Early metabolic predictors for the development of Type 2
diabetes in 1st degree relatives. Results of a 10-year
follow-up study (1984-1994), together with cross-sectional
analyses in 1994.

by

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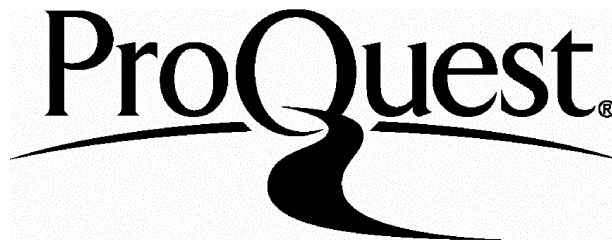
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Abstract

The relative contributions of reduced insulin sensitivity and beta-cell dysfunction to the pathogenesis of Type 2 diabetes are unknown. In addition, the influence of islet amyloid polypeptide (IAPP) and proinsulin secretion upon disease expression have not been fully characterised. In 1994 a 10 year-follow-up study of 96 previously non-diabetic, 1st degree relatives of Type 2 diabetic subjects was undertaken, with a physiological test-CIGMA(Continuous infusion of glucose with model assessment). The aims were to determine the conversion rate to diabetes, together with the most sensitive baseline predictors for disease progression. Results showed a cumulative prevalence for diabetes of 29% in the case of the siblings by mean age 60(10)y with an additional 8% having Impaired Fasting Glucose (fasting plasma glucose$\geq$6.1mmol/l (IFG), and in the offspring, 10% by mean age 44(8)y with an additional 5% having IFG. The major predictor for conversion to diabetes was the degree of fasting glycaemia at baseline. Neither beta cell function nor insulin sensitivity were predictive. In a separate study, cross-sectional analyses of plasma IAPP and proinsulin concentrations in 58 non-diabetic relatives, 19 control subjects and 39 Type 2 diabetic patients, showed both peptides increased proportionally to C-peptide from normoglycaemia (FPG<5.5mmol/l) to IFG. Once diabetes was

established, disproportionate hyperproinsulinaemia occurred, particularly in relation to the lower C-peptide concentrations in the insulin treated diabetics. IAPP levels were highest in relatives with IFG and in the diet and tablet treated diabetics and, like the C-peptide levels were significantly reduced in the insulin treated group. In summary, this study confirms the high prevalence and incidence of Type 2 diabetes in 1st degree relatives of white Caucasian diabetic patients. Raised fasting plasma glucose was the most sensitive predictor of diabetes. It is unlikely that routine assessment of beta-cell function or insulin sensitivity will give clinically useful information indicating which patients with IFG are at greater risk of developing diabetes. Disproportionate secretion of proinsulin did not occur until diabetes was established and is likely to be a secondary phenomenon.

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List of abbreviations

BMI   Body mass index

BP   Blood pressure

%B   Beta-cell function(estimated using CIGMA)

CIGMA   Continuos infusion of glucose with model assessment

CP   C-peptide

DCCT   Diabetes control and complications trial

DM   Diabetes mellitus

FPG   Fasting plasma glucose

FSIVGTT   Frequently sampled intravenous glucose tolerance test

GAD   Glutamic acid decarboxylase

IAPP   Islet amyloid polypeptide

IBW   Ideal body weight

ICA   Islet-cell antibody

IDDM   Insulin dependent diabetes mellitus

IFG   Impaired fasting glucose

List of abbreviations(continued)

IGT	Impaired glucose tolerance
ITT	Intravenous glucose tolerance test
IV	Intravenous
LADA	Late onset autoimmune Type 1 diabetes
NIDDM	Non-insulin dependent diabetes
NEFA	Non-esterified fasting acid
OGTT	Oral glucose tolerance test
PI	Proinsulin
SD	Standard deviation
%S	Insulin sensitivity (estimated using CIGMA)

Acknowledgements

My eighteen month stay at the Diabetes Research Laboratories in Oxford presented me with the ideal opportunity to study the physiological risk factors for the development of Type 2 diabetes in a large cohort of 1st degree relatives, originally studied by Professor Steve O'Rahilly in 1984. My clinical assistant was Beryl Barrow a Diabetic Nurse Specialist with boundless energy and a natural rapport with both the patients and fellow staff. On many occasions we managed to study four or five patients simultaneously-an operation requiring well orchestrated planning and a great deal of dexterity. Without her fantastic capabilities and good humour this study would never have happened. In addition I must thank all the laboratory staff but in particular Pauline Sutton who performed a number of the assays and was on all occasions kind and accommodating. Dr Jonathon Levy was frequently called upon to give advice regarding processing and analyses of the data generated during the study. I always found him willing to take time out of his busy clinical schedule to 'shed some light' on my various mathematical conundrums. A large section of this thesis deals with the role played by IAPP and islet amyloid in the pathogenesis of Type 2 diabetes. Dr Anne Clark was of immense help in the analyses of the IAPP data and also performed the histological staining of the pancreatic material dealt with in the section on Sibship BA. I would like to acknowledge

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Chapter 1

Summary of Clinical studies

The overall aim of these studies was to determine the most sensitive predictors for the development of Type 2 diabetes. The following 3 clinical studies are described in this thesis.

I.   The 10-year follow-up study:

Title: Development of diabetes and assessment of predictive factors in 10 year prospective study of siblings and offspring of white Caucasian, Type 2 diabetes patients.

Background: First degree relatives of Type 2 diabetic patients are at increased risk of developing diabetes but the incidence in white Caucasian subjects has not been determined.

Aims: To determine (I) the proportion of relatives progressing to Type 2 diabetes over a 10 year period (1984-1994), (ii) the baseline characteristics associated with progression.

Methods: 155 relatives were studied in 1984. 135 were non-diabetic: 47 siblings and 88 offspring. After 10 years, 96 non-diabetic subjects, 37 siblings

and 59 offspring, were restudied. Diabetes was defined as fasting plasma glucose (FPG) ≥ 7.8 mmol/l. Beta cell function and insulin sensitivity were assessed by continuous glucose infusion with model assessment (CIGMA).

Results: At baseline 11/62 (18%) of siblings were diabetic and by 10 years an additional 7 (11%), a cumulative prevalence of 29% by mean age 60 years. An additional 5 (8%) siblings had FPG ≥ 6.1 mmol/l (IFG) at 10 years. In 8 siblings with IFG at baseline, 4 (50%) progressed to diabetes. At baseline 5/93 (5%) of offspring had diabetes and by 10 years an additional 4 (4%), a cumulative 10% prevalence at mean age 44 years. An additional 5 (5%) had FPG≥ 6.1 mmol/l. Univariate risk factors for progression were FPG≥ 6.1 mmol/l (IFG), body mass index>26 kg/m^2, age>40 years, impaired insulin sensitivity<78% and male gender with odds ratios 9.3 (p=0.002), 12.4 (p=0.02), 4.9 (p=0.03), 0.11 (p=0.04) and 4.8 (p=0.05) respectively.

Conclusion: The high prevalence and incidence of Type 2 diabetes in first degree relatives of white Caucasian diabetic patients is confirmed. Baseline characteristics associated with progression were raised FPG, obesity, insulin resistance, age and male gender.

II. The cross-sectional study:

Title: No evidence for disproportionately raised proinsulin or IAPP, or raised NEFA, in glucose intolerant first degree relatives of Type 2 diabetic subjects.

Background: Elevated proinsulin and NEFA, together with islet amyloid formation are all features of Type 2 diabetes but their relationship to β-cell dysfunction, insulin resistance and onset of hyperglycaemia are unknown.

Aims: To determine if secretion of proinsulin and islet amyloid polypeptide (IAPP) are dissociated at any stage during the development of Type 2 diabetes. Also to determine if NEFA levels are raised prior to onset of diabetes.

Methods: 116 subjects, including 58 non-diabetic 1st degree relatives of Type 2 diabetic subjects were studied during a 1hr infusion of glucose. Non-diabetic subjects, with equivalent BMI, were assigned to three groups: i)fasting glucose, fpg <5.5 mmol/l; ii)fpg $\geq$ 5.5<6.1; iii)Impaired Fasting Glucose(IFG) fpg $\geq$6.1<7.0mmol/l. Diabetic subjects were divided according to therapy (9 diet, 19 tablet, 11 insulin). IAPP, NEFA, C-peptide and proinsulin were measured fasting and at 1hr.

