DT, Defronzo RA (1991): Effect of strict glycaemic control on renal haemodynamic responses to amino acids and renal enlargement in insulin-dependent diabetes mellitus. N Engl J Med 324: 1626-32.

243. Unger RH, Orci L (1976): Physiology and pathophysiology of glucagon. Physiological Rev 56: 778.

258. Wiseman MJ, Saunders AJ, Keen H, Viberti G (1985): Effect of blood glucose control on increased glomerular filtration rate and kidney size in insulin dependent diabetes. N Engl J Med

259. Wolffenbuttel BHR, Sels J-PJE, Jungbauer FHW, Memheere PPCA, Nieuwnhuijzen Krauseman AC (1991): The value of fasting blood glucose and serum fructosamine in the evaluation of metabolic control in NIDDM patients. Diab Nutr Metab 4: 297-303.

260. World Health Organisation, WHO (1985): Report of a WHO Study group. Diabetes Mellitus Tech Rep Ser 727, Geneva.

261. Yalow RS, Berson SA (1960): Plasma insulin concentrations in non diabetic and early diabetic subjects. Determination by a new sensitive immuno assay technique. Diabetes 9: 254-60.

262. Yue DK, Hanwell MA, Satchell PM, Turtle JR (1982): The effect of aldose reductase inhibition on motor nerve conduction velocity in diabetic rats. Diabetes 31: 789-94.

263. Zatz R, Meyer TW, Rennke HG, Brenner BM (1985): Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. Proc Natl Acad Sci, USA 82: 5963-7.

264. Zatz R, Meyer TW, Anderson S, Rennke HG, Brenner BM (1986): Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. J Clin Invest 77: 1925-30.

265. Zimmet P, Taft P, Guinea A, Guthrie W, Thoma K (1977): The High prevalence of diabetes mellitus in central pacific Island. Diabetologia 13: 111-115.

266. Zmirou D (1979): epidemiologie due diabete en cote d'Ivoire (These). Grenoble Universite de Grenoble.

APPENDIX I

ANALYTICAL EVALUATION OF RED CELL SORBITOL ASSAY IN DIABETIC NIGERIANS

DEPARTMENT OF CHEMICAL PATHOLOGY, AHMADU BELLO UNIVERSITY TEACHING HOSPITAL, ZARIA

GENERAL DATA BANK

1. Name:.....
2. (a) Lab. No. ..... (b) Hosp. No.....
3. Age (Years).....
4. Sex: ..... 5. Weight (Kg.).....
6. Height (M) ..... 7. Blood Pressure (BP) .....
8. Fasting:.....
9. Post Prandial:.....
10. Tribe: .....
11. Occupation: .....
12. Level of Education:
    (a) Tertiary: .....
    (b) Secondary: .....
    (c) Primary: .....
    (d) No Formal Education: .....
13. Any Medication:Yes (Specify) ..... No.: .....
14. Pregnant: Yes ..... (Trimester) ..... No. .....
15. Oral Contraceptive Pills Yes: ..... No:.....
16. Smoking habbit:
    (a) Less than 5 sticks/day .....
    (b) 5-10 sticks/day .....
    (c) 11-20 sticks/day .....
    (d) Duration: .....
    (e) Not applicable (NA): .....

17. Drinking habbit:

(a) Less than 5 bottles/day (beer):.....

(b) 5-10 bottles/day: .....

(c) 11-20 bottles/day: .....

(d) Others (e.g. burukutu): .....

(e) Duration: .....

(f) Not applicable: .....

18. New Diabetic: ..... Date: .....

19. Known Diabetic: .....

(a) Type: .....

(b) Duration: .....

(c) Ambulatory: .....

(d) Hospitalised: .....

(e) Drugs: .....

(f) Any other disease(s) present: .....

(g) Family history of diabetes: .....

(h) Complication(s)

i) DKA: .....

ii) Infection: .....

iii) Cataract: .....

iv) Retinopathy: .....

v) Nephropathy: .....

vi) Neuropathy: .....

vii) Vascular disease: .....

viii) History of Myocardial Infarction: .....

ix) Hospitalised for ulcers or gangrene of the lower extremities or for surgical procedures Yes: ..... No: .....

x) Impotence (sexual dysfunction) Yes (Duration) .....

No: .....

20. Any other disease may be associated with DM

(a) Duration ..... (b) Treatment .....

21. 2 Hour Blood Glucose Screen (2HBGS)

i) Fasting: ..... ii) 2 hour: .....

22. Glycaemic status and diabetic control assessments:

RBS ..... (mmol/l)

FBS ..... (mmol/l)

Fructosamine ..... (mmol/l)

Red cell Haemoglobin ..... (g/dl)

Red cell sorbitol ..... (nmol/gHb)

TC ..... (mmol/l)

TG ..... (mmol/l)

LDLC ..... (mmol/l)

HDLC ..... (mmol/l)

Urine albumin .....

Urine glucose: .....

23. Other bichemical parameters (Plasma)

Urea ..... (mmol/l)

Na^{+} ..... (mmol/l)

K^{+} ..... (mmol/l)

HCo_3^{-} ..... (mmol/l)

Cl^{-} ..... (mmol/l)

Ca^{++} ..... (mmol/l)

PO_4^{-} ..... (mmol/l)

Creatinine ..... (µmol/l)

Uric acid ..... (mmol/l)

Total protein ..... (g/l)

Albumin ..... (g/l)