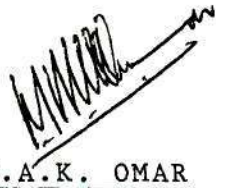
DECLARATION

I hereby declare that the whole thesis, unless specifically indicated to the contrary in the text, is my own original work and has not been submitted for a degree at any other university.



M.A.K. OMAR

MARCH 1982

A STUDY OF DIABETES MELLITUS IN YOUNG AFRICANS AND INDIANS (AGE OF ONSET UNDER 35) IN NATAL.

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN THE

FACULTY OF MEDICINE

UNIVERSITY OF NATAL, 1982

BY

MAHOMED ABDOOL KHALEK OMAR

M.B. CH.B. (NATAL), F.C.P. (S.A.), M.R.C.P. (U.K.)

1982

DEPARTMENT OF MEDICINE

UNIVERSITY OF NATAL, DURBAN.

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ACKNOWLEGEMENTS

First of all I would like to express my deepest gratitude to Professor Abdul Cader Asmal, without whose invaluable guidance and encouragement this thesis would, I doubt, have been completed. It was he who instilled in me an interest in endocrinology - particularly in the field of diabetes- while I was still a registrar working under him; it was an interest that culminated in this study and for this I am again indebted to him.

It is also a pleasure to record my thanks to Professor Y.K.Seedat, Head of the Department of Medicine, for providing me with the opportunity of undertaking this project in his Department and for putting at my disposal whatever expertise I needed. In addition I am grateful to Mrs. Linda van Rooyen for her invaluable technical assistance and to Professor Denis Pudifin, Mandla Mbatha, Kogie Naidoo,Dr J Naidoo, Mrs J. Duursma and the staff of Ward H2, all of whom have in one way or another contributed to the successful completion of this project. Thanks are also due to Professor S.M. Joubert for providing facilities in the Department of Chemical Pathology; to Dr. Bottazzo of the Department of Immunology, Middlesex Hospital for his help in doing the islet cell and other auto antibody assays; to Dr. Mike Hammond of the Natal Immunology Institute for assistance and valued comments in doing the HLA studies; to Cherilee Randall for typing the Script; to Mrs. F. Baumgaardt for the illustrations; to Mrs. N. Currie; to Dr. Truter, the Medical Superintendent of King Edward VIII Hospital, for use of hospital facilities; and to Doctor M.A. Seedat for reading the thesis.

The generous support of the South African Medical Research Council, the H.G. Brymer Research Grant and the University of Natal Research Fund is gratefully acknowledged, since this study could not have been undertaken without their assistance.

Finally my deepest gratitude to Fatima and to Imran, Ebrahim and Aziza for their patience and forbearance and the support they constantly provide. Above all, my everlasting indebtedness to the Almighty for being a perpetual source of inspiration.

CHAPTER I

INTRODUCTION

Diabetes mellitus has been known since antiquity. Detailed descriptions of the disease in two books (Charaka Samhita and Sushruta Ayurveda) written over 2 000 years ago show that the physicians of ancient India were well versed with it. Based on the following extracts taken from the translations of the two books, it can be clearly seen that they were able to recognise the two classical forms of diabetes mellitus that are known today: "That person who passes urine which is exceedingly sweet, cool, slightly viscid, turbid and resembling the juices of sugar cane . . . suffers from glycosuria . . . There are two types of urinary disorders - one natural due to genetic factors and the other due to indiscrete living or dietetic indiscretions. The patient suffering from the former is thin, pale, eats less and drinks too much. . . The patient with the latter is usually obese, eats a lot, is stout and of sedentary habits and sleeps too much". (Tulloch 1962).

In the West the first detailed study of diabetes mellitus by Aretaeus of Cappadocia (2nd Century A.D.) appeared to be of the juvenile onset type. It was the only kind generally known right up to the 17th century.

With the recognition of maturity onset diabetes occurring in the middle or older age group, it was believed that although juvenile onset diabetes (JOD) and maturity onset diabetes (MOD) presented in different ways, they represented gradations or variants of the same basic disease with quantitative differences in insulin deficiency (Marble 1971, Oakley et al 1973). This concept of diabetes, viz: that it was a homogenous entity, appeared to be supported by the observations that in both types of diabetes the same complications are found.

However, in recent years it has been clearly established that diabetes mellitus represents a heterogenous group of disorders with glucose intolerance being the common denominator. Unequivocal evidence in favour of such a concept includes:

1. The existence of more than 30 distinct genetic disorders with glucose intolerance as a feature (Rimoin 1976; Rimoin and Schimke 1971).
2. Ethnic variability in prevalence and clinical features (Rimoin 1969; Rimoin and Schimke 1971).
3. Clinical variability between ketosis-prone, insulin-dependent juvenile onset diabetes and obese, non ketosis-prone, insulin resistant adult onset diabetes (Rotter and Rimoin 1979).
4. Genetic heterogeneity in animal models (Herberg and Coleman 1977; Stauffacher et al 1976).
5. Genetic studies showing JOD and MOD to be distinct entities (Barnett et al 1981; Cudworth and Woodrow, 1976; MacDonald 1974; Nerup et al 1976; Pyke and Nelson, 1976; Simpson 1976; Tattersall and Pyke 1972).

Thus reference to diabetes mellitus as a homogenous entity was no longer tenable.

Since 1960 when Fajans recognised a group of young patients showing the typical pattern of maturity onset type diabetes, the concept that the disease in the young is also a heterogenous entity steadily gained ground. Although occasionally references to such cases can be found in the literature dating back to 1910 (Tattersall 1974), it was only in 1965 that maturity onset type diabetes in the young (MODY) was documented as a distinct entity separate from classical JOD (Fajans and Conn 1965). Subsequently several others presented unequivocal data in support of the existence of such a disease pattern in the young (Johansen, 1973 : Tattersall, 1974). Thus it was concluded that the classification of JOD or MOD on the basis of age of onset could no longer be justified, because either disease can be recognised at any age. Moreover age of onset describes the onset of symptoms and not to the onset of the metabolic abnormality which in the case of MOD may be present for years before being recognised (Fajans et al 1978; National Diabetes Data Group 1979).

In terms of the strong family history and pattern of insulin responses, MODY appeared to be akin to the classical maturity type of diabetes seen in the middle aged and elderly, (Fajans et al 1978). However, in addition to an earlier age of onset, the relatively low prevalence of obesity (Fajans et al 1976) and the paucity of complications were distinct differences (Fajans et al 1976).

Biochemical studies have shown that, unlike typical JOD where an absolute deficiency of insulin is a consistent finding (Block et al 1972; Block et al 1973; Parker et al 1968) MODY is characterised by a wide spectrum of insulin responses which may remain unchanged for many years (Fajans et al 1978).

Perhaps the major advances in elucidating the pathogenesis of juvenile onset diabetes and delineating it as an entity distinct from MOD or MODY have come from studies on the associations between insulin dependent diabetes mellitus (JOD) and the HLA system. A clear relationship has been established between JOD and HLA B8, B15, B18, DW3 and DW4 in white Caucasians (Cudworth and Woodrow 1975; Nerup et al 1974a) Studies in the Japanese (Nakao et al 1977) and American blacks (Duquesnoy et al, 1979), have highlighted differences in the specific allelic associations among these ethnic groups.

Research on other aspects of immunology has gone a long way in throwing further light on the pathogenesis of JOD. The presence of islet cell antibodies in a large proportion of juvenile onset diabetic patients at the onset of the illness underlined the importance of autoimmunity as an aetiological factor (Bottazzo et al 1974). The demonstration of cell-mediated immunity to pancreatic antigen in such patients has lent further support to this hypothesis (MaCuish et al 1975; Nerup et al 1974b)

Whilst it is evident that tremendous advances have been made in recent years in our understanding of diabetes in the young, most of the published data relate to the disease in White Caucasians. Studies in young Africans and Indians have highlighted many unusual patterns of diabetes in these people. Thus J-type diabetes occurring in lean ketosis - resistant patients and first described in Jamaica but later

in other tropical countries and the Z-type diabetes associated with malnutrition and pancreatic calcification and common in Uganda and certain parts of India came to be regarded as distinct forms of diabetes grouped together as tropical diabetes and different from the disease pattern seen in temperate climates (Tulloch 1962; West 1978).

Typical MOD, JOD and a variety of insulin independent diabetes occuring in young people (Campbell 1960, 1963) have all been reported in South Africa. JOD appears to be uncommon in the African population (Jackson 1978) with apparently no cases having been reported with onset under age 14 (Seftel 1964; West 1978). On the other hand, typical JOD has been said to be extremely rare in Indians (Jackson 1978). Non insulin-dependent diabetes in the young (MODY), however, appears to be common among Indians (Campbell 1960; Jackson 1978).

Two preliminary studies on IDDM and NIDDM in Africans and Indians in Natal (Asmal et al 1981 a; Asmal et al 1981 b) focussed on the heterogeneity of diabetes mellitus in these two populations and have highlighted their distinctiveness from the so called J-type diabetes with which they have been recently linked (McMillan and Geevarghese 1979). Notwithstanding details of the clinical, biochemical and immunological aspects of diabetes mellitus in these population groups are scanty.

Studies of diabetes mellitus and the HLA system have introduced a new field for relating the disease to HLA antigens in these population groups. Such data could determine whether there are any specific associations between JOD and the HLA system in Africans and Indians and whether the absence of any such relationship with MOD or MODY, as observed in white Caucasians (Christy et al 1979; Nelson and Pyke 1975) was also true of other groups.

These major advances in our understanding of diabetes mellitus, particularly in the young, have highlighted the need for a proper evaluation of the various aspects of the disease in the young African and Indian patient seen in this subtropical setting. Such a study would permit an inter-racial comparison of the diabetic syndrome and at the same

time define the relative frequencies of IDDM and NIDDM in the young and the possible relationship this might have with tropical diabetes mellitus. It will also provide a unique opportunity for carrying out observations on two population groups whose relatives living in other countries have shown certain unusual types of diabetes peculiar to the tropics.

The present study was thus designed to investigate African and Indian diabetic patients with age of onset under 35 years and duration of disease greater than a year, and obtain a detailed clinical evaluation of their disease with additional focus on biochemical accompaniments (insulin, C-peptide, other hormones and glycosylated haemoglobin) and certain factors of aetiological importance (HLA and islet cell antibodies).

It is envisaged that such an approach will help to define the respective patterns of diabetes in young Africans and Indians and bring to light any distinct clinical, biochemical or immunological features that may have a bearing on the pathogenesis of the disease or its complications in each of the race groups.

CHAPTER II

MATERIAL AND METHODS

POPULATIONS STUDIED

A total number of 236 diabetic patients with onset of diabetes mellitus under the age of 35 were studied. They comprised 115 Africans and 121 Indians.

The African patients were almost exclusively of Zulu descent and have lived in Natal for many centuries. The vast majority came from a poor socio-economic background and were either illiterate or semi-literate.

The Indian patients were descendants of people who left the Indian sub-continent towards the latter half of the last century. They comprise two ethnic subgroups, viz: the Aryans and the Dravidians, whose predecessors came from North and South India respectively (Mistry 1965). The vast majority of the Indians studied were of a low socio-economic status, which, however, was somewhat better than that of the Africans. Their degree of literacy also was better than that of the latter.

The diabetics were all patients of King Edward VIII Hospital in Durban. They were assessed regularly by the author himself at the Diabetic Clinic of that hospital. Informed consent was obtained from all patients in whom metabolic studies were done.

The normal subjects who underwent metabolic studies were, in general, medical students who appreciated the research nature of the studies and were able to provide informed consent. They were all healthy and denied suffering from diabetes or any other medical disorder. At the time of study they showed no evidence of recent weight loss and were consuming their normal diets.

CLASSIFICATION AND DIAGNOSIS

As recently as 1978 there was no general agreement on the classification of diabetes mellitus based on either aetiology or clinical features, in spite of accumulating evidence suggesting that the disease was a heterogenous entity. Thus it proved difficult to determine the epidemiology of the different types of diabetes and to assess the impact of the disease and its complications (National Diabetes Data Group 1979).

In order to provide a uniform framework in which to conduct clinical and epidemiologic research, the Expert Committee on Diabetes of the WHO has proposed a new classification of diabetes mellitus based on the recommendations of the National Diabetes Data Group (1979). It is hoped that this classification, shown in Table 1, will be able to provide more meaningful and comparative data on the scope and impact of the various forms of diabetes.

In the present study the same classification has been used. Thus patients have been classified as insulin dependent (IDDM) or non-insulin dependent (NIDDM) on the basis of the criteria laid down in Table 1. Patients with secondary diabetes, whether due to pancreatic disease, hormonal abnormalities, drugs or genetic syndromes have been excluded from this study. In addition patients with excess ethanol intake were also excluded.

Diagnosis of diabetes mellitus was based on the revised criteria recommended by the Expert Committee on Diabetes of the WHO, shown in Table 2. However, instead of the 75 gm glucose load as recommended, a 50 gm load was mainly used in the patients, because most of them had had diabetes mellitus long before the revised criteria appeared and this had been the method of doing a glucose tolerance test (GTT) at King Edward VIII Hospital until recently.

Based on the classification shown in Table 1, 133 patients (92 Africans, 41 Indians) were found to have IDDM and 103 (23 Africans, 80 Indians) NIDDM. All patients had been followed up for at least 12 months after diagnosis of diabetes mellitus. H.L.A. studies were

TABLE I

CLASSIFICATION OF DIABETES MELLITUS AND OTHER CATEGORIES OF GLUCOSE INTOLERANCE

(From the National Diabetes Data Group 1979)

Class	Former terminology	Associated factors	Clinical characteristics	Diagnostic criteria
		Clinical Classes		
DIABETES MELLITUS (DM)				
Insulin-dependent type (IDDM), Type I.	Juvenile diabetes, juvenile-onset diabetes, juvenile-onset-type diabetes, JOD, ketosis-prone diabetes, brittle diabetes.	Evidence regarding etiology suggest genetic and environmental or acquired factors, association with certiain HLA types, and abnormal immune responses, including autoimmune reactions.	Persons in this subclass are dependent on injected insulin to prevent ketosis and to preserve life, although there may be pre-ketotic, noninsulin-dependent phases in the natural history of the disease. In the preponderance of cases, onset is in youth, but IDDM may occur at any age. Characterized by insulinopenia. Islet cell antibodies are frequently present at diagnosis in this type.	Diagnosis of diabetes in adults should be based on: 1) unequivocal elevation of plasma glucose concentration together with the classical symptoms of diabetes. or 2) elevated fasting plasma glucose concentration on more than one occasion. or 3) elevated plasma glucose concentration after an oral glucose challenge on more than one occasion. Diagnosis of diabetes in children requires either 1) or 2) and 3).
Noninsulin-dependent types (NIDDM) Type II.	Adult-onset diabetes, maturity-onset diabetes, maturity-onset-type diabetes, MOD, ketosis-resistant diabetes, stable diabetes.	There are probably multiple etiologies for this class, the common outcome being derangement of carbohydrate metabolism. Evidence on familial aggregation of diabetes implies genetic factors, and this class includes diabetes presenting in children and adults in which autosomal dominant inheritance has been clearly established (formerly termed the MODY type, maturity-onset diabetes in the young). Environmental factors superimposed on genetic susceptibility are probably involved in the onset of the NIDDM types. Obesity is suspected as an etiologic factor and is recommended as a criterion for dividing NIDDM into two subclasses, according to the presence or absence of obesity.	Persons in this subclass are not insulin-dependent or ketosis-prone, although they may use insulin for correction of symptomatic or persistent hyperglycemia and they can develop ketosis under special circumstances, such as episodes of infection or stress. Serum insulin levels may be normal, elevated, or depressed. In the preponderance of cases, onset is after age 40, but NIDDM is known to occur at all ages. About 60-90% of NIDDM subjects are obese and constitute a subtype of NIDDM; in these patients, glucose tolerance is often improved by weight loss. Hyperinsulinemia and insulin resistance characterize some patients in this subtype.	See Table 2 for diagnostic plasma glucose standards.
Other types, including diabetes mellitus, associated with certain conditions and syndromes: 1. Pancreatic disease 2. Hormonal 3. Drug or chemical induced 4. Insulin receptor abnormalities 5. Certain genetic syndromes 6. Other types		This subclass contains a variety of types of diabetes, in some of which the etiologic relationship is known (e.g. diabetes secondary to pancreativ disease, endocrine disease, or administration of certain drugs). In others, an etiologic relationship is suspected because of a higher frequency of diabetes with a syndrome or condition (e.g. a number of the genetic syndromes). See Table 3 for a list of these conditions and syndromes.	In addition to the presence of the specific condition or syndrome, diabetes mellitus is also present.	In order to place an individual in the subclass Other Types, two diagnostic determinations must be made, the presence of diabetes (as described above) and the presence of the associated condition or syndrome.

Table 2

CRITERIA FOR THE DIAGNOSIS OF DIABETES MELLITUS

(From the National Diabetes Data Group)

Any one of the following are considered diagnostic of diabetes:

A. Presence of the classic symptoms of diabetes, such as polyuria, polydipisia, ketonuria and rapid weight loss, together with gross and unequivocal elevation of plasma glucose.

B. Elevated fasting glucose concentration on more than one occasion:-

venous plasma	≧	140 mg/dl (7.8 mmol/L)
venous whole blood	≧	120 mg/dl (6.7 mmol/L)
capillary whole blood	≧	120 mg/dl (6.7 mmol/L)

If the fasting glucose concentration meets these criteria, the OGTT is not required. Indeed, virtually all persons with FPG > 140 mg/dl will exhibit an OGTT that meets or exceeds the criteria in C. below.

C. Fasting glucose concentration less than that which is diagnostic of diabetes (B. above), but sustained elevated glucose concentration during the OGTT on more than one occasion. Both the 2-h sample and some other sample taken between administration of the 75-g glucose dose and 2 h later must meet the following criteria:-

venous plasma	≧	200 mg/dl (11.1 mmol/L)
venous whole blood	≧	180 mg/dl (10.0 mmol/L)
capillary whole blood	≧	200 mg/dl (11.1 mmol/L)

done on an additional 15 Indians with IDDM. However, since a full clinical examination was not carried out on them, they have been excluded from the rest of the study.

Of the patients grouped under the category of NIDDM, 24 (19 Indians, 5 Africans) initially presented as such but later required insulin for control of symptoms.

Five of the insulin dependent-diabetic patients (4 Africans, 1 Indian) did not show ketonuria at any time even in the absence of insulin therapy, although they needed insulin for control of symptoms. The mean age of onset of diabetes mellitus in these patients was 27.5 years (range 23 - 30) and the mean duration of disease 4.3 years (range 2 - 7). They had a mean body weight of 61 kg (range 52 - 67), whilst the percentage ideal body weight in each of the 2 patients over the age of 25 was 96. The mean daily insulin requirement was 43 Iu (range 28 - 44) or 0.73 Iu/Kg.

To all intents and purposes they could be categorised under the umbrella of J-type diabetes, except that their daily insulin requirements were not particularly high as has been found elsewhere (Hugh-Jones 1955, Kar and Tripathy 1967). Since in this study they only accounted for 2.4% and 3.2% of all the young African and Indian insulin dependent diabetic patients respectively, J-type diabetes appears to be rare in these population groups.

Although the classification shown in Table 1 does not take into account J-type diabetes, West (1979) has suggested that it can be regarded as a subgroup of insulin-dependent diabetes. Therefore the 5 patients with this type of diabetes have been included under the IDDM category.

Since the Diabetic Clinic at King Edward VIII Hospital where this study was undertaken serves mainly as a consultative clinic seeing to problem cases referred from the general medical clinics of this and other hospitals in and around Durban, the patients seen there represent a select group of subjects. Thus the young patients seen do not represent the true proportions of the patterns of diabetes present in the respective

populations. Therefore in order to obtain an estimate of the relative frequencies of IDDM and NIDDM in the young, preliminary surveys of the pattern of diabetes at the referring clinics and hospitals were carried out. Two surveys were performed on Indian and one on African patients. They involved examination of the clinical case records of all diabetics seen at the referral centres over a four week period and analysis of data on age, sex, duration of diabetes and type of therapy.

In a survey of 247 African diabetic patients, 21 had IDDM, which therefore accounted for 10.1% of the total diabetic population. Of the patients under age 35, 84% were insulin-dependent. (Addendum I).

Combined data from 2 surveys showed that out of a total of 2 420 Indian diabetic patients 267 were under the age of 35 and of these 89% were categorised as non-insulin dependent (NIDDM). Patients with IDDM accounted for only 1.1% of the total Indian diabetic population. (Addendum I).

METHODS

All the patients described in this study had the following data recorded:-

1. Sex, age, height, weight.
2. Percentage ideal body weight in patients over 25, calculated as follows: $\frac{\text{Body weight of patient}}{\text{Ideal body weight for a medium framed patient of similar height (Diem and Lentner 1970)}} \times 100$
3. Percentile for body weight and height in patients under 18 years.
4. Age at diagnosis and month of onset.
5. Duration of diabetes.
6. Family history of diabetes.
7. Mode of presentation.
8. Presence or absence of ketosis.

In addition, a full physical examination was carried out on each patient, paying particular attention to:-

Blood pressure;

Presence or absence of peripheral pulses;

Abnormalities of peripheral or autonomic nerve function;

The eyes for cataracts or evidence of retinopathy;

Nephropathy;
Ischaemic heart disease.

Peripheral Neuropathy

The following criteria were used to diagnose peripheral neuropathy:-

1. Symptoms of neuropathy such as pain, paraesthesia, hyperaesthesia, foot drop.
2. Loss of touch, pain, position or vibration sense.
3. Absent reflexes.

Patients were considered to have diabetic peripheral neuropathy if these criteria were present in the absence of other common disorders associated with neuropathy.

Autonomic Neuropathy

This was diagnosed on the basis of:-

1. Impotence.
2. Heart rate response to standing i.e., absence of a tachycardia on changing from the horizontal to the vertical position.
3. Postural Hypotension, which is considered to be present if the difference between the lying and standing systolic pressure is greater than 30 mm Hg (Ewing et al 1973).

Retinopathy

Fundal examination was carried out by first instilling a mydriatic in each eye and then 30 minutes later examining each fundus for at least 2 minutes.
Retinopathy was classified as background if there were microaneurysms, exudates or small haemorrhages; or as proliferative if there were large haemorrhages, neovascularisation, retinal or vitreous fibrosis or retinal detachment.

Nephropathy

This was deemed to be present if:-

1. Proteinuria (as measured by Albustix) was found on 3 consecutive urine samples, taken on different occasions at least a month apart.
2. The serum creatanine was elevated.

In patients showing either of these abnormalities a GFR was done using Chromium^{51} labelled EDTA.

Ischaemic Heart Disease

This was diagnosed on the basis of:-

1. Symptoms of angina.
2. Resting ECG changes, viz: ST depression, T wave inversion, LBBB.

X-Ray Abdomen

Abdominal radiographs were done on all the patients with IDDM. Only two patients, aged 15 and aged 29 and both females, showed calcification in the region of the pancreas. Neither patient gave any history of alcohol consumption. The younger patient was below the 3rd percentile for height, ketosis prone and could be categorised as Z-type diabetes which appears to be associated with malnutrition. (West, 1978).

Hormonal Levels and Antibody Assays

Blood samples from patients and normal subjects were collected in the mornings and separated within 2 hours. Sera were immediately stored in small aliquots at $-20^{o}C$, until the time of assay. Thus tests were carried out on samples that had been thawed once only.

Tests of Beta Cell Function Using Glucose or Glucagon

All studies were carried out on overnight fasted subjects. In patients with NIDDM who were on long acting sulphonylureas, drug therapy was discontinued at least 72 hours before the investigations were performed. Patients with IDDM were given no insulin on the morning of the test and soluble insulin only on the evening before the test to ensure that there would be no residual depot action of long acting insulin. "Medican" (R) 16g cannulae were introduced into forearm veins under local analgesia and kept patent with 0.9% saline infusions. Basal blood samples were taken after a rest period of at least 30 minutes. Thereafter, depending on the test, 100gm glucose dissolved in 200 ml of water was administered or glucagon (1 mg) injected intravenously. Blood samples for glucose and insulin or C-peptide were then collected at varying intervals after the stimuli.

Plasma glucose was measured by means of an autoanalyser and C-peptide (Mallinkrodt Kit) and insulin (Phadebas Kit) by radioimmunoassay.

Basal Hormonal and Lipid Profiles

Blood samples for growth hormone, glucagon, cortisol, cholesterol and triglycerides were taken after an overnight fast. The hormones were measured by radioimmunoassay (Pharmaca Diagnostica, Biodata and Gamma Coat Kits for growth hormone, glucagon and cortisol respectively). Plasma cholesterol and triglyceride levels were measured enzymatically.

HLA Studies

HLA A, B, C and DR specificities were done on the same day as the blood samples were taken. A two-stage lymphocytotoxicity test using 180 antisera was employed to determine HLA A, B and C antigens, whereas an extended incubation microlymphocytotoxicity test using B cell enriched, T cell depleted lymphocytes was employed to determine HLA DR antigens.

Islet Cell Antibodies and Other Autoantibodies

Islet cell antibodies, both IGg antibodies (ICA IGg) and complement fixing (ICA-Cf), were measured by immunofluoresence using fresh pancreas from a blood group O cadaver as antigen. Antibodies to thyroglobulin and thyroid microsome, to parietal cell and to adrenal tissue were also measured by immunofluoresence.

Immune Complexes

These were measured by the Raji cell assay and the results quantitated.

Statistical Analysis

Means, standard errors, standard deviations, paired and unpaired t-tests, X^2 tests and correlation co-efficients were calculated using programmes made out for the Hewlett Packard 67 Computer.

CHAPTER III

CLINICAL FEATURES OF INSULIN - DEPENDENT DIABETES IN AFRICANS AND INDIANS

INTRODUCTION

Published data on the epidemiology, clinical and biochemical features of diabetes mellitus in the different racial groups of South Africa mostly relate to the disease in adults with maturity-onset diabetes (Jackson and Huskisson 1965; Marine et al 1969; Seftel et al 1963) Up till 1980 apart from a preliminary survey in Ethiopia on 27 Ethiopian juvenile diabetics (Lester 1978), there had been little information on the clinical features of IDDM in the young. Typical juvenile onset diabetes has been described as extremely rare in the Indian (Jackson 1978) and "much rarer in Coloured and African than white people" in South Africa (Marine et al 1969). It has also been suggested that JOD in Southern Africa might be synonymous with the so-called J-type diabetes of the Tropics (McMillan and Geevarghese 1979).

MATERIALS AND METHODS

Ninety two African and forty one Indian patients who fulfilled the following criteria were selected and studied:-

1. Age at diagnosis under 35 years.
2. Development of symptoms and/or ketosis in the absence of insulin therapy.
3. Duration of diabetes mellitus greater than two years.

The following data were recorded from all the patients studied:-

1. Age and month of onset.
2. Duration of diabetes mellitus.
3. Mode of onset.

4. Family history (first degree relative being defined as mother, father, offspring or sibling).
5. Sex.
6. Weight, height and percentage desirable weight which was calculated thus: $\frac{\text{Actual Body Weight}}{\text{Medium-frame Ideal Body Weight for the same height}} \times 100$
7. Episodes of coma or severe ketoacidosis: diagnosed on the basis of hyperglycaemia, impaired consciousness, dehydration, acidotic breathing, ketonuria and a serum bicarbonate less than 9 mmol/L, (Bradley 1971; Oakley et al 1973).

In addition, a full physical examination was carried out on each patient. Peripheral neuropathy and autonomic neuropathy were assessed on the basis of the criteria outlined in Chapter II. Fundal examiantion was done after instillation of a mydriatic, (as described in Chapter II). Nephropathy was diagnosed on the basis of albuminuria in 3 consecutive urine specimens or elevated creatinine levels. Patients in whom either of these were positive had measurements of their glomerular filtration rate done by means of chromium^{51}-labelled EDTA.

RESULTS

Results are presented in Tables 1 - 3 and Figures 1 - 6

Age of onset

Figure 1 illustrates the ages of onset of IDDM in the African and Indian patients. The mean age of onset of the disease is lower in the Indian patients (mean 17 years) than in the African patients (mean 23.5 years), $p < 0.025$. Only one African patient presented before the age of 5, whereas 8 Indian patients presented before this age.

Sex

Although in both the African and Indian patients there was a slightly higher number of females, the difference was not significant.

Duration of IDDM

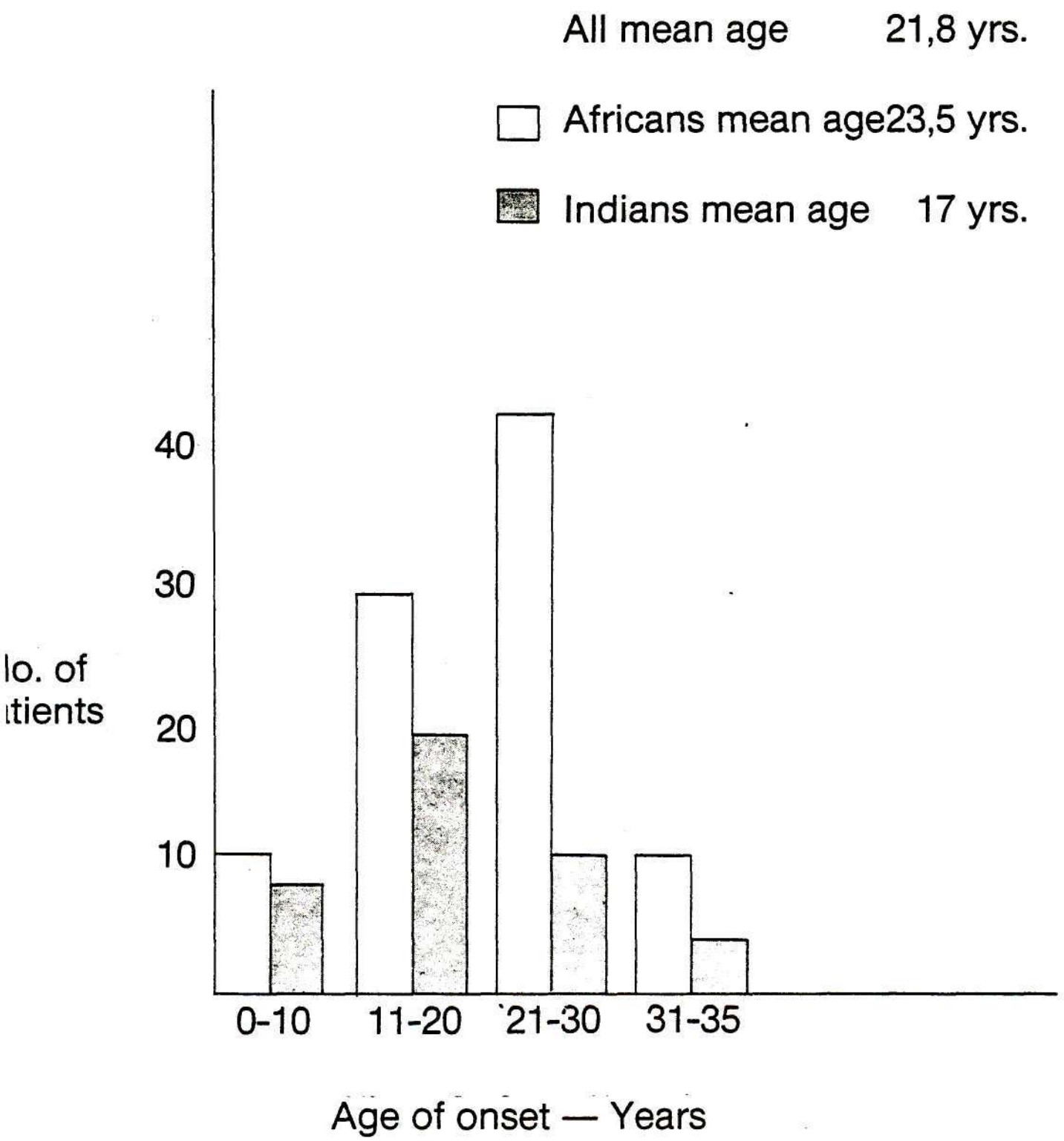
Figure 2 depicts the pattern of duration of diabetes seen in the patients studied.

TABLE 1 CLINICAL CHARACTERISTICS OF THE PATIENTS WITH IDDM

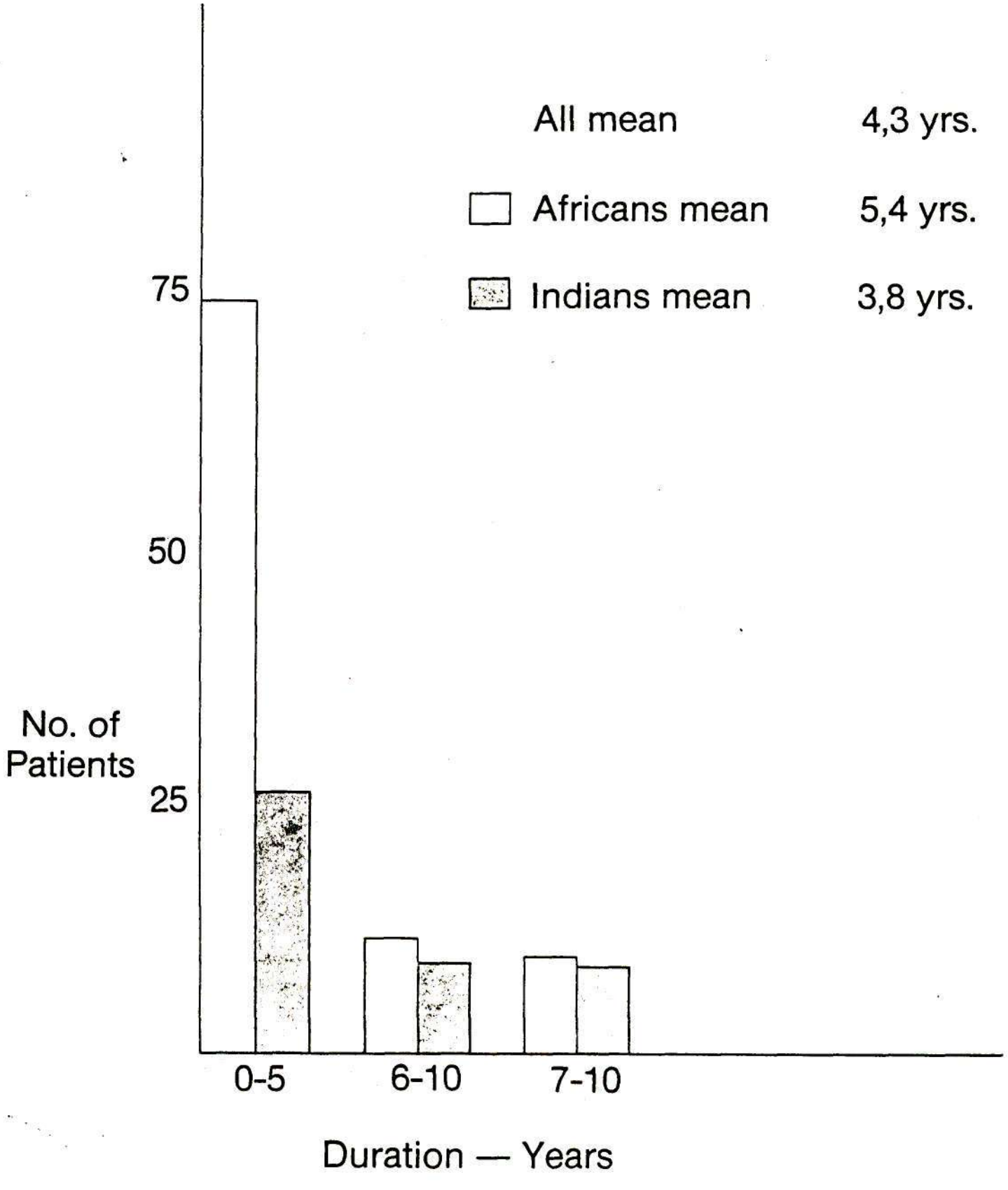
	Africans	Indians	Total
Number of patients	92	41	133
Male : female ratio	21 : 25	17 : 24	59 : 74
Mean age of onset	23.5($\frac{8}{12}$-35)*	17 ($1\frac{1}{2}$ - 35)*	
Mean duration of disease	3.8 (1-27)*	5.4 (1-22)*	4.3
Mean percentage desirable weight	106 (68-153)*	91.5 (71-136)*	101.6
Family history	12	22	34
Frequency of onset - winter : summer	1.3 : 1	2.3 : 1	1.5 : 1
( )* range			

Figure 1.

The Distribution of Patients in Relation to the Age of Onsets of I.D.D.M.



The Pattern of Duration of Diabetes in Patients with I.D.D.M.



Weight

Figure 3 depicts the distribution of percentage desirable weights in adults with IDDM. The mean percentage desirable weight of the African patients (106%) was significantly higher than that of the Indian patients (mean 91.5%), $p < 0.005$.

Of the patients under the age of 18, 50% of the Africans and 40% of the Indians fell within the 10th to the 90th percentile for height, whilst 31% and 40% respectively fell below the 5th percentile. Figure 4 illustrates the relationship between duration of diabetes and the percentile for height in the insulin-dependent diabetic patients under age 18 years. No correlation is seen between growth and duration of diabetes.

In patients under the age of 18, 77% of the Africans and 60% of the Indians fell within the 10th to the 90th percentile for weight whilst 14% and 30% respectively fell below the 5th percentile. Figure 5 illustrates the relationship between duration of diabetes and the percentile for weight in insulin-dependent diabetic patients under 18 years. No correlation is seen.

Family History

Twelve (13%) African patients gave a family history of diabetes affecting a first degree relative. It was of the non-insulin dependent type in all the affected relatives except one. In contrast a family history of diabetes affecting a first degree relative was present in 22 Indian patients (54%), in 3 of whom the affected relatives had IDDM. One of the patients had a first cousin suffering from IDDM.

Seasonal Variation in the Onset of IDDM

Figure 6 illustrates the frequency of cases of IDDM in relation to the month of onset of the disease in those patients (73 Africans and 33 Indians) in whom a reliable history was obtained. There appears to be a slightly higher rate of onset in winter than summer (1.5 : 1) if April to September are taken as the winter months. The winter predominance appears to be much more marked among Indians (2.3 : 1) than among Africans (1.3 : 1).

The Distribution of Percentage Ideal Body Weights in Adults (Age over 25) with I.D.D.M.

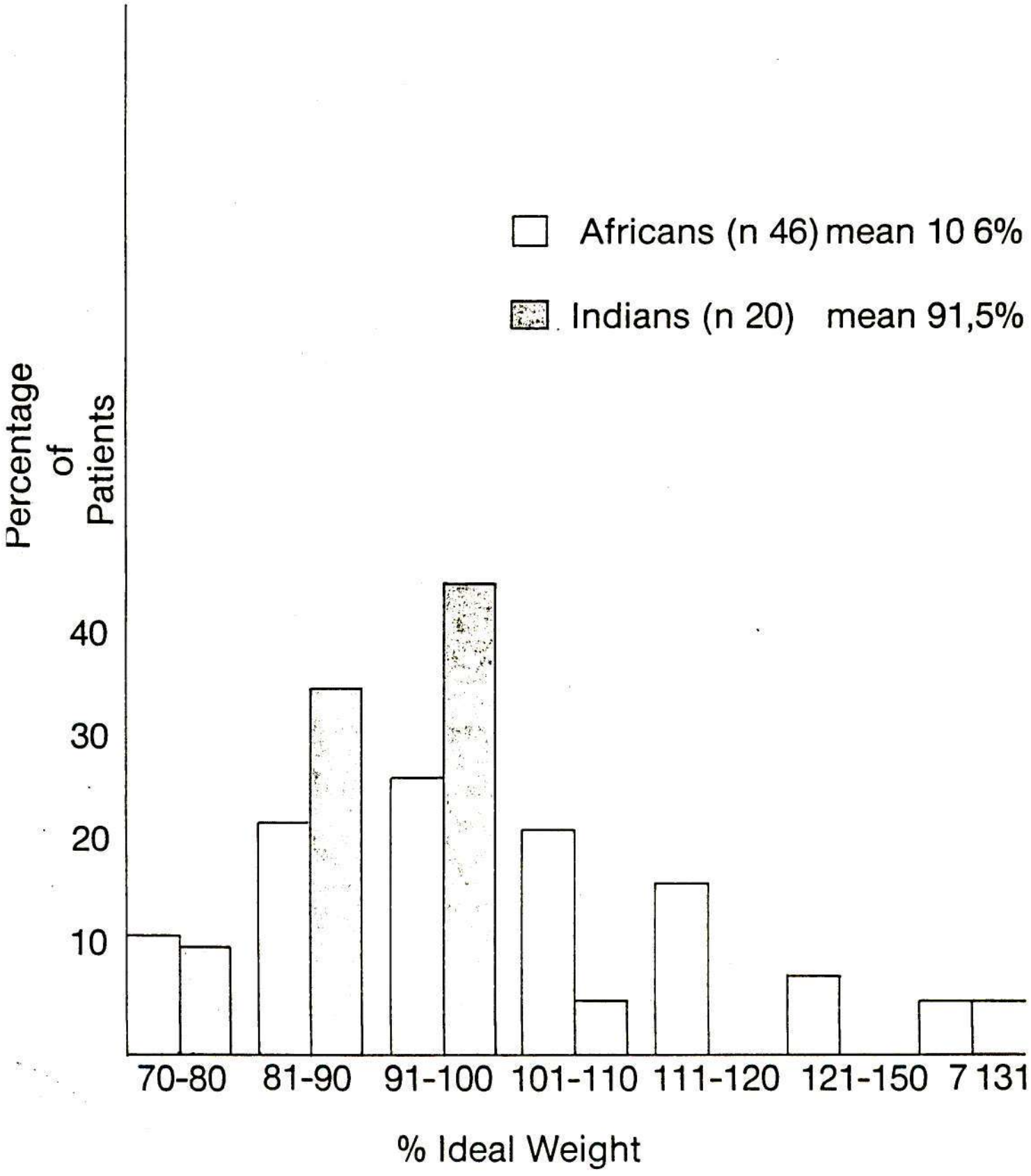


Figure 4.