Results: Basal levels of all hormones, but not NEFA were significantly elevated in non-diabetic subjects with IFG (p<0.05) compared with the other non-diabetic subjects. Fasting IAPP/CP and PI/CP were of the order of 1-2%

and were equivalent for all three non-diabetic groups. Fasting and 1-hr PI and PI/CP were significantly higher in Type 2 diabetic than in non-diabetics, even after correction for BMI (p<0.01). In contrast, IAPP and IAPP/CP were the same as in the non-diabetics with IFG apart from in the insulin treated group who had significantly lower values (p<0.01). 1hr NEFA values were significantly higher in the diabetics with no apparent correlation with level of glycaemia, obesity, insulin sensitivity or beta-cell function.

Conclusion: Co-ordinate increase in proinsulin, C-peptide and IAPP secretion occur in non-diabetics with worsening glucose tolerance but NEFA values are not significantly raised. In subjects with established Type 2 diabetes, proinsulin is disproportionately elevated even in subjects with severe β-cell failure. However, changes in IAPP secretion in nondiabetics remain linear with C-peptide. Post-glucose NEFA values are significantly higher in the diabetics but do not appear to reflect any relationship with insulin sensitivity or beta-cell dysfunction and may be an additional marker for the lack of the overall effective insulin action. Raised proinsulin and NEFA are unlikely to be related to the pathogenesis of Type 2 diabetes and probably occur as secondary phenomena.

III.   Sibship BA (cross-sectional/longitudinal+histology)

Title: Is islet amyloid formation initiated by hypersecretion of IAPP and associated with progressive beta-cell failure in Type 2 diabetes?

Background: Islet amyloid is found in the pancreas of 90% of Type 2 diabetics at postmortem compared with 20% of age and BMI matched controls. Animal studies suggest that islet amyloid formation occurs in parallel with the development of hyperglycaemia, however the situation in humans is unclear.

Aims: To relate prospective 10 year physiological data in a particular sib-ship(BA)- with a high conversion rate to diabetes, with post-mortem derived pancreatic histology in two of the diabetic siblings.

Methods: Sib-ship BA comprised 5 brothers aged 30-45y who took part in the 10 year longitudinal study of 1st degree relatives (1984-1994). The eldest siblings were identical twins, one of whom-the proband, was already diabetic at baseline. Mean BMI for the siblings was 35(4). The physiological test was a 1hr infusion of glucose with model assessment (CIGMA). C-peptide and glucose levels were measured throughout the test. In 1994, proinsulin and IAPP were also measured.

Results: The twin and middle sibling converted to diabetes during the follow-

up period (1990 and 1994 respectively). None of the siblings had impaired glucose tolerance at baseline. Progression to diabetes was accompanied by a significant decline in beta-cell function (CIGMA)(p<0.0001), but no change in insulin sensitivity. Proinsulin levels in 1994, were disproportionately raised in the two siblings who had developed diabetes. In contrast, IAPP concentrations in 1994 were only elevated in the case of the newly diagnosed middle sibling (p<0.005). Pancreatic histology subsequently became available from post-mortem examination in both of the twins.

Despite duration of diabetes less than 5 years at the time of death, histological examination confirmed the presence of significant islet amyloid deposition.

Conclusion: Development of diabetes is accompanied by a significant decline in beta-cell function but not insulin sensitivity. Accumulation of islet amyloid may play a pathological role in loss of beta-cell function. The significance of disproportionately raised IAPP concentrations in newly established diabetes needs to be established further but could serve as a marker for islet amyloid deposition.

Chapter 2

The relative roles of insulin resistance and beta-cell dysfunction in the pathogenesis of Type 2 diabetes. A review of the literature.

For the past two decades the debate has continued regarding the relative contributions of insulin resistance and reduced beta-cell function to the development of Type 2 diabetes. In the established case, both defects co-exist. However prospective studies in 'high-risk' populations have endeavoured to determine which metabolic abnormality occurs first. Despite such efforts the jury remains out as to what the primary defect is. Two of the major draw-backs of physiological studies are i) the heterogeneity of Type 2 diabetes in terms of its phenotypic expression, and ii) the different methodologies employed in determining glucose tolerance, insulin action (sensitivity) and insulin secretion (beta-cell function).

I.   Insulin resistance.

There is an approximate five-fold variation in insulin sensitivity amongst normal individuals (Hollenbeck *et al*,1987). The main factors that seem to be involved are physical fitness, body composition, fat distribution and gender. After an overnight fast, the rate of glucose release by the liver is about 8g/hr (70% from glycogenolysis and 30% from gluconeogenesis). About 30% is taken up by neural tissue by non-insulin-dependent mechanisms, 10% is taken up by splanchnic and adipose tissue and 60% by muscle(an insulin-dependent process). Hepatic glucose production is regulated by insulin, glucagon and NEFA. The beta-cell, liver and muscle make-up a sensitive counter-regulatory loop that maintains glucose homeostasis (DeFronzo, 1988). Glucose uptake into muscle and adipose tissue is via the Glut 4 glucose transporter (Bell *et al*, 1990). In muscle, sixty percent of glucose is metabolized to glycogen via glycogen synthase (non-oxidative) and 40% to CO_2 and H_2O by pyruvate dehydrogenase (oxidative). The number of insulin receptors recruited during insulin stimulated fuel uptake is approximately 10-15% ie. many are unutilized. The glucose transporter in liver is Glut-2, the same as in the beta-cell and is not under the influence of insulin. The term insulin resistance usually implies insensitivity of insulin sensitive

tissues to the actions of insulin. The mechanisms involved are not fully understood but in Type 2 diabetes the main sites of insulin resistance are thought to be muscle and liver.

Maximum aerobic power -VO2 max. is strongly correlated with insulin sensitivity. Studies in 1st degree relatives of Type 2 diabetic Pima Indians suggested that the relatives were insulin resistant regardless of physical fitness (Rosenthal et al,1983). However in relatives of Caucasian diabetic subjects, there was no evidence for insulin resistance after correcting for physical fitness (Nyholm et al, 1994). This suggests that insulin resistance may be inherited via mechanisms not related to physical fitness in certain ethnic groups.

Since muscle is the main insulin sensitive tissue, high muscle mass enhances glucose utilization and glucose tolerance regardless of physical fitness (Yki-Jarvinen and Koivisto, 1983). Premenauposal females are more insulin sensitive than males, despite lower muscle mass and 20% lower VO2 max. This difference seems dependent on female sex steroids and is abolished in the post menopausal state. Obese subjects have higher lean body mass and fat mass. However the muscle tissue comprises higher proportion of insulin resistant muscle fibres (Ravussin,1983). With regards to distribution of adiposity, both hepatic glucose output and peripheral glucose utilization are

more resistant to upper than lower body obesity (Peiris *et al*, 1988).

Centripetal distribution of fat seems to be under genetic influence (Bouchard *et al*, 1990). This may account for the insulin resistance seen in a number of ethnic groups susceptible to Type 2 diabetes. The mechanisms involved in differences in insulin resistance are not fully understood. Under basal and post-prandial conditions, the absolute rate of glucose utilization in Type 2 diabetics is normal. This is because the prevailing hyperglycaemia causes increased uptake by the glucose mass-action effect. However, under conditions of normal glucose and insulin, there are major defects in both the oxidative and non-oxidative pathways of glucose metabolism-(Eriksson *et al*,.1989; Shulman *et al*,1990). Recent studies suggest that an early event may be an impairment in the activation of glycogen synthase by insulin in skeletal muscle (Schalin-Jäntti *et al*, 1992; Vaag *et al*,1992). However recent studies using MRI spectroscopy indicate that levels of glucose-6-phosphate are reduced in subjects with Type 2 diabetes during a hyperglycaemic hyperinsulinaemic clamp (Rothman *et al*,1992). Since a reduction in glycogen synthase activity would lead to an increase in glucose-6-phosphate, this strongly suggests that the defect in glucose metabolism is at the level of glucose uptake or subsequent hexokinase. Defects in hepatic glucose

production are detectable in both the basal and post-prandial state in Type 2

diabetes (Butler *et al*, 1990). The hyperglycaemia which occurs in the fasting

and post-prandial state is due to excessive hepatic glucose production by

glycogenolysis. Del Prato and colleagues,1997 and also Welch *et al*, 1990,

have recently described the phenomenon of glucose resistance. This is the

down-regulation of glucose transporter pathways involving non-insulin-

sensitive transporter systems-mainly GLUT 1 but also GLUT 2-possibly

mediated via glucotoxicity effects.

Various candidate genes for insulin resistance have been investigated.

Mutations in the insulin receptor account for a minority of cases. In one of the

most extreme cases of this- Leprechaunism, there is an absence of the insulin

receptor (Moller and O'Rahilly, 1993). A missense mutation in the glucagon

receptor gene has been identified in up to 8% of Type 2 diabetics in Sardinia

and France (Hager *et al*, 1995) but only 2% of English subjects (Gough *et al*,

1995). No association was found for Japanese subjects (Fujisawa *et al*, 1995).

In the case of the glycogen synthase gene, a number of mutations have been

identified but none are thought to play a major role in Type 2 diabetes (Groop

et al, 1993). As mentioned previously, certain ethnic groups such as the Pima

Indians and Asian Indians, are predisposed to the development of Type 2

diabetes by virtue of their susceptibility to insulin resistance. In Pima Indians

insulin resistance is linked to the development of severe obesity at a relatively

young age. In Asian Indians, obesity is less dramatic although a centripetal

distribution of visceral adiposity predominates. In this ethnic group, insulin

resistance is associated with dyslipidaemias and a markedly raised

susceptibility for the development of coronary artery disease. In both groups,

the epidemic of Type 2 diabetes has accompanied the adoption of a more

Western lifestyle and has been termed the thrifty genotype(O'Dea and

Zimmet,1993). Hyperglycaemia induces insulin resistance-the so called

glucose toxicity phenomenon (Unger and Grundy, 1985; Rossetti *et al*, 1990).

Reversal of hyperglycaemia has been shown to improve insulin sensitivity and

is the aim of intensified treatment in both Type 1 and Type 2 diabetics.

II. Beta-cell dysfunction in Type 2 diabetes.

In simple terms, glucose intolerance develops when the beta-cell is no longer able to 'keep-up' with insulin demands. Insulin resistance is a natural occurence with increasing age. However, only a proportion of the elderly population develop impaired glucose tolerance or diabetes. This indicates the inherent ability of beta-cell to adapt to the situation of increased demand. Under normal circumstances only about 15-20% of beta-cells are actively involved in insulin secretion. In the 'stressed' situation, the phenomenon of recruitment occurs with more beta-cells secreting insulin (Pipeleers *et al*, 1994). Beta-cell mass is thought to be moderately reduced in Type 2 diabetics (Kloppel *et al*,1985). However this reduction does not appear to be sufficient to account for the secretory defect seen in established Type 2 diabetes. Islet amyloid is found in >90% of diabetics compared with <20% in age and weight matched controls (Clark,1989). Islet amyloid is an amorphous fibrillar substance which is deposited within the islets. Fibrillar amyloid is present in invaginations of the plasma membrane of the beta-cell. The resulting irregularity of the beta-cell membrane could lead to both disturbed secretory as well as sensing mechanisms.

Apart from the morphological changes that take place in Type 2 Diabetes,

secretory function is also disrupted. The inverted-U-shaped curve has been known about for decades. This shows that development of Type 2 diabetes is associated with insulin levels that are in the high/normal range compared with a matched control population and only start to decline at fasting glucose concentrations above 10.0mmol/l (Reaven and Miller,1968; Saad et al, 1989). A major draw back of these studies is the cross-reactivity of insulin with proinsulin in the radio immunoassays which were employed at the time. The amount of proinsulin that is secreted relative to insulin is very small (Horwitz et al, 1978). However the clearance rate of proinsulin is much slower, meaning that 15-25% of a normal insulin value is accounted for by proinsulin (Temple et al,1989; Ward et al, 1987). Further studies using specific proinsulin assays suggest that proinsulin concentrations in Type 2 diabetics are higher than in non-diabetics (Saad et al,1990; Ward et al,1987; Yoshioka et al, 1988) this could therefore imply that a relative state of hypoinsulinaemia exists in Type 2 diabetic subjects, more than suggested by measurement of the immunoreactive insulin concentrations.

The main regulator of insulin secretion is the ambient glucose concentration (Hedoskov,1980). When the glucose level is raised to >5.5mmol/l a bi-phasic insulin response is initiated. The first phase lasts 5-10 minutes and the second

phase for the duration of the hyperglycaemia. In Type 2 diabetes, the 1st phase insulin response is lost (Cerasi and Luft,1967; Lerner and Porte,1972). This has been demonstrated to occur at plasma glucose levels as low as 6.4mmol/l (Brunzell *et al*, 1976) although Flax *et al*,1991 did not demonstrate suppression of 1st phase response at these glucose levels. The post-prandial excursions in glucose that characterise the diabetic state are ameliorated by simulating a burst of insulin at the beginning of the meal (Bruce *et al*,1988)-suggesting that the early insulin response affects the disposal of glucose several hours later. Many other secretogogues maintain normal effectiveness on insulin secretory response in Type 2 diabetes (Deckert,1968; Simpson *et al*,1968; Ward *et al*,1986)-implying that the beta-cell defect is specific for glucose. A number of studies have demonstrated partial return of the 1st phase response following a period of improved metabolic control (Glaser *et al*,1988; Kosaka *et al*,1980; Savage *et al*,1979; Vague and Moulin,1982). However Garvey *et al*,1985, failed to demonstrate improved secretory response in diabetics following insulin administration. However the glycaemic levels post treatment in this study were of the order of ~8.6mmol/l and this modestly raised level may have accounted for this negative result. The pulsatile nature of insulin secretion is also affected in Type 2 diabetes. The small pulses which occur every 11-13

minutes are lost in Type 2 diabetes (Garvey *et al*,1985; Lang *et al*,1981), whilst

the larger pulses that occur 10-15 times a day have decreased magnitude and

a disturbed temporal pattern relative to food ingestion (Polonsky *et al*,1988).

Whilst disturbed pulsatility may occur secondary to raised glucose levels,

abnormalities have been demonstrated in non-diabetic 1st degree relatives of

Type 2 diabetic subjects (O'Rahilly *et al*,1988). This suggests that a primary

defect may exist prior to onset of hyperglycaemia. Cerasi,1995, has recently

suggested that beta-cell hyper-responsiveness may be the preliminary defect

in Type 2 diabetes-possibly mediated via increased vagal tone. This would

lead to insulin resistance secondary to sustained hyperinsulinaemia. Then,

under conditions of excessive nutritional intake, insulin levels would be raised

further leading to eventual beta-cell exhaustion and hyperglycaemia.

Correction of hyperglycaemia improves both beta-cell function and insulin

sensitivity (Glaser *et al*,1988; , Yki-Jarvinen *et al*,1988). Ilkova and

colleagues,1997, have recently treated newly diagnosed Type 2 diabetic

subjects with two week continuous infusion of insulin and shown that in a

significant proportion, hyperglycaemia was corrected for up to 26(4) months

off treatment.

Chapter 3

Diabetic Nomenclature-Diagnosis and classification:

Diagnosis

In 1979 and 1980 the National Diabetes Data Group and the World Health

Organisation made new recommendations on the diagnosis and classification

of diabetes. The 75g oral glucose tolerance test (OGTT) became the gold

standard with fasting and 2-hr values defined. Over the past decade,

particularly following the results of the DCCT, pressure has mounted for a

revision of diagnostic criteria that takes into account the risk for diabetic

complications linked to level of glycaemia.

In 1997, new guidelines for the diagnosis of diabetes were recommended by

the American Diabetes Association. A fasting plasma glucose of ≥ 7mmol/l,

rather than 7.8mmol/l was felt to be a better 'cut-off' for the diagnosis of

diabetes. Two abnormal results on two separate days are the prerequisite for

diagnosis. The FPG is felt to be preferable to the OGTT on the grounds that it

is a simpler test, cheaper and more acceptable to patients and therefore more

likely to be performed. Recommendations were also made regarding the close

monitoring of subjects with IGT who are known to be at high risk for the future

development of Type 2 diabetes and are also at risk for the development of

macrovasular disease. Two categories of IGT were defined:

i.   **impaired fasting glucose = FPG≥ 6.1mmol/l < 7mmol/l**

ii.   **IGT= FPG>7.8 <11.1 during an OGTT**

Recommendations were also made about the abandonment of the terms

NIDDM and IDDM in favour of Type 1 and Type 2 diabetes.