THE RELATIONSHIP BETWEEN DURATION OF IDDM AND PERCENTILE FOR HEIGHT IN PATIENTS UNDER 18YEARS OF AGE

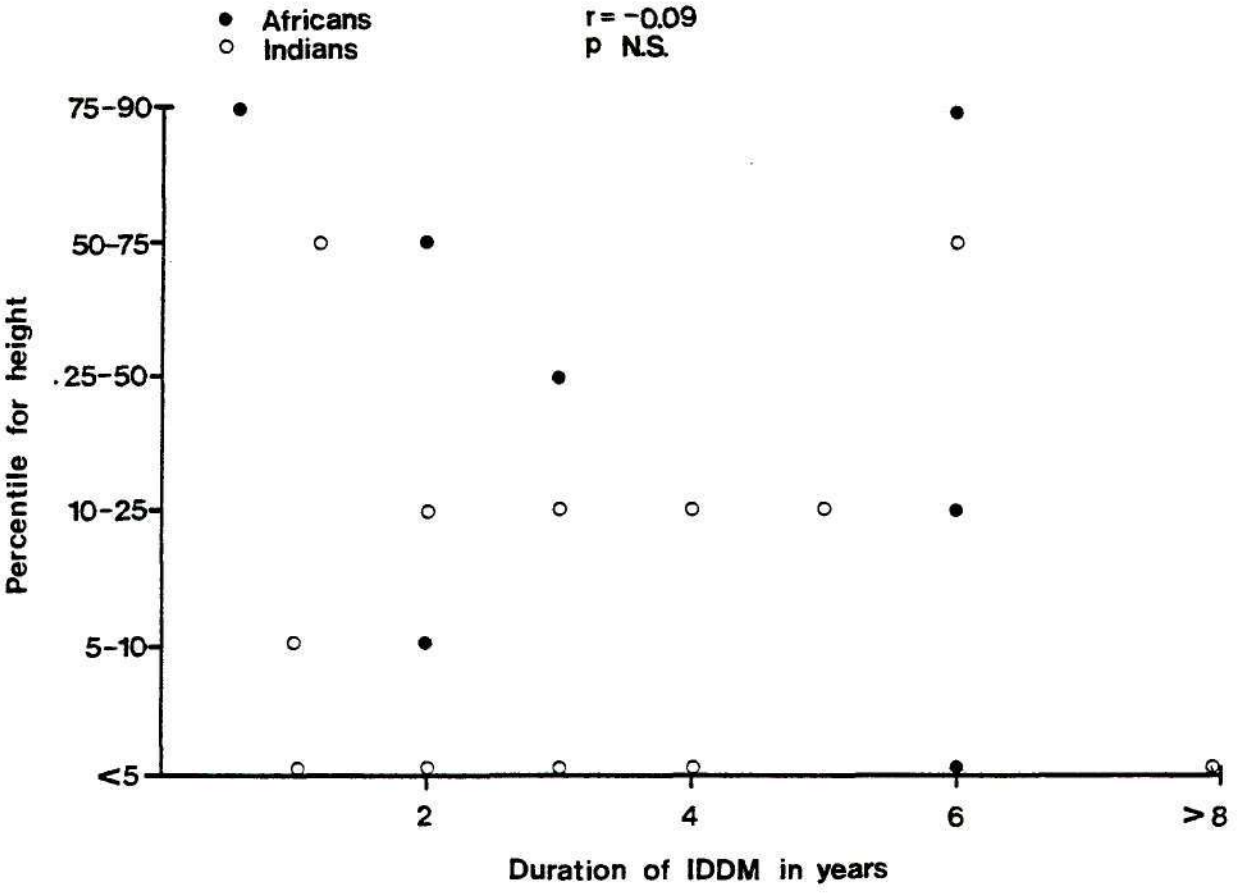


Figure 5.

THE RELATIONSHIP BETWEEN DURATION OF IDDM AND PERCENTILE FOR WEIGHT IN PATIENTS UNDER 18YEARS OF AGE

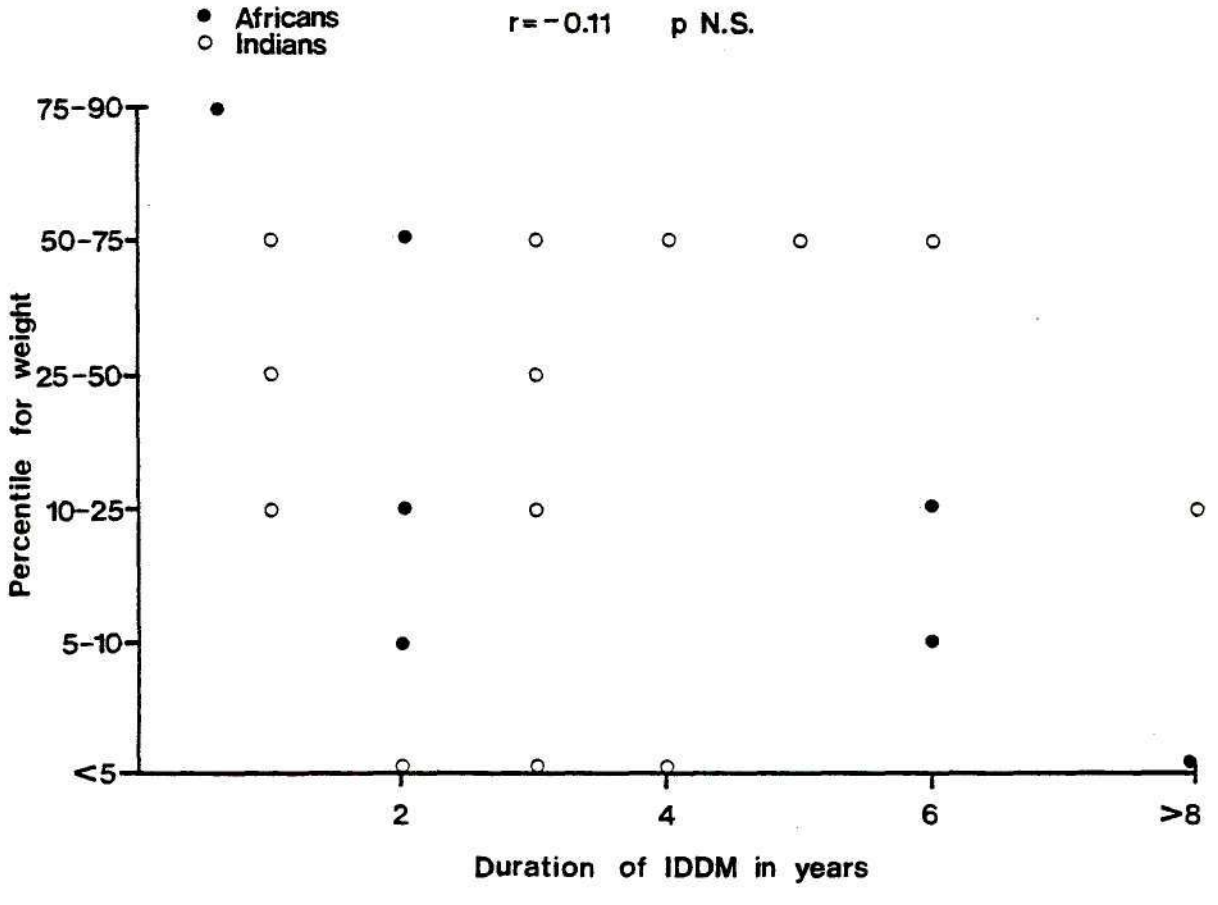
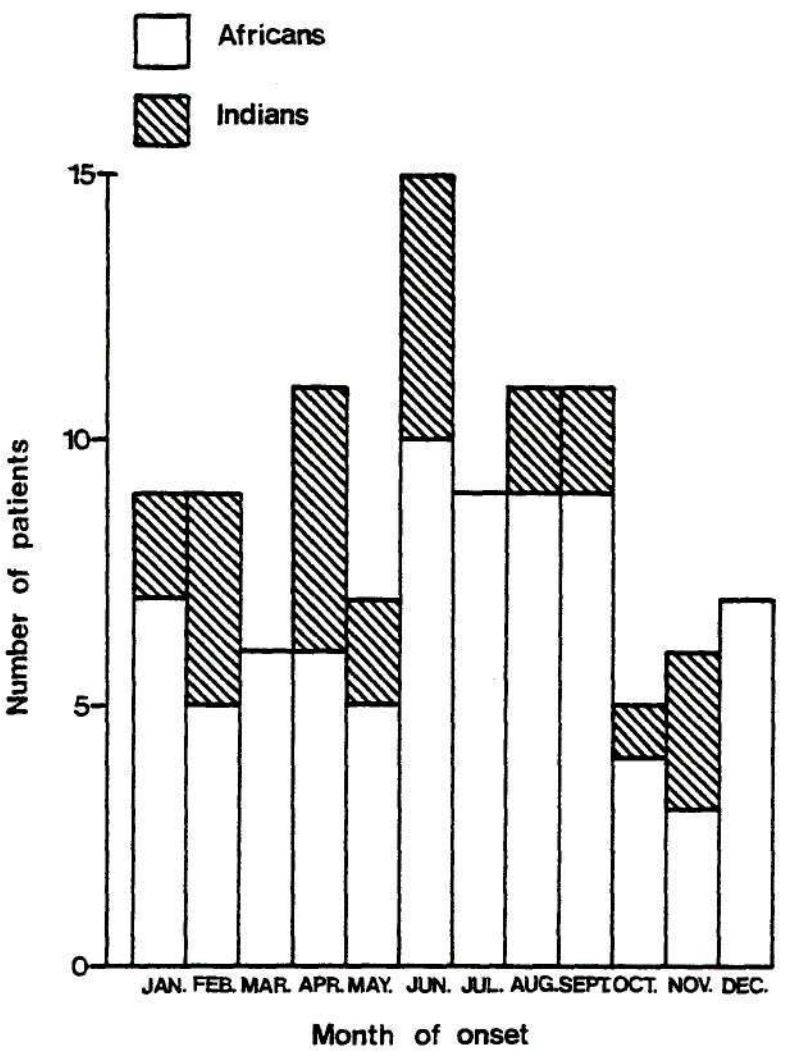


Figure 6.

THE RELATIONSHIP BETWEEN THE FREQUENCY OF PRESENTATION AND THE MONTH OF ONSET OF IDDM



Presentation

All the patients except 9 presented acutely, with symptoms of polyuria, polydipsia, tiredness, loss of weight and polyphagia being predominant and present for a short duration. The onset was fulminating in 15 patients, who presented in ketoacidotic coma. One patient presented with lactic acidosis without any previous history of diabetes or ingestion of alcohol or biguanides. Of the 9 patients (5 African and 4 Indian) with a subacute onset 5 presented with an abscess, whilst in 2 of them the disease was discovered after urine specimens showed glycosuria during routine medical examinations. Both the latter patients were under age 19 and dependent on insulin "ab initio". Ten of the 15 patients who had a severe onset of IDDM were under the age of 20.

Complications:

These are shown in Tables 2 and 3.

Ketoacidotic coma

There were 75 patients who developed ketoacidotic coma on one or more occasions. This complication was the presenting feature of the disease in almost 30% of such patients. Omission of insulin was the precipitating factor in 44% of the patients who developed ketoacidotic coma. Whilst almost 70% of the African patients manifested with ketoacidotic coma on one or more occasions, just over 50% of the Indian patients developed this complication. In 56% of the African patients omission of insulin was a contributory factor whereas it was responsible in only 26% of the Indian patients.

Neuropathy

Neuropathy was a common complication. The mean duration of the disease in all the patients with peripheral neuropathy was 5,8 years. Indian patients show a slightly longer mean duration (6 years) than the African patients (5,6), but the difference was not significant.

The duration of IDDM was less than a year in 50 per cent of the African patients manifesting with this complication. One African patient and 4 Indian patients had symptoms of peripheral neuropathy. An absent ankle jerk was present in 20 per cent of the patients and in 40 per cent of these patients IDDM was present for less than 2 years. Impairment of vibration sense and of joint position was found in 3 patients.

TABLE 2 PREVALENCE OF COMPLICATIONS IN PATIENTS WITH IDDM

Complications	Africans	Indians	Total
Ketoacidosis	53 (58%)	22 (54%)	75 (56%)
Neuropathy - Peripheral	20 (22%)	13 (32%)	33 (25%)
Autonomic	4 (4%)	2 (5%)	6 (5%)
Retinopathy	13 (14%)	9 (22%)	22 (17%)
Nephropathy	3 (3%)	3 (7%)	6 (5%)
Triopathy	1 (1%)	2 (5%)	3 (2%)
Ischaemic Heart Disease	-	-	-
Hypertension	4 (4%)	2 (5%)	6 (5%)
Cataracts	5 (5%)	2 (5%)	7 (5%)
Tuberculosis	6 (7%)	1 (2%)	7 (5%)

TABLE 3 MEAN DURATION OF IDDM IN PATIENTS WITH COMPLICATIONS

Complications	Mean Duration in Years (range)		
	Africans	Indians	All
Neuropathy	5.6 ($\frac{1}{12}$-27)	6 (1-13)	5.8
Retinopathy	10.1 (7-27)	11.7 (4-22)	10.8
Nephropathy	17 (11-27)	13.7 (13-16)	15.3
Triopathy	11	13 (12-14)	12.3
Hypertension	13 (1-27)	13 (12-14)	13
Cataracts	4 ($\frac{1}{12}$-8)	16.5 (12-24)	6
Tuberculosis	4 ($\frac{1}{12}$-8)	4	4

Abnormalities of these modalities of sensation were always associated with absent reflexes.

Autonomic Neuropathy

Impotence was present in seven patients, two of whom manifested with this complication at the onset of the disease. Four patients showed evidence of autonomic neuropathy on the basis of the screening tests used. One of them also showed other florid features of autonomic neuropathy viz: gastric and oesophageal atony, impotence, bladder atony and intractable diarrhoea. As expected he had peripheral neuropathy which has been shown to be an invariable accompaniment of autonomic neuropathy (Campbell et al 1975; Whalen et al 1969; Wheeler and Watkins 1973).

Retinopathy

Background retinopathy was found in 14% of the African and 22% of the Indian diabetic patients. The mean duration of the disease was similar in both racial groups. In 2 of the African patients retinopathy was discovered at the onset of the disease. Of the 7 African patients who had IDDM for more than 10 years, all showed evidence of retinopathy, whilst of the 8 Indian patients with a similar duration of disease, 5 showed this complication. In patients who had IDDM for less than 10 years only 7% of the Africans and 12,5% of the Indians showed evidence of retinopathy. Only one patient had proliferative retinopathy.

Nephropathy

Based on the criteria used in this study, 6 patients were found to have nephropathy. The mean duration of IDDM was 15.5 years (range 12 - 27 years). Two patients had persistent albuminuria in the presence of a normal creatinine concentration. One of them who died of acute suppurative pyelonephritis and renal papillary necrosis had typical features of diabetic nephropathy on histological examination of autopsy tissue. All 6 patients with nephropathy also had retinopathy, which has been found to be invariably associated with this complication (Bjerkelund 1951). The mean GFR, which could be done in 4 patients, was 52 ml/minute (range 35 - 75 ml).

Triopathy

Nephropathy, retinopathy and neuropathy were present together in 3 patients. The mean duration of disease in these patients was 11.3 years.

Hypertension

Hypertension was seen in 6 patients. Whilst it was associated with nephropathy in both the Indian patients, such a relationship was found in only 2 of the 4 African patients.

Cataracts

Cataracts of gradual onset, the usual form seen in diabetes of long duration, was seen in 3 patients, all of whom had the disease for at least 10 years.

Another 4 patients presented with dense subcapsular cataracts after a short history of symptoms of diabetes.

Tuberculosis

Active pulmonary tuberculosis was present in 6 of the African patients (6%) with IDDM. Only one Indian patient had this complication.

Insulin Requirements

Although the mean insulin requirements were lower in the Indian patients (mean 1.20 $\pm$ 0.10 Iu/kG/day) compared to the African patients (mean 1.50 $\pm$ 0.14 Iu/Kg/day), the difference was not significant. One of the African patients required 480 Iu monocomponent insulin daily (8.7 Iu/Kg) for stabilisation.

DISCUSSION

Studies of rates of prevalence of insulin-dependent diabetes are hampered by the fact that as the disorder is infrequent large population samples are required and it is difficult to obtain a representative sample when such large numbers are needed (West 1978). Table 3 illustrates approximate rates of prevalence in most of the populations where such studies have been done. Because methods of ascertainment are not precisely comparable in these populations, differences of

Table 4

RATES OF PREVALENCE OF IDDM IN VARIOUS COUNTRIES (WEST 1978)

COUNTRY	POPULATION	AGE	RATE PER 1000
United States	Whites	0-24	1.3
United States	Whites	0-20	1.6
United States	Blacks	0-20	1
Britain	Whites	25	3.4
Denmark	Whites	0-29	0.7
Denmark	Whites	0-35	3.9
Japan		1-15	0.1
New Zealand	Whites	0-20	1.04
Israel		2-16	0.16
PRESENT STUDY			
Natal	Africans		0.7
	Indians		0.18

moderate degree are not necessarily significant. Although the data presented are far from complete, it appears that insulin-dependent diabetes is a disease affecting people living in northern European climates and individuals whose ancestors came from those areas of the world. Cahill (1979) expresses similar views, although his conclusion that juvenile onset diabetes in the black results from racial admixture is somewhat tenuous. Similarly Pyke (1969) observed that typical juvenile onset diabetes was uncommon in all non-white racial groups.

If the overall prevalence of diabetes in the Africans is 0.7% (West 1978) then IDDM represents 0.07% of the population. The prevalence rate in Denmark is considerably higher (0.39%). Comparison whith other African populations is not possible because of the unavailability of actual data. There is a slightly higher rate among American blacks (0.1%), although the difference could be more accentuated if the age cut-off point in the latter study was higher than 20. According to several workers the prevalence of the disease appears to be intermediate between that seen in whites and that seen in black Africans from Africa, (Gorwitz et al 1976; MacDonald 1970; MacDonald 1980; Rodey et al 1979).

In contrast IDDM appears to be much less common in Indians, because if the overall prevalence of diabetes is taken as 1.8% of the Indian population (West 1978), then IDDM represents only 0.018% of the population. Such low prevalence rates have also been found in India, (Patel et al 1966; Sathe 1973; Visvanathan et al 1966), whence all our Indian patients have their origins. The disease has been found to be rare even in Indians of Singapore (Tulloch 1962) and of Fiji (Cassidy 1967).

The best-studied series on age of onset of insulin-dependent diabetes propably comes from the Joslin Clinic. The disease is rare before the age of 5 and thereafter the incidence increases with age reaching a peak at age 11 (White and Graham 1971). The maximal frequency for girls is at age 10 and for boys at age 13. Similar figures for peak frequency have been found in Denmark (Christau et al 1977). In Japan most patients were found to present with the disease between the ages of 10

and 13 (Mimura 1970). However, Bloom et al 1975, observed a bimodal distribution in the age of onset, with a peak at 11 and another in the early 20's. Similarly Cudworth et al (1977) found smaller but definite peaks between 21 to 23 years and at 5 to 7 years in addition to a large peak at age 11.

Whereas the peak frequency occurred in the third decade in Africans, it was in the second decade in the Indian patients, who showed a steady peak through all the years (11 - 19), in contrast to the sudden sharp rise found in Denmark and America. Crossley and Upsdell (1980) also observed a steady peak during the second decade among insulin-dependent diabetic patients in New Zealand. Although diabetes may be found in earliest infancy, all studies have shown much lower rates in the first 5 years, (West 1978). Only one African had an onset under age 5, whilst 8 Indians presented before this age.

In most studies of IDDM on white Caucasians there have been no significant differences in prevalence rates for boys and girls, (West 1978). Although in the African and Indian patients, there was a slightly higher number of females, the difference was not significant.

Prior to the availablility of long acting insulin poorly controlled diabetes was sometimes associated with marked retardation of growth. This condition was known as diabetic dwarfism (West 1978). Recent studies have shown that growth and skeletal maturity are normal in insulin-dependent diabetics (Birbeck 1972; Weil 1967; White and Graham 1971), and that impairment of growth only occurred with sub-optimal control (Birbeck 1972). However, Jivani and Rayner (1973) did not observe any relationship between rates of growth and degrees of control in their diabetic children, who showed subnormal growth in the three years following the onset of diabetes. In a group of Japanese juvenile diabetic children studied, growth was retarded in those who had the disease for more than four years,(West 1978).

The fact that up to a third of the African and Indian patients were below the 5th percentile for weight suggests that IDDM does retard growth in these race groups. However, growth retardation was independent of duration of diabetes.

Although considerable confusion, controversy and uncertainty exists in regard to the role of heredity in the aetiology of diabetes, it is clear that genetic factors are quite important, (West 1978). Rosenthal et al (1976) concluded that "diabetes mellitus in man, regardless of age of onset, is primarily genetic in origin with environmental factors influencing only the time of appearance of the disease". Several groups of workers felt that heredity played a greater role in the aetiology of insulin dependent diabetes than of non-insulin dependent diabetes, (Falcolner 1967; Goodman and Chung 1974; Simpson 1969). On the other hand in studies on identical twins both Gottlieb et al (1974) and Barnett et al (1981) found only a fifty percent concordance rate for diabetes in those with insulin=dependent diabetes as compared to a rate of almost hundred per cent in those pairs in whom diabetes in the index case was of the non-insulin dependent type. Thus it appears that the principal genetic mechanisms involved are to a substantial degree separate for insulin-dependent and non-insulin-dependent diabetes mellitus (Barnett et al 1981; Pyke and Nelson 1976).

The 13% prevalence of a positive family history in Africans is similar to that observed by Cudworth et al (1977). A positive family history in over 50% of the Indian patients is a most unusual finding when cognisance is taken of the fact that many studies have shown a low prevalence of positive family histories in patients with IDDM. In a study involving 210 parents of juvenile onset diabetic patients in Israel only 5 of them were found to have the disease (Cohen et al 1970). Low rates of a family history of diabetes have also been found in Japanese (West 1978), and some tropical countries (Tulloch 1962; Wicks and Jones 1974).

Since in this study the relatives of the vast majority of the patients had NIDDM, it is difficult to gauge the significance of the results

obtained. It is possible though that the gene for NIDDM in some way confers an increased susceptibility to IDDM also in the Indian patients; whereas in the African patients a different genetic mechanism operates.

Distinct seasonal patterns in the onset of IDDM have been observed in many studies. Higher rates of onset in winter than in summer have been found in British and Irish children with IDDM (Bloom et al 1975, Gamble and Taylor, 1969) and in American Blacks with juvenile onset diabetes (Al-Arif and Strong 1981). Similar observations have been made among other white Caucasian populations (Christau et al 1977; Cudworth et al 1977; Danowski 1957; White and Graham 1971). However, the rate of onset also appeared to be especially common in January in some of these studies (Gamble and Taylor 1969; White and Graham 1971). In a large series in Massachusetts Gleason et al (1977) could find no significant seasonal variation in the onset of IDDM.

A study highlighting differences in the season of onset of diabetes mellitus should include a very large number of patients. Therefore, it is very difficult to assess the significance of any differences that are found in this study where a reliable history was obtained from 73 Africans and 33 Indians with IDDM. Nevertheless, if April to September are taken as the winter months in Durban, then there is a higher rate of onset in winter than in summer, particularly in the Indian patients in whom the rate of onset (twice as high in winter as in summer) was similar to that observed by Cudworth et al (1977).

Insulin-dependent diabetes characteristically presents acutely, although in many instances the onset may be subacute or even associated with long periods of ill health, (Bloom et al 1975; Cudworth et al 1977). The vast majority of the diabetic patients in this study presented acutely.

IDDM with a fulminant onset is more common in children and adolescents than in adults (Oakley et al 1973). Thus, as expected, in this study only five of the 15 patients whose onset was severe were over the age of twenty.

Despite the fact that the use of the term complications to describe the pathological manifestations of diabetes has now been challenged (West 1978), this term has been retained in this study since the abandonment of this traditional term has not yet met with widespread acceptance.

Diabetic coma due to ketoacidosis is today largely a preventable disorder in which social, economic, administrative and medical factors play a role, (West 1978). Whilst improved public education has reduced the frequency with which diabetic ketoacidosis marks the onset of previously undiagnosed diabetes mellitus, about 8 - 20% of cases of ketoacidosis in various series are still due to new cases (Biegelman 1971; Genuth 1980). In this study, however, almost 30% of those who developed ketoacidosis presented with this complication at the onset of the disease. Inadequate public education and primary health care facilities are probably responsible for such a high rate.

Discontinuation of insulin therapy and infection used to be the most important causes of ketoacidosis in both developed and underdeveloped countries (Baker, 1936, Biegelman, 1971, Bortz and Spoont 1967; Cohen et al 1960; Lester 1980; Thandanand et al 1978). Now, however, infection appears to be the main precipitating factor in most of the patients with diabetic ketoacidosis seen in the developed countries, with omission of insulin accounting for only 4 to 7% of cases. (Genuth 1980). Among the patients reported in this study both omission of insulin and infection are equally important in precipitating ketoacidosis. Again inadequate education, poor socioeconomic conditions and lack of primary health care facilities are to a large extent responsible for such a high frequency.

The reason for the slight but significant difference in the incidence of ketoacidosis between the 2 racial groups may be related to the relatively better socioeconomic status and educational background of the Indian patients.

Studies of the prevalence of diabetic neuropathy based on clinical criteria have yielded varying results because of a inadequate standardisation of the criteria and methods of investigation. A prevalence of

40% has been reported by Goodman (1955), of 12% by Pirart (1965) and of 4% by Rundles (1945). It is rare in children and uncommon in adolescents, occurring more frequently with increasing age, (Oakley et al 1973).

Peripheral neuropathy appears to be fairly common in both groups with IDDM. Studies in other African and Indian population groups have also found a fairly high incidence of diabetic neuropathy (Chuttani and Chawla 1974; Osuntokun et al 1971).

While in the majority of the Indian patients the duration of IDDM was more than 5 years, in 50% of the African patients the disease had been present for less than a year only. Thus it appears that in addition to duration of diabetes, as suggested by Gregersen (1977) other factors play a role in the development of neuropathy. An association with hyperglycaemia has been noted by Christensen (1973), Pirart (1965) and Thomas and Ward (1975).

Symptoms such as paraesthesia and numbness are the least sensitive indices of neuropathy (West 1978). Thus the rarity of symptomatic manifestations of this complication is not at all surprising.

Nilsson et al (1967) studied the significance of the ankle reflex in a group with no diabetes. He found an absent reflex in 25% of those over the age of 60 and in none under this age. In their young diabetic patients (age 20 - 39), 34% showed an absent or partly absent ankle jerk. 20% of the patients reported in this study had an absent ankle jerk and in over 40% of these patients the disease was present for less than 2 years. In the young diabetic patients studied by Nilsson et al (1967), 34% of the patients with neuropathy had had diabetes for a short duration (mean 1.5 years).

Some workers have considered an impaired vibration sense to be the earliest sign of peripheral neuropathy, (Mouren et al 1966). However, only 2% of the patients reported in this study demonstrated an impaired vibration sense. None of the young diabetic patients in the group studied by Nilsson et al (1967) showed impaired vibration sense.

Although motor function abnormalities such as weakness of the legs and foot drop do occur, they are much less common (Faerman et al 1980; Oakley et al 1973). None of the patients in this study showed any evidence of a motor neuropathy.

Although autonomic neuropathy depends on the duration of diabetes (Annon 1976; Low et al 1975), it has also been reported in the early stages of juvenile onset diabetes (Faerman et al 1971; Jadzinsky et al 1977).

The commonest manifestation of this form of neuropathy in adult males reported in this study was impotence, which is probably the most common symptom related to autonomic neuropathy. Its prevalence has been found to be up to 50 per cent in various series (McCulloch et al 1980; Rubin and Babbott 1958; Rundles 1945; Schoffling et al 1963). It may be present without other features of autonomic neuropathy (Ewing et al 1976). When other signs of autonomic neuropathy are present, impotence is almost invariable, (Clarke et al 1979).

Although the prevalence and natural history of diabetic autonomic neuropathy have not yet been clearly elucidated (Clarke et al 1979) it is still considered relatively uncommon (Ward 1978). Thus the rare occurrence of this complication in this study is not unexpected.

Retinopathy is a major cause of morbidity in many diabetic patients. Although clinically detectable lesions of the retina eventually develop in most patients, impairment of vision of varying degrees occurs in about 50 per cent of patients with long standing diabetes (West 1978). The prevalence of retinopathy appears to vary in different population groups, (West 1978). It is rare in young Caucasian diabetic patients with duration of diabetes less than 10 years (Kahn and Bradley 1975) but common in young Kenyan diabetics whose disease was present for a similar duration, (Steel et al 1977). Low rates have been reported in Nigeria even in patients with diabetes of long duration (Osuntokun 1969), in Congo (Bourgoignie et al 1962), in Rhodesia (Gelfand and Forbes 1963) and in Uganda (Otim 1975). According to Lal et al (1968) it did not appear to be uncommon in Indian insulin-dependent diabetic patients.

IDDM of more than 10 years was very commonly associated whith retinopathy in the African and Indian patients reported in this study. Comparison with other groups who had their illness for over 10 years shows a prevalence of 80 per cent in young Kenyan diabetics (Steel et al 1977), 14% in Nigerian diabetics (Osuntokun 1969) and 22% in Uganda (Otim 1975). In Sweden a frequency of 35.7 per cent was found in young diabetics who had the disease for 8 - 15 years and 100% of those who had it for more than 16 years (Nilsson et al 1967).

In young white diabetic patients seen at Joslin Clinic retinopathy was rare (2%) if the duration of the disease was less than 10 years (Kahn and Bradley 1975) whereas it was common (48%) in young Kenyans also falling into this category, (Steel et al 1977). The African and Indian patients in this study also did not appear to be particularly prone to this complication if IDDM was present for less than 10 years, although the frequency (7% and 12,5% respectively) was somewhat higher than the Joslin Clinic patients (2%) with a similar duration of disease (Kahn and Bradley 1975).

The rarity of proliferative retinopathy in this study may be due to the disease being present for a relatively short duration in the vast majority of the patients (mean 4.3 years). Nevertheless, this form of retinopathy appears to be uncommon according to Dorf et al (1976) and Nilsson et al (1967), who found prevalence rates of 7% and 2.9% respectively in diabetic patients with retinopathy. Knowles (1971), however, estimates that almost 50 per cent eventually develop this complication during the course of 30 years of diabetes.

Diabetic nephropathy is the major cause of death in insulin-dependent diabetic patients, (West 1978). It accounts for up to 40 per cent of deaths in IDDM (Marks and Krall 1971). Although the prevalence of nephropathy varies, its frequency has been found to be up to 30 per cent in patients who have had the disease for more than 15 years (Bjerkelund 1951).

Whilst proteinuria is a useful index of diabetic nephropathy, it is not very sensitive (West 1978). Thus the rarity of nephropathy in this

study may be due to the relatively insensitive method of detecting this complication, although the relatively short duration of IDDM in most of the patients may also play a part.

The 6 patients with nephropathy constitute 10% of those who had the disease for more than 7 years. Nilsson et al (1967) found a similar frequency of proteinuria in their young diabetic patients with duration of disease longer than 7 years.

All 6 patients had retinopathy, which has been found to be invariably associated with nephropathy (Bjerkelund 1951).

In the majority of cases cataracts complicating diabetes mellitus are of gradual onset (Oakley et al 1973). Such lesions have been found in 50 per cent of young diabetic patients after 11 years of disease (Knowles et al 1965). In this study, however, only 20% of the patients who had IDDM for more than 10 years manifested with this type of cataracts. All those with this complication had had the disease for more than a decade. Dense subcapsular cataracts which are rare and develop in some young patients who have had diabetes for a short time (Oakley et al 1973) were the more common type of cataracts seen and were only observed in the African patients.

An association between hypertension and diabetes has been found in many studies (Christlieb 1973; Jarret and Keen 1975; Vaishnava and Bhasin 1969). Obesity and diabetic glomerulsclerosis probably account for the increased frequency of hypertension in diabetic patients (West 1978). In IDDM hypertension is rare at the onset of the disease, with the frequency rising precipitously after 10 years of diabetes to such an extent that about 50 per cent of patients have hypertension after thirty years, (Christlieb 1973).

In this study, of the 6 patients who had hypertension only three patients had nephropathy as judged by proteinuria. Nevertheless, the other patients would probably have shown evidence of nephropathy had a more sensitive method to estimate urinary protein been used. All the patients, except one, had had IDDM for a long time. Such a finding is in keeping with Christlieb's observation (1973).

The increased frequency of tuberculosis in diabetic patients was observed by Avicenna as long ago as the 10th century (Root 1940; Root and Bloor 1939). Since the incidence of tuberculosis in Africans is very high even in the general population, it is not surprising that this infection should manifest itself in diabetic patients more commonly than in non-endemic areas. Thus the frequency rate of 6.5% for active pulmonary tuberculosis is much higher than in Jamacian diabetic patients who had a rate of 0.9% (Tulloch 1962) and in a group of American patients who had a rate of 2% (Dillon et al 1952). In previous studies rates of 2.5% (Seftel and Schultz 1961) and 4.5% (Campbell and McNeill 1959) have been reported among Africans in South Africa. Deshmukh et al (1966) found a prevalence rate of 8.3% among known patients who had routine chest radiographs done.

The insulin requirements in both the African and Indian patients are higher than those in White Caucasians from other countries, where the average dose is about 1.00 u/kg/day. (Larsson 1977). Although it has been suggested than an insulin dose greater than 1 unit per kilogram per day may be a sign of over-treatment (Tattersall 1978), very few patients manifested with signs of overtreatment which include frank hypoglycaemic episodes, wide swings in urine and blood glucose, negative evening urine tests with 2% glycosuria next morning, refractive changes, and hepatomegaly and oedema (Tattersall 1978). A possible reason for the relatively high insulin requirements in these patients is that since many of the patients find it difficult to afford a strict diabetic diet, the dose of insulin has to be increased to improve metabolic control.

SUMMARY

Insulin dependent diabetes mellitus (IDDM) with onset below 35 years of age was studied in 92 African and 41 Indian patients. The mean age of onset was lower and the mean duration of disease longer in the Indian compared to the African patients. African patients tended to be heavier than the Indian patients. A positive family history of diabetes mellitus was obtained in over 50% of Indian, but in olny 13% of African patients. Onset of IDDM was more common in winter than in summer, particularly among Indian patients. The initial presentation in more than 90% of the patients was acute, with severe ketoacidosis in 11%. Almost 70% of the African and 50% of the Indian patients developed ketoacidotic coma on one or more occasions. It was a presenting feature in over 50% of the former and 25% of the latter groups. Most of the other complications were related to the duration of IDDM. The mean daily insulin requirements were high in both population groups.

CHAPTER IV (A)

CLINICAL FEATURES OF NONINSULIN-DEPENDENT DIABETES IN YOUNG AFRICANS AND INDIANS

INTRODUCTION

Non insulin-dependent diabetes in the young has only relatively recently been recognised as a distinct entity (Fajans and Conn 1968; Fajans et al 1978; Tattersall and Fajans 1975). It is relatively uncommon compared to the classical NIDDM seen in the middle and old age groups and has been reported from England, the United States, Denmark, India and Japan (West 1978). Though behaving like NIDDM of middle and old age (Fajans et al 1978), the disease has been shown to be associated with far less morbidity in some studies (Barbosa et al 1978; Tattersall 1974).

Reference to NIDDM in the young in South Africa as an entity distinct from the J-type or K-type diabetes of the tropics was first made in 1960 but some of its features were obscured by its inclusion with a type of diabetes mellitus considered to be due to hyperadrenalism and growth hormone deficiency (Campbell 1960; 1963).

MATERIAL AND METHODS

18 Africans and 61 Indians who fulfilled the following criteria were selected and studied:

1. Age at diagnosis under 35 years
2. Duration of diabetes greater than 12 months
3. Control of symptoms on diet with or without oral hypoglycaemic therapy only
4. Absence of ketosis without insulin therapy

All the patients described in this study had the following data recorded:

1. Age, sex, height, weight and percentage ideal bodyweight, which was

calculated by comparing the actual weight to the ideal body weight of a medium frame person of the same height and age group (Documenta Geigy 1970).

2. Age at diagnosis
3. Duration of diabetes
4. Family history
5. Mode of presentarion

In addition a full physical examination was carried out on each patient. Peripheral neuropathy was assessed on the basis of the criteria outlined in Chapter II. Fundal examination was done after instillation of a mydriatic (as described in Chapter II). Nephropathy was assessed by measurement of urinary protein and plasma creatinine levels. If either of these parameters was abnormal, a GFR using radiolabelled Chromium^{51} EDTA was done.

RESULTS

Results are shown in Table 1 - 3 and Figure 1 - 3.

Age at diagnosis and mean duration of NIDDM: Figure 1 shows the distribution of patients in relation to the various age groups at which NIDDM was diagnosed. The mean age at diagnosis in the African patients was 29 years (range 17 - 35) and in the Indian patients 27.3 years (range 17 - 35 years). The mean duration of the disease was 6.8 years (range 2 - 32) in the Indian patients and 4.3 years (range 2 - 12) in the African patients, Figure 2.

Family History:
A positive history of diabetes in a first degree relative was present in almost 80% of the Indian patients, whereas it was less common in African patients, being present in only 39% of them. All the affected relatives in both groups had diabetes of the non-insulin-dependent type.

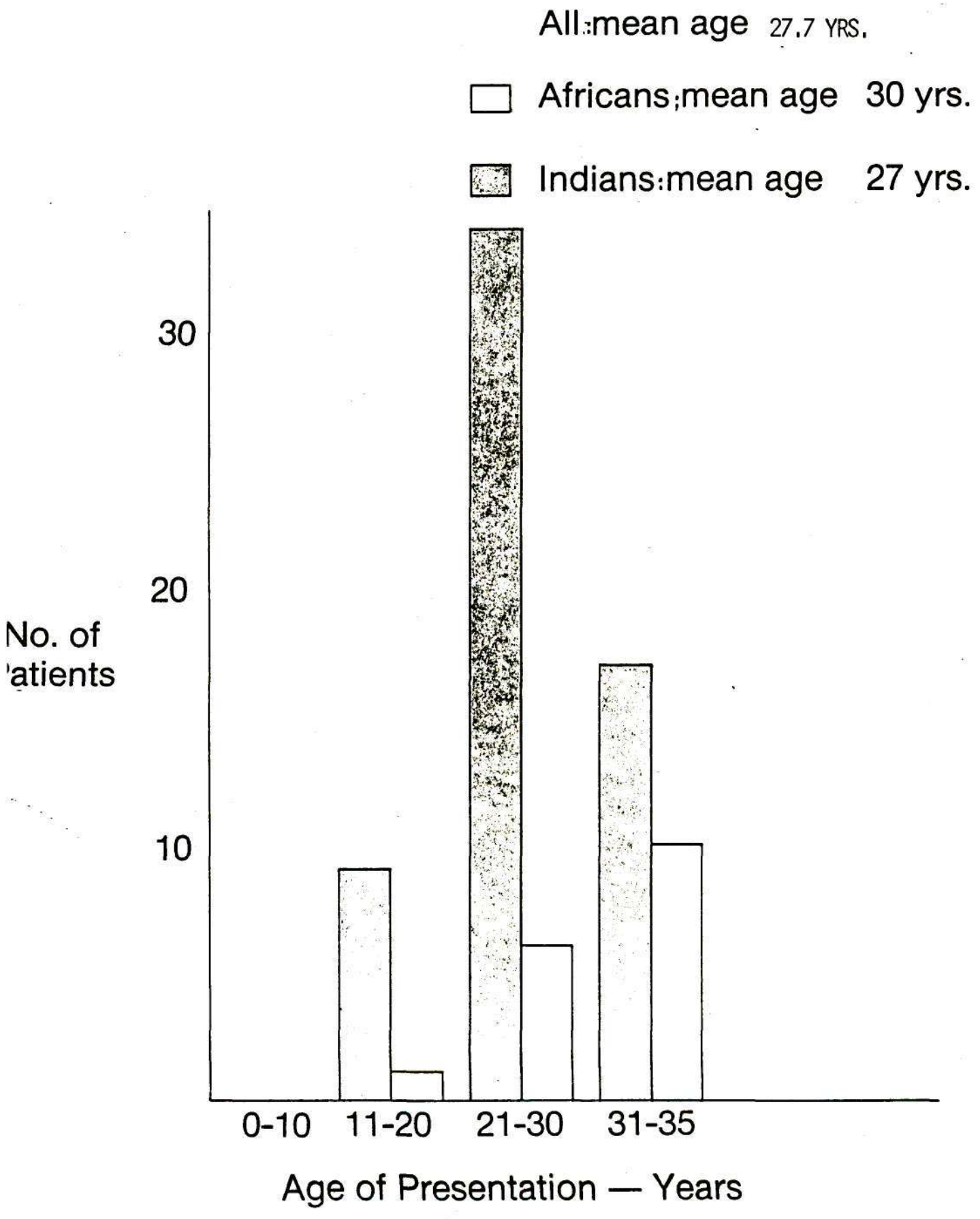
In almost two thirds of the Indian patients at least one parent had NIDDM whilst in 20% of the cases both parents had the disease. A third of the propositi had a sibling suffering from the disease. All 17

TABLE 1 CLINICAL CHARACTERISTICS OF PATIENTS WITH NIDDM

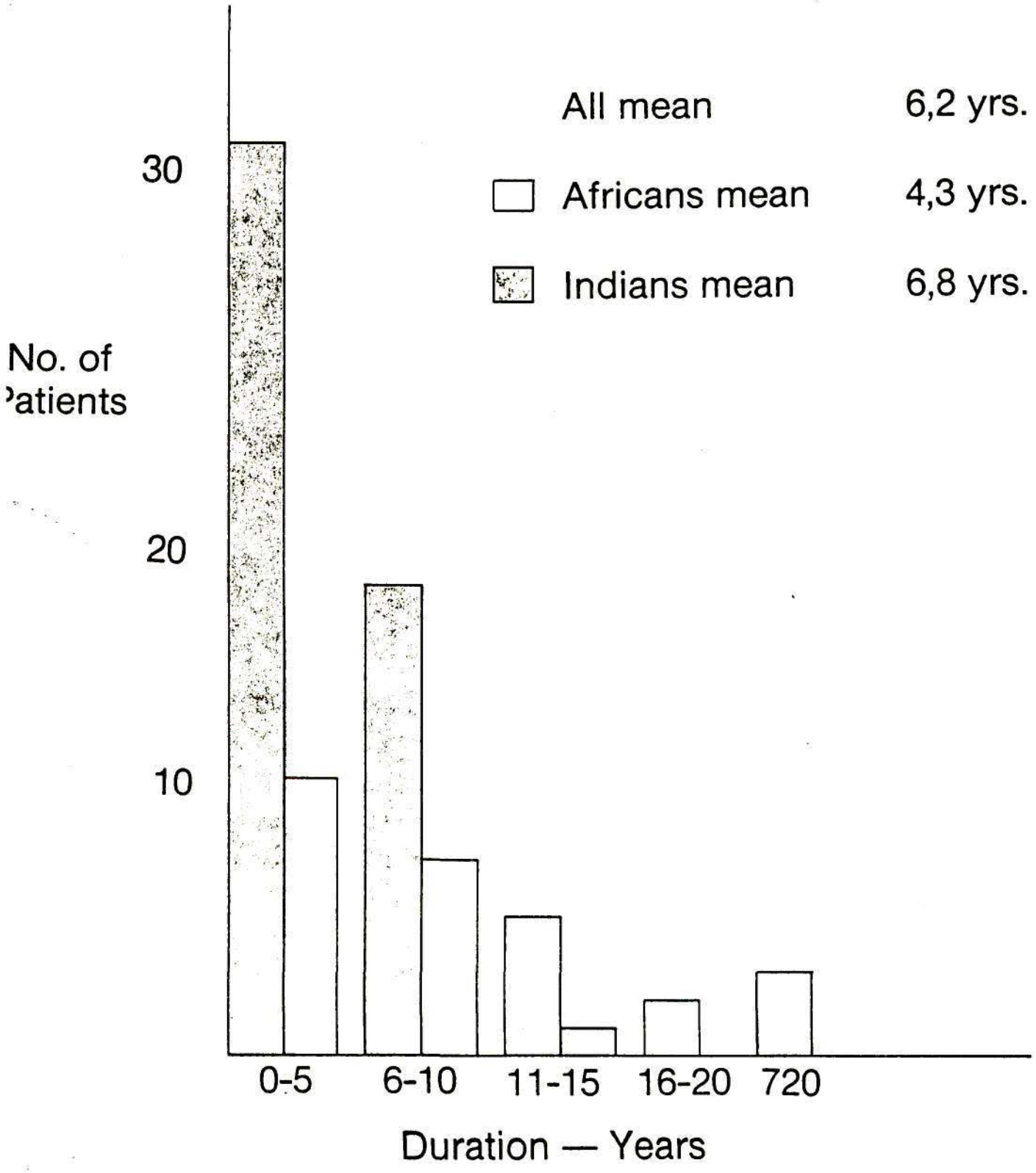
	Africans	Indians	Total
Number of patients	18	61	79
Male : female ratio	4:14	17 : 44	21 : 58
Mean age of diagnosis in years	30 (17-35)	27 (17-35)	27.7
Mean duration of disease in years	4.3 (2-12)	6.8 (2-32)	6.2
Mean percentile desirable body weight	126 (82-164)	116.8 (84-172)	118.9
Family history	7	47	54

Figure 1

The Distribution of Patients in Relation to the Age of Presentation of N.I.D.D.M.



The Pattern of Duration of Diabetes in Patients with N.I.D.D.M.



African patients had at least one parent suffering from the disease.

Sex Incidence:

There was a female preponderance (F:M = 3 : 1) in both racial groups.

Weight:

Figure 3 illustrates the distribution of percentage desirable weights in the Africans and Indians with NIDDM. The mean percentage desirable weights in the 2 groups are 126% (range 82% - 164%) and 116.8% (range 87% - 152%), but the difference falls short of statistical significance. Almost 40% of the African and Indian patients were obese, i.e. body weight greater than 20% of ideal body weight.

Both the African and Indian patients with NIDDM were significantly heavier than their corresponding counterparts with IDDM ($p < 0.0025$ and $p < 0.0005$ respectively).

Mode of Onset:

In both groups over 60% of the patients had an insidious onset characterised by non-specific symptoms of tiredness, malaise and nocturia. In 11% of the patients the onset was subacute with polydipsia and polyuria. A similar proportion of patients presented for the first time in pregnancy with the diabetic state persisting for more than a year later in all of them. In 9% of patients NIDDM was diagnosed after routine examination had shown glycosuria.

Complications:

Tables 2-3 depicts the frequency of complications in the African and Indian patients described in this study.

Peripheral Neuropathy:

16 Indian patients (27%) had neuropathy, whereas only 1 African patient showed this complication. The mean duration of the disease in all the patients with peripheral neuropathy was 10 years (1 - 32 years).

Only one patient had symptoms of neuropathy, whilst 17% showed absent ankle reflexes. Less common signs were impaired vibration sense seen in

Figure 3

THE DISTRIBUTION OF PERCENTAGE DESIRABLE WEIGHTS IN ADULTS (over age 25) WITH NIDDM

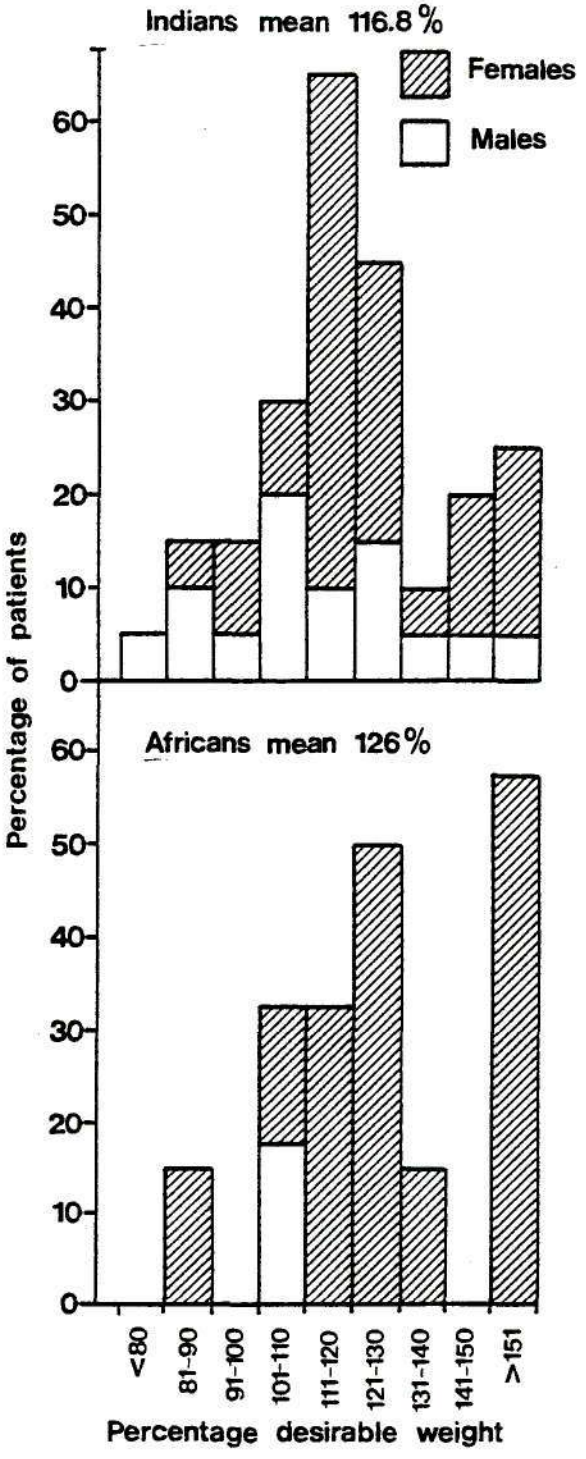


TABLE 2

PREVALENCE OF COMPLICATIONS IN AFRICAN AND INDIAN PATIENTS WITH NIDDM

COMPLICATIONS	AFRICANS	INDIANS	TOTAL
Neuropathy	1 (6%)	16 (27%)	17 (22%)
Retinopathy	3 (17%)	16 (27%)	19 (24%)
Nephropathy	1	5 (8%)	6 (8%)
Hypertension	-	1 (2%)	1 (1.3%)
Ischaemic Heart Disease	-	1 (2%)	1 (1.3%)
Peripheral Vascular Disease	-	2 (3%)	2 (2.5%)
Cataracts	-	-	-
Tuberculosis	-	-	-

TABLE 3

MEAN DURATION OF NIDDM IN PATIENTS WITH COMPLICATIONS

	Mean duration in Years (range)		
	AFRICAN	INDIAN	ALL
Neuropathy	1/12	10.9 (0-32)	
Retinopathy	2	10.5 (2.32)	
Nephropathy	6	10.6 (5-19)	
Hypertension	1	5	
Ischaemic Heart Disease		18	
Peripheral Vascular Disease		14.5 (14-15)	

4 patients and impaired position sense found in 2 patients, whilst motor deficit was not present in any of the patients.

Autonomic Neuropathy:
None of the patients had evidence of autonomic neuropathy.

Retinopathy:
Background retinopathy was present in 3 African patients (17%) and 16 Indian patients (27%). The mean duration of known disease in the 2 groups was 2 years (range 2 - 4 years) and 10.5 years (range 2 - 32 yrs) respectively. Proliferative retinopathy was present in 1 patient only.

Nephropathy:
Nephropathy was present in 5 Indian and 1 African patient. All the patients had albuminuria. The mean duration of NIDDM in these patients was 10.6 years (range 5 - 19 yrs). In 4 patients the duration of disease was less than 7 years. The mean glomerular filration, which could be done on 3 of the patients, was 51 ml/minute (range 21 - 76).

Large Vessel Disease:
Only 3 patients had evidence of macrovascular disease, 2 showing peripheral vascular disease and 1 ischaemic heart disease.

Hypertension:
Hypertension was rare, being present in only one patient.

DISCUSSION

As NIDDM in the young has been known for a relatively short time, it is hardly surprising that little information is available on the epidemiology of this entity, in contrast to the extensive information on prevalence and incidence rates of NIDDM in the adult (West 1978). A crude estimate of the actual prevalence rates of NIDDM in the young may be obtained by analysis of studies which give information on the percentage of diabetics who are not on insulin (West 1978). In the National Health Survey of 1964 - 1965 almost 27% of diabetic patients under 25 years of age were not on insulin (Bauer 1967) whilst in a survey of 2 816 school children with the disease in Michigan, 15.5% were noninsulin-dependent (Gorwitz et al 1976). According to the data from the National Centre for Health Statistics only a maximum of 34% of diabetic patients under

the age of 39 were on insulin (Sayetta and Murphy 1979).

In the series of 207 Indian diabetic patients documented by Campbell (1960), 16% fell into the category now known as NIDDM in the young.

In this study NIDDM in the young has been found to be rare in Africans, accounting for only 1.7% of the total diabetic population, but much more common in Indians accounting for 11% of all diabetic patients. Thus if the overall prevalence of diabetes in Africans and Indians is taken as 0.7% and 1.8% respectively (West 1978), then the prevalence rate for NIDDM in the young is 0.01% in Africans and 0.2% in Indians. In a survey done in Erfurt, East Germany, the prevalence rate for the disease was found to be .15% (Panzram and Adolph 1981).

Since NIDDM in the young usually presents insidiously, just as in adults, the age of diagnosis does not necessarily coincide with the onset of the disease. Hyperglycaemia may be present for a long time prior to diagnosis. The age of presentation appears to be earlier in Indians, as 50% of the African patients presented with the disease in the 4th decade whereas a similar proportion of Indian patients did so in the 3rd decade. An attempt to compare the age of diagnosis of the patients in this study with those elsewhere is hampered by the fact that different workers have chosen their own arbitary age cut-off level for categorising diabetes in the young. Thus 14 - 20 years (West 1979), 25 years (Fajans et al 1976), 29 years (Christau et al 1977), 31 years (Deckert et al 1978) and 40 years (West 1978; Seltzer et al 1967) have all been chosen as age cut-off levels for diabetes in the young. Nevertheless the mean age of onset of NIDDM in Africans (29 years) and Indians (27 years) is well within the 35-year cut off level selected for this study.

Since the first detailed description of NIDDM in the young (Fajans and Conn 1968) several workers have observed a striking familial occurrence of this entity (Fajans et al 1976). Tattersall (1974) described in detail 3 families with 69 offsprings and proposed that NIDDM in this group of patients was inherited in an autosomal dominant fashion.

Although a positive family history of diabetes in the African with NIDDM was less common than in the Indian patients, it was still much more common when compared to African insulin-dependent diabetic patients. NIDDM in Indians also was much more often associated with a family history of diabetes than IDDM.

In a group of 26 young patients with NIDDM Tattersall and Fajans (1975) found diabetes in at least one parent in 85 per cent of the propositi. In addition, 53% of the siblings were found to be diabetic. Studies on 58 such patients by Panzram and Adolph (1981) showed the presence of diabetes in at least one parent in 74% of the propositi. Although diabetes in either parent or in siblings appears to be less frequent in the Indian or African patients in this study, the frequency may be higher if all the family members are subjected to a glucose tolerance test, as had been done by Tattersall and Fagans (1975) and Panzram and Adolph (1981). In this study, data on family history was obtained by questionnaire alone and as NIDDM can often be asymptomatic, cases of undiagnosed IDDM in family members were not accounted for by this method. Thus it is quite possible that NIDDM in the young Indian particularly is associated with an autosomal mode of inheritance. Vague et al (1981) postulate that heredity is compatible with autosomal dominance not only in typical NIDDM of the young (MODY) but in NIDDM presenting at any age irrespective of the presence or absence of obesity.

NIDDM in middle age (classical maturity onset diabetes) is associated with female dominance of varying degrees (West 1978). The W.H.O. (1964) found a mean male : female ratio of 1 : 1.5. In some communities however, no difference of a male predominance has been found (West 1978). Among Indian diabetics a slightly higher male predominance was observed (West 1978). In Uganda also the rate among males is higher (Ajgaonkar 1970).

In contrast to the extensive data on maturity onset type diabetes, little information is available on the sex differences in NIDDM in the young. Spiegelman and Marks (1946) found a slightly higher rate of diabetes among young women than young men in Massachesettes in U.S.A. In the U.S.S.R. the rate was four times as high in females between 30 - 40 years

of age as in males of the same age group, (West 1978).

In a series of 26 young non-insulin-dependent diabetics reported by Tattersall et al (1975), only 34% were females. In this study, however, the disease appeared to be much more common in females.

NIDDM in the young shows a wide variation in the weights of individual patients (West 1979). In the 78 patients with the disease studied by Fajans et al (1976) only 18 (23 per cent) were found to be obese, i.e. body weight greater than 20 per cent of ideal body weight. However, Vague et al (1981), found obesity in 50% of their patients. In this study also obesity was common in both African and Indian patients with NIDDM. Cosnett (1959) and Campbell (1960, 1963) also commented on the frequency of obesity in the young Indian diabetic patients they had studied.

It is possible that a diet leading to obesity is also an important environmental factor in the aetiology of NIDDM in the young, as has been suggested for the typical maturity onset type diabetes of middle and old age (Fajans et al 1978). In this regard studies have been done showing that small increases in weight can exacerbate glucose intolerance and precipitate fasting hyperglycaemia (Tattersall and Fajans 1975; Radder and Terpstra 1975). Hence it is likely that obesity together with genetic factors play an important role in the pathogenesis of NIDDM in many of the young Africans and Indians reported in this study.

Although NIDDM in the middle age may present with symptoms, the presentation is insidious and unlike the abrupt onset characteristic of the classical juvenile onset type diabetes mellitus (IDDM) (Tattersall and Fajans 1975). Such an insidious onset is also true of NIDDM in the young, (Tattersall 1974). Thus it is hardly surprising that the vast majority of African and Indian patients also presented in such a fashion.

Of the 9 young patients with NIDDM described by Johansen (1973), 3 patients presented with mild symptoms, 5 were asymptomatic (NIDDM

being discovered on routine urine examination and 1 patient presented in pregnancy. It is difficult to compare the presentation in these patients to Fajans's group of 78 patients (Fajans et al 1976) because many of his patients were discovered after glucose tolerance tests had been done in the family members of the index cases.

Tattersall (1974) and Barbosa et al (1978) have commented on the rarity of vascular complications in their young patients with NIDDM. Others, however, have found that such complications are by no means uncommon (Fajans et al 1978; Steel et al 1976) and that the frequency may be the same as in NIDDM presenting in the middle age or the elderly (Fajans et al 1978). In Fajans' study, over 50% of the young non-insulin dependent diabetics who had the disease for more than 15 years had evidence of microvascular or macrovascular disease, thus showing that NIDDM in the young is not necessarily a benign disease (Fajans et al 1978).

As stated in Chapter III, the prevalence of diabetic neuropathy based on clinical criteria has varied from 4 per cent to 40 per cent owing to a lack of uniformity in the definition of neuropathy and in methods of investigation.

Although in 3 of the patients with peripheral neuropathy NIDDM had been diagnosed less than 2 years previously, it is possible that the metabolic derangement had been present for a much longer time. Hence the difference between the duration of the disease in the young insulin dependent and the non-insulin-dependent diabetics with peripheral neuropathy becomes all the more significant, since the mean duration in the former (4.5 years) is less than half of that in the latter.

Based on simple screening tests as outlined in Chapter II, none of the patients showed evidence of autonomic neuropathy, which is still considered relatively uncommon (Ward 1978). Nevertheless, many patients, although asymptomatic have been found to show evidence of autonomic dysfunction using more sensitive methods (Thandroyen et al 1980).

Retinopathy was present in almost 60% of Indian patients who had NIDDM for more than 10 years. Fajans et al (1978) found a prevalence of 18

per cent in patients who had the disease for more than 15 years. In Tattersall's series (1974) however, only 2 out of 14 patients with a mean duration of NIDDM of 24 years had evidence of retinopathy. Thus retinopathy appears to be particularly common among young Indians with NIDDM. Only one patient, an Indian who had known disease for 12 years, showed evidence of proliferative retinopathy. This complication has also been found to be rare in other studies (Dorf et al 1976; Nilsson et al 1967).

Nephropathy is a less common cause of death in late onset diabetes than in childhood diabetes (Marks and Krall 1971). Data on the frequency of this complication in NIDDM in the young suggest that it is very rare (Tattersall 1974). In the series of 69 patients reported by Fajans et al (1978) only one had nephropathy. The duration of disease in him was more than 15 years. None of Tattersall's patients (1974) had nephropathy as gauged by the presence of proteinuria, despite a mean duration of disease of 24 years.

It would appear that nephropathy tends to occur earlier in young Indian patients with NIDDM, than in those with IDDM, since none of the 16 insulin-dependent patients who had the disease for 5 to 10 years developed this complication, compared to 19% of the non-insulin dependent diabetic patients with a similar range of duration of illness. It is unlikely that poorer metabolic control in patients with NIDDM could account for this difference, because in general they show lower glycosylated haemoglobin values than patients with IDDM (Chapter VIII). Thus the development of nephropathy may be independent of metabolic control in such patients and may be related to other causes, e.g., genetic factors. However, the longer interval between onset of metabolic abnormality and diagnosis of NIDDM could also account for the higher frequency of this complication in such patients. In older non-insulin-dependent diabetic patients also nephropathy has been found to develop earlier when compared to patients with IDDM (Martin and Stocks 1968).

Macrovascular disease appeared to be uncommon in the African and Indian diabetics with NIDDM reported in this study as only 2 patients had peripheral vascular disease and 1 had ischaemic heart disease. In the

series reported by Fajans et al (1978), 13 per cent of their young patients with NIDDM had peripheral vascular disease,with a similar proportion developing myocardial infarction. However, almost 50 per cent of their patients had had the disease for more than 15 years and most of the complications were present in this group. On the other hand in this study there were only 6 patients with duration of disease more than 15 years and this could explain the rarity of ischaemic heart disease and peripheral vascular disease. Nevertheless, large vessel disease has been found in one study to be uncommon in young non-insulin-dependent diabetic patients (Tattersall 1974).

SUMMARY

Non insulin dependent diabetes mellitus (NIDDM) with onset below 35 years of age was studied in 60 Indian and 18 African patients. There was a female preponderance in both groups. The mean age at diagnosis was 27.3 years in the Indian and 29 years in the African group. The former group had a longer mean duration of NIDDM than the latter (6.8 years and 4.3 years respectively). A positive family history of diabetes mellitus was obtained from over 80% of the Indian and less than 40% of the African patients. Obesity was common in both racial groups, almost 40% of patients having a percentage ideal body weight of more than 120%. The mode of presentation was symptomatic in most patients, although the onset was often insidious. Microvascular complications were detected in almost a third of the patients, but macrovascular complications were rare.

CHAPTER IV (B)

NON INSULIN - DEPENDENT DIABETES PROGRESSING TO INSULIN DEPENDENCE

INTRODUCTION

In the course of our study on diabetic patients with age of onset under 35 years, we found a considerable number who could not be categorised as either insulin dependent or non-insulin dependent according to the criteria used in this study. These were patients who to all intents and purposes presented initially like the typical NIDDM, i.e. with an insidious onset and were responsive to diet with or without oral hypoglycaemic drugs for a number of years (mean 9.8 years) without developing ketosis during that period. However, because of development of symptoms during later years they had to be treated with insulin. These patients were thus insulin-dependent but unlike the typical ketosis prone IDDM described in Chapter III, became so much later.

It is quite likely that the major difference between the typical NIDDM and this entity (NIDDM progressing to IDDM) is one of duration, the latter representing progression of the disease in a way similar to the typical NIDDM of older age group decompensating to insulin-dependent diabetes with time. However, since there may be other factors as well, they will be discussed separately.

MATERIALS AND METHODS

19 Indian patients and 5 African patients fell into this group according to the following criteria:

1. Age at presentation of diabetes under 35 years.
2. Control of symptoms by diet with or without oral hypoglycaemic therapy for at least 2 years after diagnosis (Fajans et al 1976).

3. Absence of ketosis without insulin therapy during the entire period of dietary therapy with or without oral hypoglycaemic drugs.
4. Eventual dependence on insulin because of symptoms.

The following data were recorded in all the patients:-

1. Sex, age, height, weight and percentage of ideal body weight, calculated as shown earlier in the chapter.
2. Age at diagnosis.
3. Duration of diabetes.
4. Interval between diagnosis of diabetes and commencement of insulin therapy.
5. Episodes of ketosis (after commencement of insulin therapy).
6. Family history of diabetes.
7. Mode of presentation.

In addition a full physical examination was carried out on each patient. Peripheral and autonomic neuropathy were assessed according to the criteria outlined in Chapter II. Fundal examination was done after instillation of a mydriatic (as described in Chapter II). Nephropathy was assessed on the basis of proteinuria and elevated serum creatine levels.

RESULTS

Results are shown in Figures 4 - 7 and Tables 4 - 5. A comparison of the various clinical parameters and complications between patients belonging to this group and those who have always been non-insulin dependent is shown in Table 4.

The mean age of onset in African and Indian patients was 27 years (range 14 - 34). The disease tended to occur earlier in the Indian (mean 25 years, range 14 - 35 years) than African patients (mean 29 years, range 22 - 34). The mean age of onset of the disease in these patients was similar to that of patients who had always been non-insulin-dependent (mean 26 years). However, as can be seen in Figure 5 the mean duration of diabetes in the former (mean 14 years, range 5 - 26 years) was much longer than in the latter (mean 6.2 years), range 1 - 32 years). The mean interval between diagnosis and commencement of insulin therapy was 9.8 years (range 4 - 25 years). Figure 6.

TABLE 4 COMPARISON OF THE CLINICAL CHARACTERISITCS AND PREVALENCE OF COMPLICATIONS IN PATIENTS WHO HAVE ALWAYS BEEN NON-INSULIN DEPENDENT (NIDDM) AND THOSE WHO WERE INITIALLY NON-INSULIN DEPENDENT BUT LATER REQUIRED INSULIN.

	NIDDM Progressing to Insulin Dependence			NIDDM (always)		
	Africans	Indians	Total	Africans	Indians	Total
Number of patients	5	19	24	18	61	79
Male : female ratio	1 : 4	6 : 13	7 : 17	2 : 7	19 : 44	21 : 58
Mean age of diagnosis in years	29	25	25.8	30	27	27.7
Mean duration of disease in years	6.2	14	12.4	4.3	6.8	6.2
Mean percentile desirable weight	135	122	125	126	116.8	118.9
Family history	2(40%)	15(79%)	17	7(39%)	47(79%)	54
Complications (Prevalence)						
Neuropathy: Peripheral	1(20%)	12(63%)	13(52%)	1(6%)	16(27%)	17(22%)
Autonomic	-	2(11%)	2(8%)	-	-	-
Retinopathy	1(20%)	15(79%)	16(67%)	3(17%)	16(27%)	19(24%)
Nephropathy	-	6(32%)	6(25%)	1(5.5%)	5(8%)	6(8%)
Hypertension	-	6(32%)	6(25%)	-	1(2%)	1(1.3%)
Ischaemic Heart Disease	-	2(11%)	3(8%)	-	1(2%)	1(1.3%)
Peripheral Vascular Disease	-	2(11%)	2(8%)	-	2(3%)	2(2.5%)
Cataracts	-	1(5%)	1(4%)	-	-	-
Ketoacidosis	2(40%)	6(32%)	8(33%)	-	-	-
Tuberculosis	2(40%)	-	2(8%)	-	-	-

Figure 4

THE DISTRIBUTION OF PATIENTS IN RELATION TO THE AGE OF PRESENTATION IN THOSE PROGRESSING FROM NIDDM TO INSULIN DEPENDENCE

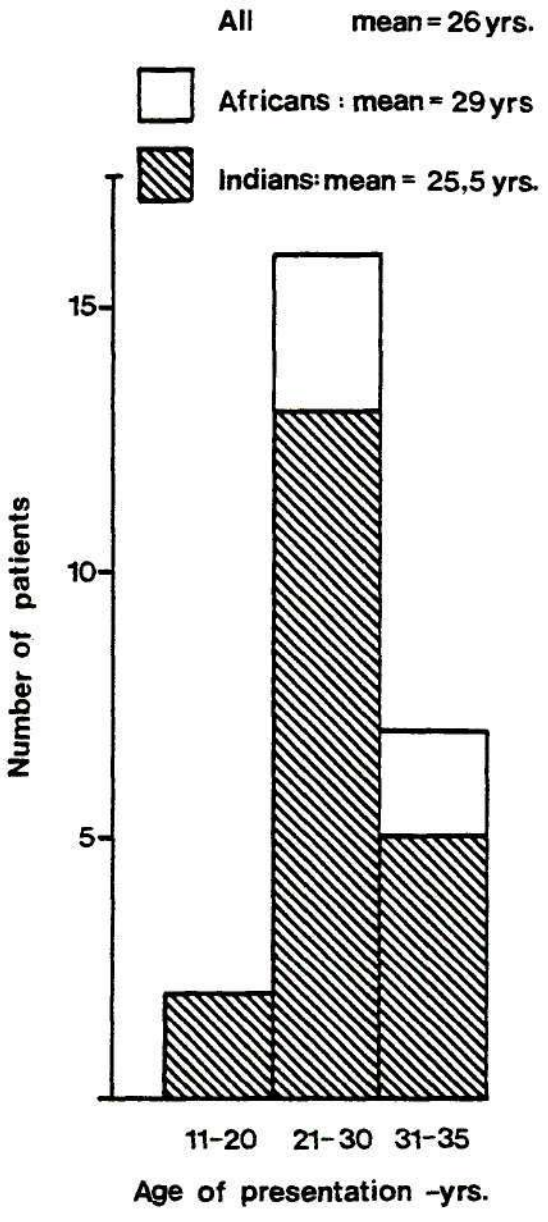


Figure 5

The Distribution of Percentage Ideal Body Weights in Adults Presenting with N.I.D.D.M. and later progressing to Insulin Dependence

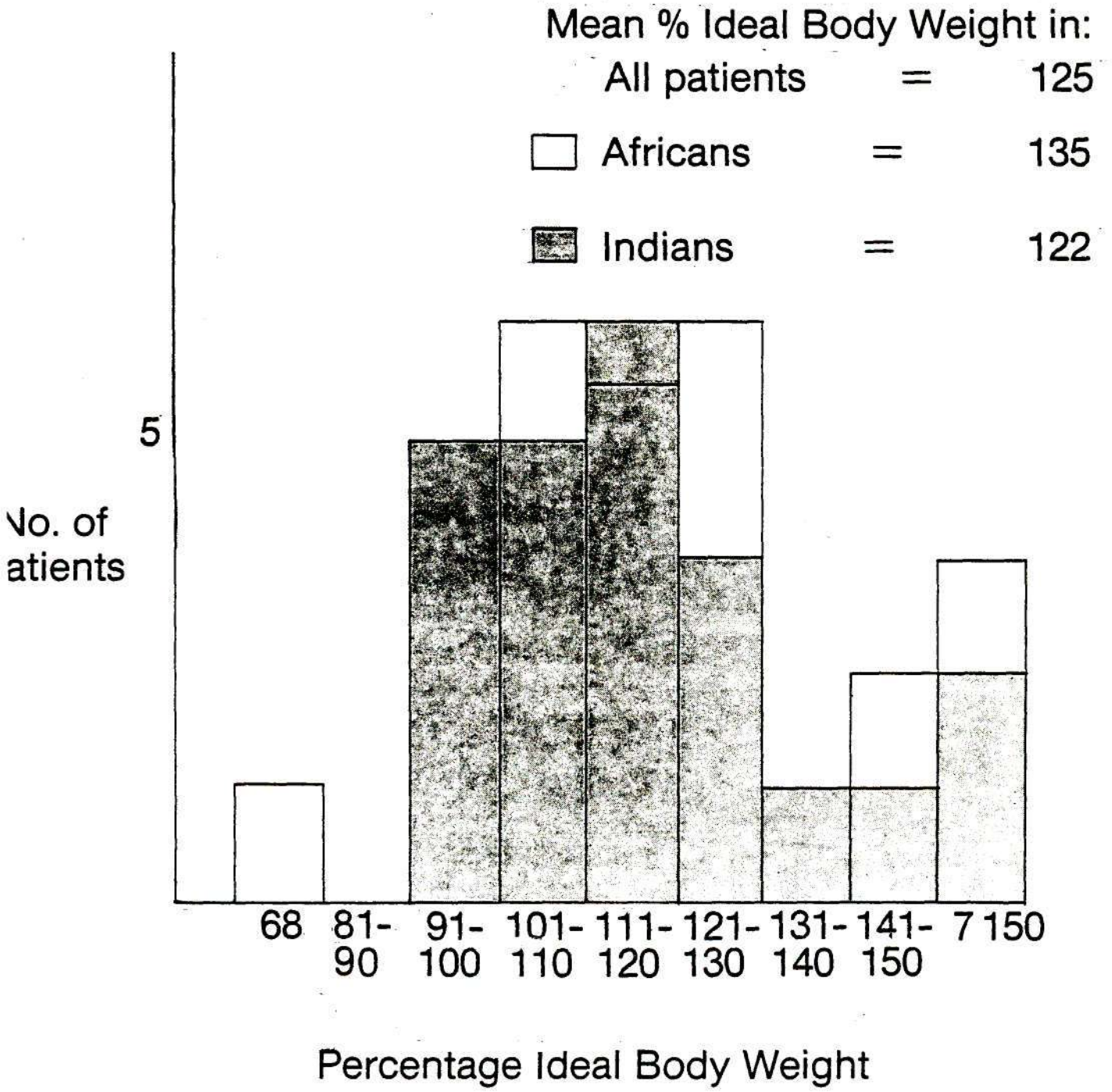
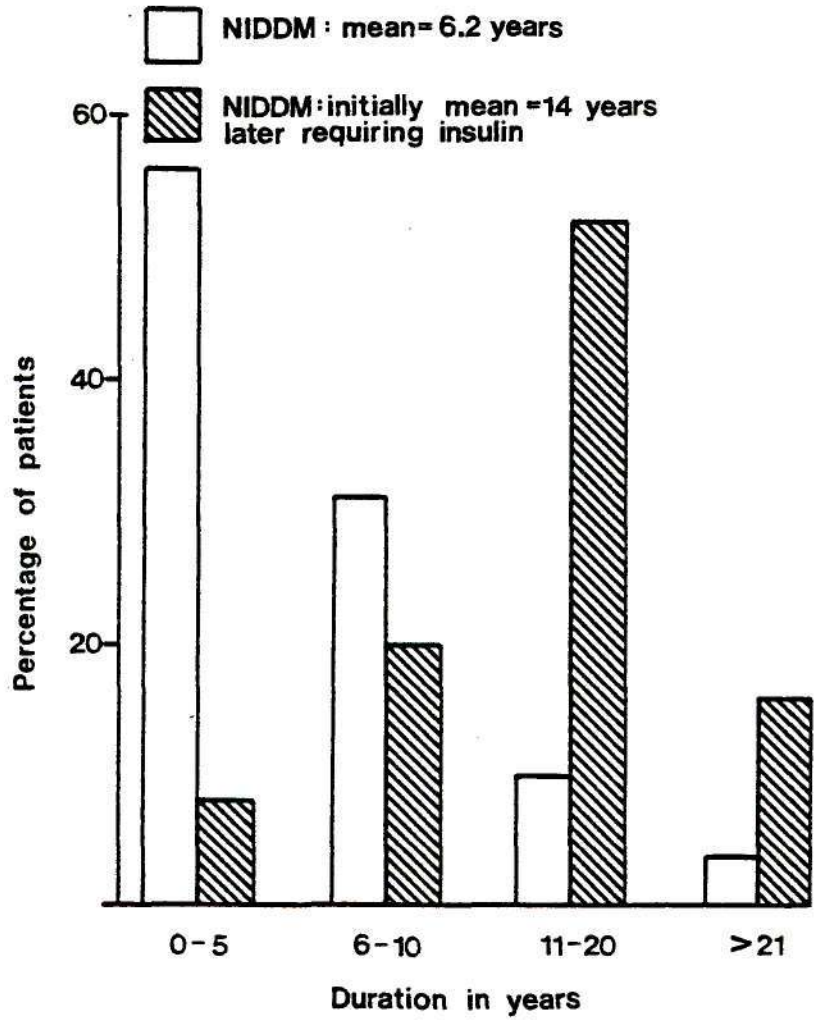
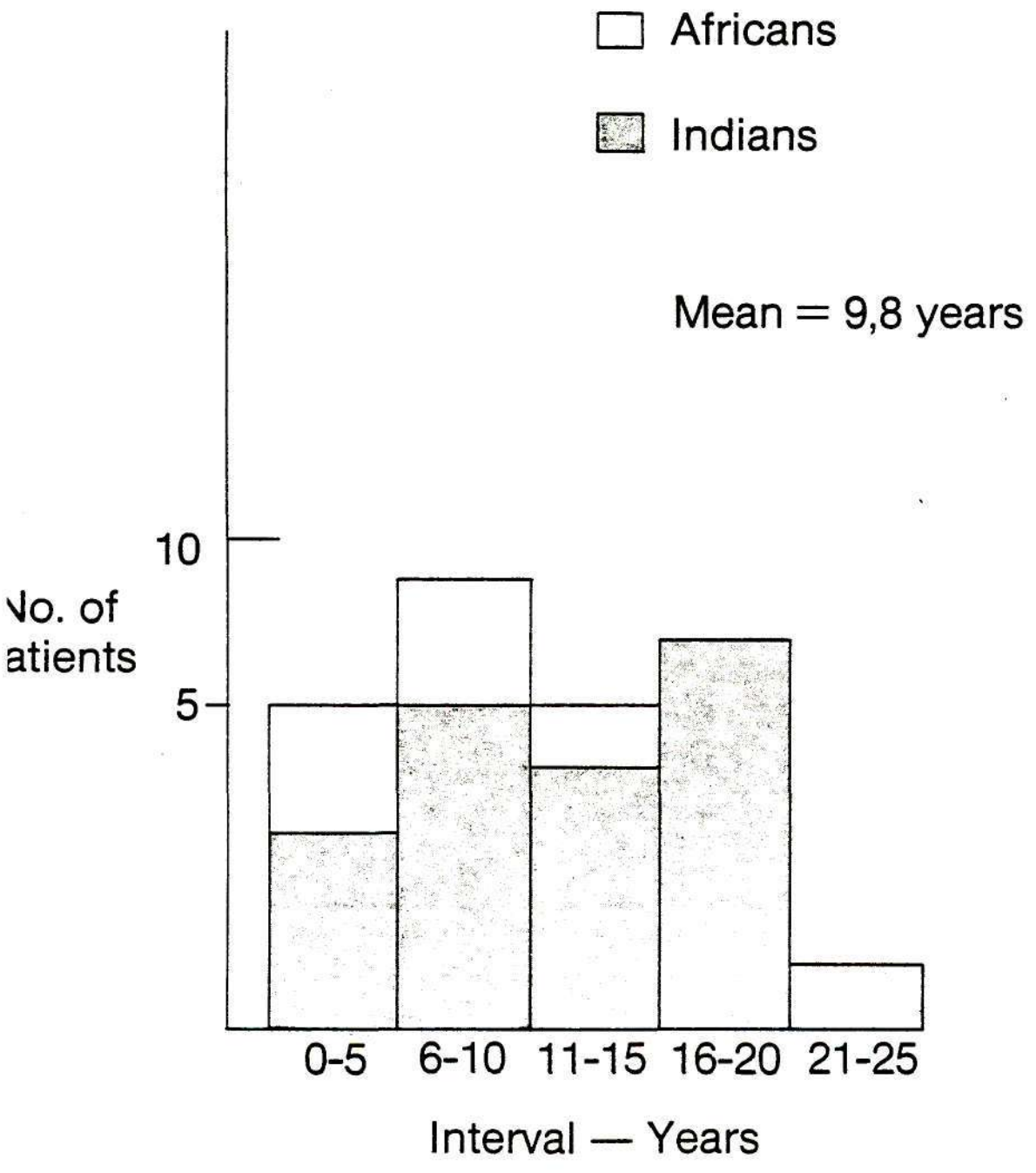


Figure 5

COMPARISON OF THE DURATION OF DIABETES IN PATIENTS WHO HAVE ALWAYS BEEN NON INSULIN-DEPENDENT (NIDDM) AND THOSE REQUIRING INSULIN LATER



The Distribution of the Intervals between Presentation (with N.I.D.D.M.) and Commencement of Insulin Therapy



In 71% of the patients there was at least one first degree relative suffering from diabetes mellitus, which was of NIDDM type in all of them. All patients with a positive family history had at least one parent suffering from NIDDM. Whilst in over 50% of the patients both parents were suffering from the disease, the mother was affected in everyone of them.

Obesity was common, as can be seen from the distribution of percentage desirable weights depicted in Figure 6. Over 40% of the patients were found to have a weight greater than 120% of ideal body weight. Even in patients who had always been non insulin-dependent a similar proportion had a body weight greater than 20% of IBW, although they had a lower mean percentage ideal body weight (116.8% vs 125%).

Complications

Table 5 shows details of the complications found in the patients belonging to this group. Comparison of the frequencies of complications between these patients and those who have always been non insulin-dependent (NIDDM) shows that they are much more common in the former group, Table 4.

Peripheral Neuropathy

Although an absent ankle jerk was the commonest abnormality of peripheral nerve function (present in 56% of patients), impaired vibration sense was seen in nearly 20% of patients.

Retinopathy

Of the 16 patients with retinopathy, 12 had the background type and 4 the proliferative type. Only 2 of the 11 patients who had diabetes mellitus for more than 15 years did not show evidence of retinopathy. In the group with proliferative retinopathy the disease was present for more than 20 years in 2 patients (50%).

Nephropathy

Nephropathy was common, being present in 6 patients (24%), all of whom had the disease for over 10 years. The mean duration of disease was 16 years (range 11 - 26 years). The mean GFR in the patients was 48 ml per minute, (range 28 - 75 ml).

TABLE 5 MEAN DURATION OF DISEASE IN PATIENTS (INITIALLY NIDDM, LATER INSULIN DEPENDENT) WITH COMPLICATIONS

Complications	Mean Duration in years (range)		
	Africans	Indians	All
Neuropathy	7	15.5 (6-22)	14.8
Retinopathy	7	15.2 (5-26)	14.7
Nephropathy	-	16 (11-26)	
Hypertension	-	17 (11-21)	
Ischaemic Heart Disease	-	14 (8-20)	
Peripheral Vascular Disease	-	14 (12-16)	
Cataracts	-	1 (20)	

and the mean serum creatinine concentration 281 mE/L (range 88 - 955 mE/L). In 2 patients the creatinine concentration was normal, despite the presence of persistent albuminuria. All the patients with nephropathy also had retinopathy.

Hypertension

Hypertension was present in 5 patients, being associated with nephropathy in 3 of them. The mean duration of disease was 17 years (11 - 21 years).

Macrovascular Disease

According to the criteria used in this study, ischaemic heart disease and peripheral vascular disease were not common.

DISCUSSION

Fajans et al (1976) described a group of young diabetic patients who were initially controlled on diet with or without sulphonylureas but who later became insulin-dependent. In their proposed classification this group is described as being more akin to non-insulin dependent diabetes mellitus as regards inheritance, provided that the patients become insulin-dependent more than 2 years after diagnosis.

If the 24 patients described in this study are included with the patients who have always been non-insulin dependent, then they constitute about 28 per cent of all the patients initially presenting with NIDDM. In the series described by Fajans et al (1976) 20 per cent of the patients who presented with NIDDM progressed to insulin dependence with time.

There was no significant difference between the ages of onset in patients not requiring insulin and those who eventually became insulin dependent. In Fajan's (1976) series of 9 patients who were initially diagnosed as NIDDM but later became insulin dependent at least 2 years later, the mean age of onset of the disease was 21 years.

Whilst the mean interval between discovery of diabetes and commencement of insulin therapy was 10 years in the patients described in this study

a much shorter interval (mean 6 years) was observed by Fajans et al (1976). Thus it appears that the NIDDM that progresses to IDDM is of a milder type in African and Indian patients than in the group described by Fajans.

The high frequency of a positive family history of diabetes, similar to that seen in the African and Indian patients who have always been non-insulin dependent, corroborates the observations of Fajans et al (1976) who concluded that patients initially diagnosed as NIDDM and later requiring insulin usually show the pattern of inheritance seen in classical NIDDM of the young. Another striking finding was the presence of a much higher frequency of diabetes in either parent (100%) or in both parents (55%), compared to the patients who have always remained non-insulin dependent.

Vascular as well as neurological complications were frequently seen in this group of patients and were much more common than in patients with either NIDDM or IDDM. The longer duration of diabetes in many of these patients as compared to the other groups probably accounts for this increase. Nevertheless, the less likely possibility that this group is particularly prone to complications irrespective of the duration of disease cannot be ignored.

Retinopathy was present in 68 per cent of the patients. Whilst 82% of the patients who had the disease for more than 15 years showed evidence of retinopathy, a similar percentage of the non-insulin dependent diabetics reported by Fajans et al (1978) did not have this complication. Comparison with Fajans et al's 11 patients who later became insulin dependent is not possible because the frequency of retinopathy in all the patients was documented and not shown separately.

Nephropathy appeared to be common in this group of patients, presumably because of the long duration of disease in most of them. In contrast only one out of 69 patients reported by Fajans et al (1978) had this complication, despite a duration of NIDDM of over 15 years in almost 50 per cent of them.

The rarity of ischaemic heart disease in this group of patients is rather surprising in view of the long history of diabetes in many of them. In the series reported by Fajans et al (1978) there was an equal incidence of ischaemic heart disease and retinopathy in patients who had the disease for more than 15 years.