In July 1998 Part 1 of a WHO consultation was published with preliminary

recommendations for diagnosis and classification of diabetes. Basically this

agrees with the new lower FPG value for the diagnosis of diabetes which is felt

to be of equal diagnostic significance to that of the 2 hr post load concentration.

This equivalence has been determined by a number of population based studies

(Engelgau *et al*,1997; Finch *et al*,1990;McCance *et al*,1994). Microvascular

disease complications have been shown to be increased at FPG values ≥

7.0mmol/l (McCance *et al*,1994), and similarly macrovascular complications

at the same level even when 2-hr values are <7.8mmol/l (Charles *et al*,1996).

Classification

Table 1 (Taken from the WHO Provisional Report,1998 Part 1:

Diagnosis and Classification of Diabetes Mellitus)

Aetiological classification of disorders of glycaemia

Type 1 (beta -cell destruction, usually leading to absolute insulin

deficiency)

    Autoimmine

    Idiopathic

Type 2 (may range from predominantly insulin resistance with   relative

insulin deficiency to a predominantly secretory defect with   or without insulin

resistance)

Other specific types

    Genetic defects of beta-cell function

    Genetic defects in insulin action

    Diseases of the exocrine pancreas

    Endocrinopathies

    Drug- or chemical-induced

    Infections

    Uncommon forms of immune-mediated diabetes

    Other genetic syndromes associated with diabetes

Gestational Diabetes

Type 2 diabetes

From the above classification, it is clear that a diagnosis of Type 2 diabetes applies when other causes have been excluded. Type 2 diabetes usually has an onset in subjects >40 years and is more common in subjects who are obese and take little physical exercise (Harris *et al*,1995; Zimmet,1992). Ethnicity and positive family history are also significant risk factors (de Courten *et al*,1997; Harris *et al*,1995; Zimmet,1992; Valle *et al*,1997). Approximately 4% of white Caucasian subjects are Type 2 diabetic by 60y. This figure rises to about 10% by age 70y. Often abnormal glucose tolerance is found in subjects with one or more component of the Metabolic Syndrome (Reaven,1988; Zimmet,1992). Each metabolic defect confers considerable cardiovascular risk but combined the effect is magnified (Kaplan,1989). Features of the Metabolic Syndrome can present up to a decade before abnormalities in glucose tolerance occur (Mykkanen *et al*,1993). Distinct genetic forms of diabetes-most importantly MODY, account for <5% of subjects previously diagnosed as Type 2 diabetic. Young age of onset and autosomal dominant inheritance-provide useful clues to the diagnosis. In the case of MODY, three genetic loci on different chromosomes have now been identified. A mutation in HNF 1α on chromosome 12 is the most common form-MODY 3 (Yagamata

et al,1996). A second form is associated with mutations in the glucokinase

gene on chromosome 7p (Froguel *et al*,1992). Both of these defects lead to

disturbance of insulin secretion rather than action. Glucokinase converts

glucose to glucose-6 phosphate, the metabolism of which stimulates insulin

secretion by the beta-cell. Elucidation of these monogenetic varieties of

diabetes has fuelled large scale genetic studies aimed at identifying the major

gene defects that contribute to the expression of Type 2 diabetes. The genetic

defects of beta-cell function and insulin action are listed below in Table 2.

Table 2: Genetic forms of diabetes (Taken from the WHO Provisional Report, 1998 Part 1: Diagnosis and Classification of Diabetes Mellitus)

Genetic defects causing diabetes

Genetic defects of beta-cell function

    Chromosome 20, HNFα (MODY 1)

    Chromosome 7, glucokinase (MODY 2)

    Chromosome 12, HNF1α (MODY 3)

    Chromosome 13, IPF-1 (MODY 4)

    Mitochondrial DNA 3243 mutation

    Others

Genetic defects in insulin action

    Type A insulin resistance

    Leprechaunism

    Rabson-Mendenhall syndrome

    Lipoatrophic diabetes

    Others

Late-onset Type 1 diabetes

It is estimated that 40% of Type 1 diabetics develop the disease before age 15 years, 30% between the ages of 15 and 34years and 30% after age 30 years. Most Type 2 diabetics have relative rather than absolute insulin deficiency. The characteristics that distinguish subjects with late onset Type 1 diabetes are:

i.   Low basal and stimulated C-peptide concentrations

ii.   Low BMI

iii. ICA and anti-GAD antibodies

iv.   High frequency of HLA DR3 and DR4

v.   Presence of other autoimmune antibodies

Tuomi *et al*,1993, studied 102 Type 2 diabetics diagnosed age $\geq 34y$ who were non-ketotic and maintained on diet or oral-hypoglycaemic agents for ≥ 6 months. Subjects were classified as insulin deficient or non-insulin deficient on the basis of stimulated C-peptide response to IV glucagon. 33 patients were insulin deficient (stimulated C-peptide<0.6mmol/l). 69 patients were non-insulin deficient (stimulated C-peptide>0.6mmol/l). BMI was significantly lower in the insulin deficient group (p<0.001). Anti-GAD(glutamic acid decarboxylase) antibodies were positive in 76% of the insulin deficient

diabetics as opposed to 12% of the non-insulin deficient group (p<0.001). ICA

(islet-cell antibodies) were available on a sub-set of the cohort. Results

showed that 52% of the anti-GAD positive insulin deficient group were also

positive for ICA, compared with 13% of the anti-GAD negative insulin

deficient group. In the case of the non-insulin deficient diabetics, 25% of the

anti-GAD positive were also positive for ICA whilst 7% of the ant-GAD

negative were ICA positive. Previous studies have shown that presence of ICA

antibodies correlates with progression to insulin requirement in Type 2

diabetics in cross-sectional and prospective studies(Gleichmann *et al*,1984;

Groop *et al*,1986). This study concluded that the presence of anti-GAD

antibodies was a more sensitive and specific marker for latent onset Type 1

diabetes. Both ICA and anti-GAD antibodies are usually present at the time

of diagnosis. ICA antibodies disappear before anti-GAD, the latter usually

persisting for 15-20 years (Rowley *et al*,1992). The recognition of LADA as

a distinct pathological entity from Type 2 diabetes has raised concern regarding

the inclusion of these subjects in studies examining the physiological markers

for progression to Type 2 diabetes. Recognition of the phenotypic

characteristics together with anti-body screening allows these subjects to be

excluded from future analyses. Another interesting phenotypic subgroup is

black, male North Americans who present in a fulminant manner similar to

Type 1 diabetes but are subsequently managed on low-dose sulphonylureas

(Banerji *et al*,1995). Whether this sub-group represents a distinct genetic

entity, remains to be determined.

Chapter 4

Physiological tests used to assess insulin resistance and beta-cell function.

A variety of tests have been devised over the past twenty years for the measurement of insulin sensitivity (an inverse measurement of insulin resistance) and beta-cell function. These two aspects of diabetes are separate determinants of disease expression and are governed by different genetic and environmental factors (Prospective Diabetes Study V,1988; Rudenski *et al*,1988; Rudenski *et al*,1991). Assessment of insulin sensitivity *in vivo* is based upon quantifying the relationship between plasma insulin and some measurable metabolic process (usually plasma glucose concentration). Assessment of beta-cell function usually consists of measuring insulin and C-peptide following oral or intravenous glucose challenge (Bergman *et al*,1985; Davis *et al*,1992).

Insulin sensitivity.