SUMMARY

Nineteen Indian and five African patients who were intially non-insulin dependent but later required insulin were studied. They constitute 28% of all the patients initially presenting with NIDDM. The mean age of presentation was lower and the mean duration of disease much longer in the Indian compared to the African patients. A positive family history of diabetes was obtained in over 70% of patients. The mean interval between presentation of NIDDM and commencement of insulin therapy was 10 years. Obesity was common, over 40% of patients having a percentage ideal body weight of over 120%. Microvascular complications were common and appeared to be related to duration of disease. Macrovascular complications were infrequent.

CHAPTER V

BETA CELL FUNCTION IN INSULIN-DEPENDENT DIABETIC PATIENTS

INTRODUCTION

Studies of endogenous insulin secretion based on C-peptide measurements have provided valuable information on residual pancreatic beta cell function in insulin-dependent diabetic patients (Block et al 1972; Faber and Binder 1977a; Grajwer et al 1971; Heding and Rasmussen 1975). Almost 100 per cent of patients with duration of diabetes under two years have been shown to have some residual beta cell function and with longer duration the prevalence decreases progressively, reaching a level of about 15 per cent after 15 years of disease (Madsbad et al 1978). Greatly reduced beta cell function has been shown to be associated with poor control of diabetes (Faber and Binder 1977a) and with elevated plasma ketone bodies (Madsbad et al 1977). Even the prevalence of retinopathy appears to be lower in long-standing diabetic patients with persisting beta cell function (Eff et al 1978).

Virtually all the published data on C-peptide measurements have been confined to White Caucasian populations and there have certainly been no reports of residual beta cell function in Indian and African diabetic patients in South Africa. The present study was undertaken to evaluate beta cell secretory reserve following glucagon or glucose provocation in insulin dependent African and Indian diabetic patients.

MATERIALS AND METHODS

The study was carried out in 2 parts.
In the first part the C-peptide response to glucagon was assessed in 35 insulin-dependent diabetic patients, all of whom were part of the group described in Chapter III, and 22 normal adults.

The healthy adults who served as controls comprised 11 Africans and 11 Indians. The male : female ratio in each group was equal. The mean age and weight of the healthy controls were 23 years and 61 Kg respectively. None of them had diabetes mellitus or a family history of the disease.
Table 1 lists the clinical features of the diabetic patients studied. Patients and healthy subjects were studied in the mornings after an overnight fast (the last meal having been taken at 1830 hrs the previous day with a snack at 20H00). No insulin was given on the morning of the test day and only soluble insulin was used on the evening before the test to ensure that there would be no residual depot action of long acting insulin.

The patients and controls remained seated throughout the procedure and were not permitted to eat, chew or smoke. Informed consent was obtained from all the subjects. Blood samples were collected through indwelling cannulae inserted under local anaesthesia 30 minutes prior to the test. In initial studies free flowing venous blood was collected 5 minutes before and at 2, 4, 6, 8, 10, 15 and 20 minutes after an intravenous injection of glucagon (Img) in 13 African and 5 Indian patients, and 10 normal subjects (6 Africans and 4 Indians).

As a significant correlation was found between the maximal rise in the C-peptide level and the level observed at 6 minutes (Figure 1), in subsequent studies blood for C-peptide assays was taken before, and 6 minutes after glucagon injection in a further 17 patients and 12 normal subjects.

Plasma C-peptide was measured by radioimmunoassay (Byk-Mallinckrodt kit). The effective detection limit of C-peptide in this assay was 0.055 nmoles/litre and the cross-reaction with human pro-insulin about 10%.

TABLE 1 CLINICAL CHARACTERISTICS OF THE DIABETIC PATIENTS UNDERGOING THE GLUCAGON TEST

	Total	Africans	Indians
Number of patients	35	25	10
Male : female ratio	10 : 25	8 : 17	1 : 4
Mean weight		50.6(34-69)	53.2(30-80)
Mean age in years	23	24 (9-41)	22 (11-36)
Mean duration in years	4	3.5 (0-01)	5.5 (0-26)
Mean insulin dose in Iu/Kg/day $\pm$ S.E.	1.49 $\pm$ 0.12	1.57 $\pm$ 0.15	1.32 $\pm$ 0.21

TABLE 2 CLINICAL CHARACTERISTICS OF THE PATIENTS WITH IDDM UNDERGOING A GLUCOSE TOLERANCE TEST

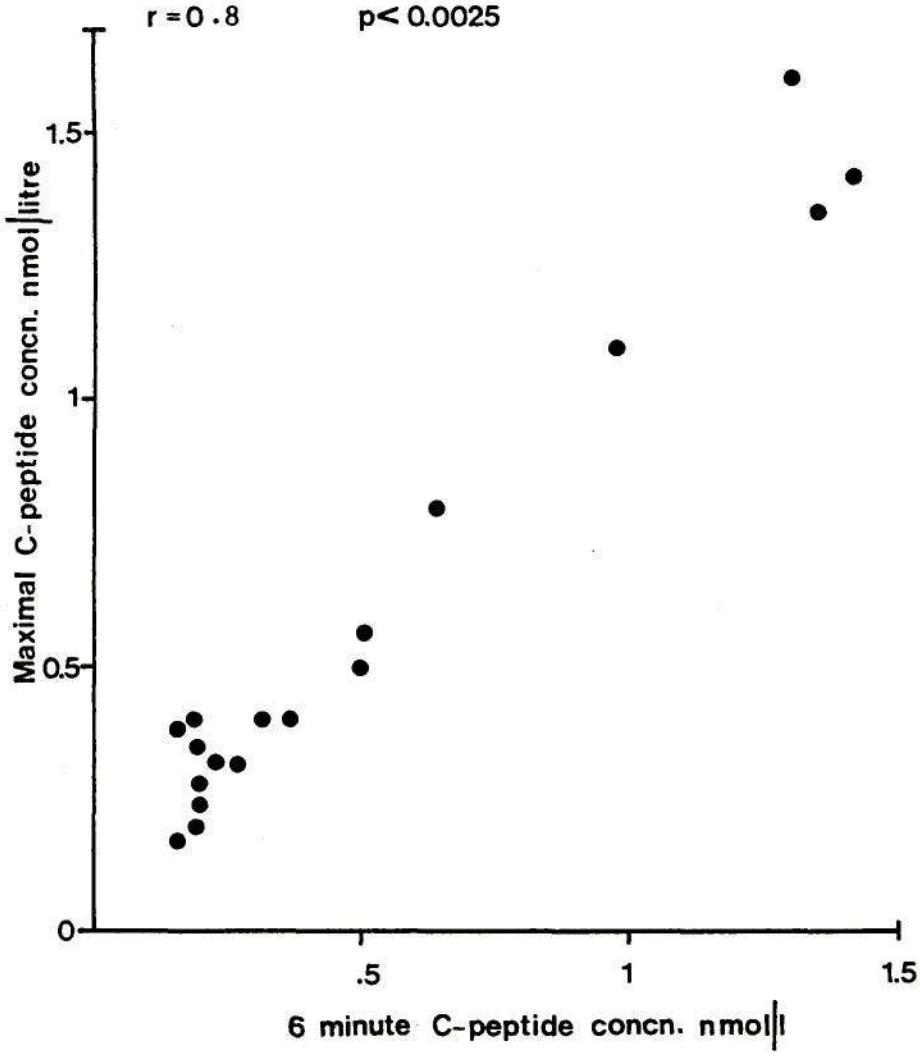
	Africans	Indians	Total
Number of patients	27	12	39
Mean w ght (Kg)	55 (34-68)	53.5 (30-90)	
Male : female ratio	13 : 14	2 : 1	7 : 6
Mean age in years (range)	24 (12-38)	25 (12.37)	24.3
Mean duration in years	3.3 ($\frac{1}{12}$-9)	7.2 ($\frac{1}{12}$-18)	4.5
Mean insulin dose (Iu/Kg) $\pm$ S.E.	1.34 $\pm$ 0.15	1.36 $\pm$ 0.16	1.35 $\pm$ 0.11

TABLE 3 CLINICAL CHARACTERISTICS OF PATIENTS PRESENTING WITH NIDDM AND LATER PROGRESSING TO INSULIN DEPENDENCE

Number of patients (all Indians)	6
Mean weight in Kg	62.7 (45-68)
Male : female ratio	1 : 2
Mean age in years (range)	39.5 (35-51)
Mean duration in years (range)	14 (7-20)
Mean duration of insulin therapy in years (range)	4 (3-8)
Mean insulin dose (Iu/Kg) $\pm$ S.E.	1.35 $\pm$ 0.08

Figure 1

CORRELATION BETWEEN THE 6 MINUTE C-PEPTIDE AND THE MAXIMAL C-PEPTIDE LEVELS AFTER GLUCAGON STIMULATION



In the second part of the study the C-peptide response to a glucose load was measured in 27 African and 12 Indian patients who had always been insulin-dependent and in 6 patients who had initially been non-insulin-dependent but later became insulin-dependent. The 39 patients formed part of the patients described in Chapter III whilst the latter group was part of the subgroup described in Chapter IV. In addition 18 healthy adults (9 Africans and 9 Indians) served as a control group. The mean age and weight of the healthy subjects who served as controls were 23 years and 58 Kg respectively. There was no significant difference in the male : female ratio in both African and Indian control groups.

Table II lists the clinical characteristics of the subjects studied.

Patients and healthy subjects were studied in the morning after an overnight fast. They remained seated throughout the procedure and were not permitted to eat, chew or smoke. Informed consent was obtained from all the subjects. Indwelling cannulae were introduced into antecubital veins under local anaesthesia and kept patent with slow infusions of 0.9% NaCl. After a rest period of 30 minutes blood samples for basal glucose and C-peptide levels were collected. A drink of 100 gm glucose dissolved in 300 ml water was then given slowly. Blood for glucose and C-peptide was taken at 15, 30, 45, 60, 90, 120 and 150 minutes after the first sip of glucose.

Plasma C-peptide was measured by radioimmunoassay (Byk-Mallinkrodt kit). The method used was a modification of that used for the samples taken during the glucagon test. The reason for the change was that the kits using the first method had become unavailable and those with the modification were introduced. The latter was less sensitive, the effective detection limit being 0.1 mmol/L.

In all the patients and controls plasma glucose concentrations were measured by means of an autoanalyser, using a ferricyanide method.

RESULTS

1. C-Peptide Response to Glucagon: The basal and 6 minute post-glucagon C-peptide results in the patients and controls are depicted in Figure 2. The mean fasting C-peptide level in the control group is 0.49 ± 0.20 nmoles/L and the mean value 6 minutes after glucagon is 1.24 ± 0.48 nmoles/L. This is a more than two-fold increase and is highly significant ($p < 0.001$). The mean basal and 6 minute C-peptide concentrations in African controls (0.42 ± 0.05 nmol/L and 1.17 ± 0.14 nmol/L respectively) were lower than those in Indian controls (0.61 ± 0.06 nmol/L and 1.40 ± 0.12 nmol/L respectively), but the differences were not significant.

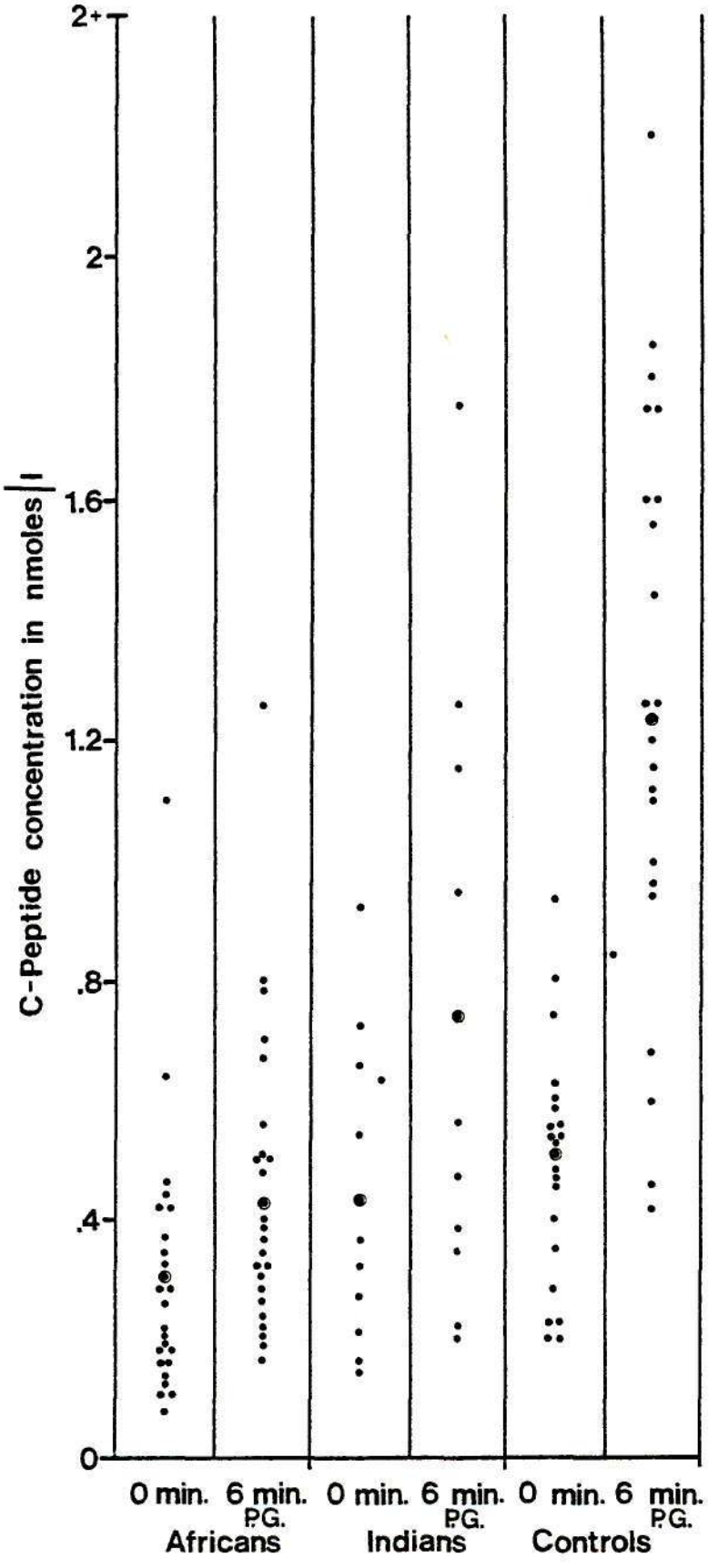
The mean basal C-peptide level in the patients is 0.32 ± 0.23 nmoles/L. The mean 6 minute post glucagon value is 0.56 ± 0.47 nmoles/L and represents a 75% increase over the basal level. In 7 patients the rise in the C-peptide level after glucagon injection is not significant, if a rise of 16,2% or greater is considered significant, (Eff et al 1978; Faber and Binder 1977a). On the other hand 4 patients have markedly elevated basal and post glucagon C-peptide levels. Compared to the controls, the diabetic patients show significantly lower basal C-peptide levels ($p < 0.01$) and in addition a significantly less relative increase after glucagon ($p < 0.001$).

The African patients demonstrate significantly lower basal C-peptide level (mean 0.30 ± 0.22 nmoles/L) than the Indian patients (0.43 ± 0.27 nmoles/L) ($p < 0.001$). The corresponding post-glucagon values of 6 minutes are also lower in Africans (0.43 ± 0.25 nmoles/L) than Indians (0.73 ± 0.53 nmoles/L, $p < 0.0001$).

Figure 3 illustrates the basal and maximal post-glucagon C-peptide levels in the diabetic patients and the controls who had C-peptide assays done at -5, 2, 4, 6, 8, 10, 15 and 20 mintues after glucagon injection. It is evident that the mean basal and the mean maximal values in the diabetics are significantly lower than in the controls ($p < 0.04$). The 6 minute values are significantly correlated with the maximal levels ($r = 0.80$; $p < .001$), Figure 1.

Figure 2.

C-PEPTIDE CONCENTRATIONS BEFORE AND 6 MINUTES AFTER GLUCAGON (I.V.) IN THE DIABETIC PATIENTS AND CONTROLS



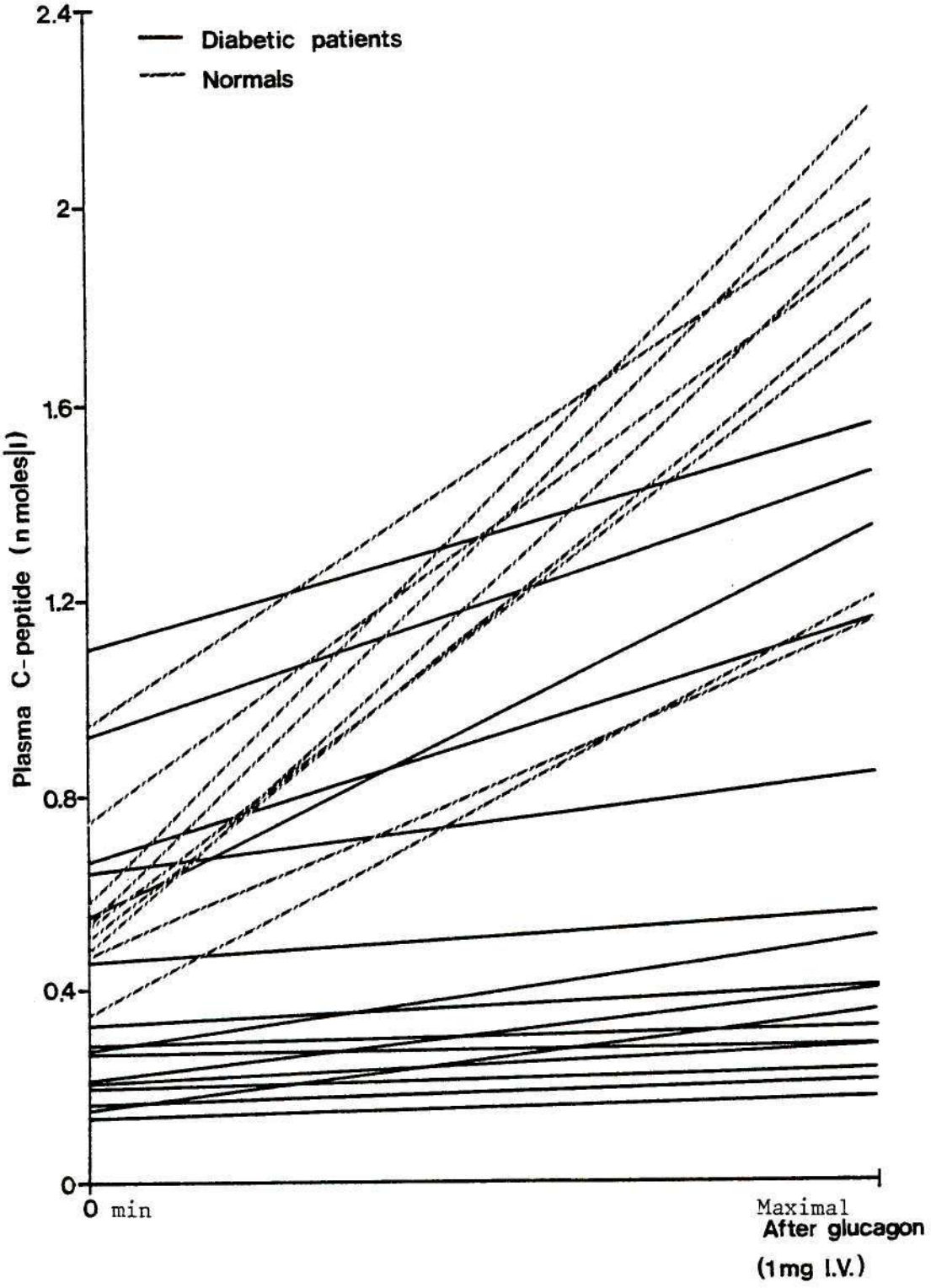
● - Mean values

• - Individual values

P.G.-Post glucagon

Figure 3

FASTING C-PEPTIDE CONCENTRATIONS AND THE MAXIMAL C-PEPTIDE LEVELS AFTER GLUCAGON STIMULATION IN DIABETICS AND CONTROLS.



The mean fasting blood glucose level is 17.8 nmoles/L, and shows no correlation with either the basal or maximal post-glucagon C-peptide level, Figure 4.

2. C-Peptide Response during a GTT: The basal C-peptide levels and the levels during a GTT in the patients and controls are depicted in Figures 5 and 6.

The mean fasting C-peptide level in the control group is 0.22 ± 0.04 nmol/L. The mean maximal value is 2.15 ± 0.31 nmol/L, representing almost a 10-fold increase, which is highly significant ($p < 0.0005$), Figure 7. The maximal C-peptide level in Africans (1.45 ± 0.20 nmol/L) is significantly lower than the mean maximal level in Indians (2.59 ± 0.49 nmol/L), $p < 0.05$, Figure 8.

The mean basal C-peptide level in the patients is 0.26 ± 0.04 nmol/L. The mean maximal value during the GTT is 0.53 ± 0.08 nmol/L and represents a 2-fold rise over the basal level, Figure 7. Compared to the controls the diabetic patients show significantly lower maximal C-peptide levels ($p < 0.0005$). The peak C-peptide response occur later in the diabetic patients (mean 137 minutes after glucose ingestion) than in the controls (mean 54 minutes), $p < 0.0005$, Figure 6.

Although the mean basal and maximal C-peptide levels are lower in the African patients compared to the Indian patients, the difference is not significant, Figure 7. Similarly there is no significant difference in the time taken for the maximal C-peptide to manifest between the 2 groups, Figure 8.

In 10 patients (5 African and 5 Indian) C-peptide levels are undetectable in the serum and remained so during the GTT. They include 3 patients with IDDM of recent onset (less than 6 months). The remainder had a mean duration of diabetes of 15 years.

The Indian patients who had always been insulin-dependent had mean basal C-peptide levels of 0.27 ± 0.07 nmol/L and mean maximal values of 0.51 ± 0.15 nmol/L, whereas Indian patients who were initially non-

Figure 4.

THE RELATIONSHIP BETWEEN FASTING PLASMA C-PEPTIDE LEVELS AND FASTING PLASMA GLUCOSE AND BETWEEN MAXIMAL PLASMA C-PEPTIDE LEVELS AND FASTING PLASMA GLUCOSE IN INSULIN DEPENDENT DIABETIC PATIENTS

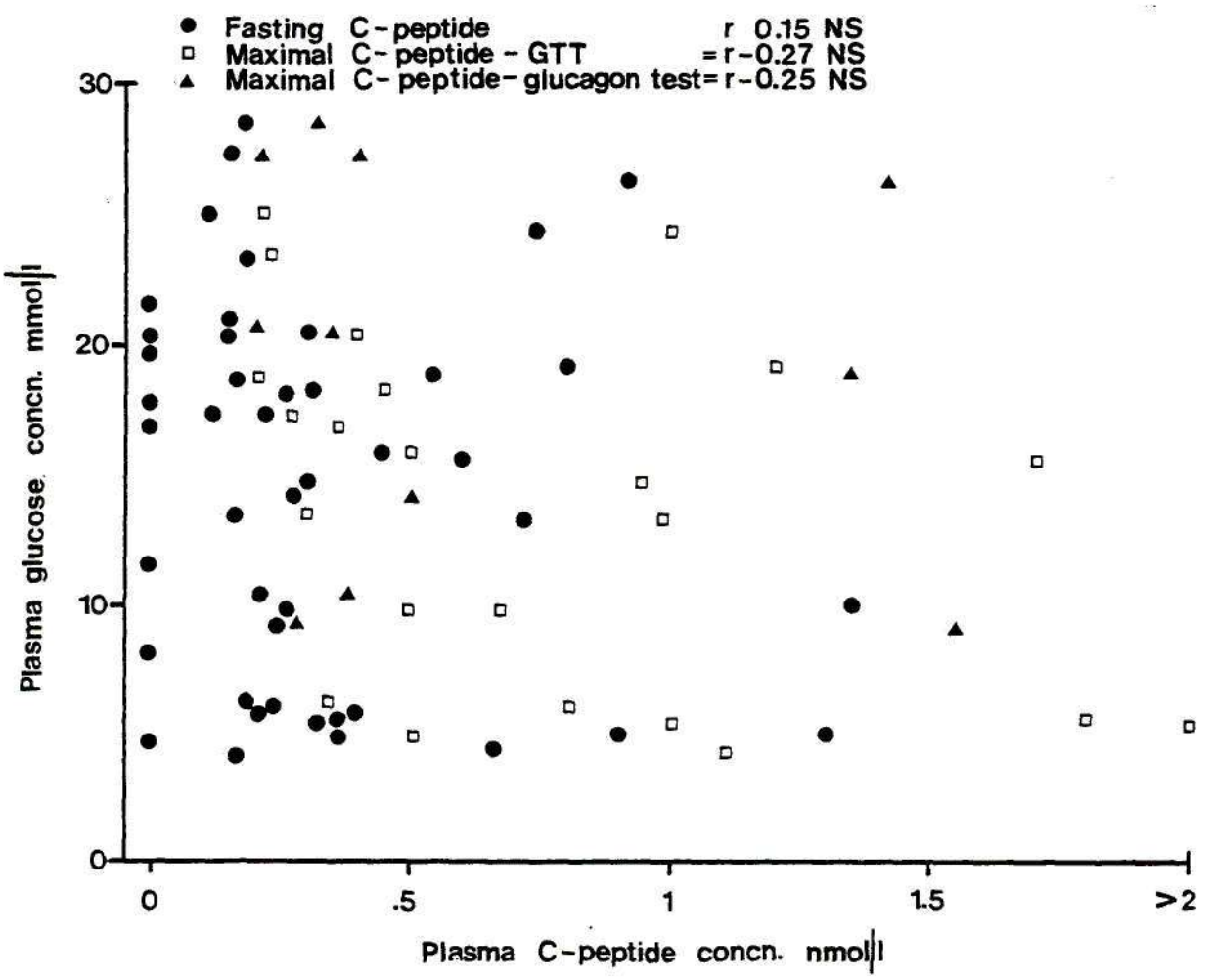


Figure 5.

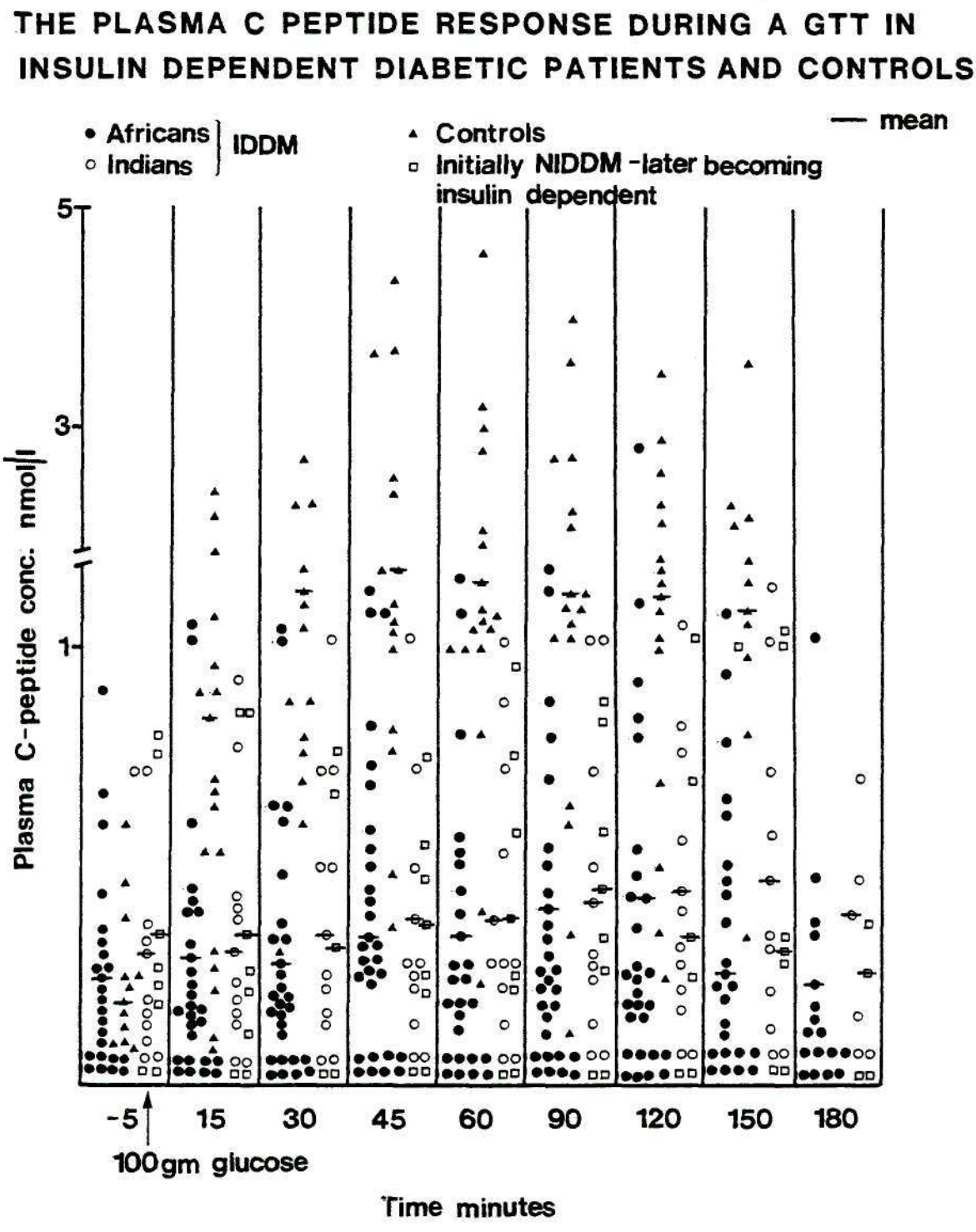

THE PLASMA C PEPTIDE RESPONSE DURING A GTT IN INSULIN DEPENDENT DIABETIC PATIENTS AND CONTROLS
• Africans
○ Indians
IDDM
▲ Controls
▫ Initially NIDDM -later becoming insulin dependent
— mean
Plasma C-peptide conc. nmol/l
5
3
1
-5
15
30
45
60
90
120
150
180
100gm glucose
Time minutes

Figure 6

THE PLASMA C-PEPTIDE AND GLUCOSE CONCENTRATIONS (mean±SEM) IN INSULIN DEPENDENT DIABETIC PATIENTS AND CONTROLS BEFORE AND AFTER 100gm GLUCOSE

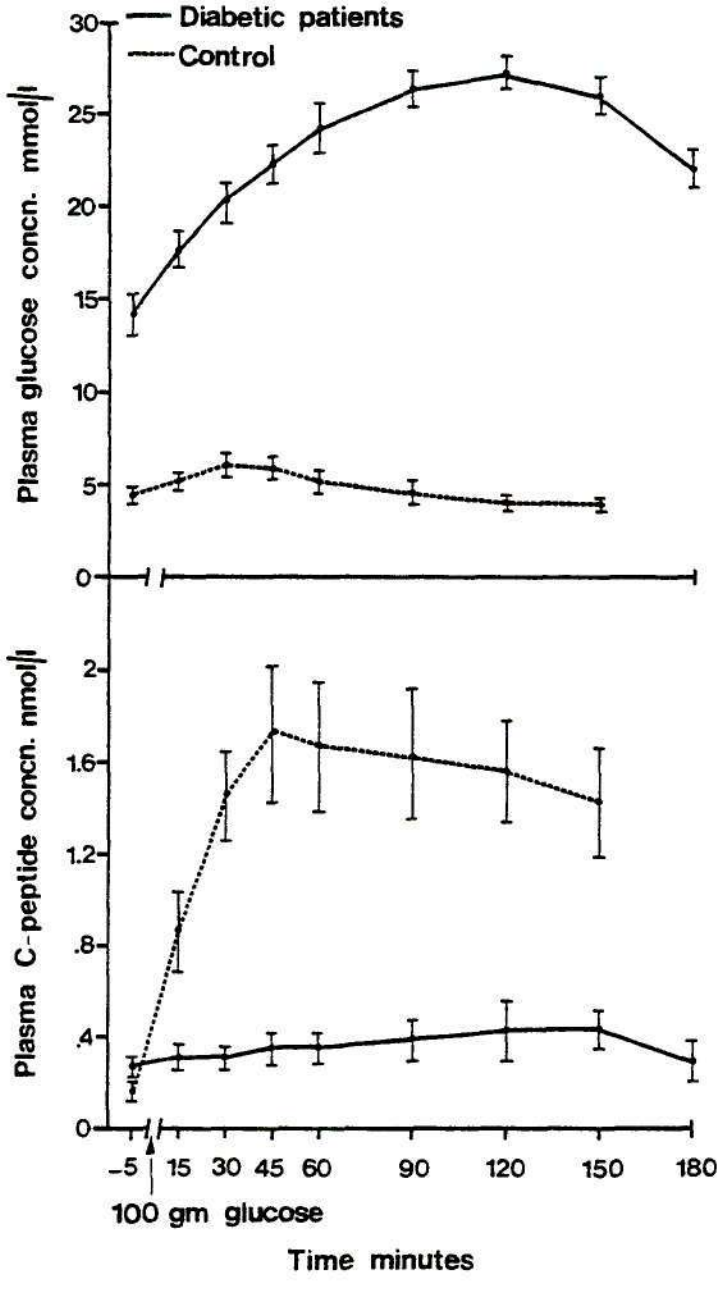


Figure 7.

THE FASTING AND MAXIMAL PLASMA C-PEPTIDE CONCENTRATIONS (mean±SEM) DURING A GTT IN INSULIN DEPENDENT DIABETIC PATIENTS AND CONTROLS

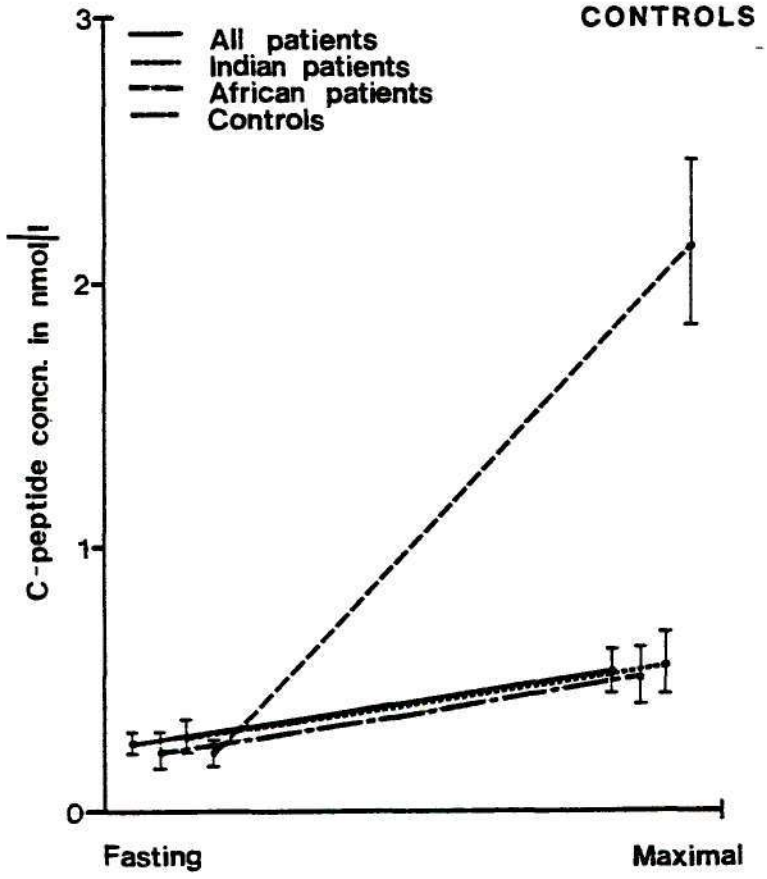
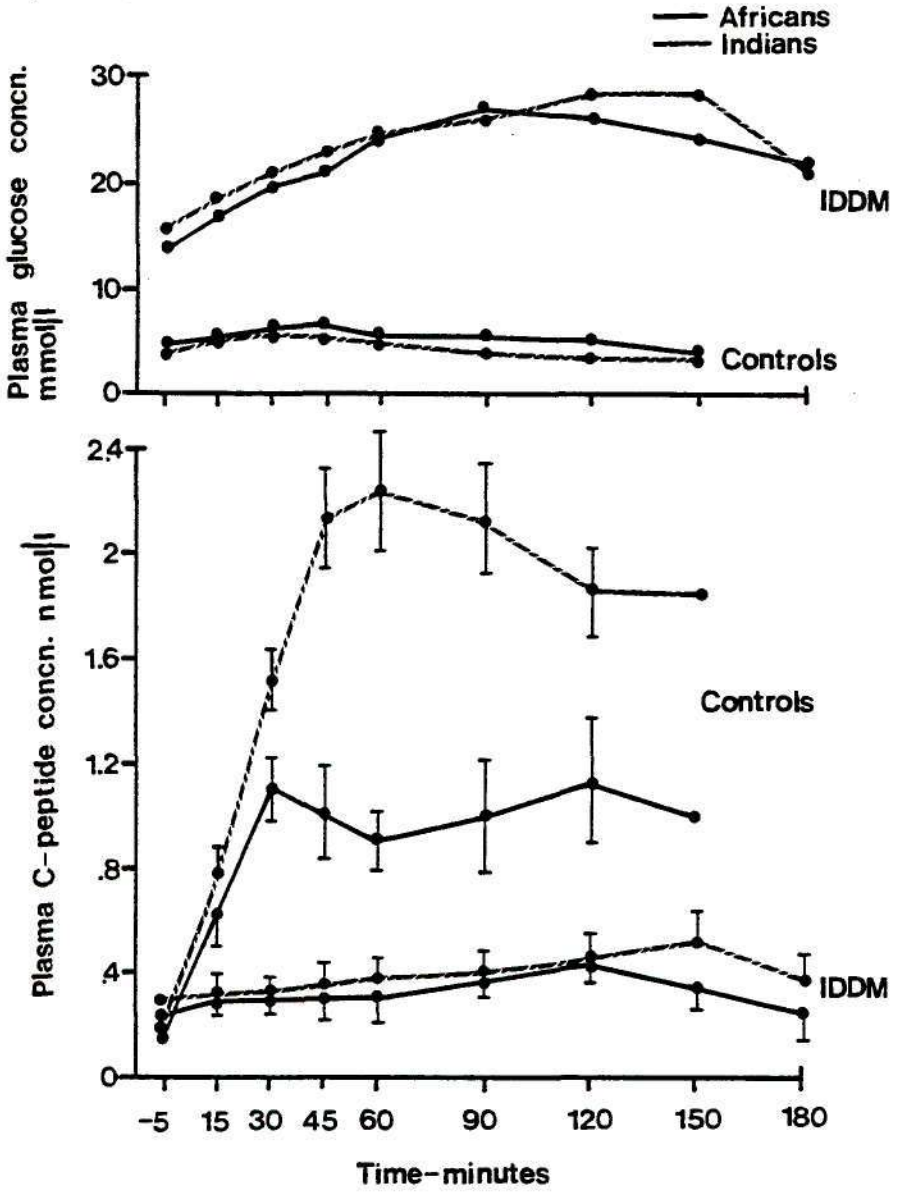


Figure 8.

COMPARISON OF THE C-PEPTIDE RESPONSE (mean ±SEM) TO A GLUCOSE LOAD IN AFRICAN AND INDIAN INSULIN DEPENDENT DIABETIC PATIENTS AND CONTROLS



insulin-dependent but later needed insulin show mean basal levels of 0.34 ± 0.14 nmol/L and mean maximal levels of 0.53 ± 0.21 nmol/L. There is no significant difference between the 2 groups.

Fasting plasma glucose concentrations show no significant correlation with the basal or maximal C-peptide levels, Figure 4. Similarly no significant correlation is seen between the daily insulin requirements and the basal or maximal C-peptide concentrations, Figure 9.

Duration of insulin therapy also shows no significant correlation with the basal or maximal C-peptide levels, Figure 10. However, a significant correlation is seen between the ages of onset of IDDM and the fasting C-peptide levels, Figure 11.

DISCUSSION

The determination of C-peptide levels in response to a variety of stimuli has been documented in many studies. The stimuli used included glucose (Block et al 1972; Block et al 1973; Heding and Rasmussen 1975; Ikeda et al 1975; Kuzuya et al 1976; Reynolds et al 1977), arginine (Reynolds et al 1977) and glucagon (Faber and Binder 1977a; Henriksen et al 1977; Ludvigsson and Heding 1977). In the present study residual beta cell function was demonstrated in most insulin-dependent diabetics both by the detection of basal C-peptide levels, as well as by the augmentation of C-peptide secretion in response to glucose or glucagon.

C-peptide levels were undetectable in only 12 per cent of the patients. In other large studies, however, over 50% of insulin-dependent diabetic patients have been found to show no detectable C-peptide levels (Binder and Faber 1978; Hendriksen et al 1977). A possible reason for this discrepancy is that the duration of diabetes of most of the patients reported in the other studies was much longer than that of the patients described here. Beischer et al (1976) and Ludvigsson and Heding (1977) have also found a much lower frequency of undetectable C-peptide concentrations if diabetes was present for a shorter duration.

Figure 9.

THE RELATIONSHIP BETWEEN INSULIN REQUIREMENTS AND BASAL AND MAXIMAL PLASMA C-PEPTIDE LEVELS IN INSULIN DEPENDENT DIABETIC PATIENTS

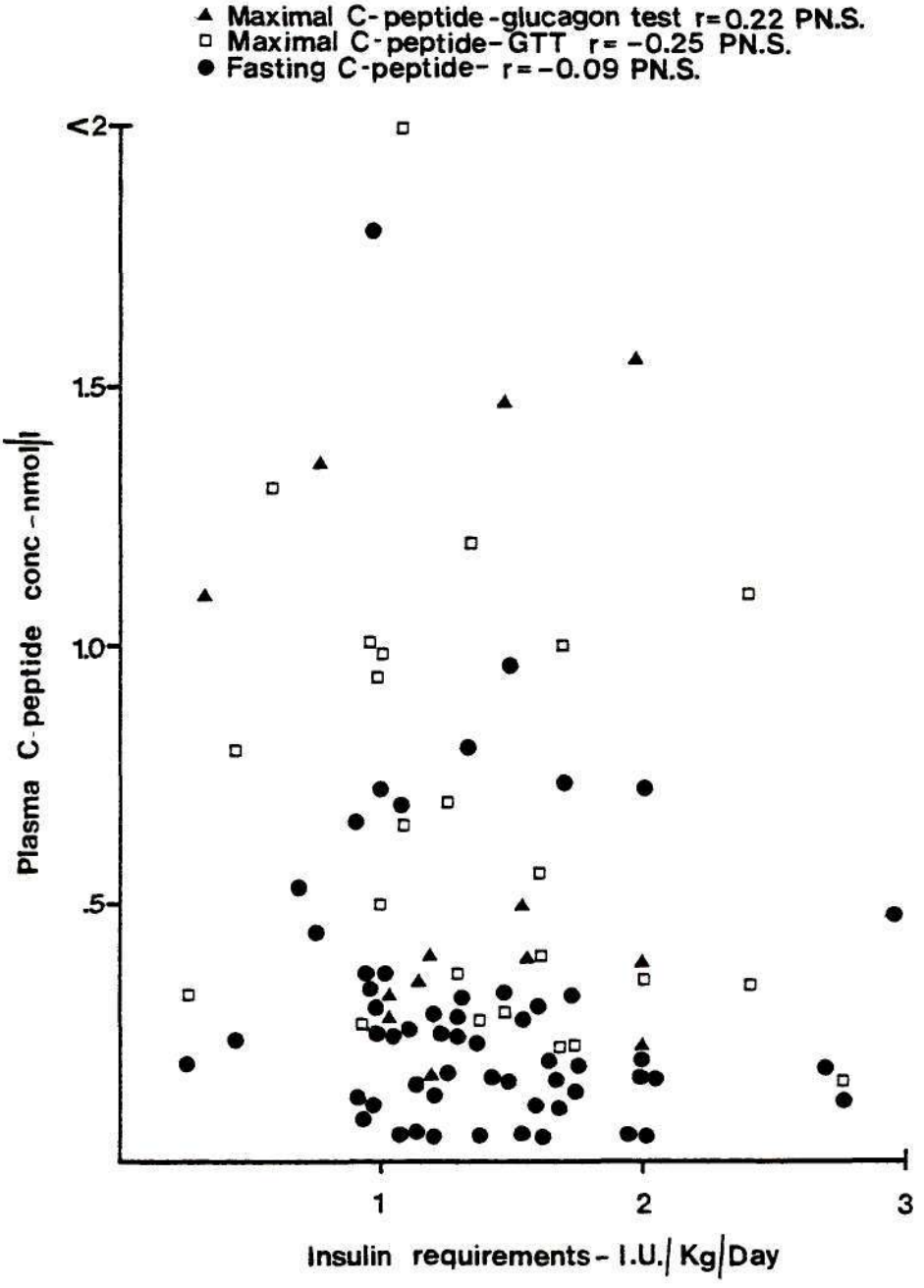


Figure 10.

THE RELATIONSHIP BETWEEN DURATION OF INSULIN THERAPY AND FASTING AND MAXIMAL PLASMA C-PEPTIDE LEVELS IN INSULIN DEPENDENT DIABETIC PATIENTS

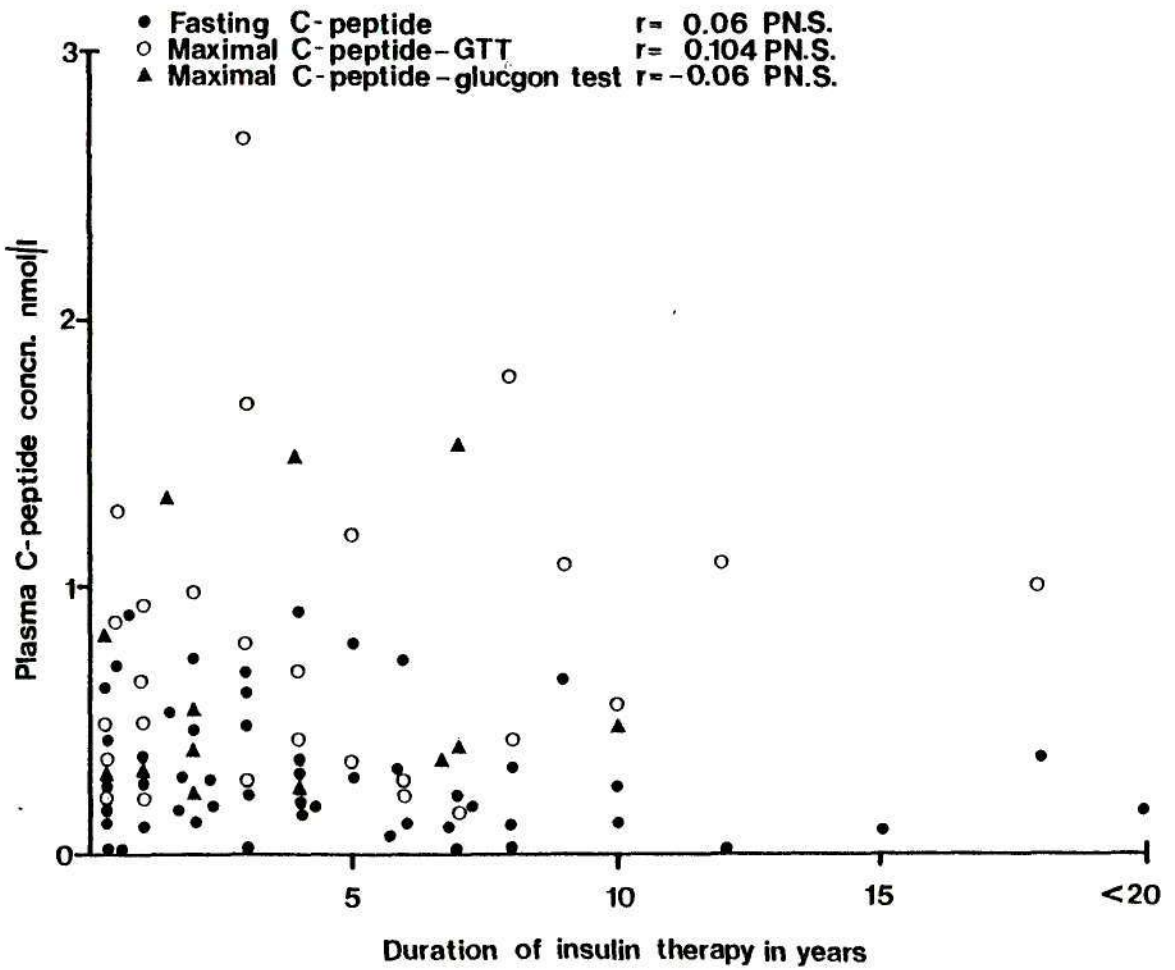
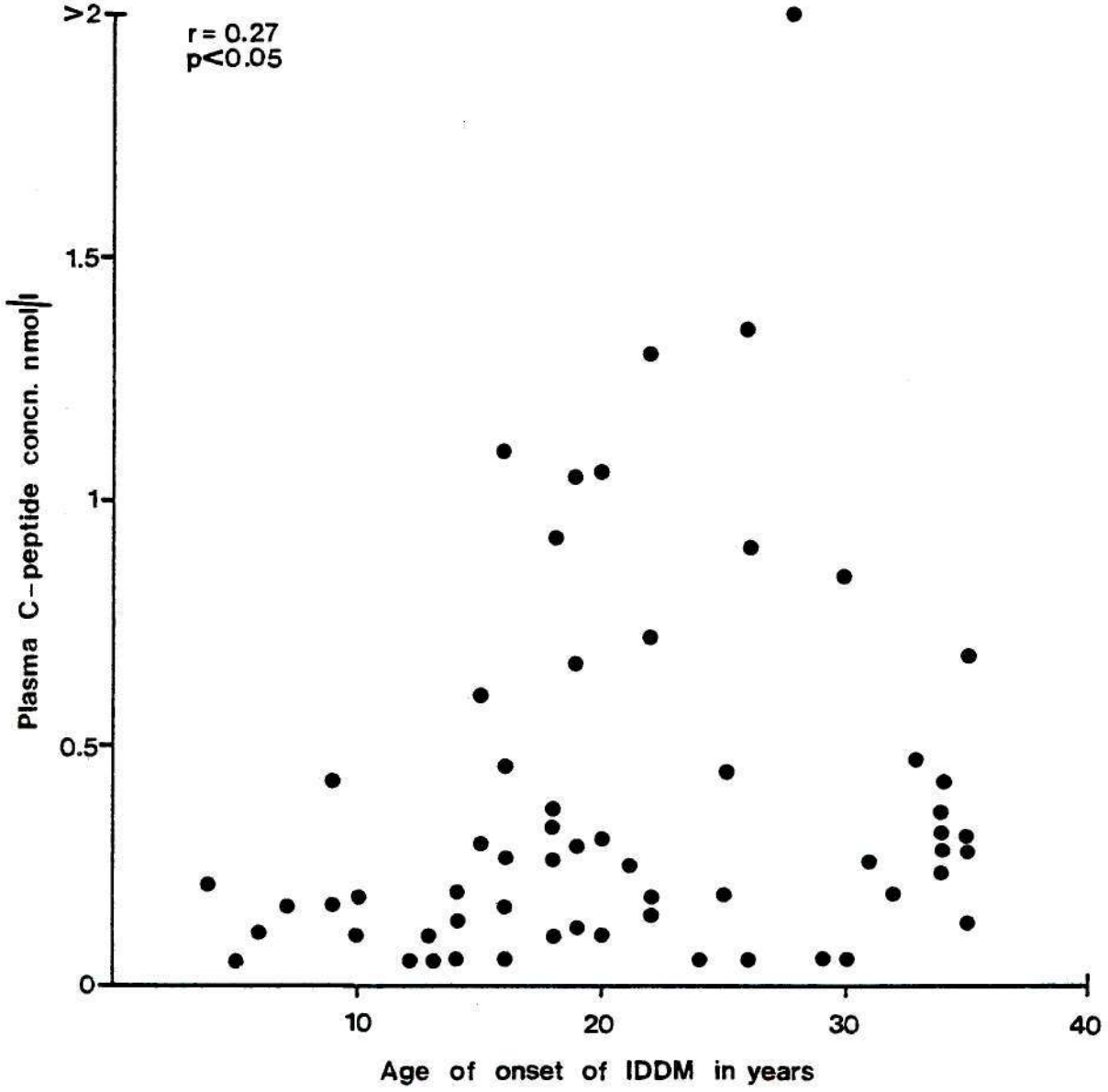


Figure 11.

THE CORRELATION BETWEEN AGE OF ONSET OF IDDM AND FASTING PLASMA C-PEPTIDE CONCENTRATIONS



Patients with undectable basal C-peptide levels very seldom demonstrate a significant response to stimuli (Hendriksen et al 1977). Thus it is not surprising that only one of the 11 patients with no detectable basal plasma C-peptide showed a significant response during a GTT.

Although the mean C-peptide level was significantly lower in the diabetic patients than the controls, a significant response to glucose or glucagon was seen in most of the patients. However, 20% of the patients undergoing the glucagon stimulation test failed to show a significant response to glucagon. In Hendriksen's Series (Hendriksen et al 1977), 15% of the patients who had detectable basal C-peptide levels did not show a significant response to glucagon. Of the patients who underwent a glucose tolerance test less than 10 per cent did not show a significant increase in the C-peptide level. Interestingly these were the very patients who required high doses of insulin for control.

Although both the glucagon test and the glucose tolerance test was done in 15 patients, comparison between the responses of the two tests is not possible, since the 2 tests were done a few months apart in each of the cases. In addition, differences in the methods of C-peptide assay between the 2 tests would invalidate the significance of such a comparison.

Studies on the correlation between insulin requirements and the basal or maximal C-peptide levels have yielded conflicting results (Faber and Binder 1977a, Grajwer et al 1971; Ikeda et al 1975). In this study an inverse correlation could not be established over the entire range of insulin doses and C-peptide concentrations. In fact there were some patients who had low C-peptide levels and yet needed relatively small doses of insulin and were controlled satisfactorily, confirming the view that a low level of the hormone is not necessarily associated with poor control (Grajwer et al 1971; Ikeda et al 1975). On the other hand it would appear that insulin dosage might bear a close relationship with an absent C-peptide response to stimuli like glucagon. This is borne out by the large amounts of insulin needed for control in the patients who failed to respond to glucagon or glucose.

Although some workers have observed an inverse correlation between the degree of beta cell function and the duration of diabetes (Faber and Binder 1977b; Grajwer et al 1971; Madsbad et al 1978), such a relationship could not be shown in this study; most probably because in the majority of patients studied the disease was present for a short period only (mean duration 4 years). Nevertheless, if 5 years is taken as the cut off level for duration of diabetes then over 90% of the patients whose duration of disease fell within this period had residual beta cell function as judged by detectable C-peptide levels, whereas the prevalence in those with a longer duration of illness was 60%. In other series the prevalence of detectable C-peptide levels has also been very high (over 75%) in patients whose diabetes was present for 5 years or less, whereas it was only 11% - 20% in those with a longer duration of disease (Grajwer et al 1971; Hendriksen et al 1977; Ludvigsson and Heding 1976).

The positive correlation found between basal C-peptide levels and age of onset of diabetes has been observed in other studies (Ludvigsson and Heding 1976; Madsbad et al 1977). Thus it would appear that the older the patient is at the time of presentation of IDDM, the greater the degree of residual beta cell function.

The significantly lower basal and maximal C-peptide levels observed in the African patients after glucagon provocation may reflect a greater degree of beta cell destruction in them as compared to the Indian patients. However it is also possible that the African patients have a lesser degree of beta cell function even before the development of IDDM.

The fact that the basal and maximal C-peptide levels, though being lower in Africans, did not show significant differences between the 2 control groups would tend to negate this hypothesis. On the other hand significantly lower maximal C-peptide levels seen in African controls during the glucose tolerance test would tend to favour such a possibility.

The lack of a significant difference in the C-peptide levels after a glucose load between Africans and Indians with IDDM may seem surprising

in the light of the differences shown during glucagon provocation. A possible reason to explain this paradox is that the African patients undergoing the glucose tolerance test had a much shorter duration of IDDM (mean 3.3 years) compared to the Indian patients (mean 8 years) and hence any difference in C-peptide level may be offset by differences in duration of disease. Duration of disease in patients undergoing the glucagon test, however, was similar between the two groups and hence the difference assumes great significance.

SUMMARY

Residual beta cell function based on C-peptide assays was estimated in 35 young patients with IDDM and 22 controls using glucagon as a provocative agent, and in 45 insulin dependent diabetic patients and 18 controls using glucose as a stimulus. The basal and 6 minute post-glucagon C-peptide levels were significantly lower in the diabetic patients compared to controls. Residual beta cell function as gauged by the presence of significant C-peptide increments in response to glucagon was present in most patients. The diabetic patients showed significantly lower maximal C-peptide values and far more delayed peak levels compared to controls. Just over 10% of patients had no residual beta cell function as gauged by undetectable basal C-peptide levels which failed to rise after a glucose load. A significant correlation was seen between the age of onset of IDDM and the fasting C-peptide levels. No correlation was seen between the fasting or maximal C-peptide levels; and the daily insulin requirements, duration of insulin therapy and fasting plasma glucose. C-peptide levels tended to be lower in Africans compared to Indians.

CHAPTER VI

INSULIN RESPONSE TO ORAL GLUCOSE IN YOUNG NONINSULIN-DEPENDENT DIABETIC PATIENTS

INTRODUCTION

Studies of endogenous insulin secretion in response to a glucose load have shown significantly delayed and lower increments in most young noninsulin-dependent diabetic patients. (Fajans et al 1976; Fajans et al 1978; Johansen 1973). However, there is a marked variation in the magnitude of the individual responses (Fajans et al 1978) just as has been found in late onset NIDDM (Asmal and Leary 1975; Fajans et al 1974; Fajans et al 1976). Lower levels of insulin have been found in late onset noninsulin-dependent African patients when compared to similar Indian patients (Asmal and Leary 1975).

MATERIALS AND METHODS

Twenty healthy medical students comprising 10 Africans and 10 Indians with an equal sex distribution in each group and 48 young noninsulin-dependent diabetic patients participated in the study. The mean age and weight of the healthy subjects were 23 years and 57 Kg respectively. The diabetic patients all had the disease before the age of 35 and included 14 Africans and 24 Indians. They formed part of the group described in Chapter IV. Table 1 shows the clinical characteristics of the patients studied.

In patients on oral hypoglycaemic agents, the drugs were discontinued at least 72 hours before the clinical investigations were carried out. Patients and healthy subjects were studied in the morning after an overnight fast. They remained seated throughout the procedure and were not permitted to eat, chew or smoke. Informed consent was obtained

TABLE 1 CLINICAL CHARACTERISTICS OF THE PATIENTS

	Africans	Indians	Total
Number of patients	14	24	38
Male : female ratio	3 : 11	7 : 17	10 : 28
Mean weight in Kg (range)	23.5 (47-106)	69 (52-102)	70.6
Mean age in years (range)	33.2 (19-40)	32.4 (23-40)	32.7
Mean duration in years (range)	4 (1-9)	4.5 (1-10)	5.1

from all the subjects. Indwelling cannulae were introduced into ante cubital veins under local anaesthesia and kept patent with slow infusions of 0.9% Na Cl. After a rest period of 30 minutes blood samples for basal glucose and insulin levels were collected. A drink of 100 gm glucose dissolved in 300 ml water was then given slowly. Blood for glucose and insulin was taken at 15, 30, 45, 60, 90, 120 and 150 minutes after the first sip of glucose.

Glucose was estimated using an autoanalyser and insulin levels were done by radioimmunoassay using Phadebas kits. The "insulinogenic index" or insulin : glucose ratio was obtained by dividing the plasma insulin increments above fasting value by the corresponding net increase in plasma glucose, (Seltzer et al 1967).

RESULTS

The individual insulin concentrations of all patients and controls during the GTT are depicted in Figure 1.
The mean basal level in the controls is 24.7 $\pm$ 3.3 µu/ml rising to a mean maximum value of 129.2 $\pm$ 15.3 µu/ml, Figure 2. This represents almost a 5 fold increase over the basal level and is highly significant ($p < .0005$). Although the mean basal insulin levels are higher in the African (29.1 $\pm$ 5.4 µu/ml) than Indian controls (20.8 $\pm$ 3.7 µu/ml), the difference is not significant, Figure 3.

The diabetic patients show a mean basal insulin level of 20.2 $\pm$ 1.7 µu/ml which rises by 120% to a mean maximum value of 48 $\pm$ 6.6 µu/ml, Figure 2. The difference between the basal and maximal values is highly significant ($p < 0.0005$). The mean maximal insulin level in the diabetic patients is significantly lower than in the controls ($p < 0.0005$). The mean percentage elevation in insulin is also significantly lower in the diabetic patients ($p < 0.01$). The peak insulin response occurred significantly later in the diabetic patients (mean 84.6 minutes) when compared to controls (mean 46 minutes), $p < 0.005$, Figure 4. The controls show a greater and more rapid rise in the insulin : glucose ratio compared to a much slower rate and magnitude of increase in the diabetic patients, Figure 5.

Figure 1.

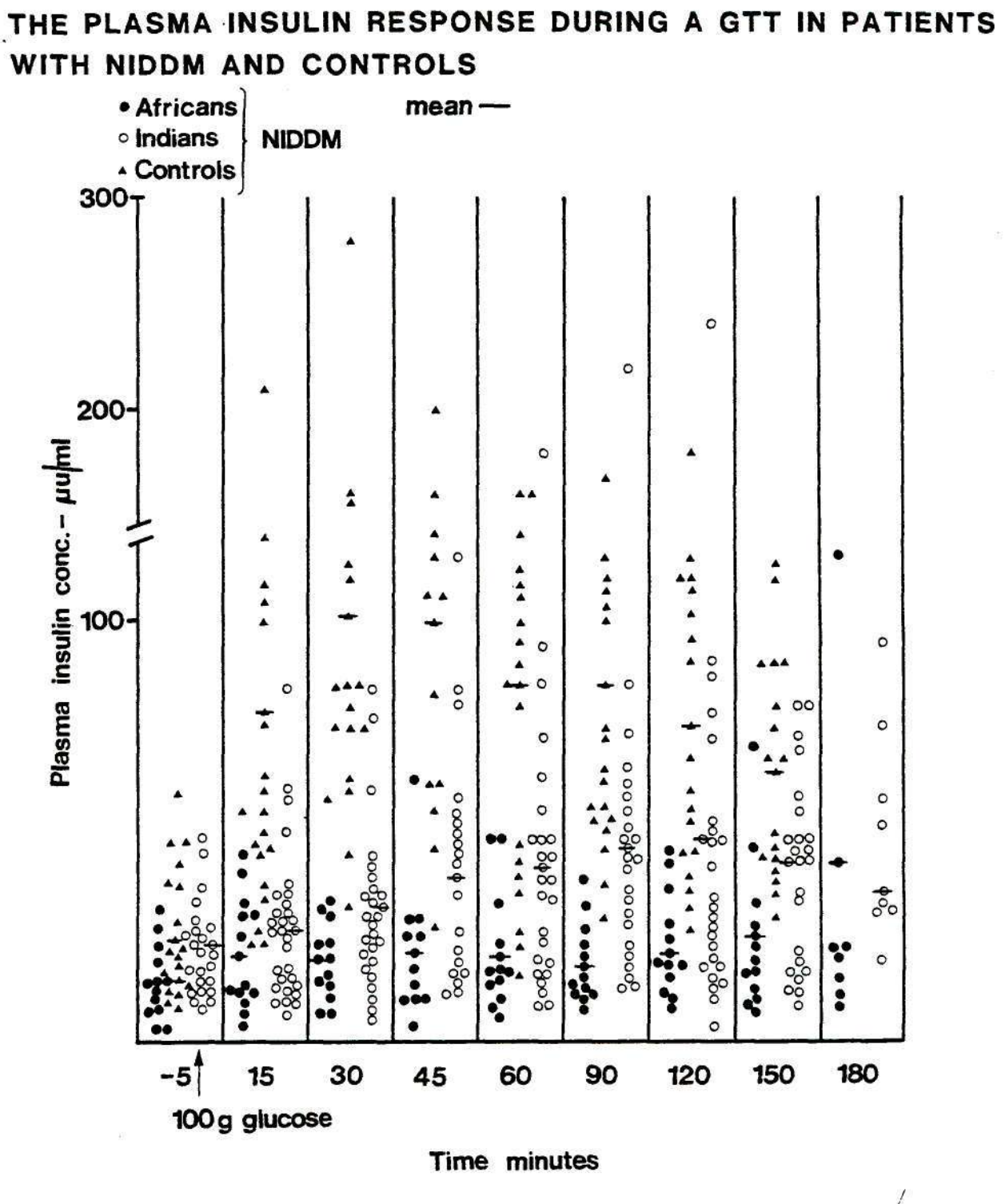

THE PLASMA INSULIN RESPONSE DURING A GTT IN PATIENTS WITH NIDDM AND CONTROLS
• Africans
○ Indians
▲ Controls
NIDDM
mean —
300
200
100
Plasma insulin conc. - μu/ml
-5
15
30
45
60
90
120
150
180
100g glucose
Time minutes

Figure 2.

THE FASTING AND MAXIMAL PLASMA INSULIN RESPONSES (mean±SEM) IN THE PATIENTS WITH NIDDM AND CONTROLS

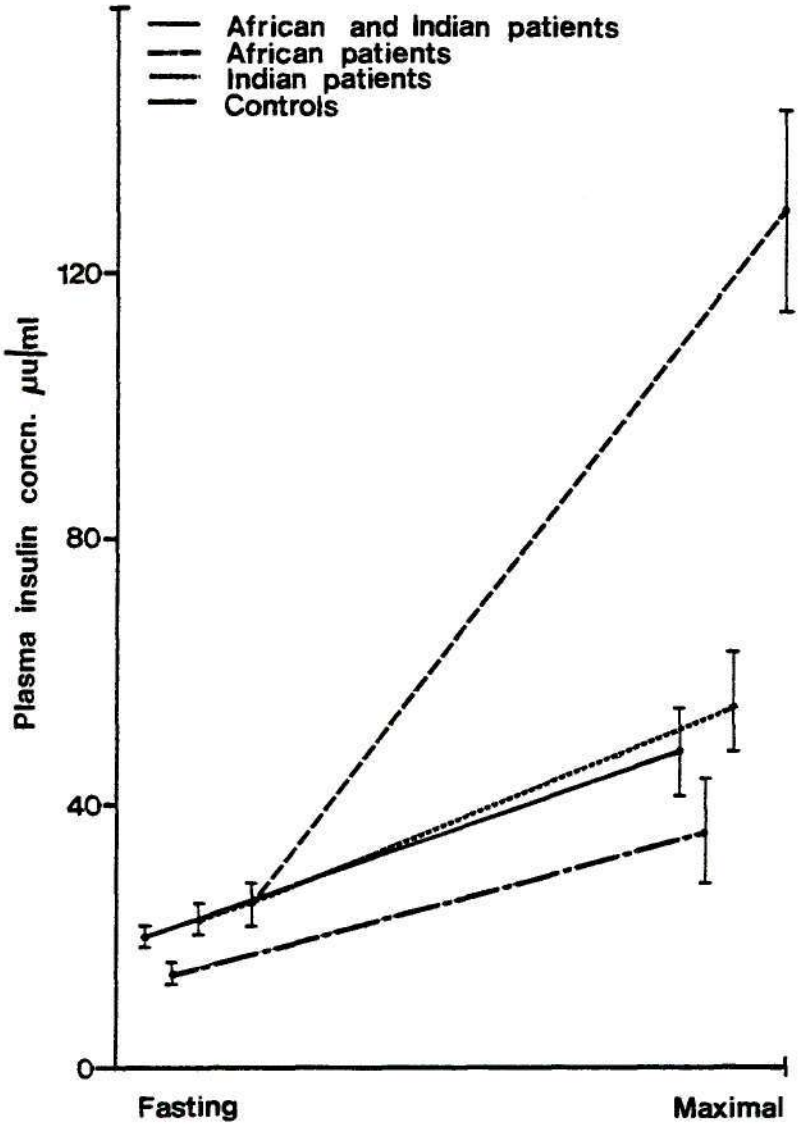


Figure 3.

COMPARISON OF THE PLASMA INSULIN RESPONSES TO A GLUCOSE LOAD IN AFRICANS AND INDIANS WITH NIDDM AND CONTROLS

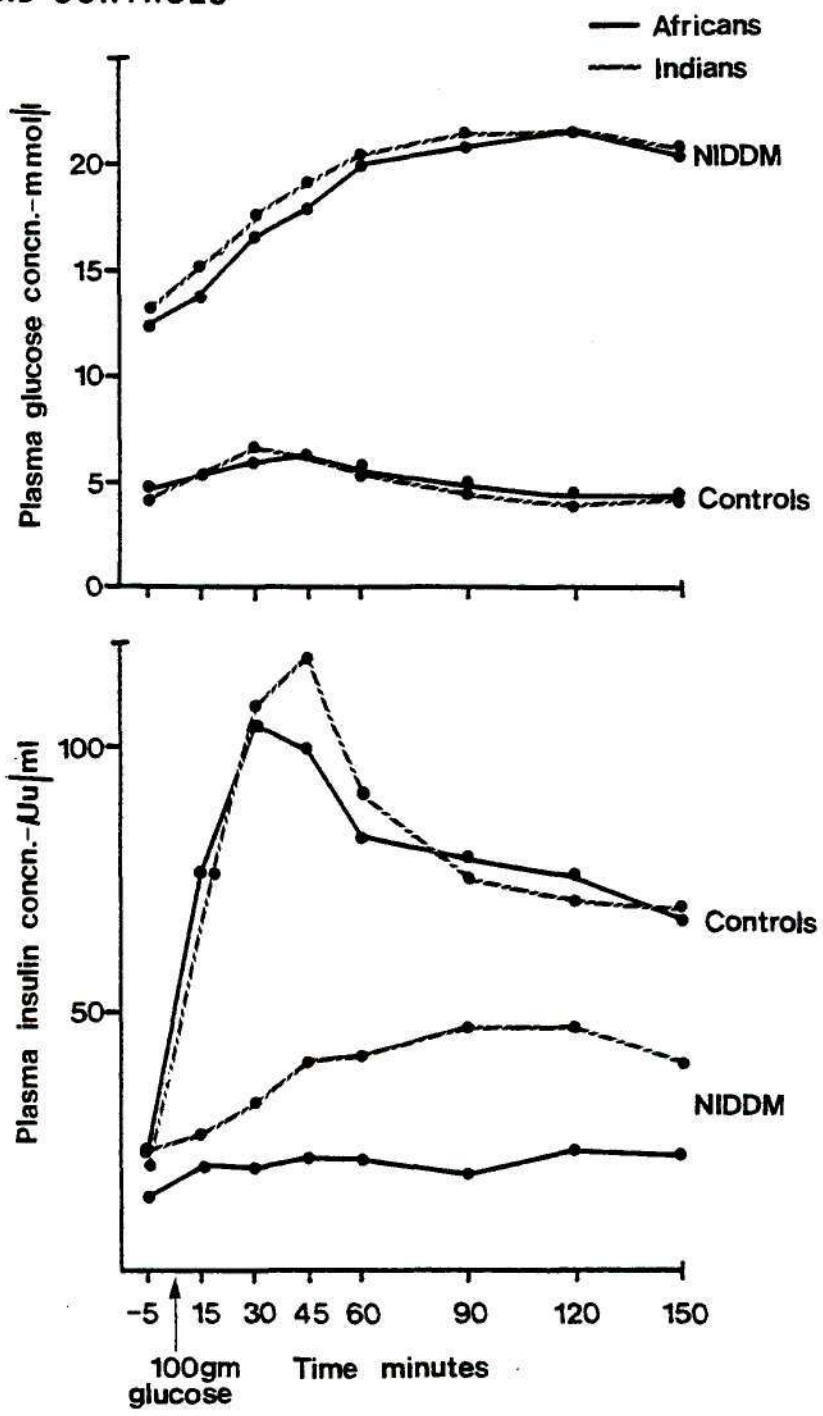


Figure 4.

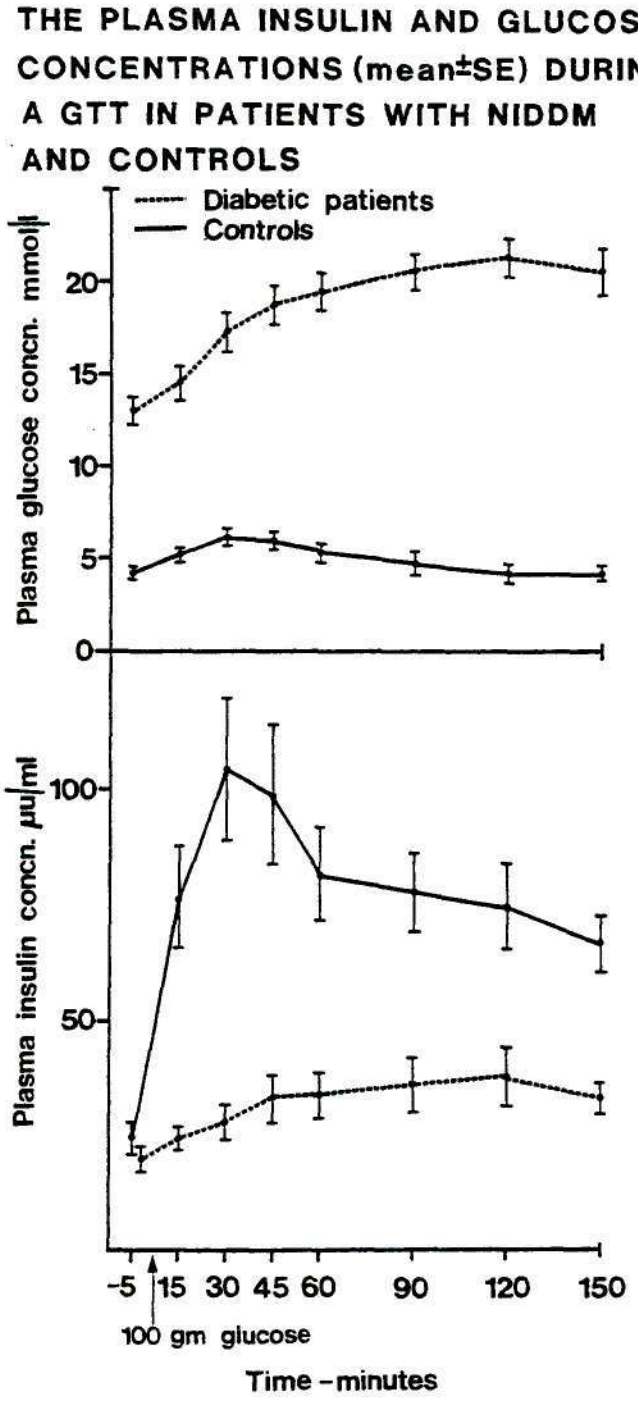
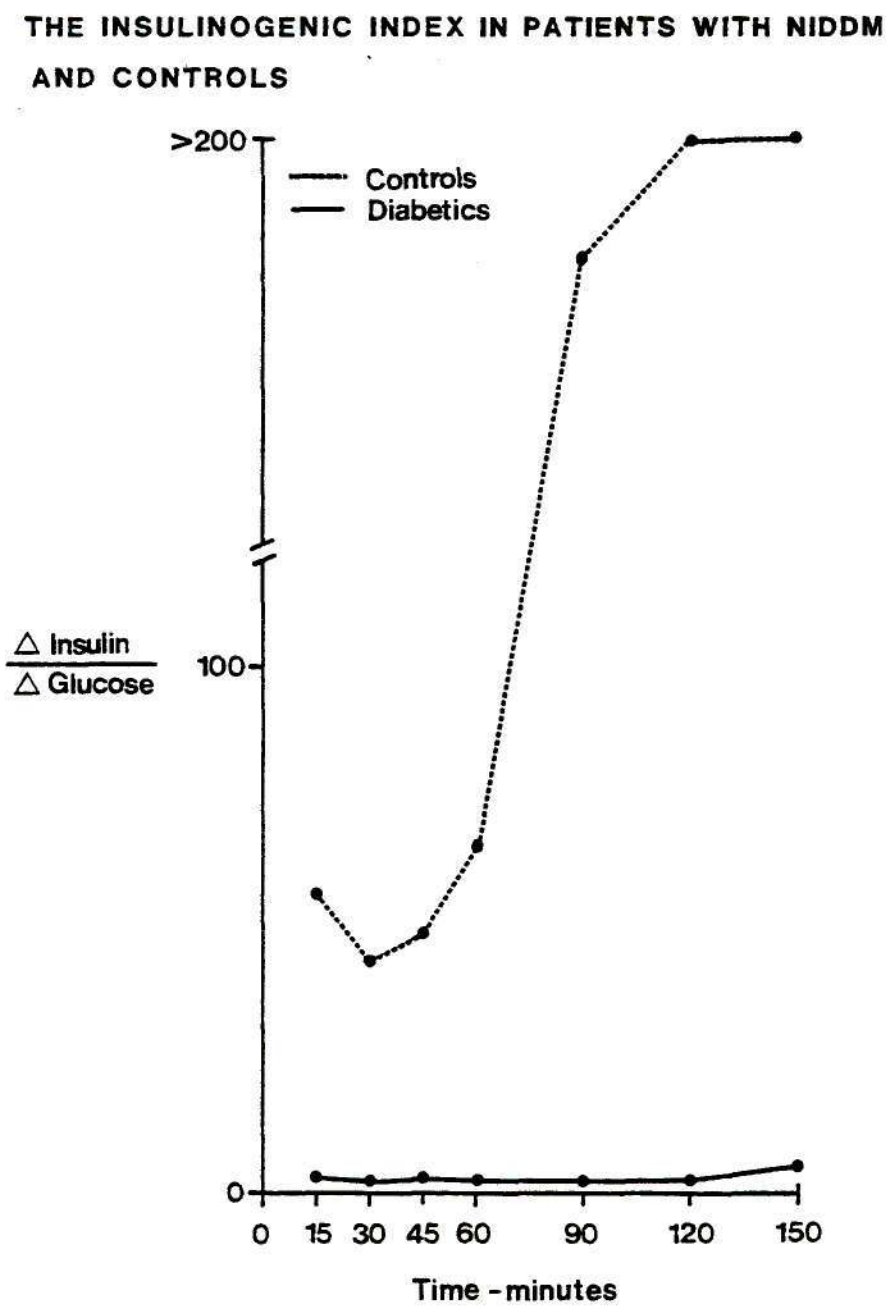

THE PLASMA INSULIN AND GLUCOSE CONCENTRATIONS (mean±SE) DURING A GTT IN PATIENTS WITH NIDDM AND CONTROLS
Diabetic patients
Controls
Plasma glucose concn. mmol/l
20
15
10
5
0
Plasma insulin concn. μu/ml
100
50
-5
15
30
45
60
90
120
150
100 gm glucose
Time - minutes

Figure 5.


THE INSULINOGENIC INDEX IN PATIENTS WITH NIDDM AND CONTROLS
>200
100
0
Controls
Diabetics
Δ Insulin / Δ Glucose
0 15 30 45 60 90 120 150
Time - minutes

There is no correlation between the basal glucose levels and the maximal insulin levels, Figure 6.

Duration of NIDDM also shows no correlation with the basal or maximal insulin levels, Figure 7.

The African patients show significantly lower basal insulin levels (mean 14.8 $\pm$ 2.1 µu/ml) compared to Indian patients (mean 23.3 $\pm$ 2.1 u/ml), $p < 0.01$, Figure 2. Although the mean maximal value is also lower in the former group (35.7 $\pm$ 8.3 µu/ml) compared to the latter (mean 54.9 $\pm$ 8.9 µu/ml), the difference falls short of statistical significant ($p < 0.1$). The Indian patients show significantly higher insulin values at 30, 45, 60, 90, 120 and 150 minutes than the African patients, Figure 3.

The insulinogenic indices in the 2 race groups show that plasma insulin rises relatively higher in Indians with NIDDM ($p < 0.01$), Figure 8. Only 2 African patients showed a maximal insulin level of greater than 50 µu/ml compared to 10 Indian patients.

The 2 racial groups do not show any significant differences in the plasma glucose values taken at various points of the glucose tolerance test, Figure 3.

DISCUSSION

In this study it has been clearly shown than NIDDM in young Africans and Indians is characterised by a blunted and delayed insulin response to a glucose load and that in this respect it does not differ much from the pattern reported by Asmal and Leary (1975) in older non-insulin-dependent diabetic patients belonging to the same race groups. Fajans et al (1976); Fajans et al (1978) have also observed a similar insulin response in most of their young patients with NIDDM. Studies by Panzram and Adolph (1981) showed similar patterns of insulin secretion in younger and older patients with NIDDM.

Figure 6.

THE CORRELATION BETWEEN FASTING PLASMA GLUCOSE AND MAXIMAL PLASMA INSULIN LEVELS IN PATIENTS WITH NIDDM

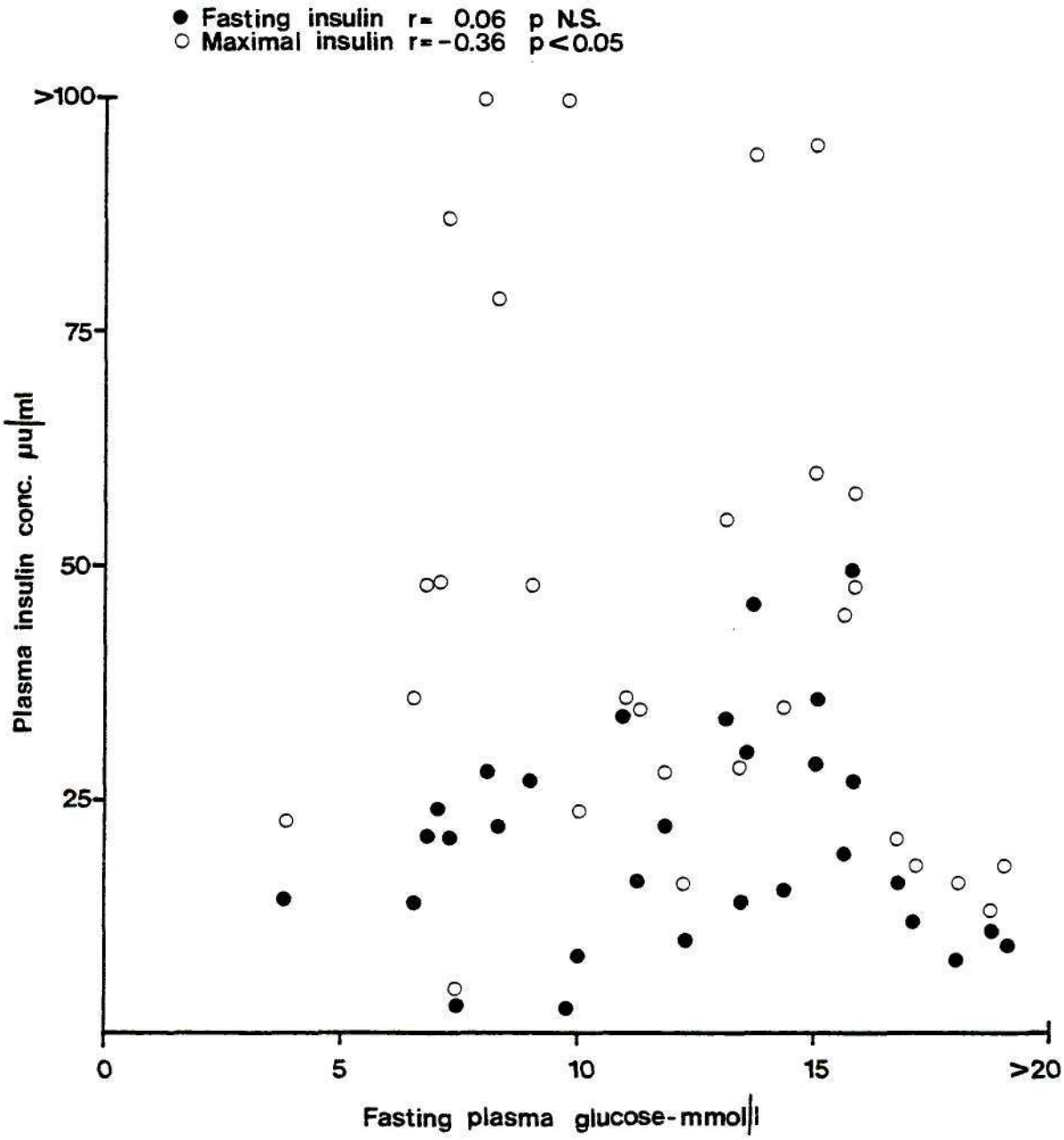


Figure 7.