The euglycaemic hyperinsulinaemic clamp is the relative 'gold standard' to evaluate insulin sensitivity in the intact organism (DeFronzo *et al*,1979). The reason for this is that measurements are carried out at a fixed glucose and insulin concentration. Another benefit of this method is that it can easily be combined with measurement of hepatic glucose production using infusion of labelled glucose. However, because of the labour intensive nature of the clamp technique, investigators have sought more 'user friendly' methods that can be applied to large cohorts of subjects. The glucose-insulin interactions used to assess insulin sensitivity are sufficiently complex that mathematical models are required to analyse the interactions. Two independent analytical models have been developed:

- A "Minimal Model" approach. This evaluates the relationship between glucose and insulin during a bolus intravenous glucose tolerance test (Bergman *et al*,1985; Steil *et al*,1993). FSIVGTT is modified to include bolus exogenous insulin to allow it's use in diabetic subjects with impaired insulin secretion. Although modified FSIVGTT is easier to operate than glucose clamps, it requires>30 timed specimens and a significant amount of blood. A reduced (12 points) sampling regimen

was proposed to provide acceptable estimates of insulin sensitivity (Steil

et al,1993), but retention of the full sampling procedure was later

advised (Coates *et al*,1993). The computer modelling of each patient's

results is a skilled and time consuming process. For these reasons,

FSIVGTT also proves to be labour intensive and is relatively difficult to

administer.

- A "Structural Model" of metabolism has been devised in Oxford. This

uses known physiological responses of each major organ as a set of

simultaneous equations. This assesses the degree to which combinations

of impaired beta-cell function and insulin resistance induce diabetes

(Levy *et al*,1991; Matthews *et al*,1985; Prospective Diabetes Study

V,1988; Rudenski *et al*,1988; Rudenski *et al*,1991). Two physiological

methods are derived from the structural model. The basal, steady state

glucose and insulin measurements can be assessed by HOMA

(Homeostasis Model Assessment) (Matthews *et al*,1985; Prospective

Diabetes Study V,1988; Rudenski *et al*,1988; Rudenski *et al*,1991), and

by the glucose-insulin response to a slow glucose infusion, mimicking

the physiological post-prandial rise in blood glucose (CIGMA) (Levy *et*

al,1991). CIGMA and HOMA have both been validated against

independent measures of insulin resistance and beta-cell function. Both

models are practical, less labour intensive and cheaper to operate than modified FSIVGTT and only require limited blood sampling. The near-steady state response also means that results can be read off a nomogram rather than computed.

- A third non-mathematical, direct and rapid estimate of in vivo insulin action consists of measuring the initial fall in plasma glucose following IV insulin injection in the fasting state. This short Insulin Tolerance Test or ITT has been validated against the glucose clamp and is suitable for large-scale studies of insulin sensitivity (Akinmokum *et al*,1992; Bonora *et al*,1989; Hirst *et al*,1993). The test, however does not estimate beta-cell function.

Beta-cell function

The gold standard is the hyperglycaemic clamp (DeFronzo *et al*,1979). Methods used for assessment of insulin sensitivity can also be used to estimate insulin secretory capacity- these include IVGTT (Bardet *et al*,1989), CIGMA and HOMA. Others include the glucagon stimulation test (Scheen *et al*,1996) and the glucose dependent arginine stimulation test (Larsson and Ahrén,1996).

Criteria required when assessing insulin sensitivity and beta-cell function using in-vivo tests.

Groop *et* al,1993, described the criteria required for accurate assessment of insulin sensitivity and beta-cell function using *in vivo* tests.

insulin sensitivity

For assessment of insulin sensitivity , 5 criteriae should be met:.

i. Insulin levels should be high enough to stimulate glucose metabolism and detect small differences in insulin resistance between subjects. In the case of the clamp, infusion rates of insulin of $1mU.Kg^{-1}$ min^{-1} achieve steady state insulin levels of 400-500 pmol/l. This affords an ability to differentiate systemic differences in insulin sensitivity of ~ 10%. In the case of CIGMA (Levy *et al*,1991), insulin concentrations in the steady state are about half this. This is therefore a somewhat weaker stimulus for peripheral glucose uptake and could lead to underestimation of insulin resistance, particularly in more obese subjects.

ii. It is desirable to distiguish between hepatic and peripheral insulin sensitivity. In non-diabetic as well as Type 2 diabetic subjects, hepatic glucose production is 'turned -off' at insulin concentrationsan of 100-300pmol/l. Therefore in more insulin resistant subjects-the rate of

glucose uptake is underestimated.

iii. Measurements should be made under steady state conditions otherwise the 'fitted' algorithms do not properly apply (Cobelli *et al*,1987).

iv. The assumptions about the body glucose system must be physiologically sound. For example the minimal model (Bergman *et al*,1979) 'falls-down' in its assumptions regarding the mono-compartmental kinetics of glucose action and also by implying that insulin action takes place in a remote compartment. In the case of the latter, this could apply to insulin action on the periphery or for insulin action on the liver-but in vivo, interplay with both occurs (Cobelli *et al*,1987).

v. Many of the tests result in hyperglycaemia. Glucose clearance is affected by the ambient glucose concentration (glucose mass-action effect), especially at low insulin concentrations. This was demonstrated by Vaag *et al* in 1992 when they suggested that glycogen synthase activity in muscle was reduced in young, nonobese 1st degree relatives of white Caucasian Type 2 diabetic subjects. This defect however can be overcome by raising the plasma glucose level as demonstrated using the hyperglycaemic clamp technique (Eriksson *et al*,1989). Under these conditions, the difference between 1st degree relatives and normal

controls was ~14% and was not statistically significant. (Mitrakou *et al*,1992).

Beta-cell function.

Measurement of insulin secretory dynamics is complex owing to the fact that although glucose is the principal secretogogue, a number of others have an influence on insulin secretion, most importantly, amino-acids and NEFA. Also in the normal physiological process of ingesting food, certain GI hormones such as GLP1 exert an incretin effect. Peripheral plasma concentrations are assumed to correlate directly with insulin output from the pancreas. For this assumption to 'hold true', it is necessary to measure both insulin and C-peptide kinetics and apply them to deconvolution or system model methods of analysis (Ferrannini and Pilo,1979). Insulin secretion responds not only to the ambient glucose concentration but also to the rate of change (Ferrannini and Pilo,1979). Insulin secretion is bi-phasic. Whilst both of these phases are discernible in a continuous infusion of glucose, only the 1st phase is reliably determined in IVGTT (Galvin *et al*,1992). In the case of the hyperglycaemic clamp, a plasma glucose of ~7.0mmol/l, does not stimulate concentrations of insulin which are considered to be maximal. Thus defects in insulin secretion may be missed using this technique (Ward *et al*,1984).

The CIGMA model

The clinical studies described in this thesis use CIGMA. This is a structural model of the glucose/insulin feedback system. It incorporates mathematical descriptions of the function of the various organs involved in plasma glucose control based on empirical data. The mathematical structure of this model was outlined by Hosker *et al* in 1985 who also described the correlation of CIGMA estimations of %S and %B in 23 subjects compared with independent measurements derived from euglycaemic and hyperinsulinaemic clamp studies in the same subjects. The correlation coefficients for %S and %B for the two methods were R_s =0.87, p<0.0001 and R_B =0.64, p<0.002. The CIGMA coefficient of variability was 21% for %S and 19% for %B. In 1988, Rudenski *et al*, described the details of that part of the model dealing with insulin and C-peptide secretion and kinetics. In 1991, Levy *et al*, looked at the glucose-insulin and glucose- C-peptide vector slopes derived during CIGMA and found them to generate an empirically linear relationship which afforded reliable prediction of insulin levels achieved by the hyperglycaemic clamp. The vector slope agreed closely with the beta-cell coefficient generated by the CIGMA model. Also in 1991, Hammersley *et al*, showed that the CIGMA test was more precise than the OGTT in determining glucose tolerance. Hermans *et al*,

1995, showed that CIGMA has better discriminatory power over the minimal model approach using FSIVGGT and HOMA in determining differences in beta-cell function over a range of glucose tolerance. The same group also demonstrated that for insulin sensitivity testing, the best discriminatory power was achieved using CIGMA and the reciprocal concentration of fasting plasma insulin. In practical terms, the CIGMA model has been calibrated for a young, lean reference population and is available as a computer model or in nomogram form. 1hr paired insulin and glucose or C-peptide-glucose values allow % S and %B for that individual to be estimated. Insulin concentrations used in the calibration are in pmol/l; a choice is available of using insulin measured by the standard radio immunoassay with a virtual 100% cross-reactivity for proinsulins or by specific insulin assay method, which is assumed to have no cross reactivity with proinsulin. Note %B and %S are not conceptually independent. However to enhance 'measurement independence' %B can be estimated using glucose/ insulin pairs and %S using glucose/C-peptide pairs (or vice-versa). This will prevent assay noise from being transmitted simultaneously to both parameters. The model is best used to compare values in two groups, or in a single group under different conditions, using the same biochemical assays for each group.

Chapter 5

Prospective studies: a review of the literature.

Over the past 15 years a number of prospective studies have been performed looking at markers for progression to Type 2 diabetes. To improve the power of such studies, high risk populations are studied. Such populations include ethnic groups with an increased prevalence of Type 2 diabetes, such as the Pima Indians, Japanese Americans and Nauruans, 1st degree relatives of Type 2 diabetic subjects and women with previous gestational diabetes. Kobberling et al,1985 estimated that the life time risk for development of diabetes in the siblings of affected subjects was 40% and for the offspring~35%. This gives an approximate three-fold increased risk for the development of Type 2 diabetes in the 1st degree relatives of Type 2 diabetic subjects compared with the general population. In this chapter some of the major prospective studies in Caucasian populations and in other ethnic groups are reviewed.

I.   Prospective studies in Caucasian subjects.

- Skarfors *et al*,1991, studied 2,000 middle aged Swedish men over a mean time period of 10 years. The study method employed was the intravenous glucose tolerance test(IVGT). If the baseline FPG was ≥ 5.7mmol/l an OGTT was performed to determine glucose tolerance. Subjects with FPG≥ 6.7mmol/l were excluded from the study. In 1984 subjects were tested using an FPG (and OGTT if FPG≥ 5.7). 4.2% of subjects who were normoglycaemic at baseline had converted to Type 2 diabetes. A further 2% had IGT. The latter were excluded from the statistical analyses. Stepwise logistic regression analysis was performed with the dependant outcome being development of Type 2 diabetes. Significant predictive variables were the 1hr glucose, the fasting insulin at baseline, the 1st phase insulin response, BMI, systolic BP and treatment with anti-hypertensive agents-with odds ratios of 5.9, 2.1,1.7,1.4, 1.2 and 1.7 respectively. The conclusions of the overall study were that features of the Metabolic Syndrome were present at baseline in the subjects who converted to diabetes-namely raised insulin, increased BP, increased W/H ratio and BMI. Positive family history was also a risk factor but did not reach statistical significance, possibly

reflecting that insulin resistance or insulin increment are the risk factors that are inherited. Approximately 40% of the diabetics were on antihypertensive treatment at follow-up in keeping with previous reports suggesting that diabetes is more common in subjects treated with β-blockers and diuretics (Bengtsson *et al*,1984).

- Ohlson *et al*,1985 looked specifically at anthropometric risk factors for the development of Type 2 diabetes in a cohort of 800 middle aged Swedish men. Glucose tolerance was assessed by a plasma glucose sample taken in the afternoon after a 6-8hr fast. FPG≥7.0mmol/l was taken as diabetes. The follow-up period was 13.5y. 88.7% of subjects survived and were re-examined. 6.3% of subjects had developed Type 2 diabetes. Subjects with W/H ratio in the top 5% of the distribution, at baseline, had over 16-fold risk for developing diabetes compared with those whose W/H ratio was in the lowest quintile. In a bi-variate analysis where BMI was accounted for as a background variable, waist circumference and BMI remained significant predictors for the development of diabetes. The overall conclusion of this study was that not only the degree of obesity but also its android localization is a risk factor for diabetes mellitus. These two factors seem to be independent

of each other and to potentiate each other as risk factors for the development of the disease.

- Lundgren *et al*,1990, studied ~350 middle aged women over 10 years. The aim was to test whether 1st phase insulin response, adiposity, raised BP and the use of antihypertensive medications were significant risks for the development of diabetes. Subjects underwent an IVGTT at baseline(500g/l at a concentration of 0.5g/kg/ infused over 2.5-3.0min). Samples were taken at 4,6,8,25 minutes and then every 5 minutes until 60 min. Glucose tolerance was expressed as a k value, representing disappearance rate expressed as percent/min. between 25-60 min.(Hamilton and Stein,1942). Early insulin response was calculated as the area under the insulin curve during the first 8 minutes (Thorell *et al*,1973). Low early insulin response was defined as belonging to the lowest fifth of the total series. They were further subdivided into one of two groups depending whether k value > or < 1.0. At follow-up subjects with $FPG \geq 6.0mmol/l$ underwent two further estimations of capillary blood glucose at a special diabetes unit. Values $\geq 7.0mmol/l$ were taken as diabetic in accordance with the World Health Organizations 1980 recommendations. 5% of subjects developed diabetes during the follow-

up period. Significant risk factors for conversion to diabetes were a raised fasting insulin level, low early insulin response, obesity and antihypertensive treatment.

- In the Bedford Survey, Keen *et al*,1982, followed 241 subjects with 'borderline diabetes' for 10 years. Borderline diabetes was defined as a 2 hour capillary blood glucose level of 6.7-11.1mmol/l following a 50g oral glucose load. This was essentially also an intervention study in which subjects were randomly allocated to treatment with tolbutamide versus placebo, dietary intervention to restrict carbohydrate to 120g/day or brief advice simply to restrict the use of table sugar. The study took place1962-1972. 36 subjects converted to Type 2 diabetes (based on 2hr glucose value >11.1mmol/l). The effects of intervention were not significant in predicting outcome. The most significant factor was the degree of baseline hyperglycaemia. Baseline obesity was not significant but subjects who developed diabetes tended to gain weight in the second half of the follow-up period. IGT did not progress or reverted to normoglycaemia in a significant proportion of subjects. It was therefore concluded that for the most-part IGT should not be equated with early diabetes.

- Charles *et al*,1991 carried out the Paris Prospective study in which 5,000 middle aged men were followed over a three year period. Glucose tolerance was assessed using a 75gm OGTT and three categories were assigned- i) normoglycaemic (FPG & 2hr glucose <7.8mmol/l), ii)IGT (FPG<6.1, 2hr glucose >7.8mmol/l,), iii)IFG(FPG≥.6.1mmol/l, 2hr value <7.8mmol/l). At baseline examination ~400 were IGT and ~400 had IFG. At follow-up~ 2% of subjects were diabetic. Subjects with IFG were at five-fold risk for conversion to diabetes. Subjects with IGT were at an equivalent level of risk. Factors predicting conversion to diabetes were analysed separately for each glucose tolerance group. In the normoglycaemic group, conversion to Type 2 diabetes was associated with increased BMI, positive family history and increased FPG ($p<0.05$). In the IFG group, 2hr insulin response appeared to be reduced in the subjects who converted to Type 2 diabetes but this was not statistically significant. In the IGT group, BMI, increased fasting insulin and reduced 2hr insulin were significant risk factors for conversion on a univariate analysis ($p<0.001$, $p<0.001$, $p<0.01$). In a stepwise multiple regression analysis, BMI and positive family history remained significant in the normoglycaemic group ($p<0.05$); in the IFG

group, no factors were of predictive significance and for the IGT group, BMI, fasting insulin and 2hr insulin were significant predictors (p<0.001, p<0.05 and p<0.05) respectively. In summary therefore this study indicates that conversion to Type 2 diabetes in IGT subjects is associated with raised baseline insulin concentrations and low 2hr values. This suggests that conversion to diabetes from IGT occurs when the pancreas is not able to respond to insulin resistance with adequate secretion of insulin. This study has also subsequently examined serum NEFA as a risk factor for conversion to Type 2 diabetes. Prospective analyses carried out by Charles *et al*,1997, over 2 years, showed that raised NEFA were an independent risk factor for the development of IGT or conversion from IGT to Type 2 diabetes.

- Martin *et al*,1992 performed a study on 1st degree relatives of Type 2 diabetic subjects. This study is similar to the follow-up study outlined in this thesis but the results and conclusions are somewhat different. The study group comprised 155 offspring of 43 pairs of diabetic parents. All of the subjects were normoglycaemic at baseline on the basis of a 100g OGTT (FPG< 5.56mmol/l, 2hr glucose <6.67mmol/l and <10.0mmol/l at all intervening times)- (National Diabetes Data Group, 1979). The

mean age of the offspring at baseline was 32 (SD 9). 97% of study

participants underwent a 3hr IVGTT within 1yr of recruitment (0.5g/kg

of 15-20% glucose over 3-5 min). The study recruitment interval was

1963-1983. Follow-up occurred between 1983-1985 and 16% of

subjects had developed diabetes (mean time from study entry to

diagnosis of diabetes was 13.7yr). Verification of absence of diabetes

was based on the result of an OGTT(78%) or fasting plasma glucose

(18%) and normal post-prandial glucose (5%). The minimal model

approach was used to determine the S_I, S_G, Phi-1 and Phi-2 (Bergman

et al,1979). The results showed that the mean insulin sensitivity (S_I) was

significantly lower in the subjects who converted to diabetes ($p<0.0001$).