THE RELATIONSHIP BETWEEN DURATION OF NIDDM AND FASTING AND MAXIMAL PLASMA INSULIN LEVELS IN PATIENTS WITH NIDDM

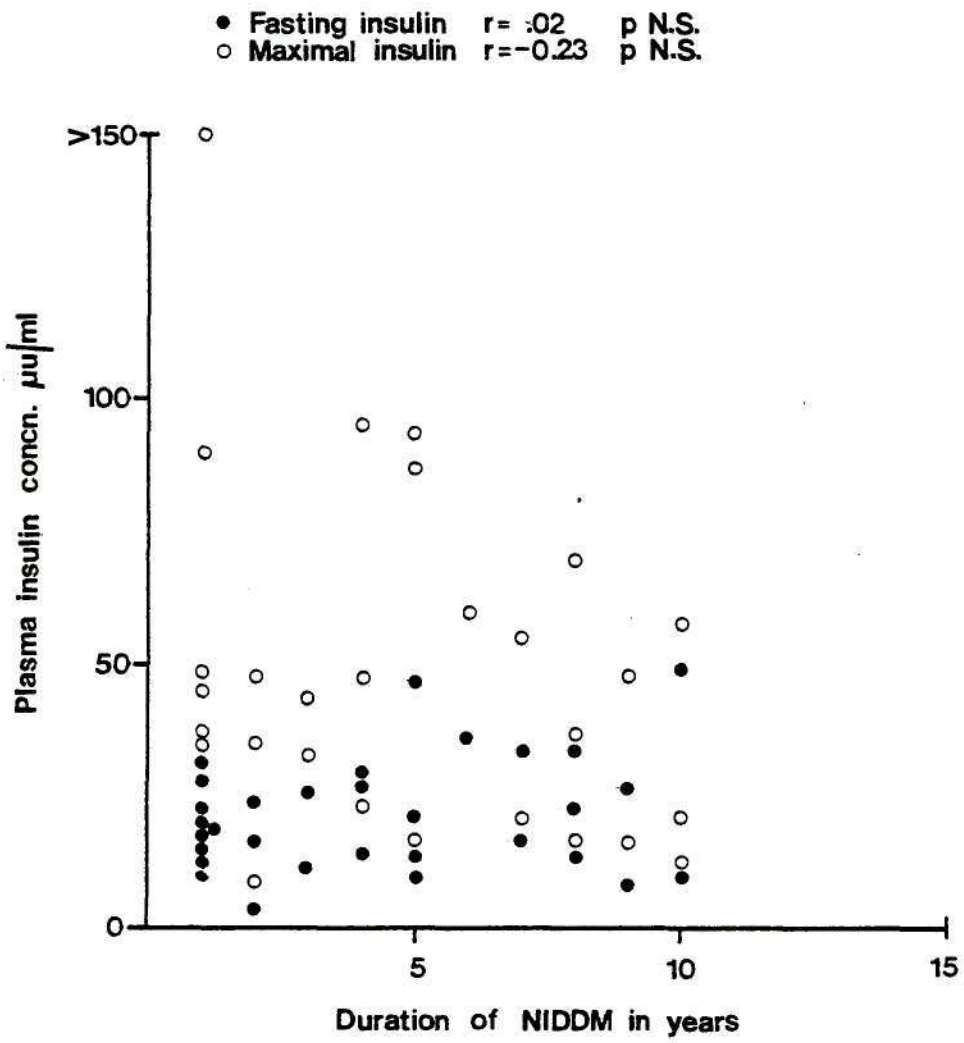
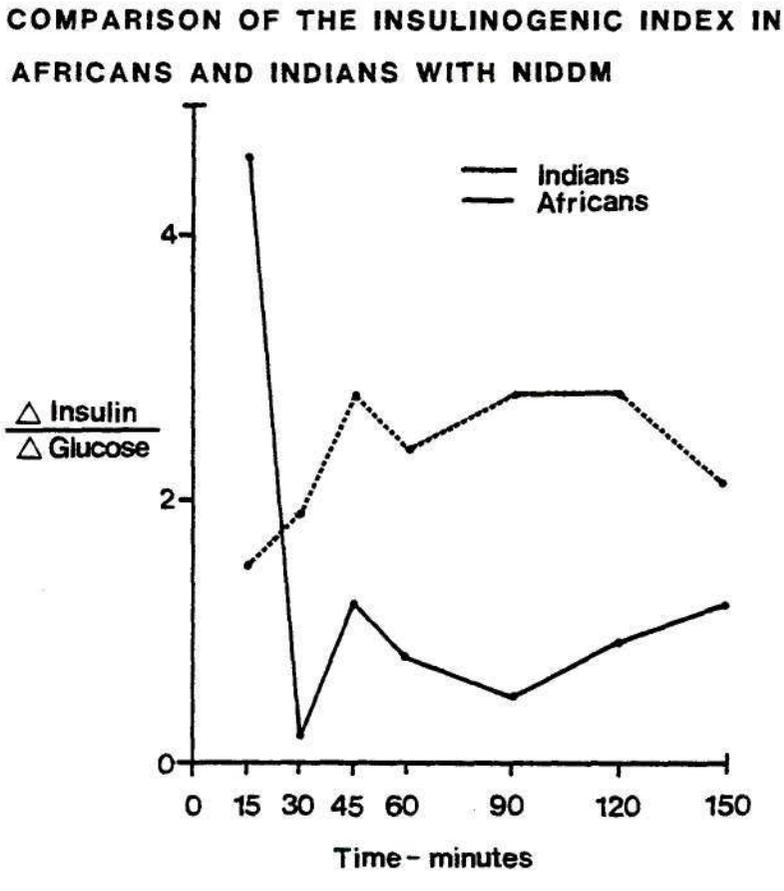


Figure 8.


COMPARISON OF THE INSULINOGENIC INDEX IN
AFRICANS AND INDIANS WITH NIDDM
Indians
Africans
△ Insulin
△ Glucose
4
2
0
0 15 30 45 60 90 120 150
Time - minutes

Heterogeneity of insulin responses to glucose shown by wide variations in the individual patterns is a recognised feature of NIDDM in the young (Fajans et al 1976 , Fajans et al 1978) and in the older age group (Kobberling 1976). Thus the wide spectrum of insulin responses that was observed in the Indian patients in particular is not at all surprising.

The lower insulin levels observed in African patients as a group compared to Indian patients points to the existence of racial differences in the pattern of insulin secretion in NIDDM in the young. This difference is accentuated by the greater mean weight of the African compared to the Indian patients. Asmal and Leary (1975) have also observed a more attenuated insulin response in older Africans with NIDDM compared to a similar group of Indians with the disease. Low insulin responses to a glucose load in Africans with NIDDM have been well documented in previous studies, (Keller et al 1972; Shires et al 1978; Wicks and Jones 1973). Even in White Caucasians Fajans et al (1978) observed a familial aggregation of patients, with low insulin response in some families and high responses in others. These observations suggest that racial and genetic factors play a part in delineating subgroups within the broad entity of NIDDM on the basis of insulin responses.

The lack of a significant difference in insulin levels between African and Indian controls is at variance with the findings of Rubenstein et al (1969) who found lower levels in normal Africans compared to Indians and of Wapnick et al (1972) who found an attenuated response in healthy African cleaners compared to whites. On the other hand Keller et al (1972) observed similar insulin levels in healthy Cape Africans and Indian students, whilst Wapnick et al (1972) found higher values in healthy African students compared to African cleaners. Since the controls used in this study were nearly all students, it is quite possible that differences among the various studies are based on variations in the category of the control groups selected.

Despite markedly decreased insulin responses the African patients show similar plasma glucose values to the Indian patients. In healthy African subjects also normal glucose homeostasis in the presence of

attenuated insulin responses has been observed (Rubenstein et al 1969, Wapnick et al 1972).

The lack of a significant correlation between the fasting plasma glucose and the fasting or maximal insulin level, which was also observed by Asmal and Leary (1975) in older patients, is not unexpected in the light of recent evidence implicating defects at the receptor level rather than insulin secretion per se in the pathogenesis of NIDDM, (Olefsky and Reaven 1974; 1976). Nevertheless, Fajans et al (1976) did observe higher mean glucose levels in patients with low insulin responses, particulary at the time of diagnosis.

The lack of correlation between duration of NIDDM and the individual insulin responses suggest that over a short period of time a decline in beta cell function may not be seen with increasing duration of disease, as has been found in IDDM. Similar observations have been made by Fajans et al (1976) who found that although 90% of their young patients with NIDDM had subnormal and delayed insulin responses to glucose, many of them did not show any deterioration in the responses over periods of up to 12 years. Therefore it would appear that it is the pattern of insulin response in, rather than the duration of, NIDDM that determines progression to insulin dependence, as had been found by Fajans et al (1976); Johansen (1971, 1973); Rosenbloom et al (1973).

SUMMARY

The insulin response to an oral glucose load was studied in 24 Indian and 14 African patients with NIDDM and 20 controls. The maximal insulin levels attained during the glucose tolerance test were significantly lower, and the peak insulin response occurred later in the diabetic patients compared to controls. The insulinogenic indices were also much lower in the patients. African patients showed lower basal and maximal insulin levels and lower insulinogenic indices than Indian patients, despite similar plasma glucose values. No correlation was seen between the fasting or maximal insulin levels and duration of NIDDM.

CHAPTER VII

BASAL HORMONAL AND LIPID PROFILE IN YOUNG DIABETIC PATIENTS

INTRODUCTION

The role of hormones other than insulin in the pathogenesis of diabetes or its complications has been the subject of much debate. The bihormonal theory of the pathogenesis of diabetes mellitus, viz: too little insulin and inappropriate or exaggerated secretion of glucagon, first propounded by Unger et al (1970) and later supported by Gerich (1976) held sway until recently. However, studies by Barnes et al (1975), Lundbaeck et al (1976), Sherwin et al (1976, 1978) provided enough evidence to refute this hypothesis. For instance, infusion of large amounts of glucagon into maturity or juvenile onset diabetics had very little, if any, adverse affect on diabetic control (Sherwin et al 1976).

Similarly although elevated levels of growth hormone have been found in many diabetic patients (Molnar et al 1968; Prange-Hansen A. 1970, 1971, 1973; Unger et al 1965), others have found normal values (Baker et al 1969; Luft and Cerasi 1968). Thus the latter came to the conclusion that growth hormone is diabetogenic only in such instances where the beta cells show an inherited defect in responding to the insulinogenic effect of growth hormone and in producing adequate quantities of insulin. Whilst growth hormone has also been implicated in the pathogenesis of some of the complications of diabetes, notably retinopathy, (Knopf et al 1972), any direct line of evidence to support this hypothesis is lacking (Luft and Guillemin 1974).

The role of plasma lipids in accelerating atherosclerosis in diabetic subjects can also be seen in a similar light (West 1978). In Framingham Garcia et al (1974) could not find any significant differences

in cholesterol and triglyceride levels between diabetics and controls. Bowyer et al (1974) and Westlund and Nicolaysen (1972) came to the same conclusion. However, Chase and Glasgow (1976) and Wilson et al (1970) observed elevated lipid levels in Caucasian diabetics. Whilst high levels of lipids have been found in Indian (Asmal and Leary 1975) and African diabetics (Shapiro et al 1973) such investigations have been done mainly on older diabetic patients. Data on lipid levels in young African and Indian diabetics are at best scanty.

MATERIALS AND METHODS

The diabetic patients studied all had the disease before the age of 35. They included patients with IDDM, patients with NIDDM and patients who presented with NIDDM but later became insulin dependent. All patients formed part of the groups mentioned in Chapters III and IV. None of the patients had had ketosis in the previous 2 months. Blood samples for glucagon, growth hormone, cortisol, cholesterol and triglyceride were taken in the basal state, after an overnight fast of 12 - 14 hours. Blood samples for glucagon were taken in tubes containing 9 mg of EDTA-Na. 3 000 Iu of Trasylol was added as soon as the blood was taken and the samples were immediately centrifuged, after which the plasma was kept frozen until the time of assay.

Glucagon, growth hormone and cortisol were measured by radioimmuno-assay (Biodata diagnostica, Serono Laboratories, England for glucagon and Gamma Coat and Phadebas Kits respectively for cortisol and growth hormone). Cholesterol and triglyceride concentrations were measured enzymatically.

RESULTS

Glucagon: Glucagon levels were estimated in 40 patients with IDDM (27 Africans, 13 Indians), 16 patients with NIDDM (14 Indians, 2 Africans), 7 patients presenting with NIDDM but later requiring insulin and 18 healthy controls (9 Africans, 9 Indians). The mean basal glucagon levels in the various groups of diabetics and controls are

depicted in Figure 1. There is no significant difference in the mean glucagon levels betwen African and Indian controls. The basal levels in controls (mean 159 ± 25 pg/ml) are significantly lower than in patients with IDDM (mean 306 ± 26 pg/ml, $p < 0.0005$), NIDDM (mean 270 ± 28 pg/ml, $p < 0.005$) and NIDDM prgressing to insulin-dependent diabetes (mean 385 ± 7), $p < 0.0005$).

Although the Indian patients with IDDM show higher glucagon values (mean 313 ± 45 pg/ml) than their African counterpart (mean 303 ± 33 pg/ml), the difference is not significant. Similarly there is no significant difference in glucagon values between patients with IDDM and NIDDM.

The highest mean glucagon levels was seen in the patients initially noninsulin-dependent but later requiring insulin for control.

Growth Hormone: Growth hormone assays were done on 67 patients with IDDM (46 Africans, 21 Indians); 34 patients with NIDDM (7 Africans, 27 Indians); 14 patients progressing from NIDDM to insulin-dependence and 18 controls.

The mean basal growth hormone levels in the various groups of diabetics and controls are depicted in Figure 2. The patients with IDDM show significantly higher levels (mean 2.32 ± 0.31 ng/ml) than controls (mean 0.77± 0.23 ng/ml), $p < 0.01$. However, the values in patients with NIDDM (mean 1.82 ± 0.52 gn/ml) do not show a significant difference from the control values. In patients and controls there are no significant differences in growth hormone levels between Africans and Indians. The mean value in patients with retinopathy (1.67 ± 0.44 ng/ml) showed no significant difference when compared to those without this complication (mean 2.31 ± 0.30 ng/ml).

Cortisol: Cortisol levels were estimated in 82 patients with IDDM (59 Africans, 23 Indians); 52 patients with NIDDM (9 Africans, 27 Indians); 16 patients with NIDDM progressing to insulin dependence; and 18 controls (9 Africans, 9 Indians).

Figure 1

THE BASAL PLASMA GLUCAGON CONCENTRATIONS (mean ± SEM) IN THE DIABETIC PATIENTS AND CONTROLS

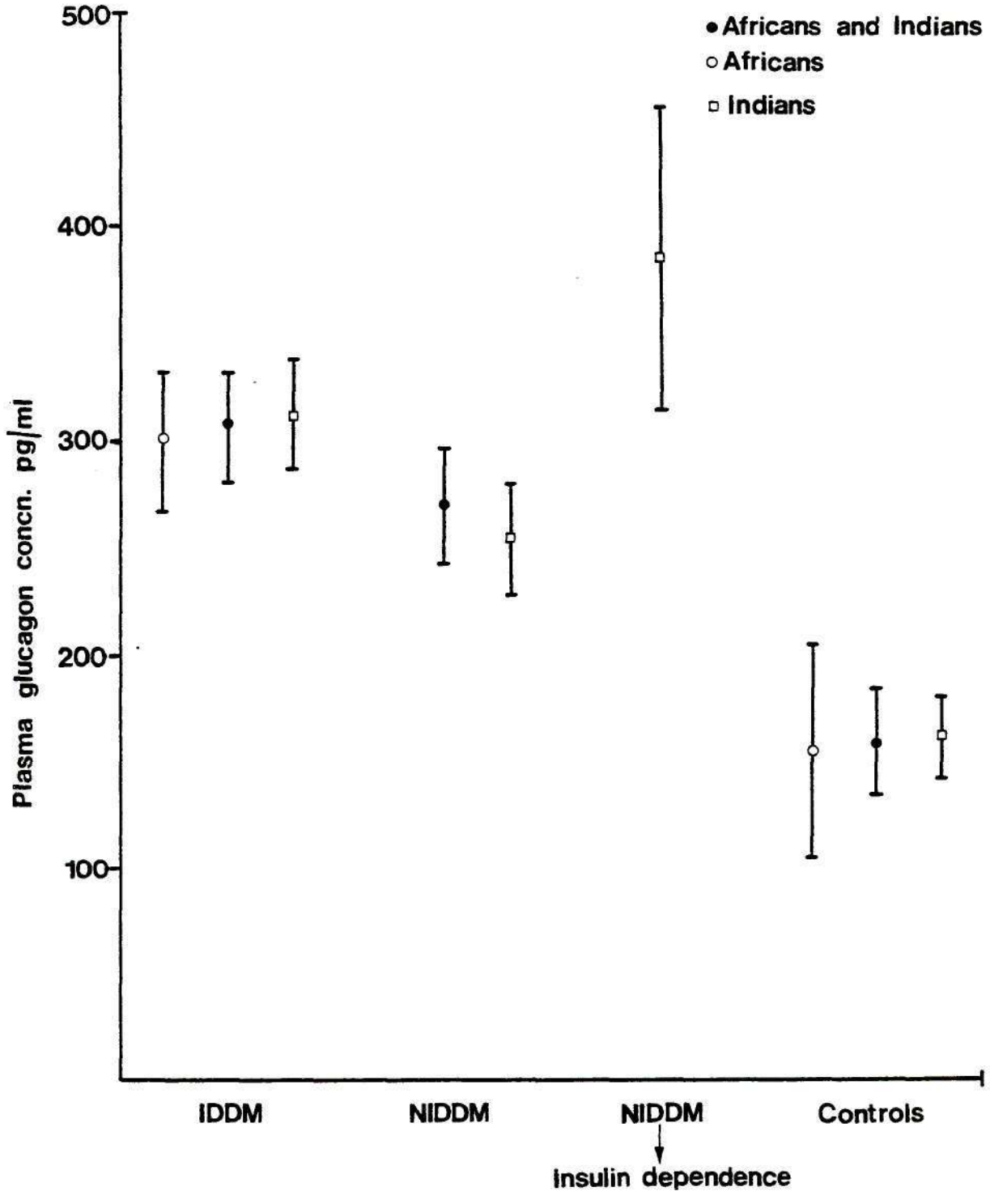


Figure 2.

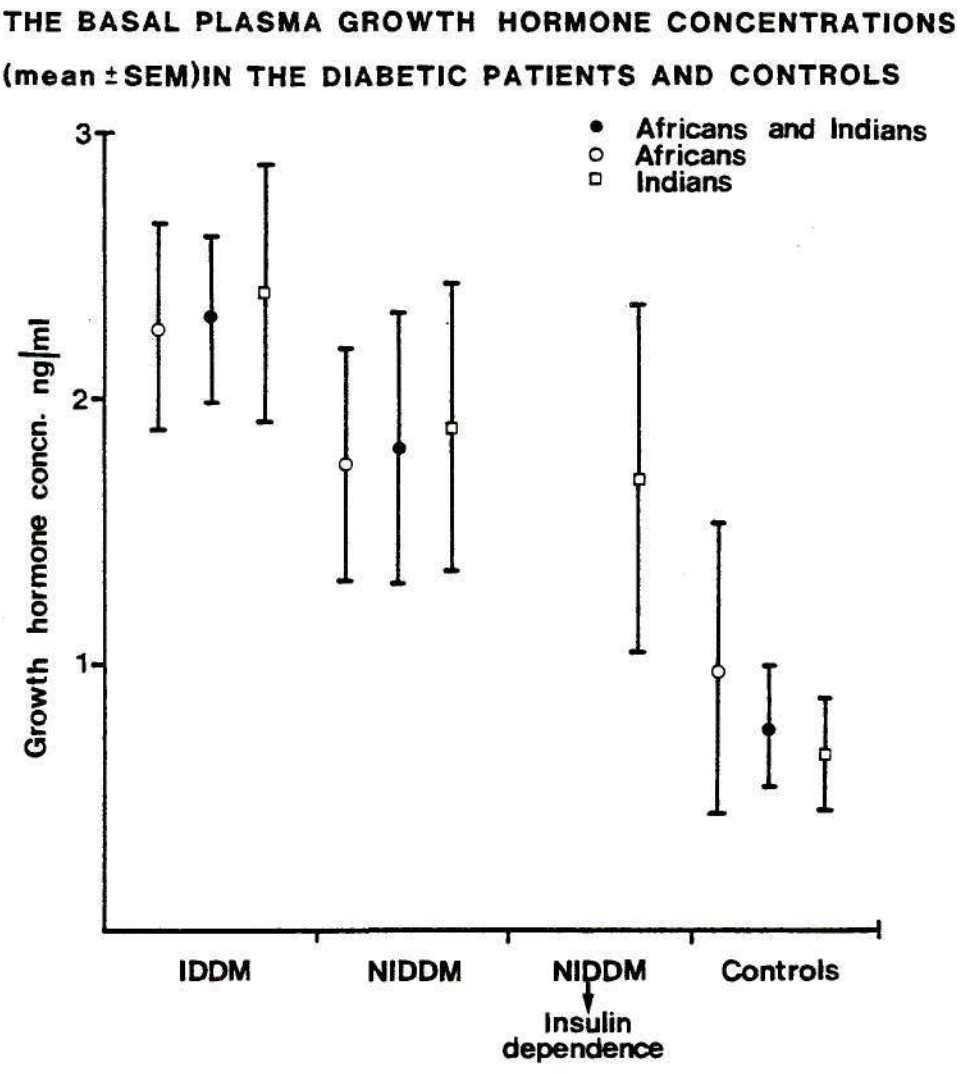
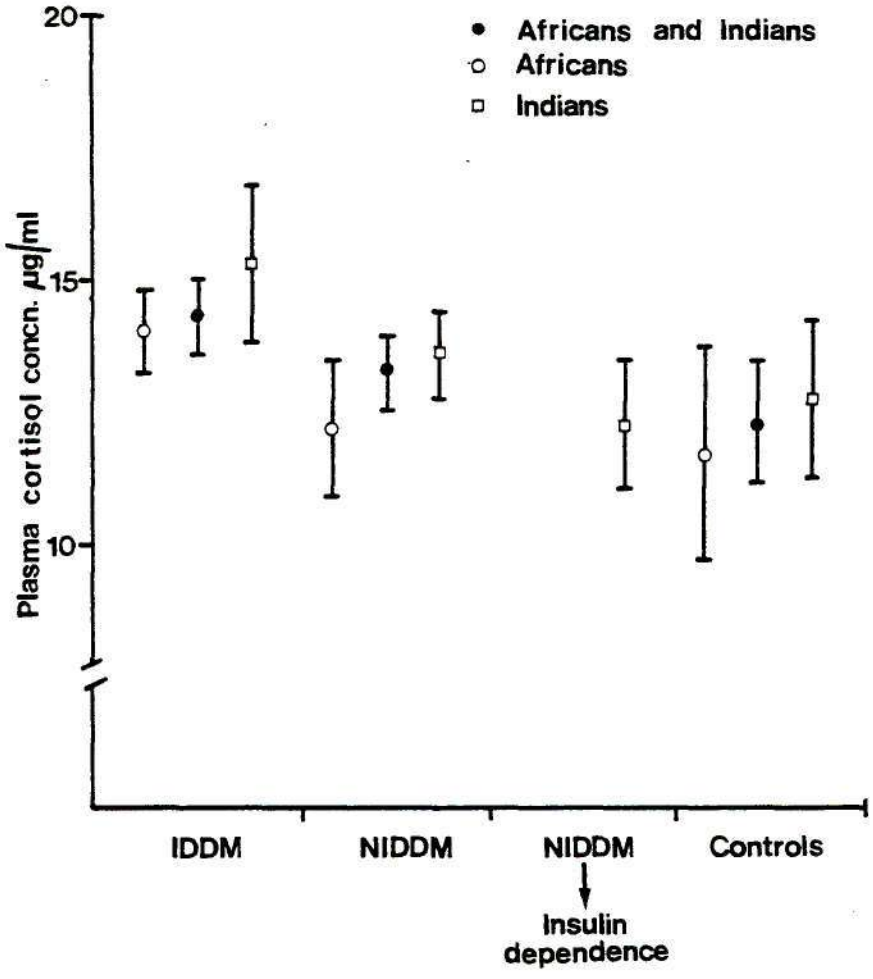

THE BASAL PLASMA GROWTH HORMONE CONCENTRATIONS
(mean ± SEM)IN THE DIABETIC PATIENTS AND CONTROLS
Africans and Indians
Africans
Indians
Growth hormone concn. ng/ml
3
2
1
IDDM
NIDDM
NIDDM
Insulin dependence
Controls

Figure 3.

THE BASAL PLASMA CORTISOL CONCENTRATIONS (mean ±SEM) IN THE DIABETIC PATIENTS AND CONTROLS



The mean cortisol levels in the various groups of diabetics and controls are shown in Figure 3. Although the mean value seen in controls (12.24 ± 1.2 µg/ml) is lower than the means in patients with IDDM (14.23 ± 0.7 µg/ml), NIDDM (13.2 ± 0.7 µg/ml) and NIDDM progressing to insulin-dependence (12.24 ± 1.26 µg/ml) the differences are not significant. Similarly the levels among the various groups of diabetics do not differ significantly from one another. In addition there were no racial differences in cortisol levels among patients and controls.

Cholesterol: Plasma cholesterol concentrations were measured in 98 patients with IDDM (73 Africans, 25 Indians); 55 patients with NIDDM (16 Africans, 32 Indians); 17 patients presenting with NIDDM but later becoming insulin-dependent (all Indians); and 17 controls (9 Africans, 8 Indians).

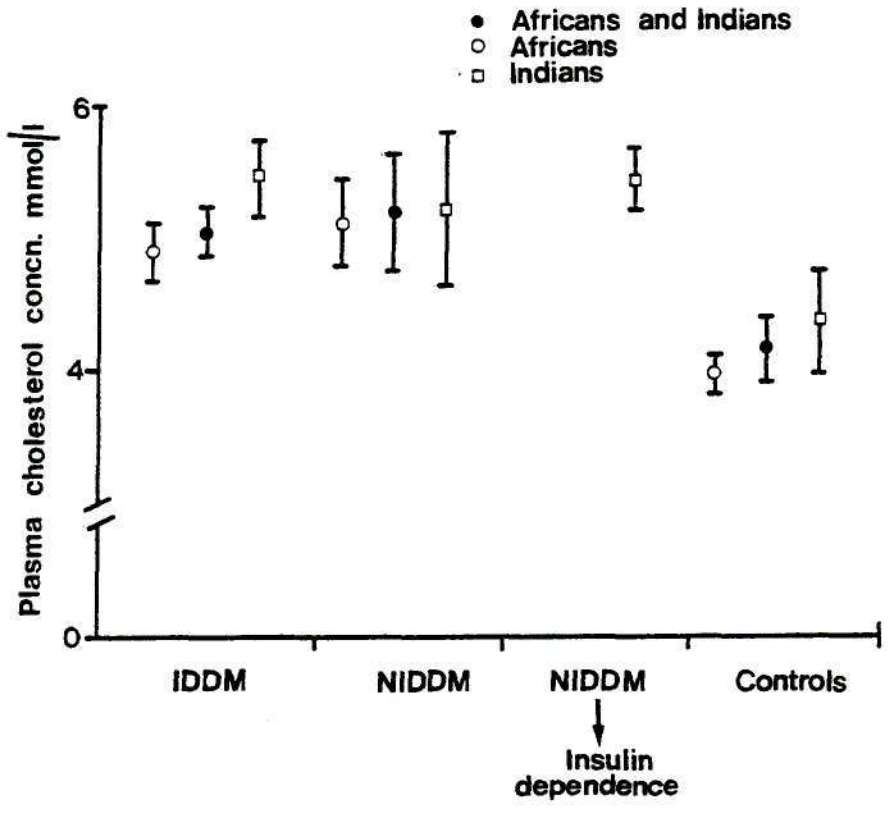
The mean cholesterol concentrations in the various groups of diabetic patients and controls are depicted in Figure 4. The mean value in controls (4.19 ± 0.23 mmol/L) is significantly lower than in patients with IDDM (mean 5.10 ± 0.18 mmol/L, $p < 0.025$); NIDDM (mean 5,23 ± 0.59 mmol/L, $p < 0.025$); and NIDDM progressing to insulin-dependence (mean 5.44 ± 0.31 mmol/L, $p < 0.05$).

The Indian patients with NIDDM show significantly higher cholesterol levels (mean 5.50 ± 0.28 mmol/L) than the Indian controls (mean 4.39 ± 0.44 mmol/L), $p < 0.05$. Those with NIDDM (mean 5.25 mmol/L ± 0.59) also show a significant difference compared to controls, $p < 0.025$.

There is no significant difference seen between African controls (mean 3.98 ± 0.17 mmol/L) and African patients with IDDM (mean 4.95 ± 0.22 mmol/L). The African patients with NIDDM, however, show significantly higher cholesterol values (mean 5.15 ± 0.36 mmol/L) compared to the African controls, $p < 0.025$. Although the mean cholesterol levels were higher in the Indian patients with IDDM and NIDDM when compared to the corresponding African groups, the differences were not significant.

Figure 4.

THE PLASMA CHOLESTEROL CONCENTRATIONS (mean ± SEM) IN THE DIABETIC PATIENTS AND CONTROLS



Triglyceride: Plasma triglyceride concentrations were done on 89 patients with IDDM (63 Africans, 29 Indians), 41 patients with NIDDM (10 Africans, 31 Indians), and 17 controls (9 Afircans, 8 Indians).

The mean plasma triglyceride levels in the various groups of diabetic patients and controls are depicted in Figure 5. The mean value seen in the controls (1.27 $\pm$ 0.73 mmol/L) is significantly lower than in patients with NIDDM (mean 3.07 $\pm$ 0.60 mmol/L, $p < 0.05$). However, the levels in patients with IDDM (mean 1.92 $\pm$ 0.20 mmol/L) do not differ significantly from those in controls. Similarly patients with NIDDM progressing to insulin dependence (mean 2.68 $\pm$ 0.47) do not show a significant difference from the controls.

Although African patients with IDDM show a lower mean triglyceride level (1.79 $\pm$ 0.24 mmol/L) than the Indian patients (mean 2.24 $\pm$ 0.30 mmol/L) the difference is not significant. In patients with NIDDM also no significant difference is seen between the 2 racial groups.

The mean triglyceride concentration in African controls (0.91 $\pm$ 0.10 mmol/L) is lower than that in Africans with IDDM, but the difference falls short of statistical significance. The Africans with NIDDM, however, show significantly higher values (mean 1.76 $\pm$ 0.27 mmol/L) than African controls, $p < 0.01$.

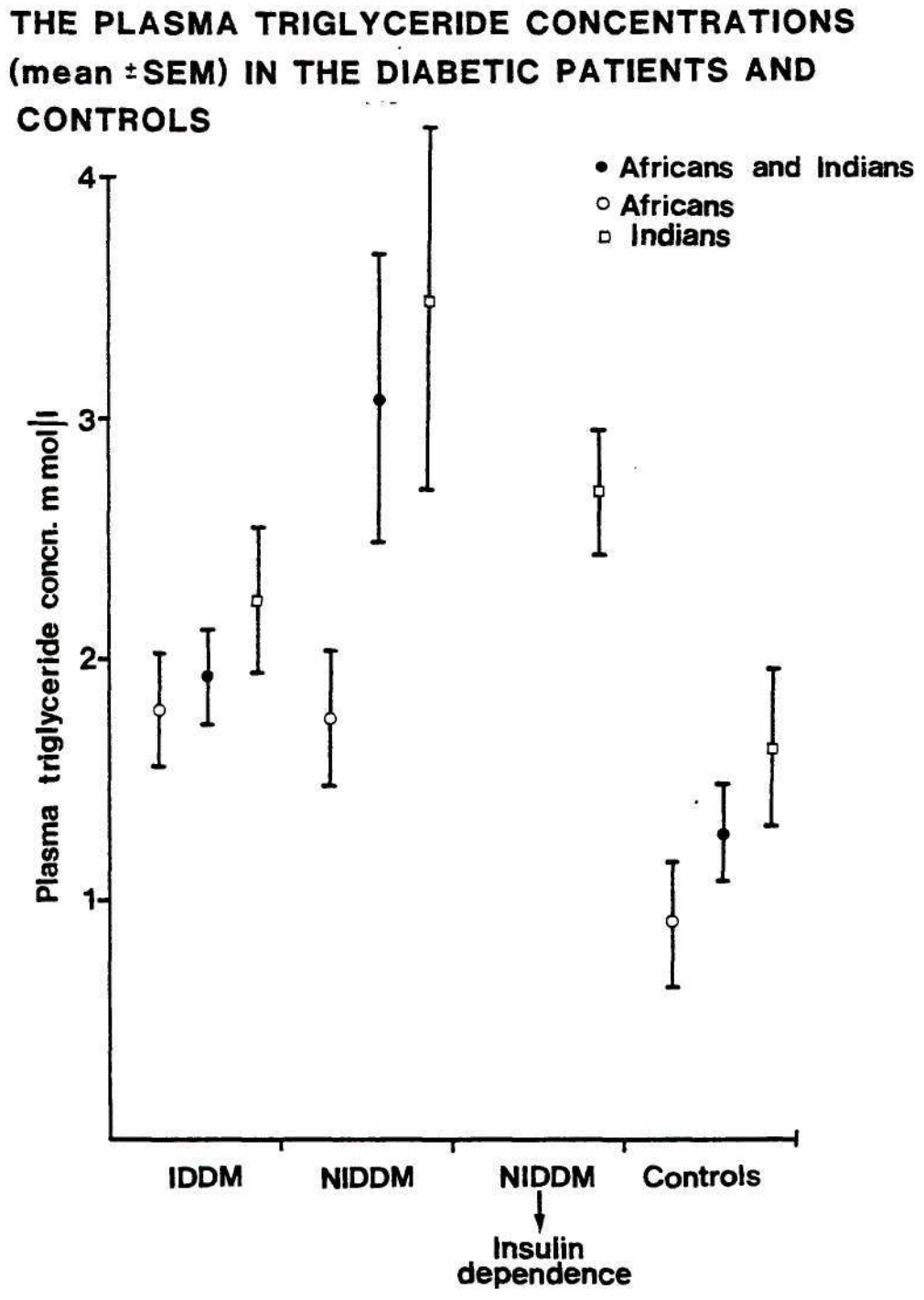
Indians with IDDM and NIDDM, although showing higher mean levels (2.20 $\pm$ 30 mmol/L and 3.49 $\pm$ 0.78 mmol/L respectively) do not differ significantly from Indian controls (mean 1.63 $\pm$ 0.34 mmol/L).

Comparison of the 2 control groups shows that the Indians have significantly higher triglyceride levels than the Africans, $p < 0.05$.

DISCUSSION

The status of diabetes is affected in some way or the other by almost all hormones (Williams 1972). Although it is well known that permanent diabetes may result from administration of large amounts of growth hormone, glucocorticoids or glucagon and that less insulin is

Figure 5.


THE PLASMA TRIGLYCERIDE CONCENTRATIONS (mean ± SEM) IN THE DIABETIC PATIENTS AND CONTROLS
• Africans and Indians
○ Africans
□ Indians
Plasma triglyceride concn. m mol/l
4
3
2
1
IDDM
NIDDM
NIDDM
Insulin dependence
Controls

required for control of diabetes in the absence of growth hormone, cortisol or glucagon (Williams 1975), their role in the aetiology of primary diabetes is obscure.

The markedly elevated glucagon levels found in the diabetic subjects compared to controls have been observed in several studies (Aguilar-Parada et al 1969; Alford et al 1976; Day and Anderson 1973; Heding and Rasmussen 1972). That this elevation probably represents a true excess of secretion in the diabetic patients is supported by the work of Alford et al (1976) who found that the metabolic clearance of glucagon is not decreased in diabetic subjects.

High levels of glucagon in non ketotic diabetic patients, as has been observed in the patients with NIDDM described in this study, led Alford et al (1976) to speculate that this hormone may be responsible for the decreased effectiveness of circulating insulin which was thought to be the mechanism operating in non-ketotic diabetes (Reaven et al 1976). However, Olefsky et al (1977) found no difference in glucagon levels between normal and diabetic subjects and therefore discounted the role of this hormone in causing insulin resistance.

Whilst the unequivocal demonstration of elevated glucagon levels in the African and Indian diabetic patients in this study may appear to support Unger's bihormonal hypothesis in the causation of diabetes most evidence now suggest that hypergluconaemia is a result of the impaired metabolic balance seen in this disease (Assan et al 1969; Matsuyama et al 1975; Muller et al 1973; Pek 1977). Relatively lower glucagon levels have been observed in patients treated with sulphonylureas compared to those on insulin (Loreti et al 1974; Ohneda et al 1975). Such a trend was also observed in this study, since insulin-dependent diabetic patients tended to have higher values than those with NIDDM.

Elevated growth hormone levels in diabetes, which were observed in this study, have been found by various workers (Burday et al 1968; Molnar et al 1972). However, other studies have shown normal levels of this hormone in diabetes, particularly in children, (Baker et al 1967; Baker et al 1969; Parker et al 1967). Thus the role of growth hormone

itself in the pathogenesis of diabetes mellitus can be seriously questioned. Another drawback to such a hypothesis is that in acromegaly only a minority of patients develop diabetes mellitus despite the marked increase in growth hormone production.

Based on the observations that patients with retinopathy have high growth hormone levels (Knopf et al 1972; Knowles 1975) and that hypophysectomy may ameliorate this condition, this hormone has been implicated in the pathogenesis of such complications. However, the fact that acromegalic patients are relatively free of angiopathy argues against such a postulate (Luft and Guillemin 1974). In this study the mean growth hormone level in patients with retinopathy was lower than in those without this complication, although the difference was not significant. Thus the findings in this study are at variance with those of Knopf et al (1972) who observed higher growth hormone levels in patients with retinopathy.

Elevated growth hormone levels and normal cortisol values in Indians with NIDDM provide enough scientific evidence to refute Campbell's postulate (made without any scientific evidence) that the young Natal Indian diabetic suffered from a form of diabetes characterised by a relative insulin deficiency, overproduction of glucocorticoids and relative lack of growth hormone. (Campbell 1960; Campbell and McKechnie 1961).

Although earlier work suggested that the secretion of adrenal hormones was considerably higher in diabetics compared to controls (Forbes et al 1947; Fraser et al 1941; Miller and Mason, 1945), subsequent studies did not confirm this finding (Dancaster et al 1963).

The mean cholesterol and triglyceride levels in African controls are almost identical to the values found in a group of adolescent African girls by Walker et al (1980). The lack of significant difference in cholesterol levels between the small numbers of African and Indian controls is not entirely unexpected, since Walker (1973) also found similar mean values between urban Indians and urban African teachers and clerks in a study involving a fairly representative group of healthy adults between the ages of 30 to 39.

Although the cholesterol levels were higher in the diabetic patients compared to controls, the mean values would, according to Walker's observations (Walker 1966), be well within the normal range for these population groups. Taking 5.78 mmol/L as the cut-off point, Walker (1973) found that 25% of healthy urban African and 55% of healthy Indians had a cholesterol concentration in excess of that figure. In this study less than 20% of African and only 25% of Indian diabetic patients had a cholesterol level above 5.78 mmol/L. Similarly less than a third of African and Indian patients with NIDDM had higher values. Studies involving White Caucasians have also shown relatively normal levels of cholesterol in diabetic patients (Garcia et al 1974; Kenien et al 1977; Lundbaeck and Petersen 1953). In middle aged black diabetic patients also relatively low levels have been observed (Shapiro et al 1973). However, Asmal and Leary (1975) showed elevated levels in middle aged African and Indian patients, whilst others have found high values in White Caucasians with juvenile diabetes (Chase and Glasgow 1976; Sterky et al 1963).

Elevated triglyceride levels in diabetes mellitus have been documented in numerous studies involving White Caucasians and older African and Indians with the disease (Asmal and Leary 1975; Shapiro et al 1973; Walker 1966; Wilson et al 1970). Similar findings have been observed in this study. NIDDM in Indians in particular appears to be associated with high triglyceride levels.

Since the prevalence of large vessel disease was low in the patients studied, probably because of the relatively short duration of diabetes in most of them, it is difficult to draw any conclusions on the relationship of serum lipids to such complications.

SUMMARY

Basal glucagon, growth hormone, cortisol, cholesterol and triglyceride concentrations were estimated in patients with IDDM and NIDDM and those initially presenting with NIDDM but later becoming insulin dependent. All the groups of diabetic patients showed significantly higher glucagon levels compared to controls. Higher growth hormone levels were found in the patients compared to controls, the difference being significant in those with IDDM. No significant difference in growth hormone levels were seen between those with and those without retinopathy. Mean cortisol levels in the diabetic patients and the controls did not differ significantly from each other. There were no racial differences in the levels of these hormones among patients and controls. Although the mean cholesterol and triglyceride levels in the patients were significantly higher than in the controls, they were well within the range of normal values.

CHAPTER VIII

GLYCOSYLATED HAEMOGLOBIN LEVELS IN THE YOUNG AFRICAN AND INDIAN DIABETIC PATIENTS

INTRODUCTION

Haemaglobin A_1 is a glycosylated haemoglobin formed by the binding of glucose to the N-terminal valine of the beta-chain of adult haemoglobin. The process is a non-enzymatic one, being dependent on the degree and duration of hyperglycaemia. Possessing the property of rapid mobility on electrophoresis, Hb A_1 constitutes a minor component of adult haemoglobin.

After Huisman and Dozy (1962) had observed elevated glycosylated levels in diabetic patients, several other groups reported on the correlation between diabetic control and haemoglobin A_1 levels (Gabbay et al 1977; Gonen et al 1978; Koenig et al 1976). It soon became apparent that this haemoglobin moiety is a useful objective indicator of the integrated plasma glucose level over prolonged periods of time (Gabbay 1976; Peterson et al 1977; Anon. 1977). Subsequently, however, it was shown that even short term fluctuations in blood glucose may alter Hb A_1 levels, but in a reversible fashion. (Bolli et al 1981; Karamanos et al 1977; Widness et al 1980). Thus the process of glycosylation occurs in two ways, viz: a reversible one due to acute changes in blood glucose and an irreversible one due to chronic hyperglycaemia. (Franklin Bunn 1981).

However, the difference between the glycosylated haemoglobin level that included the reversible component and the level consisting of the stable moiety only was relatively small, with the maximal fluctuation being not more than 1.5% over 2 - 12 hours (Bolli et al 1981; Botterman 1981). Moreover, the maximal changes only occurred in the range from

14% to 15%, wheras at lower levels the differences were very small and only slightly above the range of error of methodology, (Botterman 1981). Thus it was concluded that in the clinical context greater fluctuations of glycosylated haemoglobin values in the range of 14% to 15% are of little significance because patients with such high values are already in poor control and require close attention regardless of any minor variations, (Botterman 1981).

PATIENTS AND METHODS

Ninety seven patients with insulin-dependent diabetes and twenty one patients with noninsulin-dependent diabetes were studied. All patients formed part of the group described in Chapter II. They were followed up regularly at the Diabetic Clinic of King Edward VIII Hospital. The age, race, sex, duration of diabetes and dose and frequency of insulin therapy were recorded for each patient, Tables 1 and 2.