Similarly glucose effectiveness (S_G)was also significantly lower in the

converters ($p<0.0001$). By contrast 1st phase insulin response Phi-1 was

reported to be equivalent for the two groups (0.62 (0.82) *vs* 0.34 (0.37))

(no *p*- value reported). There was a negative correlation between S_I and

Phi-1 for the whole group(no correlation coefficient reported). There

was a positive correlation with baseline BMI and conversion to diabetes

($p<0.0005$). To test whether reduced S_I was a consequence of increased

weight, %IBW was divided into tertiles. Within each weight group, the

mean S_I in the subjects who converted to diabetes was lower than for

those who did not. Thus low S_I was associated with the development of diabetes independently of its association with obesity. Logistic regression analysis with development of Type 2 diabetes as the dependant outcome was performed. The full model included %IBW, 2hr glucose during OGTT, fasting insulin, S_I and S_G The results showed that S_I and S_G remained significant whilst the rest did not ($p<0.04$ and $p<0.003$ respectively). The study concluded that the offspring of diabetic parents had significant defects in insulin sensitivity and glucose disposal prior to onset of diabetes or impaired glucose tolerance and that these served as significant predictors for conversion to diabetes up to a decade before development of the disease. Although baseline fasting insulin concentrations were significantly higher in the converters than in the nonconverters, first and second phase insulin responses were not significantly impaired. Thus defects in insulin sensitivity seem to predate defects in beta-cell function in 1st degree relatives of two diabetic parents.

- The Hoorn study, Heine *et al*,1996, has looked at the conversion rate from IGT to diabetes in a random sample of the population aged 50-75years in the Dutch town of Hoorn. 158 IGT subjects have been

followed-up over 2 years. 13.8% converted to diabetes per annum. The

most sensitive predictors for conversion to diabetes were the 2hr glucose

level and the fasting proinsulin level. Parameters that did not seem to

influence conversion were the FPG, BMI,W/H ratio, age, gender,

HbA_{1c}, fasting insulin and 2hr insulin and 2hr proinsulin concentrations.

This study therefore concludes that defective beta-cell function rather

than insulin sensitivity is responsible for the conversion to diabetes from

IGT.

II. Prospective studies in other ethnic groups.

- Haffner *et al*, 1997, compared the risk factors for conversion to Type 2

  diabetes in a high risk group-the Mexican Americans vs a low risk group

  of non-Hispanic whites. Over the course of an 8yr follow-up period,

  ~10% of Mexican Americans converted to diabetes compared with ~5%

  in the case of the non-Hispanic whites. Age was a significant factor

  associated with conversion in both groups, however gender was not.

  BMI was an associated risk for the development of diabetes in the case

  of the Mexican Americans but not in the case of the non-Hispanic

  whites, whereas W/H ratio was predictive for both. IGT was a

  significant predictor in both groups (relative risk,$p<0.001$, for both).

Similarly high fasting insulin (>78.7pmol) had the same predictive

significance for both groups as glucose tolerance status at baseline. Low

1st phase insulin response ($\Delta I_{30}/\Delta G_{30}$) was of significant predictive value

in the case of the Mexican Americans but not for the non-Hispanic

whites. In a multiple regression analysis including age, gender, BMI,

IGT vs normoglycaemic, fasting insulin and $\Delta I_{30}/\Delta G_{30}$; - fasting insulin

was the only significant predictor with an odds ratio of 2.8 for the

Mexican Americans and 5.12 for the non-Hispanic whites. In conclusion

risk factors for Type 2 diabetes are similar in different populations

having differing risks of Type 2 diabetes suggesting that for most

subjects, similar pathophysiological mechanisms apply.

- Kadowaki *et al*, 1984, reported on the results of a 5-12yr follow-up of

  ~300 Japanese subjects with IGT(on 100g OGTT). The main predictors

  for conversion to Type 2 diabetes were raised fasting and 2hr glucose

  levels at baseline, reduced early insulin response ($\Delta I_{30}/\Delta G_{30}$), and high

  maximal body mass index.

- In a more recent Japanese study, Inoue *et al*,1996, followed-up 37

  subjects with IGT over a 2.5 yr period. Over half of the subjects

remained IGT, 30% reverted to normoglycaemia and 13.5% converted

to Type 2 diabetes. Baseline glucose and 2hr values did not correlate

significantly with glycaemia or conversion to diabetes at follow-up

whereas 2hr proinsulin level did.

- Saad *et al*,1989, reported on the sequential changes in insulin secretion

  with changes in glucose tolerance in a cohort of Pima Indians. The

  inverted- U-shaped relation of fasting insulin secretion to plasma glucose

  concentrations ("Starling's curve of the pancreas"-(DeFronzo,1988)-

  was confirmed. The authors concluded that the primary abnormality in

  the development of Type 2 diabetes in Pima Indians was increased

  insulin resistance, probably genetically determined (Bogardus *et*

  al,1988). The beta-cells respond by increasing insulin secretion both

  basally and following a glucose challenge, maintaining normoglycaemia.

  However with increasing age, obesity, sedentary lifestyle and possibly

  other factors, insulin resistance increases further and IGT occurs. At this

  stage insulin levels are still high but not relative to the level of glycaemia.

  Progression to Type 2 diabetes is accompanied by a further increase in

  insulin resistance, decrease in beta-cell function or a combination of the

  two. Once diabetes progresses, insulin levels fall dramatically.

Improved glycaemic control can partially restore the defective beta-cell response (Glaser *et al*,1988; Savage *et al*,1979). However the defect in beta-cell response eventually becomes irreversible (Nagulesparan *et al*,1981) and basal insulin secretion falls.

• Lillioja *et al*,1988 conducted a prospective study on Pima Indians to determine the relative roles of insulin resistance, insulin secretory dysfunction and excess hepatic glucose production in the development of Type 2 diabetes. The subject group comprised 200 non-diabetic Pima Indians who were studied over a mean time interval of 5yrs. At baseline various anthropometric measurements were taken, in addition an OGTT and IVGTT were performed and a euglycaemic hyperinsulinaemic clamp. At baseline 151 subjects were normoglycaemic on a 75g OGTT and 49 had IGT. The acute plasma insulin response to glucose was determined by the response to a 25g bolus injection of dextrose injected for 3.6 minutes(Chen and Porte 1976). The acute insulin response was defined as the incremental area under the glucose curve between 3-5 minutes, divided by two. A two step euglycaemic hyperinsulinaemic clamp was carried out. The insulin concentrations achieved were ~780pmol/l and ~2,000pmol/l. During the low-dose insulin infusion, labelled glucose was infused in order to calculate the rate of glucose disappearance

(Steele,1959). The effects of variations in plasma glucose concentrations during the clamp study were adjusted to 5.6mmol/l (Best et al,1981). 38 subjects developed diabetes during the follow-up period. The single strongest predictor was the reduced glucose disposal rate at a low insulin concentration. This remained so when corrected for obesity and the level of glycaemia. Reduced suppression of hepatic glucose production was not predictive in the post-absorptive state. However hepatic glucose production at low insulin concentrations was significantly impaired in the converters to diabetes and was predictive. Taking into account the degree of body fat however, this predictive significance was lost. The acute phase insulin response was significantly impaired in the subjects who converted and remained so when insulin resistance and body fat were accounted for. However a direct comparison of rate of glucose disposal at low insulin concentrations vs acute phase insulin response, revealed that the former accounted for the major risk in conversion to Type 2 diabetes.

Chapter 6

The 10-year follow-up study: Development of diabetes and assessment of predictive factors in 10 year prospective study of siblings and offspring of white Caucasian, type 2 diabetes patients.

I.   Background to the original study-performed in 1984.

In 1984 Steve O'Rahilly studied 154 1st degree relatives of Type 2 diabetic subjects using CIGMA (O'Rahilly *et al*, 1986). The relatives comprised 82M:72F, mean age 40±16y, 118±20% IBW.