All patients were studied in the post-prandial state (between 08.00 and 12.00). Venous blood was taken for estimation of HbA_1 and plasma glucose in all the patients. Where more than one HbA_1 levels were done, the mean value was recorded. In 80 patients at least 2 measurements were performed over a 12 month period.

Plasma glucose was measured using an autoanalyser. HbA_1 levels were measured using the Helena Glycosylated (Fast Fraction) Haemoglobin Quik Column Kit which utilises a microchromatographic technique for rapid quantitation of glycosylated haemoglobin.

RESULTS

The mean HbA_1 level in a group of 49 healthy subjects was 7.50% $\pm$ 0.49% (S.D.). The distribution of HbA_1 levels in the patients with NIDDM and the insulin-dependent diabetic patients are depicted in Figures 1 and 2. The levels are significantly lower in patients with NIDDM (mean $\pm$ SE - 10.6 $\pm$0.4%) than the insulin-dependent diabetic patients (mean 12.1% $\pm$ 0.2%) $p < 0.0025$. There were 57 patients on a single daily dose of insulin and they had a mean HbA_1 level of 11.75 $\pm$ 0.29% respectively. In the

TABLE 1 CLINICAL CHARACTERISTICS OF THE INSULIN DEPENDENT DIABETIC PATIENTS

	Africans	Indians	Total
Number of patients	54	43	97
Male : female ratio	23 : 31	17 : 26	40 : 57
Mean weight in Kg (range)	53.9 (31-76)	53.2 (30-87)	53.6
Mean age in years (range)	25.5 (6-49)	29.5 (12-44)	27.2
Mean duration of insulin therapy in years (range)	4.6 (0.3-16)	5.9 (1-12)	5.1
Mean insulin requirement in Iu/Kg/day (S.E.)	1.42 $\pm$ 0.11	1.18 $\pm$ 0.08	1.33 $\pm$ 0.07
Patients on twice daily insulin	23	17	40

TABLE 2 CLINICAL CHARACTERISTICS OF THE PATIENTS WITH NIDDM

	Africans	Indians	Total
Number of patients	7	14	21
Male : female ratio	1 : 6	4 : 10	5 : 16
Mean weight in Kg (range)	73.5 (54-105)	62.7 (52-91)	66.3
Mean age in years	31.3 (19-35)	32.8 (27-40)	32.3
Mean duration of NIDDM in years	2.2 (1-3)	3.6 (1-9)	3.13

Figure 1.

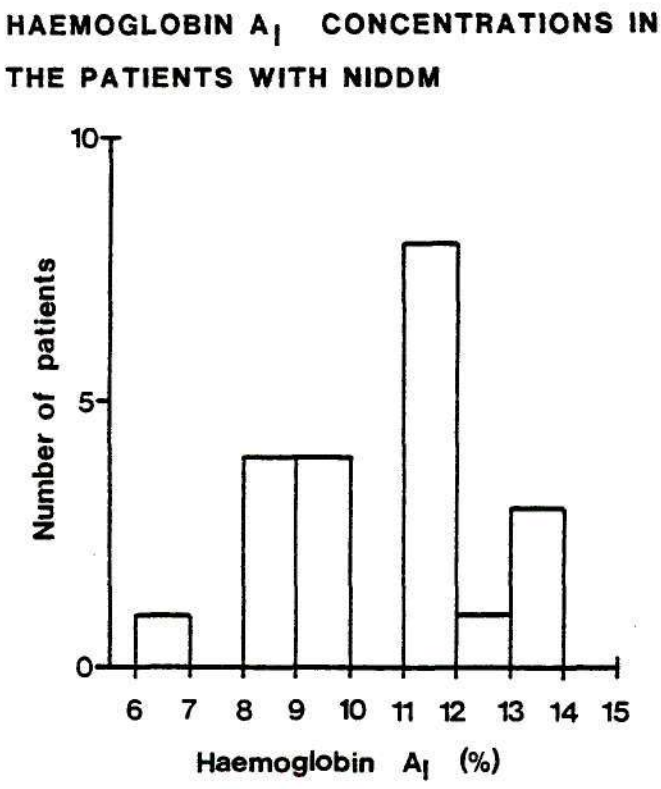
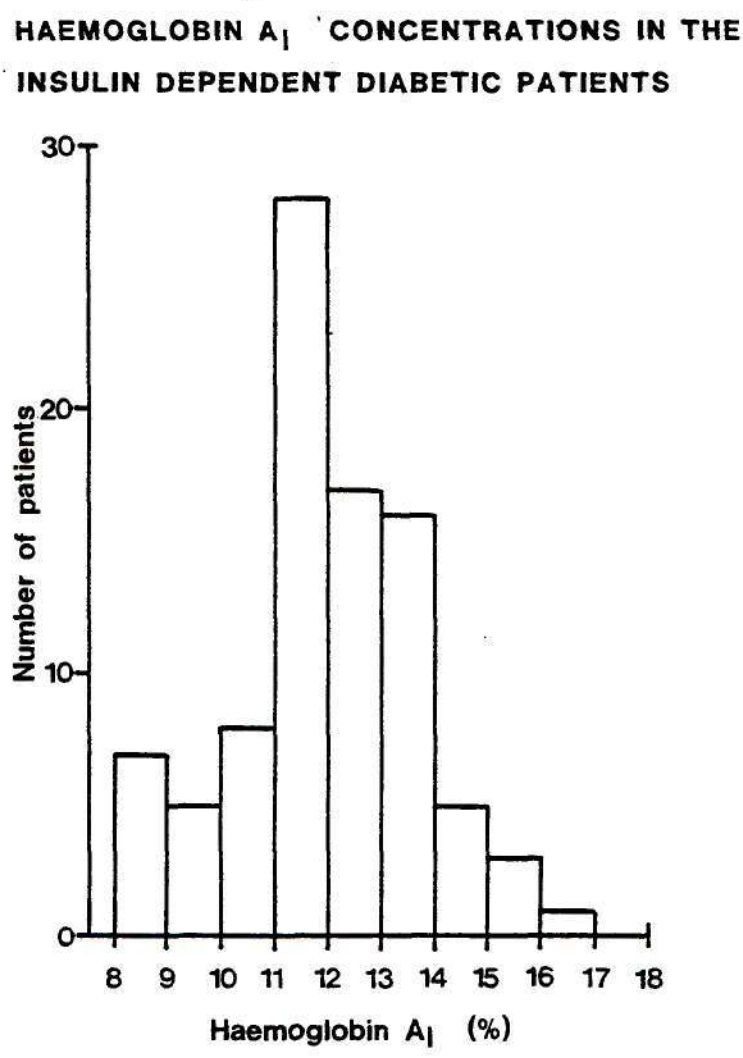

HAEMOGLOBIN A_1 CONCENTRATIONS IN THE PATIENTS WITH NIDDM
Number of patients
10
5
0
6 7 8 9 10 11 12 13 14 15
Haemoglobin A_1 (%)

Figure 2.


HAEMOGLOBIN A_1 CONCENTRATIONS IN THE INSULIN DEPENDENT DIABETIC PATIENTS
Number of patients
30
20
10
0
8
9
10
11
12
13
14
15
16
17
18
Haemoglobin A_1 (%)

40 patients on a twice daily insulin regime the mean HbA_1 was 12.7 ± 0.24%. Twelve patients had a HbA_1 level below 9% which is slightly higher than three standard deviations above the normal mean value. They comprised 5 patients with NIDDM (25%) and only 7 insulin dependent diabetic patients (7%).

The African patients with insulin-dependent diabetes had a significantly lower HbA_1 level (mean 11.8% ± 3%) than their Indian counterparts (mean 12.4% ± 3%). In addition, the random plasma glucose levels in the former (mean 12.9% ± 0.9%) were significantly lower than in the latter (mean 15.2% ± 1.1%), $p < 0.05$.

Hb A_1 and Beta Beta Cell Function

In the insulin-dependent diabetic patients there is a significant negative correlation between the Hb A_1 levels and the maximal C-peptide concentrations attained during a GTT ($r = -0.47$ $p < 0.05$), Figure 3. However no correlation is seen between the Hb A_1 levels and the fasting C-peptide. In patients with NIDDM no correlation could be established between HbA_1 levels and the fasting or maximal insulin levels observed during a glucose tolerance test, Figure 4.

HbA_1 and Duration of Diabetes

In patients with both NIDDM and IDDM no correlation is seen between HbA_1 values and the duration of diabetes.

HbA_1 and Type of Insulin-Dependent Diabetes

In the 14 patients who initially presented as NIDDM but later required insulin, the HbA_1 values (mean 12.5% ± 0.5%) do not differ significantly from those of patients who have always been insulin-dependent (mean 12.0% ± 0.21%).

Serial Measurements of HbA_1

In the 68 patients with IDDM who had HbA_1 values done more than once (at intervals of at least 2 months between consecutive estimations) during a one year period no change (defined as more than 1% above or below the initial value), (Gonen et al 1979), was seen in 10 patients. A decrease was observed in 43 patients whilst an increase was seen in

THE CORRELATION BETWEEN HAEMOGLOBIN A_1 LEVELS AND FASTING AND MAXIMAL PLASMA C-PEPTIDE CONCENTRATIONS IN PATIENTS WITH IDDM

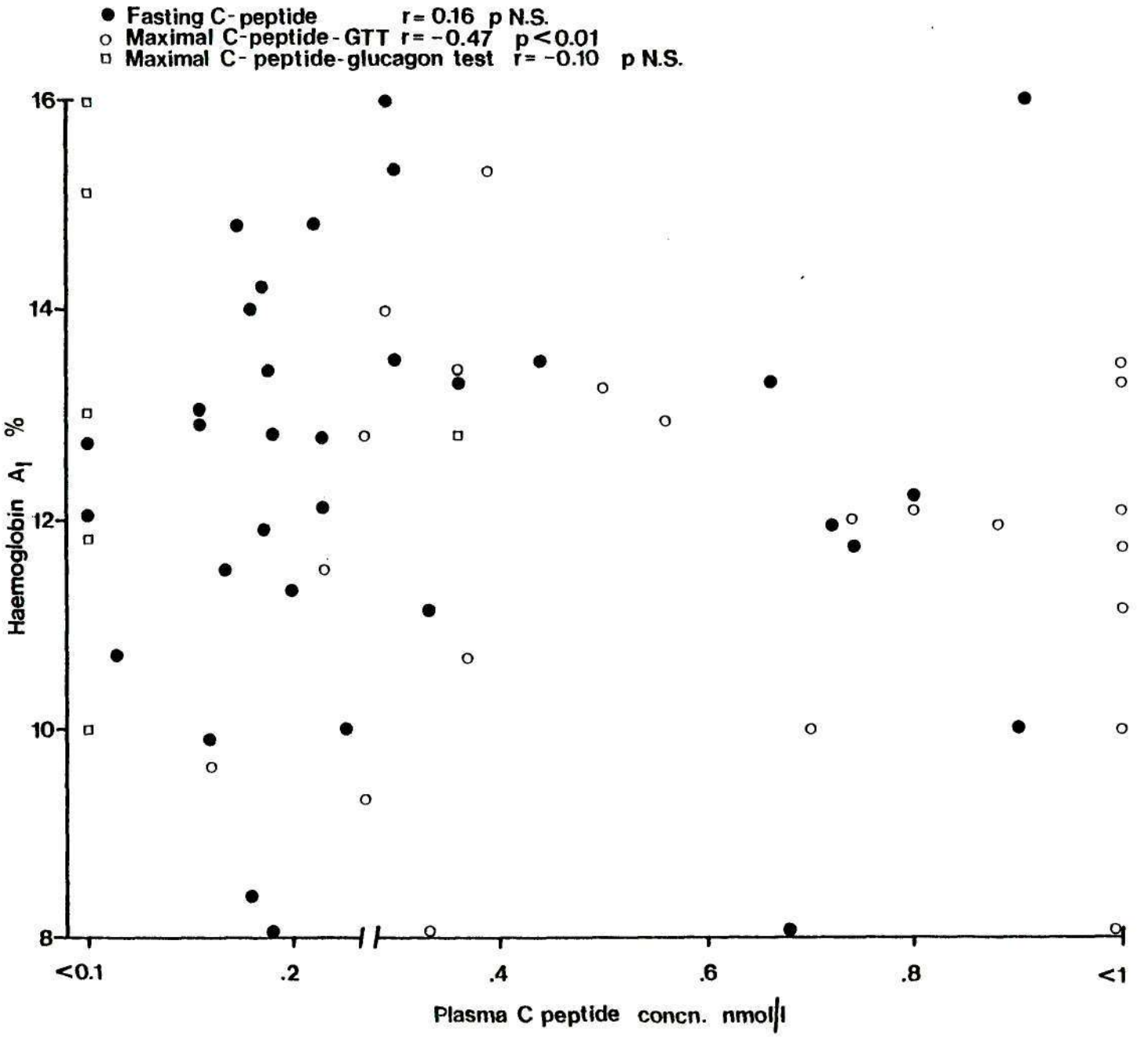
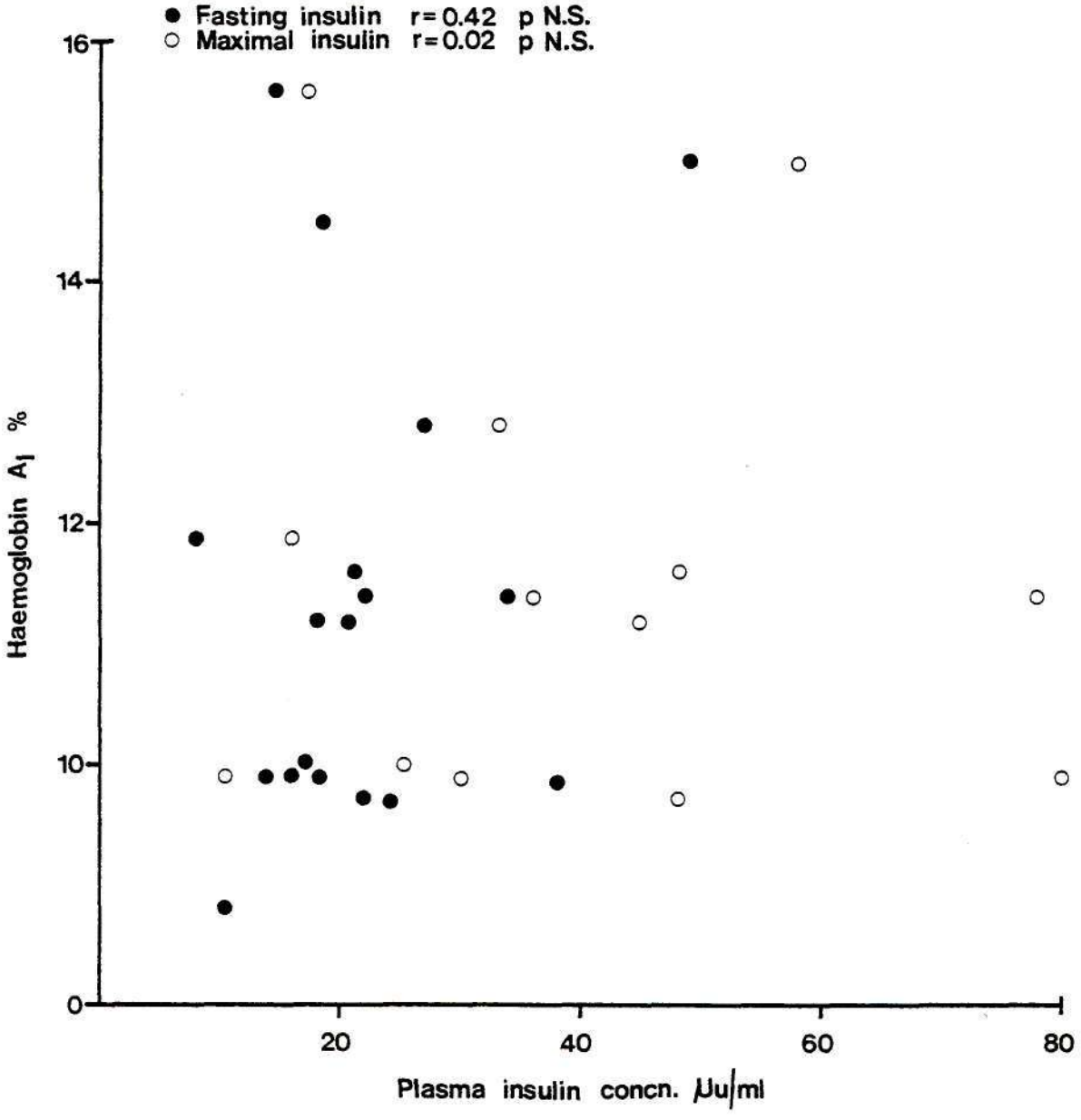


Figure 4

THE CORRELATION BETWEEN HAEMOGLOBIN A_1 LEVELS AND FASTING AND MAXIMAL PLASMA INSULIN CONCENTRATIONS IN PATIENTS WITH NIDDM



only 2 patients. Among the 34 patients with an initial value of less than 14, no change was observed in almost 25% of them, whilst a decrease was observed in 50% and an increase in the remainder.

HbA_1 and Random Plasma Glucose Levels

A significant correlation is seen between HbA_1 levels and random plasma glucose concentrations taken at the same time, Figure 5. In 13 patients there is a discrepancy between HbA_1 levels and random plasma glucose concentrations. All of them showed poor control on the basis of HbA_1 levels (mean 14.2% $\pm$ 0.6%), but at the same time had glucose values in the near normal range.

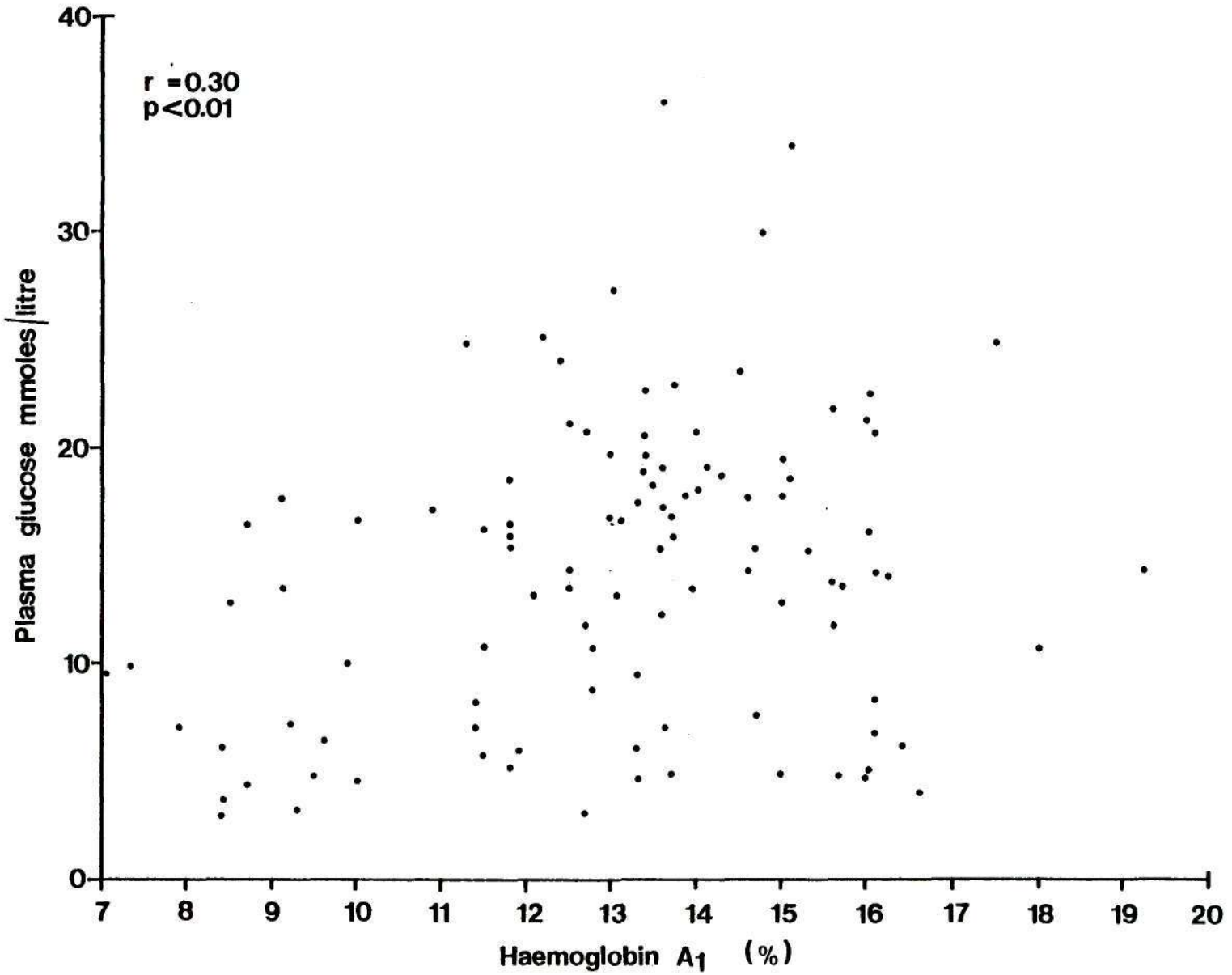
DISCUSSION

The aim of therapy in diabetic patients is to control the blood glucose level to as near normal as possible. However, in practice this objective is achieved in very few patients as shown in recent studies, (Gonen et al1979, Tchobroutsky et al 1980).

Studies on metabolic control based on HbA_1 levels have highlighted the poor quality of diabetic control in insulin-dependent diabetic patients even in referral centres where the establishment of good metabolic control is of primary concern. Taking three standard deviations below the mean to be indicative of excellent control, Gonen et alia (1979) found that only 13% of their insulin-dependent diabetic patients fell into this category. A similar study done by Tchobroutsky et alia (1980) showed that 19% of their insulin-dependent diabetics could be regarded as being in excellent control. The results in this study show that a very small percentage (7%) of insulin dependent diabetic patients were found to be in excellent control (HbA_1 < 9.0%). Even if a level of 12% is arbitrarily chosen as the upper limit for acceptable though by no means good metabolic control, (Gonen et al 1979), then only 44% of the patients fell into this category. Thus the majority of the patients did not measure up even to a satisfactory standard of diabetic control. Several reasons could account for such findings. Firstly in many of our patients primary health care facilities are conspicuous by

Figure 5

CORRELATION OF RANDOM PLASMA GLUCOSE CONCENTRATION AND THE PERCENTAGE HAEMOGLOBIN A_1 IN THE DIABETIC PATIENTS.



their absence. In some because of poor socio-economic status a diabetic diet is difficult to follow. Moreover, because of poor education other patients found it difficult to administer the correct dose of insulin.

The significantly lower HbA_1 levels in patients with NIDDM shown in this study has also been observed by others (Gonen et al 1979) and reflects the greater ease with which this type of diabetes can be controlled.

As there was a significant negative correlation between HbA_1 levels and maximal C-peptide concentrations it would appear that glycosylated haemoglobin estimations may provide a rough index of beta cell function in insulin-dependent diabetic patients. In patients with NIDDM, however, the absence of a significant correlation between HbA_1 levels and the maximal insulin value may point to a defect at the receptor level serving to accentuate the metabolic abnormality.

The significance of serial estimations of HbA_1 levels in this study as an indicator of improved metabolic control is hampered by the presence of reversibly glycosylated components of glycosylated haemoglobin that are formed by acute elevations of plasma glucose, (Bolli et al 1980). However, since the reversible moiety only makes a significant contribution to the glycosylated haemoglobin level in the range 14% - 15% (Botterman 1981), sequential HbA_1 levels in patients in whom initial values were less than 14% may provide a useful index of the ease with which metabolic improvement may be achieved. Thus a trend towards improvement was seen in only 50% of the insulin dependent diabetic patients, illustrating the difficulty with which good metabolic control can be attained in IDDM.

Tchobroutsky et al (1980) found a significantly lower HbA_1 level in patients who were on a twice daily insulin regime. In this study, however, patients on a once daily insulin regime had a significantly lower HbA_1 level than those on a twice daily regime. It is difficult to draw any conclusions from this, since in many of the patients (except the very young) a single daily dose of insulin therapy was instituted

initially and only if control was poor, were they changed to twice daily therapy.

Although both the African and Indian patients reported in this study came from poor socio-economic background and hence found it difficult to follow a diabetic diet strictly, the former had significantly lower HbA_1 and plasma glucose levels. A possible explanation for the difference is that the traditional African diet, which contains more fibre, itself helps improve glucose homeostasis.

In patients who progressed from noninsulin-dependent diabetes to an insulin-dependent state, it would be expected that insulin requirements and glycosylated haemoglobin levels will be lower because of residual endogenous insulin secretion. This, however, was not the case in the 13 patients who fell in this category. Their mean HbA_1 level was not significantly different from the diabetic patients who were insulin dependent ab initio.

The correlation demonstrated between single random glucose levels and HbA_1 levels is encouraging, in view of the fact that it is still probably the most widely used test in assessing diabetic control. While the measurement of HbA_1 has been used as a cumulative index of the adequacy of control of diabetic patients over given periods of time, several cautionary remarks have been made about its use as the sole arbiter of control, (Asmal 1980; Gonen et al 1979). For instance, it does not take into account the occurrence of hypoglycaemic episodes or daily fluctuations in plasma glucose levels. Thirteen patients exhibited such a phenomenon. Despite this drawback HbA_1 estimation permits an easy assessment of what the mean blood glucose in a given patient has been in the preceding 2 - 4 weeks. In this way, particularly in situations where reliance cannot be placed on regular urinalysis or blood glucose values, comparison of HbA_1 levels from visit to visit will allow an assessment of the quality of control. This aspect assumes particular importance in socio-economically deprived populations.

The diabetic patients that attend the King Edward VIII Hospital are generally from poor socio-economic groups whose social and other

problems often prevent proper supervision of their disease at home. The urine results brought to the clinic may not be done accurately at home or are conjured up to satisfy the physician. The random blood glucose done when patients attend the clinic in the mornings suffer from the disadvantage that they are drawn at variable intervals after meals at different visits and do not provide any measure of certainty of the quality of control. Measuring HbA_1 levels would eliminate this problem.

SUMMARY

Glycosylated haemoglobin levels (HbA_1) were measured in 97 insulin dependent and 21 non insulin dependent diabetic patients. Patients with NIDDM had significantly lower HbA_1 levels compared to insulin dependent diabetic patients. Excellent control as gauged by a HbA_1 level below three standard deviations above the mean value (9%) was seen in 25% of patients with NIDDM and only 7% of the insulin dependent diabetic patients. A significant negative correlation was seen between maximal C-peptide levels attained during a GTT and the HbA_1 levels in the insulin dependent diabetic patients. Random plasma glucose levels showed a sginificant correlation with HbA_1 levels. Serial measurement of HbA_1 levels showed a trend towards improvement in 50% of patients. African patients had significantly lower HbA_1 and plasma glucose levels compared to Indian patients. Poor socioeconomic conditions are probably responsible for the unsatisfactory state of metabolic control in the majority of patients.

CHAPTER IX

THE HLA SYSTEM AND DIABETES MELLITUS IN YOUNG AFRICANS AND INDIANS

INTRODUCTION

The HLA system, also known as the major histocompatibility system, constitutes a complex group of antigens located on the surfaces of all nucleated cells and platelets. These antigens are determined by genes located on the short arm of the sixth chromosome where they are closely linked with genes controlling various immune responses and some components of the complement cascade. HLA, A, B, C and D antigens are determined by four different loci, each with a large number of alleles. The recently described DR antigens are possibly controlled at the same locus as the HLA D antigens. An individual inherits one set of A, B, C, D and DR antigens (called HLA-haplotype) from each parent, usually without any recombination taking place (Platz et al, 1978; Rotter et al, 1978; Rubinstein et al, 1977). As the genes are codominant, the phenotype of each individual may express two specificities of the A-locus, two of the B-locus, etc. (Nerup et al, 1977).

The HLA system is characterised by extreme polymorphism at each locus. In addition pronounced linkage disequilibrium occurs between the various loci, that is, certain pairs of HLA antigens are found together in a population in greater frequency than would be expected from multiplying their individual frequencies together. For example, as HLA A1 at the A locus is found in about 16% of white Caucasians and HLA B1 at the B locus in about 13%, they would be expected to be found together in about 2% of the population. However, they are found together 10% of the time and therefore are in linkage disequilibrium (Bodmer and Thomson 1977; McMichael and Mc Devitt 1977; Rotter and Rimoin 1979).

There is considerable variation in the prevalence of specific HLA antigens among different population groups throughout the world (Dausset and Svejgaard 1977; Hammond et al 1975; Hammond et al 1977; Menozzi et al 1978; Ryder et al 1978).

In recent years numerous studies have shown clear associations between the HLA systems and various diseases, (Ryder et al, 1979). Although the mechanisms through which HLA antigens confer disease susceptibility are obscure, several hypotheses have been suggested: (Christy et al 1979.)

1. direct effects of the HLA antigens (e.g. interference with ligand-receptor interaction on all surfaces).
2. effects of different but closely linked or functionally related genes in the HLA region (e.g. immune response genes).
3. effects of genes in linkage disequilibrium with HLA by pure co-incidence, the HLA antigens here being 'inert' markers.

The term relative risk (Woolf 1955) is used to indicate how much more frequently the disease in question occurs in carriers of a particular antigen as compared to those lacking this antigen. It refers to the strength (but not the statistical significance) of HLA and disease associations, (Christy et al, 1979).

Studies on the possible association between the HLA system and diabetes were apparently undertaken almost simultaneously by several groups of investigators. Finkelstein et al (1972) could find no association between HLA and juvenile diabetes. Singal and Blajchman (1973) however, did show an increase of HLA BW15 in a small group of insulin dependent diabetic patients, but owing to technical difficulties their conclusions had to be interpreted with caution. It was Nerup et al (1974a) who finally produced definite evidence to show a relationship between IDDM and the presence of HLA B8 and BW15. Not long after, Cudworth and Woodrow (1974) confirmed these findings. Since then many studies, using two approaches, viz: population studies and family studies, have provided unequivocal evidence in support of an association between IDDM and the HLA system.

The antigens associated with IDDM in White Caucasian populations include HLA C3, C4, B8, B15, B18, DW3, DW4, DR W3 and DR W4. HLA B7 and DW2, however, show a negative correlation with IDDM (Christy et al 1979). None of the studies on White Caucasians has found an association between NIDDM and HLA antigens (Christy et al 1979; Nelson and Pyke 1975).

Data on the relationship between diabetes mellitus and the HLA system in non-Caucasian populations are scanty (Christy et al 1979a). However, they do show definite associations between IDDM and certain HLA antigens. In the Japanese the disease has been associated with HLA DYT and HLA BW54 (Kawa et al 1977; Nakao et al 1977; Okimoto et al 1978), whilst in American Blacks an association with DR3 and DR4 has been shown. (Duquesnoy et al 1979; Rodey et al 1979). It is thus evident that there are differences in the specific allelic associations among various ethnic groups.

In this chapter the association between diabetes mellitus and the HLA system in Africans and Indians will be described.

MATERIALS AND METHODS

HLA A, B and C antigens were determined on 208 patients who were classified as follows:

Insulin-dependent (always):	70 Africans
	56 Indians (29 Dravidians and 27 Aryans)
Noninsulin-dependent initially later becoming insulin-dependent:	
	7 Africans
	14 Indians
Noninsulin-dependent:	61 Indians (33 Dravidians and 28 Aryans)

The Aryans originated from North India and the Dravidians from South India. DR specificities were determined on 53 diabetic patients of whom 30 were Africans (always insulin-dependent) and 23 were Indians. In the latter group 16 patients had always depended on insulin for control of symptoms and 7 were initially controlled on oral agents but later required insulin.

The frequencies of the HLA antigens were compared to a group of healthy controls comprising 1 000 Africans and 768 Indians (271 Aryans and 497 Dravidians), in all of whom HLA A, B and C antigens were determined. HLA DR antigens were determined in 118 African controls and 73 Indian controls.

A total of 180 antisera were used in a two-stage microlymphocytotoxicity test to determine HLA A, B and C specificities. Lymphocytes were isolated on a Ficoll-Hypaque density gradient, (Boyum 1968).

HLA DR specificities were determined by means of an extended incubation microlymphocytotoxicity test using T cell depleted B cell enriched lymphocytes.

The frequency differences between the patients and controls were tested for significance by means of the chi-square test (without Yeates' correction). (Woolf 1955). The resulting probabilities were multiplied by the the number of specificities tested in order to determine the corrected P value (Svejgaard et al 1974).
Relative risk was calculated according to the method of Woolf (1955):

$$\frac{\text{Number of patients positive} \times \text{Number of controls -ve}}{\text{Number of patients negative} \times \text{Number of controls +ve}}$$

RESULTS

Tables 1 and 2 shows the antigen frequencies in the African patients with IDDM and the African controls.

IDDM and HLA Antigens in Africans

At the A and C loci there is no significant difference in the frequency of any of the antigens between the African patients with IDDM and the African controls. The frequency of HLA B8 is increased in the African patients with IDDM compared to the controls (21,4% vs 14,1%) but this was not significant after correcting for the number of antigens being tested. Similarly the frequency of HLA B14, although increased in the Africans with IDDM (15,7% vs 6,22%), does not attain a significant level after correction had been made for the number of antigens tested.

TABLE 1 PERCENTAGE FREQUENCIES OF HLA A AND B ANTIGENS IN AFRICANS WITH IDDM

HLA Antigen	% Frequency in controls (n = 1000)	% Frequency in patients with onset				
		< 10 n = 8	< 20 n = 32	< 30 n = 62	< 35 n = 70	
A1	6.5	0	0	8.1	8.6	
A2	21.3	37.5	28.1	24.2	24.3	
A3	13.3	12.5	9.4	13	12.9	
A11	0.1	0	0	0	0	
AW23	18.4	50	37.5	27.4	25.7	
AW24	3.9	0	3.1	8.1	7.1	
A25	15.3	0	9.4	9.7	8.6	
A26	8.5	12.5	6.2	6.5	5.7	
A28	20.3	12.5	18.8	19.4	22.9	
A29	16.3	12.5	9.4	11.3	12.9	
AW30	37.6	25	31.2	29	27.1	
AW31	10.9	12.5	6.3	4.8	5.7	
AW32	1.8	0	3.1	8.1	7.1	
AW33	0.7	12.5	3.1	1.6	1.4	
One antigen	25.1	12.5	34.3	29	30	
B7	18.2	12.5	15.6	19.4	21.4	
B8	14.1	0	21.9	22.6	*26.4*	
B14	6.2	25	15.6	16.1	*15.7	*P < 0.005
B8/14	19.7	25	37.5	**38.7*	**37.1*	*Pc < 0.02
B13	4.8	12.5	9.4	4.8	4.3	
B15	5.2	0	0	3.2	2.9	
B16	2.3	0	0	1.6	1.4	
B17	38.5	37.5	31.3	35.5	34.3	
B18	4	0	0	6.5	7.1	
BW21	1.8	0	3.2	3.2	2.9	
BW22	0	0	0	0	0	
B27	0.3	0	0	0	0	
BW35	6.5	0	3.2	3.2	4.3	
B37	0	0	0	0	0	
B40	1.2	0	0	0	0	
BW41	2.5	12.5	9.4	9.7	8.6	
BW42	*25.5*	12.5	9.4	**9.7*	**8.6*	*P < 0.005
BW44	16.4	0	9.4	9.7	11.4	
BW45	7.6	0	6.3	8.1	10	
BW51	1.8	0	0	0	0	
BW52	0	0	0	0	0	
BW53	3.2	0	6.3	3.2	5.7	
BU	13.7	0	2.5	9.7	8.6	
One antigen	26.2	87.5	43.8	32.3	31.4	

Pc Corrected p value

TABLE 2 PERCENTAGE FREQUENCIES OF HLA C AND DR ANTIGEN IN AFRICANS WITH IDDM

HLA Antigen	% Frequency in controls	% Frequency in patients with onset				
		< 10	< 20	< 30	< 35	
CW1	0.7	0	0	16.1	1.4	
CW2	19.6	25	31.3	24.2	22.9	
CW3	14.4	12.5	25	17.7	17.1	
CW4	17.2	0	12.5	8.1	12.9	
CW5	3.5	0	6.3	9.7	8.6	
DR1	3.4	0	6.7	8	10	
DR2	20.3	16.7	6.7	8	10	
DR3	44.9	33.3	46.7	36	40	
DR4	9.3	**66.7*	$^{+}40$	**36*	**36.7*	*Pc < 0.02 $^{+}$Pc < 0.04
DR5	31.4	0	13.3	16	16.7	
DR7	11	0	20	32	26.8	
One antigen	44.1	83.8	60	52	50	

Since HLA B8 and B14 form a part of a cross reacting group of antigens, the presence of either of these antigens in the patients was compared to that in the controls. The difference is highly significant even after correction for the number of antigens tested (37,1% vs 19,7%), Pc < 0,02.

There appears to be a negative correlation between the presence of HLA BW42 and IDDM in Africans, since it was found in 25.5% of controls and only 8.4% of patients. However, the corrected p value fails to attain a level of significance. At the DR locus IDDM in Africans is associated with a significant increase in the frequency of DR4 (36,7% vs 9,3%), Pc < 0,04.

IDDM and HLA Antigens in Indians

Tables 3 and 4 shows the antigen frequencies in the Indian patients with IDDM and the Indian controls. At the A locus there is an increase in the frequency of HLA AW24 in those with onset of IDDM under the age of 20 (51,3% vs 27,6%). However after correction for the number of antigens the difference falls short of statistical significance. At the B locus there is a significant increase in the frequency of B8 (21,4% vs 6,1%) Pc < 0,02.

Comparison between the 2 subgroups, viz: Aryans and Dravidians, shows that the relative risk is higher in the former group (4,9 vs 3,6) and that the relationship between IDDM and HLA B8 fails to attain a statistical significance in the latter group after correcting the p value, Table 5. The Aryans, however, show a significant increase in HLA B8 even after correction has been made for the number of antigens being tested.

There are no differences in the frequencies of any of the antigens at the C Locus.

The frequency of DR4 is significantly increased in the Indians with IDDM (43,8% vs 10,8%), Pc < 0,04.

NIDDM and HLA Antigens in Indians

Table 6 shows the antigen frequencies in the Indian patients with NIDDM and the Indian controls. In the Indian patients with NIDDM there is a significant increase in the frequency of HLA BW61 (41% vs 16,8%) Pc < 0,02.

TABLE 3 PERCENTAGE FREQUENCIES OF HLA A AND B ANTIGENS IN INDIANS WITH IDDM

HLA Antigen	% Frequency in controls (n = 768)	% Frequency in patients with onset:-				
		< 10 yrs	<20 yrs	<30 yrs	<35 yrs	
A1	27.5	12.5	17.9	23.1	10.7	
A2	23.1	12.5	28.3	30.8	33	
A3	14.3	12.5	7.7	11.5	10.7	
A11	26.8	12.5	20.5	19.2	19.6	
AW23	0.7					
AW24	27.6	75	**51.3*	42.3	41.1	*P < 0.005
A25	2.0		5.1	5.8	5.4	
A26	6.6	25	10.3	9.6	8.9	
A28	13.4	12.5	12.8	13.5	16.1	
A29	0.9	12.5	2.6	1.9	1.8	
AW30	3.8	12.5	5.1	3.8	3.6	
AW31	3.1		2.6	1.9	1.8	
AW32	2.1		2.6	1.9	1.8	
AW33	8.5	12.8	15.4	17.9		
One antigen		12.5	20.5	19.2	17.9	
B7	12.1	12.5	12.8	13.5	14.3	
B8	6.1	50	**25.6*	**23.1*	**21.4*	*Pc < 0.02
B13	6.6	12.5	5.1	5.8	5.4	
B14	0.3					
B15	10.7		7.7	5.8	7.1	
B16	2.5		2.6	1.9	1.8	
B17	21.2		10.3	11.5	12.5	
B18	3.3		2.6	3.8	3.6	
BW21	2.0					
BW22	2.9		2.6	1.9	1.8	
B27	2.2	12.5	5.1	3.8	3.6	
BW35	21.5	12.5	10.3	11.5	10.7	
B37	4.7		5.1	7.7	8.9	
BW41	0.1					
BW42	0					
BW44	11.7	12.5	17.9	17.3	17.9	
BW45	0.3					
BW51	20.8	12.5	15.4	11.5	10.7	
BW52	10.4		10.3	9.6	10.7	
BW53	1.7					
B5 IND	3.0	25	5.1	5.8	5.4	
BW60	16.5		5.1	5.8	5.4	
BW61	13.2	37.5	20.5	25.0	26.8	
BU						
One antigen		12.5	35.9	34.6	32.1	

TABLE 4 PERCENTAGE FREQUENCIES OF HLA C AND DR ANTIGENS IN INDIANS WITH IDDM

HLA Antigens	% Frequency in controls		% Frequency in patients with onset				
			<10 yrs	<20 yrs	<30 yrs	<35 yrs	
CW1	3.0		2.6	1.9	1.8	1.4	
CW2	1.4		0			2.9	
CW3	12.2		12.8	13.5	14.3	14.3	
CW4	22.3		10.3	11.5	10.7	8.6	
CW5	1.0		2.6	1.9	1.8	1.4	
DR1							
DR2	35.5		23.1	20.0	18.8	21.7	
DR3	19.4		38.5	33.3	31.3	30.4	
DR4	10.8		*38.5*	*46.7**	*43.8*$^{+}$	*39.1*$^{+}$	$^{*}Pc < 0.02$ $^{+}Pc < 0.04$
DR5	12.9		23.1	20.0	25.0	17.4	
DR6							
DR7	26.9		27.1	20.0	18.8	21.7	
DR8	1.1						
DR10	5.4						
One antigen	51.6		53.8	60.0	62.5	60.9	
Blank	18.3					4.3	

TABLE 5 PERCENTAGE FREQUENCIES OF HLA B8 IN ARYANS AND DRAVIDIANS WITH IDDM

ARYANS					DRAVIDIANS				
% Frequency in controls n = 271	% Frequency in patients with age of onset				% Frequency in controls n = 497	% Frequency in patients with age of onset			
	<10	<20	<30	<35		<10	<20	<30	<35
5.5	40	26.7	24	22.2	6.4	66.7	11.7	19.5	20.7

TABLE 6 PERCENTAGE FREQUENCIES OF HLA A and B ANTIGENS IN INDIANS WITH NIDDM

HLA ANTIGEN	% FREQUENCY IN CONTROLS	% FREQUENCY IN PATIENTS	
	n = 768	n = 61	
A1	27.5	13.1	
A2	33.1	37.7	
A3	14.3	16.4	
A11	26.8	23	
AW23	0.7	0	
AW24	27.6	44.3	
A25	2	3.3	
A26	6.6	3.3	
A28	13.4	6.6	
A29	0.9	1.6	
AW30	3.8	4.9	
AW31	3.1	0	
AW32	2.1	4.9	
AW33	8.5	8.2	
One antigen		32.8	
B7	12.1	19.7	
B8	6.1	11.5	
B13	6.6	6.6	
B14	0.3	0	
B15	10.7	11.5	
B16	2.5	3.3	
B17	21.2	8.2	
B18	3.3	1.6	
BW21	2.0	8.2	
BW22	2.9	0	
B27	2.2	4.9	
BW35	21.5	19.7	
B37	4.7	1.6	
BW41	0.1	0	
BW42	0	0	
BW44	11.7	11.5	
BW45	0.3	0	
BW51	208	13.1	
BW52	10.4	14.8	
BW53	1.7	1.6	
B5	3.0	3.3	
BW60	16.5	1.6	
BW61	13.2	41	Pc < 0.02
One antigen		16.4	

NIDDM Progressing to Insulin-Dependent Diabetes Mellitus and HLA Antigens in Africans and Indians

Table 7 shows the antigen frequencies in the patients who were initially noninsulin-dependent but later became insulin-dependent. It is difficult to draw any conclusions about their relationship to the HLA system because of the small number of patients tested. Nevertheless there is certainly no preponderance of HLA B8 or BW 61 or B14.

DISCUSSION

In White Caucasians two distinct forms of IDDM have been recognised and these may be distinguished on the basis of HLA studies (Rotter and Rimoin 1978). There is an auto-immune variety which is associated with DW3 and less strongly with B8; the presence of islet cell antibodies (Morris et al 1976; Nerup et al 1977), an increased risk of micro-angiopathy (Barbosa et al 1976; Bottazzo et al 1978; Schernthaner et al 1976). The other type, which is associated with B15 and C3, appears to have an earlier age of onset (Christy et al 1979), and to show an increased antibody response to exogenous insulin. It shows a stronger association with DW4 (Christy et al 1979; Rotter and Rimoin 1978) and is not associated with autoimmune disease or persistence of islet cell antiboides. The presence of both B8-DW3/B15-DW14 is characterised by an increased relative risk, i.e., the presence of both allelic groups confers an additive risk for developing the disease.

In the present study a significant association between IDDM and B8 had been found in Indians only. The relative risk, which is 4,2, is higher than in European Caucasians. In African patients, although the relative risk due to B14 is 2,8, the increased frequency falls short of statistical significance. However there was a significant increase in the presence of either B8 and B14, which are cross-reacting antigens. It is probable that the same susceptibility gene is associated with either of these antigens in Africans.

Neither the African patients nor the Indian patients with IDDM showed any increase in the frequencies of CW3, B15 and B18 as has been found in European Caucasians (Ryder et al 1979), or of BW54 and B12 which

TABLE 7 FREQUENCIES OF HLA A AND B ANTIGENS IN PATIENTS PRESENTING WITH NIDDM AND LATER BECOMING INSULIN-DEPENDENT

AFRICANS			INDIANS		
HLA	% Frequency in controls n = 1000	% Frequency in patients n = 5	HLA	% Frequency in controls n = 768	% Frequency in patients n = 14
A1	6.5	0	A1	27.5	14.3
A2	21.3	0	A2	33.1	50
A3	13.3	0	A3	14.3	7.1
A11	0.1	0	A11	26.8	28.6
AW23	18.4	20	AW23	0.7	
AW24	3.9	0	AW24	27.6	14.3
A25	15.3	20	A25	2.0	
A26	8.5	10	A26	6.6	
A28	20.3	10	A28	13.4	28.6
A29	16.3	10	A29	0.9	
AW30	37.6	80	AW30	3.8	7.1
AW31	10.9	0	AW31	3.1	
AW32	1.8	0	AW32	3.0	
AW33	0.7	10	AW33	13.9	21.4
One antigen	25.1	20	One antigen		28.6
B7	18.2	20	B7	12.1	28.6
B8	14.1	80	B8	6.1	7.1
B14	6.2	0	B13	6.6	7.1
B8/14	19.7	80	B14	0.3	
B13	4.8	10	B15	10.7	7.1
B15	5.2	0	B16	2.5	
B16	2.3	10	B17	21.2	14.3
B17	38.5	10	B18	3.3	
B18	4	0	BW21	2.7	
BW21	1.8	0	BW22	2.9	7.1
BW22	0	0	B27	2.2	7.1
B27	0.3	0	BW35	21.5	7.1
BW35	6.5	0	B37	8.1	7.1
B37	0	0	BW41	0.1	
B40	1.2	0	BW42	o	
BW41	2.5	0	BW44	11.7	21.4
BW42	25.5	20	BW45	o.3	
BW44	16.4	10	BW51	20.8	7.1
BW45	7.6	0	BW52	10.4	7.1
BW51	1.8	0	BW53	3.0	7.1
BW52	0	0	B5 IND	30	7.1
BW52	3.2	0	BW60	16.5	7.1
BU	13.7	10	BW61	13.2	21.5
One antigen	26.2	10	BU	0.1	
			One antigen		28.6

have been observed in Japanese (Kawa et al 1977; Nakao et al 1977; Wakisaka et al 1976). Studies in American blacks have not shown any significant associations at the B locus (Duquesnoy et al 1979; Rodey et al 1979). Patel et al (1977) did find an increased frequency of B8, but the corrected p value was not significant, as has been the case with the Afircan patients reported in this study.

The negative correlation between B7 and IDDM shown in white Caucasians by Ludwig et al (1976) who combined data from several centres and by van de Putte et al (1976) in family studies was not seen in the African or Indian patients described here, and has also not been observed in American Blacks and Japanese. BW42 which has been detected only in black populations (Hammond et al 1980) appears to show a negative correlation with IDDM. It is difficult to gauge the significance of such a finding at present, since a decreased frequency of an antigen as opposed to an increased frequency requires a much larger sample size to become evident (Christy et al 1979).

The close correlation between DR4 and IDDM seen in both the African and Indian patients has been observed in all the ethnic groups studied thus far (Svejgaard et al 1980). However an association with DR3 could not be shown, unlike the findings in European Caucasians and American blacks.

A significant negative correlation between IDDM and HLA B7 or DR2 which has been observed in white Caucasians was not seen in the African and Indian patients. In contrast a significant decrease in the frequency of B7 has been observed in Indians of North India (Srikanta et al 1981).

Studies in white Caucasians have thus far been unable to establish a significant relationship between NIDDM and HLA system in White Caucasians (Christy et al 1979; Nelson and Pyke 1975). Other population groups, however, do show such associations. An increase in the frequency of B35 has been shown in a small study involving South African Xhosas with NIDDM (Briggs et al 1980). In the Pima Indians with NIDDM an association with HLA-A2 has been shown, particularly in those with onset of disease under age 35 (Knowles et al 1981).

The clear association between NIDDM in young Indians and HLA BW61 shown in this study assumes particular significance when comparison is made with the findings of Serjeantson et al (1981), who also showed the same relationship in Fiji Indians with NIDDM. Since both the Fiji Indians and the Natal Indians have similar origins, the findings of identical HLA associations is not unexpected.

Unlike Fiji Indians, Natal Indians do not show any linkage disequilibrium between BW61 and AW24. In fact there was a high frequency of the latter in Indians with onset of IDDM before age 20, although the corrected p value fell short of statistical significance.

This study has shown a clear association between diabetes mellitus and the HLA system in Africans and Indians. It has also served to highlight differences in the specific allelic associations among various population groups.

SUMMARY

HLA A, B and C antigens were determined in 126 patients (70 Africans, 56 Indians) with IDDM, 61 Indians with NIDDM, 14 patients initially presenting with NIDDM but later becoming insulin dependent, and 1 768 (1 000 Africans, 768 Indians) controls. 30 Africans and 16 Indians with IDDM, and 118 African and 73 Indian controls were also typed for DR specificities. HLA DR4 was associated with IDDM in both races. In the African patients a relationship was seen between IDDM and the cross-reacting antigens B8 or B14. IDDM in Indians was associated with HLA B8, whereas NIDDM in the same race group showed a clear relationship with HLA BW61.

CHAPTER X

IMMUNOLOGICAL ASPECTS OF IDDM IN YOUNG AFRICANS AND INDIANS

INTRODUCTION

The role of immunological factors in the aetiology of IDDM has aroused considerable interest in recent years. Although the association between IDDM and certain organ-specific autoimmune diseases had been known for some time, it was only after the demonstration by Bottazzo et al (1974) of islet cell antibodies in the sera of such patients that a direct line of evidence was produced supporting the part played by autoimmunity in the pathogenesis of the disease. Since then numerous studies have shown a high frequency of these antibodies in newly diagnosed cases of IDDM irrespective of whether other associated organ-specific autoimmune endocrinopathies were present or not, (Irvine 1977; Irvine et al 1980; Lendrum et al 1975; Lendrum et al 1976; Madsbad et al 1980; Neufeld et al 1980). On the basis of these findings Cudworth (1978, 1980) was able to classify Type I diabetes (IDDM) into:

a) Type 1a (the classical juvenile variant) in which ICA appears transiently at the onset of illness.
b) Type 1b (the autoimmune variant) which is associated with thyroid, gastric and adrenal autoimmunity, the presence of HLA B8 and persistence of ICA.

Various types of ICA have been detected although they are all specifically directed at the islets of Langerhans. Complement-fixing islet cell antibodies (ICA-IgG) react with autoantigens present in the cytoplasm of the islet cells whereas islet cell surface antibodies (ICSA) are directed at the islet cell surface (Bottazzo and Doniach 1978; Bottazzo et al 1980; Lernmark et al 1978).

Since 1977 when increased levels of circulating immune complexes were reported for the first time in patients with IDDM (Irvine et al 1977) their significance as regards pathogenesis of the disease or its complications has provoked much controversy which has as yet not been resolved. Like islet cell antibodies, immune complexes are found in high concentrations at the onset of IDDM and tend to disappear with time, (Irvine et al 1977; Irvine et al 1980a). Thus it may well be that islet cell-antibody reaction provides a substantial contribution to the presence of circulating immune complexes seen at the time of diagnosis (Irvine et al 1980).

Although data on the prevalence of islet cell antibodies and immune complexes in populations other than white caucasians are scanty, it does seem that American blacks and Japanese have a lower propensity to develop organ-specific autoimmunity (Irvine et al 1980b; Neufeld et al 1980.)

PATIENTS AND METHODS

Blood samples for islet cell antibodies, thyroid antibodies, parietal cell antibodies and adrenal antibodies were obtained from 44 African and 27 Indian patients with IDDM. They all formed part of the group of patients with IDDM described in Chapter II. The race, sex, and age distribition, together with the mean age of onset and the mean duration of disease are shown in Tables 1 and 2.

In addition sera from 37 healthy controls (19 Africans, 18 Indians) were tested for these antibodies.

Islet cell antibodies (ICA-IgG) were detected by indirect immunofluorescence in unfixed blood group O human pancreas obtained from cadaver kidney donors (Bottazzo at al 1981).

Complement-fixing islet-cell antibodies (Cf-ICA) were assayed by the immunofluorescent complement-fixation test as described by Bottazzo et al (1981).

TABLE 1 THE CLINICAL CHARACTERISTICS AND AUTOANTIBODY PROFILE OF AFRICANS WITH IDDM

Patient No.	Sex	Age of onset	Interval between onset of IDDM and time of taking sample in months	ICA		3 GPC	4 TGHA	5 MCHA	6 ADR
				1 IGg	2 -C3				
1	F	19	60	+	-	++	-	-	-
2	F	9	3	-	-	-	-	-	-
3	M	18	4	-	-	-	-	-	-
4	M	27	4	+	+	-	-	-	-
5	F	34	40	+	-	-	-	-	-
6	M	16	16	-	-	-	-	-	-
7	M	22	2	-	-	-	-	-	-
8	M	22	6	-	-	-	-	-	-
9	M	27	9	+	-	-	-	-	-
10	M	19	120	+	+	-	-	-	-
11	M	25	26	-	-	-	-	-	-
12	F	16	3	+	+	-	-	-	-
13	F	34	29	+	+	-	-	-	-
14	F	20	9	-	-	-	-	-	-
15	F	23	15	-	-	-	-	-	-
16	M	35	5	-	-	+	-	-	-
17	F	16	3	-	-	-	-	-	-
18	F	27	12	-	-	+	-	-	-
19	F	21	81	-	-	-	-	-	-
20	M	9	48	-	-	-	-	-	-
21	M	27	84	+	-	-	-	+	-
22	M	16	14	-	-	-	-	-	-
23	F	13	180	-	-	+	-	-	-
24	F	12	2	+	-	-	-	-	-
25	F	21	4	-	-	-	-	-	-
26	F	10	70	-	-	-	-	-	-
27	M	23	84	+	-	-	-	-	-
28	F	8	72	++	+	-	-	-	-
29	F	10	60	+	-	-	-	-	-
30	M	18	36	-	-	-	-	+	-
31	F	11	1	+	-	++	-	-	-
32	M	30	24	-	-	-	-	-	-
33	M	30	50	+	-	+	-	-	-
34	F	2	3	-	-	+	-	-	-
35	F	18	3	-	-	-	-	-	-
36	F	27	84	-	-	+	-	-	-
37	F	29	38	-	-	-	-	-	-
38	M	31	125	-	-	-	-	-	-
39	M	19	3	+	-	-	-	-	-
40	F	6	75	-	-	++	-	-	-
41	F	25	3	-	-	-	-	-	-
42	F	25	3	-	-	-	-	-	-
43	F	22	100	+	-	-	-	-	-
44	F	27	3	-	-	-	-	-	-
Mean		20.4	39						
TOTAL				16	5	9	-	2	-

Key : 1. Islet Cell Antibodies
2. Complement-fixing Islet Cell Antibodies.
3. Gastric Parietal Cell Antibodies.
4. Thyroglobulin Haemagglutinating Antibodies
5. Thyroid Microsomal Haemagglutinating Antibodies
6. Adrenal Antibodies.

TABLE 2 THE CLINICAL CHARACTERISTICS AND AUTOANTIBODY PROFILE OF THE INDIANS WITH IDDM

Patient No.	Sex	Age of onset	Interval between onset of diabetes and time of taking sample in months	ICA 1 IGg	ICA 2 -C3	3 GPC	4 TGHA	5 MCHA	6 ADR
1	M	26	2	+	-	-	-	-	-
2	F	11	48	-	-	-	-	-	-
3	F	9	168	-	-	-	-	-	-
4	M	19	156	-	-	-	-	-	-
5	F	27	24	+	+	+	-	-	-
6	F	9	60	++	++	-	-	-	-
7	F	5	180	-	-	-	-	-	-
8	M	17	144	-	-	-	-	-	-
9	F	14	30	-	-	-	-	-	-
10	M	17	130	-	-	-	-	-	-
11	F	6	20	+	-	-	-	-	-
12	M	22	9	+	-	+	-	-	-
13	F	21	18	-	-	+	+	-	-
14	F	18	30	+	-	-	-	+	-
15	F	20	24	-	-	-	-	+	-
16	F	33	80	-	-	+	-	+	-
17	M	32	96	-	-	-	-	-	-
18	M	5	96	-	-	-	-	-	-
19	F	30	36	-	-	-	-	-	-
20	M	34	24	+	-	-	-	+	-
21	M	28	8	-	-	-	-	-	-
22	F	19	90	-	-	-	-	-	-
23	F	27	9	-	-	-	-	-	-
24	F	16	2	+	-	++	-	-	-
25	F	21	2	-	-	-	-	-	-
26	F	2	64	-	-	-	-	-	-
27	F	18	40	-	-	-	-	-	-
Mean		15	59						
TOTAL				8	2	5	1	4	-

KEY : Same as Table 1.

Thyroid microsomal haemagglutinating antibody, thyroglobulin haemagglutinating antibody, gastric parietal cell antibody and adrenal antibodies were determined by immunofluorescence using human tissue sections.

Sera from immune complexes were obtained from 31 patients with IDDM (19 Africans and 12 Indians) and 29 healthy controls (19 Africans and 10 Indians). The age and sex distribution together with the mean age of onset and mean duration of disease are shown in Table 3.

Immune complexes were determined by means of the Raji cell assay and the method used was Cunningham-Rundles et al's (1980) modification of the one described by Theofilopoulos et al (1976).

RESULTS

Results are shown in Tables 1 and 2.

Islet Cell Antibodies

Islet cell antibodies were detected in over a third of patients with IDDM, but in only 5% (2/37) of controls, the difference being highly significant ($p < 0.005$).

Although the frequency of ICA's was slightly higher in the African patients (34% vs 30%) the difference was not significant. Similarly there was no significant difference in the frequencies of ICA's between males and females of both racial groups.

ICA-IgG and ICA-Cf were found together in 10% of patients (5 Africans, 2 Indians) but in none of the controls. Complement-fixing islet cell antibodies were found only in patients who were positive for ICA-IgG. They were not detected in any of the controls.

The prevalence of ICA was significantly higher (30% vs 17%) in patients who had IDDM for less than a year than in those with a longer duration of disease ($p < 0.025$). Persistence of ICA beyond 3 years was significantly more frequent in the African group, being found in almost 50% (10/21) of these patients and in only 1 (1/16) Indian patient, $p < 0.025$.

TABLE 3 CLINICAL CHARACTERISTICS OF THE PATIENTS WITH IDDM ON WHOM IMMUNE COMPLEXES WERE DONE.

	Africans	Indians	Total
Number of patients	19	12	31
M : F ratio	11 : 8	5 : 7	16 : 15
Mean age in years (range)	25 (16-35)	24.7(16-30)	24.7
Mean duration of IDDM in years (range)	6 ($\frac{2}{12}$-2.1)	6 ($\frac{2}{12}$-17)	3.7

Other Autoantibodies

Gastric Parietal Cell antibodies were detected in 13 patients with IDDM (18%) and in only 2 controls (5%), but the difference was not significant.

Six patients (8%) had thyroid microsomal antibodies, whilst none of the controls was positive. They tended to be more common in Indian (4/27) than African (2/44) patients. Thyroglobulin haemagglutinating antibodies were detected in 1 patient and 1 control.

None of the patients or controls showed the presence of adrenal autoantibodies.

ICA and other autoantibodies were found together in 9 patients (12%) of whom 6 had IDDM for over a year.

Immune Complexes

The mean circulating immune complex levels in Afican patients and controls were 277 ± 127µg/ml and 200 ± 78 µg/ml respectively. In the Indian patients and controls the values were 156 ± 35 µg/ml and 257 ± 35 µg/ml respectively.

Owing to a lack of an absolute standard of normal values, CIC levels below 100 µg/ml are regarded as normal; 100 - 500 µg/ml as mild elevation; 501 - 1000 µg/ml as moderate elevation and those above 1 mg/ml as gross elevation (Pudifin and Duursma 1981). Among Africans immune complexes were found to be mildly elevated in 53% of patients and 68% of controls and normal in 47% of patients and 32% of controls. In the Indian group 83% of patients and 80% of controls had normal CIC levels, whereas mildly elevated levels were found in 17% and 20% respectively.

None of the patients or controls had moderately elevated or grossly elevated CIC levels.

DISCUSSION

Islet cell antibodies have been detected in up to 80% of white Caucasians with IDDM at the time of diagnosis. Thereafter their prevalence falls steadily, reaching 45% at one year and 25% at 2 years (Lendrum et al 1976). In this study over 60% of the patients had ICA within 2 months of diagnosis and by one year the rate had fallen to 30%, thus providing further support for the suggestion that the immunogenic parameters in blacks with IDDM are similar to those found in white Caucasians (Neufeld and Blizzard 1980).

Studies in American blacks with a mean duration of IDDM of 3.2 years have shown an ICA prevalence rate of 22%, which was significantly lower than that found in white Caucasians (Neufeld et al 1980). In Natal African patients, however, the overall frequency is higher (34%), in spite of a similar mean duration of disease, and appears not to differ much from the 36% prevalence rate found in white Caucasians by Neufeld et al (1980). In addition, persistence of ICA beyond 3 years appears to be much more common in the Natal African diabetic patient (48%) than in his Indian (4%) or American counterpart (15%) (Neufeld et al 1980), although the fact that far more American blacks (53 vs 21) had a duration of disease falling in that range calls for caution in reaching any definite conclusions.

In contrast ICA's have been found to be rare in Nigerian and Pima Indian diabetic subjects, even if assays were done at the time of diagnosis (Knowler et al 1979., Oli et al 1980). Such findings serve to highlight differences in the immunological aspects of the disease among the various population groups and to emphasise the heterogeneity of the disorder in blacks themselves.

In this study the ICA frequency within 3 months of diagnosis of IDDM in Africans and Indians (29%) is similar to that reported in American blacks (33%), but considerably lower than the 74% rate found in white Caucasians (Christy et al 1977; Lendrum et al 1976; Neufeld et al 1980). Thus it appears that ICA's persisting for longer than 3 months continue to do so for a long time in the Natal African.

The observation that only 48% of patients with ICA-IgG also had Cf-ICA is not surprising, as Bottazzo et al (1981) have also found a similar rate (50% - 55%). A likely explanation for this discrepancy is that complement-fixing ICA tend to disappear sooner than ICA-IgG (Bottazzo et al 1981). The former type of ICA has also been found to be a better monitor of active beta cell damage (Bottazzo et al 1981).

High frequencies of antibodies to thyroid, adrenal or gastric tissues in patients with IDDM have been shown in numerous studies (Bosteni and Gept 1974; Christy et al 1977; Drash 1971) Irvine et al 1970; Neufeld et al 1980). Although the African and Indian patients reported in this study showed higher frequencies of parietal cell and thyroid antibodies compared to controls, none of them had adrenal antibodies. Neufeld et al (1980) also were unable to detect adrenal antibodies in American blacks, whilst finding increased frequencies of the other autoantibodies, which, however, were lower when compared to White Caucasians. Interestingly the 4% prevalence rate for thyroid antibodies in American blacks (Neufeld et al 1980) was similar to that found in the Natal African. However, parietal cell antibodies were less common in the former group (10%) when compared to Africans (18%) or Indians (20%).

Despite the small number of patients studied, the presence of parietal or thyroid antibodies appeared to be associated with ICA positive sera, particularly when the latter persisted beyond a year.

The lack of significantly higher levels of circulating immune complexes in African and Indian patients with IDDM may seem surprising in view of the relatively high frequency of islet cell antibodies, which have been shown to correlate with such complexes (Irvine et al 1980b) Several reasons could be advanced to explain this paradox. First and foremost is that the number of newly diagnosed diabetics studied were few and even in these cases sera were not taken in the first week of diagnosis, as had been done in Irvine's large series of 114 cases. In addition, circulating immune complexes disappear earlier than ICA and are found only in about a

third of newly diagnosed cases of IDDM (Bottazzo et al 1981). Thus the conclusion that CIC are not elevated in African and Indian patients with IDDM may not be valid.

The findings in this study certainly support Neufeld and Blizzard's (1980) contention that immunological mechanisms similar to those in Caucasians play a part in the pathogenesis of IDDM in other race groups. However, whilst their conclusion that there is a lower propensity to organ-specific autoimmunity among blacks may apply to America, it may not be the case with the Natal African insulin-dependent diabetic patient, in whom a relatively high frequency of persistent ICA has been found.

SUMMARY

The presence or absence of islet cell antibodies and other autoantibodies was determined in 44 African and 27 Indian patients with IDDM and 37 controls. Tests for immune complexes were done on 19 African and 12 Indian patients with IDDM, and 19 African and 10 Indian controls. Islet cell antibodies (ICA-IgG) were found in over a third of the patients and in only 2 controls. Complement fixing antibodies (ICA-Cf) were also found in 10% of patients, but in none of the controls, Persistence of ICA beyond 3 years was more frequent in African compared to Indian patients. Parietal cell and thyroid antibodies were found more often in patients than controls. None of the patients or controls had adrenal antibodies. Mild elevation of immune complex concentrations were found in both patients and controls with no significant difference between the 2 groups. Neither the patients nor the controls showed moderate or gross elevation of immune complex levels.

CHAPTER XI

SUMMARY AND CONCLUSIONS

In conclusion, this thesis set out to describe the patterns of diabetes mellitus in young Africans and Indians, identify differences between the racial groups and probe into the role of known aetiological factors in producing the disease in these populations.

The data presented have described the pattern of IDDM and NIDDM in the 2 groups. Thus both types of diabetes mellitus occur in each of the races, although the prevalence of IDDM is greater in Africans than Indians and that of NIDDM greater in the latter than the former.

There are no distinctive features in the mode of onset, duration of disease and tendency to complications between the two groups, but interestingly the peak age of onset of IDDM tends to be later in Africans. Although Indians with the disease also show a later peak when compared to White Caucasians, the difference is not as pronounced as in Africans. It is difficult to ascribe a reason for such a difference, particularly when viewed against a background in which the aetiology of the disease remains obscure. Suffice it to say that if the same environmental factor is implicated in the aetiology of the disease in all population groups, Africans in particular appear to be protected at an age when susceptibility is greatest in White Caucasians.

The demonstration of a significant association between IDDM and the HLA system in both Africans and Indians highlights the importance of genetic susceptibility to the disease, as has been observed in numerous studies on White Caucasians. This study also confirms the existence of ethnic variability in the specific allelic associations of the disease. Thus, as in Whites HLA B8 is a significant risk factor for

IDDM among Indians whereas the same is not true for Africans. The presence of DR4, which has been associated with IDDM in Whites, also confers increased susceptibility to the disease in Africans and Indians.

The association between HLA BW61 and NIDDM in young Indians, although an unexpected finding, serves to emphasise the heterogenous nature of the disease because as yet no such relationship has been observed in white Caucasians (Christy et al 1979; Nelson and Pyke 1975).

The high prevalence of NIDDM in first degree relatives of young patients with this disease suggests an autosomal dominant mode of inheritance, as shown in studies on similar patients elsewhere (Fajans et al 1978; Tattersall 1974).

IDDM in young Indians was also associated with a relatively strong family history of NIDDM in first degree relatives. Whether it reflects the high prevalence of NIDDM in the population group (West 1978) or whether some aetiological role could be ascribed to it is difficult to determine. However, it certainly represents a unique finding.

The presence of islet cell antibodies in a fair proportion of African and Indian patients with IDDM, in spite of the relatively long time after onset of the disease that specimens were taken, serves to highlight the important role played by autoimmunity in the pathogenesis of IDDM in both population groups.

NIDDM in young Indians shows certain distinct differences from the classical descriptions of the disease by Fajans et al (1976) and Tattersall (1974). Obesity is much more common and complications are by no means rare in patients reported in this study. Thus the disease in this population group is more akin to the typical NIDDM of the middle aged and elderly.

Chronic complications of the disease appear to be related to duration of diabetes in both population groups. Similar findings have been documented in numerous studies (West 1978).

Ketoacidotic coma, however, was much more common in patients reported in this study, often being due to omission of insulin. Lack of primary health care facilities, inadequate education and poor socio-economic conditions appear to be primarily responsible for such a high prevalence of this complication.

This study has served to highlight the poor state of metabolic control in most of the insulin-dependent diabetic patients. Once again the inadequate social and education circumstances appear to play a major role for this state of affairs. Hence better education of the patients and the provision of more primary health care facilities should occupy top priority.

Although the hormonal pattern shows no striking differences when compared to the disease patterns seen elsewhere, there are certain racial differences. Thus insulin and C-peptide repsonses are lower in Africans withNIDDM and IDDM, suggesting a greater degree of beta cell destruction in these patients.

The relatively high insulin requirements found in Africans and Indians with IDDM represent a feature of the disease that is commonly seen in J-type diabetes mellitus. However, the tendency to ketosis characteristic of IDDM in both population groups is a distinct difference that makes it more akin to the disease as seen in temperate climates.

NIDDM in young Africans and Indians also has been shown to be quite distinct from J-type diabetes, in that obesity is quite common and control could be easily achieved by means of diet with or without oral hypoglycaemic agents, in contrast to the leanness and high insulin requirements characteristic of J-type diabetes.

The rarity of J-type diabetes and virtual absence of Z-type diabetes in both Africans and Indians is somewhat surprising, because both varieties of tropical diabetes are common in India and other parts of Africa. Judging by the high prevalence of kwashiokor in South Africa (Davel 1965, Schlemmer and Stopforth 1974), there is little doubt that malnutrition is rife. Since tropical diabetes is generally associated with

malnutrition, the disease would be expected to be common here, but this is not borne out by the results of this study. A possible explanation for this anomaly is that some other environmental factor peculiar to the areas of high prevalence of the disease, e.g. cassava consumption (McMillan and Geevarghese 1979) plays a part in its pathogenesis. This may also account for its virtual absence among Indians, although the hypothesis that the state of nutrition of young Indians, being better than that of their cousins in India, acts as a protective mechanism also sounds reasonable.

The data presented, it is hoped, have more than covered the scope of the thesis. They have served to show that diabetes mellitus affecting young Africans and Indians in Natal is similar to the disease seen in temperate climates and no less heterogenous in its appearance. This study has been instrumental in highlighting the role of known aetiological factors elsewhere as being of equal importance in the pathogenesis of the disease in the local populations. It has also helped to raise more questions than could be answered, namely, questions on the association between the HLA system and NIDDM, the high prevalence of NIDDM in first degree relatives of Indian patients with IDDM, and the later peak in age of onset of IDDM in Africans.

Further studies will undoubtedly have to be undertaken to define and evaluate the role of these and other factors in the pathogenesis of IDDM and NIDDM in Africans and Indians in Natal.

APPENDIX A

Determination of Plasma C-Peptide Concentrations.

This was assayed by radioimmunoassay using a Byk-Mallinckrodt RIA-mat C-Peptide kit. (Radiopharmazeutika-Diagnostica).

Reagents

1. ^{125}I-C-Peptide.
2. C-Peptide standards.
3. Rabbit anti-C-peptide serum.
4. Goat anti-rabbit-gamma-globulin.
5. Control serum.

Method

1. 0.1 ml of C-peptide standards, test sera and control sera were pipetted into appropriate test tubes, each one being done in duplicate.
2. 0.1 ml ^{125}I-C-peptide was added to each test tube.
3. 0.1 ml C-peptide antiserum was then added to each tube, mixed well and covered with Parafilm.
4. The test tubes were incubated at 4°C for 48 hours.
5. 0.5 ml goat anti-serum (reconstituted just before use) was added to each test tube and mixed well.
6. The tubes were incubated at 4°C for 24 hours.
7. They were then centrifuged for 30 minutes at 2000g.
8. The supernatant in each tube was aspirated.
9. The radioactivity in each tube was determined using a gammacounter.
10. The count rate for each standard was expressed as a percentage of the mean rate of the zeros and a standard curve constructed plotting these values against the concentration of the C-peptide. The count rates of the test sera were also expressed as percentages of the mean count rate of the zeros and the resulting values used to read off the C-peptide concentrations from the standard curve.

Modified Method

Byk-Mallinckrodt Radiopharmazeutika-Diagnostica introduced a slight modification of the original method in their kits from April, 1981. In these kits the reagents were identical. The changes in the methodology involved:-

a. Step 4 (above), where incubation was only done for 24 hours at 25°C instead of for 48 hours.

b. Step 6 (above), where the tubes were now incubated for 30 minutes only at 25°C, instead of for 24 hours.

APPENDIX B

Determination of Plasma Insulin Concentrations.

Insulin was assayed by radioimmunoassay, using Pharmacias Phadebas Insulin Test Kits.

Reagents

1. Sephadex-Anti-Insulin complex, antibodies rasied in guinea pigs.
2. Insulin standard 320 μU/ml after reconstitution.
3. Insulin ^{125}I 8ng - 3 μCi.
4. Buffer subtance 42 gm dry powder.

Method

1. Insulin standard solution was diluted with buffer solution in order to obtain suitable concentrations within the range 3 - 320 μU/ml. Buffer was used as zero sample.
2. 0.1 ml of standard (of varying dilutions ranging from 0 - 320 μU/ml) were pipetted, each one in duplicate, into siliconized glass centrifuged tubes with round bottoms.
3. 0.1 ml of the test sera were pipetted each one in duplicate into similar tubes.
4. 0.1 ml of the Insulin ^{125}I were then pipetted into each of the tubes mentioned in (2) and (3).
5. 0.1 ml of the Insulin ^{125}I were pipetted into 2 tubes which were then stoppered immediately. These were used to determine the total activity and were therefore not incubated, centrifuged or washed.
6. 1 ml sephadex-Anti-Insulin Complex suspension was pipetted into each of the tubes mentioned in (2) and (3).
7. The tubes were then incubated for 3 hours in a shaker.
8. The tubes mentioned in (2) and (3) were then centrifuged twice and the supernatant aspirated.
9. They were then washed by adding 2 ml 0.9% saline and centrifuged, and the supernatant aspirated. The washing procedure was done thrice.

10. The radioactivity was determined in all the tubes using a gamma-counter.
11. The count rate for each of the standards was expressed as a percentage of the mean count rate of the zeros. A standard curve was contructed plotting the percentage values obtained for the standards against the logarithm of insulin concentrations. The count rate for each of the test samples was also expressed as a percentage of the mean count rate of the zeros and from the values obtained the concentrations of insulin were read off directly from the curve.

APPENDIX C

Determination of Plasma Glucagon Concentrations.

Glucagon was assayed by radioimmunoassay using Biodata Glucagon Kits.

Reagents

1. Anti-Glucagon serum.
2. Glucagon standard 2 000 pg/ml after reconstitution.
3. ^{125}I-Glucagon.
4. Polyethylenglycol solution.
5. Trasylol.
6. Buffer.
7. Carrier serum.

Method

1. Four groups of tubes are prepared, representing zero (cold hormone) concentrations); controls; glucagon standards; and sample tubes.
2. 0.1 ml Trasylol was added to all the tubes.
3. 0.1 ml antiglucagon serum was then added to the standard, sample and zero tubes.
4. All the tubes were then incubated at 4^{o}C for 24 hours.
5. 0.1 ml ^{125}I-Glucagon was added to all the tubes and the contents mixed.
6. The tubes were then incubated at 4^{o}C for 24 hours.
7. 0.1 ml Carrier serum was added to all the tubes and the contents mixed.
8. The total counts of the zero tubes were determined.
9. 1 ml polyethylenglycol solution was then added to all the tubes and the contents mixed by Vortex.
10. All the tubes were centrifuged at 3 000 g for 15 minutes.
11. The supernatant was decanted and the precipitate washed by adding 2 ml distilled water to each tube.
12. The tubes were centrifuged again at 1 500 - 3 000 g for 2 - 3 minutes.

13. The supernatant was decanted and the tubes left inverted on a sheet of filter paper.
14. The radioactivity in all the tubes was now counted for at least a minute.
15. The percentage binding in absence of unlabelled hormone (maximum binding) is obtained using the mean c.p.m. bound in the zero tubes:-

$$\frac{\text{Mean c.p.m. bound in zero tubes}}{\text{Total radioactivity}} \times 100 = \% \text{ binding}$$

16. The inhibition percentage for each standard ans sample level was then calculated by expressing the mean c.p.m. in these tubes as a percentage of the mean c.p.m. in the zero tubes. By plotting the % inhibition against the different standard concentrations, the standard curve was obtained. The % inhibition values of the sample tubes were then used to read off the hormone concentrations from the standard curve.

APPENDIX D

Determination of Plasma Growth Hormone Concentrations.

Plasma growth hormone levels were assayed by radioimmunoassay, using Phadebas Kits (Pharmacia Diagnostics.

Reagents

1. Human Growth Hormone (hGh) standard.
2. Anti hGH ^{125}I (antibodies raised in rabbits).
3. Anti-hGH discs (antibodies raised in sheep).
4. hGH-free diluent (horse serum).

Method

1. 3 groups of tubes are prepared (each tube in duplicate), representing total activity, standards, and samples.
2. One Anti-hGH disc is added to the bottom of all tubes except the total activity ones.
3. 100 µl of each standard is pipetted in duplicate onto the discs in the standard tubes.
4. 100 µl of the sample sera are pipetted in duplicate onto the discs in the sample tubes.
5. The tubes are covered with plastic film and incubated for 3 hours at room temperature.
6. The supernatant from each tube is decanted and 2.5 ml saline added to all the tubes except the total activity tubes. Each tube is allowed to stand for 10 minutes. This procedure is repeated twice.
7. 100 µl of the anti-hGH ^{125}I solution is pipetted into the bottom of all tubes.
8. The total activity tubes are capped and set aside, whilst the other tubes are covered with plastic and incubated overnight at room temperature.
9. The supernatant in each tube is decanted.
10. 2.5 ml saline is added and the tubes allowed to stand for 10 minutes. All the liquid is again aspirated and the rinsing procedure repeated twice.

11. The tubes are capped and the radioactivity determined in all of them including the total activity tubes for 1 minute.
12. The count for each of the standards and unknowns is expressed as a percentage of the mean count of total activity. A standard curve is obtained by plotting the percentage values of the standards against the hGH concentrations. The growth hormone concentrations of the samples are then read off from the graph.

APPENDIX E

Determination of Plasma Cortisol Concentrations.

Plasma Cortisol levels were done by radioimmunoassay, using Gamma-Coat Kits (Travenol Laboratories).

Reagents

1. ^{125}I Cortisol Tracer.
2. Rabbit anti-cortisol serum coated tubes.
3. Phosphate buffered saline.
4. Processed human serum (blank).
5. Cortisol serum standards.

Method

1. 4 groups of Gamma Coat tubes are prepared, (each tube in duplicate), representing the Total Counts; the blanks, the standards and the samples.
2. 1 ml of Tracer-Buffer Reagant is added to each tube and the contents mixed.
3. The tubes are incubated in a water birth for 45 minutes at $37^{\circ}C$.
4. All the tubes, except the Total Counts, are then decanted.
5. The radioactivity in each tube is then determined for one minute.
6. A standard curve is drawn by plotting the CPM Bound for the cortisol concentrations. The CPM Bound for the samples are used to read off the concentrations from the standard curve.

APPENDIX F

Determination of Islet-Cell Antibodies.

Islet cell antibodies were detected by a standard indirect immunofluorescent method.

A. Islet Cell Antibodies (ICA-IgG)

Reagents

1. Human pancrease-obtained from group O cadaver kidney donor.
2. Anti-IgG Fluorescine Isothiocyanate Conjugate (FITC).

Method

1. The patient's serum is applied for 30 minutes at room temperature to the pancreas sections.
2. Specific anti-IgG FITC conjugate is then added.
3. The section is observed for immunofluorescence using an ultraviolet microscope equipped with epi-illumination.

B. Complement-Fixing Islet-Cell Antibodies (Cf-ICA)

Reagents

1. Human pancrease - obtained from group O cadaver kidney donor.
2. Fresh normal human serum.
3. Anti-human C3 conjugate.

Method

1. The patient's serum is applied for 30 minutes at room temperature to the pancreas sections.
2. After washing, a drop of fresh human normal serum is applied as a source of complement.
3. Anti-human C3 conjugate is then added.
4. The section is observed for immunofluorescence as above.

APPENDIX G

Determination of Circulating Immune Complexes.

Circulating Immune complexes were determined by a modified Raji cell assay.

Reagents

1. Raji cells.
2. .05% Tween 20 in saline.
3. Anti-human IgG.
4. Nitrophenyl phosphate solution.
5. 10% sodium hydroxide.

Method

1. Raji cells propagated in RPMI 1 640 + 10% fetal calf serum are washed 3 times, counted and resuspended in medium at a cell concentration of 40 000/µl.
2. 25 µl of a 1-in-4 dilution of test serum in saline is added to .50 µl of the Raji cell suspension.
3. The mixture is incubated at 37°C for 30 minutes.
4. The cell pellet is then washed 3 times in saline containing 0.05% Tween 20.
5. An enzyme-linked immunosorbent assay is then used to detect IgG adhering to the surface of the cells.
6. 1 ml of rabbit anti-human IgG (previously conjugated with alkaline phosphate is added).
7. The mixture is incubated at 37°C for 2 hours and then washed thriced in saline-Tween 20.
8. 1 ml nitrophenyl phosphate solution is then added to the disaggregated pellet of Raji cells.
9. Colour change is allowed to proceed until a fairly deep yellow is observed in the high reading control tube.
10. The reaction is stopped by adding 100 µl of 10% sodium hydroxide.
11. The tubes are centrifuged and the supernatant decanted for reading in a spectrophotometer at 400 mm.

12. The results are quantitated by means of a standard curve made each day using serial dilutions of an aliquot of serum (from a patient with systemic lupus erythematous) which has been standardized against a known concentration of aggregated IgG. High-reading and low-reading control sera are also included with each test batch.

APPENDIX H

List of abbreviations used :

ADA	Adrenal Antibodies
CIC	Circulating Immune Complexes
GPC	Gastric Parietal Cell Antibodies
GTT	Glucose Tolerance Test
HbA1	Glycosylated Haemoglobin
IBW	Ideal Body Weight
ICA IgG	Islet Cell Antibodies
ICA - cf	Complement-fixing Islet Cell Antibodies
IDDM	Insulin-dependent Diabetes Mellitus
IU/L	International Units per litre
JOD	Juvenile-onset Diabetes Mellitus
MCHA	Thyroid Microsomal Haemagglutinating Antibody
MMOL/ℓ	Millimoles per litre
MOD	Maturity-onset type Diabetes Mellitus
MODY	Maturity-onset type Diabetes Mellitus of the Young
NIDDM	Non-insulin-dependent Diabetes Mellitus
nmoles/ℓ	Nanomoles per litre
pg/mℓ	Picograms per millilitre
Uu/mℓ	Microunits per millilitre
TGHA	Thyroglobulin Haemagglutinating Antibody

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ADDENDUM I

A survey of African diabetic patients was done at the outpatient's department of King Edward VIII Hospital (Durban), whilst surveys of Indian patients were carried out at the outpatient's department of RK Khan Hospital (Durban) and at the Beatrice Street Clinic (Durban). The clinical case records of all diabetic patients seen at these centres over a four-week period were scrutinised in order to determine their ages and types of diabetes. The results were are follows:

	Africans	Indians
Total No. of Patients	247	2420
Patients with IDDM	21	27
Patients with NIDDM	226	2393
Patients under age 35	25	267
Patients under 35 with IDDM	21	29
Patients under 35 with NIDDM	4	238

ADDENDUM II

Oral Hypoglycaemic Therapy in Patients with NIDDM

	Africans	Indians
Diet		3
Diet + Tolbutamide	2	3
Diet + Chlorproamide	9	21
Diet + Glibenclamide	2	17
Diet + Phenformin		2
Diet + Phenformin + Chlorpropamide	4	11
Diet + Phenformin + Glibenclamide	1	4