64 healthy volunteers were used as controls. 38M:26F, mean age 44±20y and 109±14% IBW. None had a first degree relative with Type 2 diabetes.

Establishment of a normal range to CIGMA.

This was based on the results of the 1-hr glucose in the 64 control subjects corrected for age.

Results

The controls had a normal distribution of 1-hr glucose values but the relatives almost had a bi-modal distribution (insufficient numbers to allow resolution of two peaks). 20% of the relatives had a 1-hr glucose which was >2SD above the mean for the controls. These were termed CIGMA intolerant. The

metabolic characteristics of the CIGMA intolerant relatives were compared with the characteristics of the relatives who had a 1-hr glucose within 1SD of the mean for the control group. There was no significant difference in BMI or age between the groups. Both the 1st and second phase insulin and C-peptide responses were significantly lower in the CIGMA intolerant group. %B was markedly reduced in the CIGMA intolerant group but %S was only reduced in the subjects with highest glucose levels (top tertile for FPG). From this study it was therefore concluded that beta-cell dysfunction was the major defect accounting for abnormal glucose tolerance in this particular cohort of 1st degree relatives of white Caucasian Type 2 diabetic subjects.

II.   Introduction to the 10 year follow-up study in 1994:

First degree relatives of patients with Type 2 diabetes are at high risk of developing diabetes. The lifetime risks of developing diabetes in the siblings and offspring of white Caucasian Type 2 diabetic patients have been estimated to be 38% and 32% respectively (Kobberling *et al*,1982). Prospective studies have not specifically examined the development of diabetes in the first degree relatives of white Caucasian patients, although Martin *et al*,1992, studied the offspring of two diabetic parents. Most studies in white Caucasian populations have examined a random selection of middle-aged subjects in the general population, evaluating the progression from IGT to diabetes (Charles *et al*,1991; Heine *et al*,1996; Keene *et al*,1982; Skarfors *et al*,1991). Other prospective studies have examined ethnic populations who are particularly susceptible to the development of diabetes (Bergstrom *et al*,1990; Dowse *et al*,1996; Haffner *et al*,1997; Lillioja *et al*,1988). These studies, together with prospective studies in Japanese (Inoue *et al*,1996; Kadowaki *et al*,1984), have indicated that the main predictor for the development of Type 2 diabetes is a raised fasting or stimulated glucose (Skarfors *et al*,1991).

The relative contributions of increased insulin resistance versus diminished beta cell function for the development of abnormal glucose tolerance and

subsequently Type 2 diabetes have been controversial. Both defects are present in most subjects with IGT, although ethnic differences may occur, with insulin resistance appearing to have a greater role in Mexican Americans (Haffner et al,1997) and Pima Indians (Lillioja et al,1988), and impaired beta cell function a greater role in Japanese (Inoue et al,1996; Kadowaki et al,1984) and white Caucasians subjects (Charles et al,1991; Heine et al,1996; O'Rahilly et al,1986). Progression to diabetes has been shown to be predominantly due to deterioration of beta cell function (Kadowaki et al,1984; Lillioja et al,1988), as is deterioration of glucose control in patients with Type 2 diabetes (UKPDS Group (1991) UK Prospective Diabetes Study VIII), but both factors have been described to be predictors for the development of diabetes in the general population (Lundgren et al,1990; Skarfors et al,1991). We have prospectively studied a cohort of siblings and offspring of 46 patients with Type 2 diabetes who were initially evaluated in 1984 (O'Rahilly et al,1986; O'Rahilly et al,1988) with ten years' follow-up of all available subjects. This allowed assessment of the incidence and cumulative prevalence of diabetes in both siblings and offspring and whether baseline clinical and glycaemic variables, including beta cell function and insulin sensitivity, are predictors of disease progression.

III.   Subjects and methods

Study subjects. In 1984, we studied the families of 46 white Caucasian, Type

2 diabetic patients, mean(SD) age was 54(8) y, 46% male, BMI 30(4) kg/m^2.

Of 62 siblings and 93 offspring, 8 siblings had already died, of whom 4 were

known to have had diabetes. Of the living relatives, 7 siblings and 5 offspring

had diabetes. Of the 135 living non-diabetic subjects, 125 (93%) were tested,

42 siblings, age 48(10) y, 48% male, BMI 27(5) kg/m^2, and 83 offspring, age

28(8) y, 48% male, BMI 26(4) kg/m^2.

In 1994, 96 of these subjects were restudied, 37 siblings and 59 offspring. In

addition 56 non-diabetic subjects were recruited as controls, age 61(10) y,

16% male, BMI 25(5) kg/m^2. Study flow diagrams are presented in Figures 1

and 2.

Study procedures. The study was conducted with approval by the Central

Oxford Research Ethics Committee and informed consent was obtained from

all study participants. Subjects attended the department between 8.00 and 9.00

a.m. having fasted from 10.00 p.m the night before. Subjects were asked to

ingest their normal diet for the three days preceeding the test and to refrain

from excessive exercise, smoking or alcohol consumption. On the morning of the test, study participants were brought to the centre by either public transport or taxi. Body adiposity was determined from the body mass index (BMI) calculated as weight (kg)/height ^{2}(m). With the subjects resting supine on a bed, two intravenous cannulae were inserted, one in the antecubital fossa for glucose infusion and one at the wrist for sampling with the hand kept warm to arterialise blood samples. The physiological test was a 1-hr infusion of glucose at a concentration of 180mg/m^2/min-(equivalent to 54*surface area(m ^{2})/hr of a 20% glucose solution). In the original study in 1984, the glucose concentration infused had been 5mg/kg ideal body weight. The modified glucose concentration gave the same glucose infusion rates in normal weight subjects as in 1984, but slightly higher rates in obese subjects to compensate for their greater extra cellular fluid volume. To account for this slight modification in infused glucose load in obese subjects, a small increment was added to the 1984 1h glucose values using the formula:

$\Delta G_{corrected}$ (1984) = ΔG_{actual}(1984) x G $_{INF}$(1994) / G $_{INF}$(1984)

where ΔG is the glucose increment from baseline to 1 hour and G_{INF} is the subject's glucose infusion rate.

Samples were collected for glucose, insulin and C-peptide measurement at:-10, -5, 0 mins (basal), 6, 8, 10 mins (1st-phase) and 50, 55, 60 mins (1h). Unless otherwise specified, diabetes was defined by the WHO fasting criterion of $FPG \geq 7.8$ mmol/l. Additional analyses were performed using the revised ADA fasting definition of $FPG \geq 7.0$ mmol/l (1997) as indicated in the text. Abnormal glucose tolerance was defined either as impaired fasting glucose (IFG) as $FPG \geq 6.0$ mmol/l by the ADA criterion. Insulin sensitivity and beta cell function were assessed from 1h insulin and C-peptide measurements respectively using continuous infusion of glucose with model assessment (CIGMA) (Levy et al,1991).

Biochemical methods. Plasma glucose was measured using a hexokinase method using Glucoquant glucose kit, (Boehringer Mannheim, Lewes, E. Sussex, UK). Insulin was measured by radioimmunoassay using Pharmacia Insulin RIA 100 kit (Pharmacia Ltd., Milton Keynes, Bucks, UK) in which there is 100% cross-reactivity with intact proinsulin and <20% with split proinsulin moieties. The inter-assay coefficient of variation was 4.5% at 108 pmol/l and 873 pmol/l, and 3.6% at 350 pmol/l (range 22.2-1781 pmol/l). C-

peptide was measured using the Linco human C-peptide RIA kit (Biogenesis Ltd., Poole, UK) with an assay range from 0.06-3.3 nmol/l. The interassay coefficient of variation was 6.2% at 0.5 nmol/l, 4.2% at 1.13 nmol/l and 4.4% at 2.26 nmol/l. The results of the 1984 and 1994 assays for glucose, insulin and C-peptide were realigned by reference to quality control data collected over the 10 year period, applied as necessary(Cull *et al*,1997).

Statistical methods. Data are shown as mean (standard deviation (SD)) except for insulin, C-peptide, beta-cell function and insulin sensitivity which were log transformed to provide a normal distribution and shown as geometric mean (SD range). Ages of living and dead subjects at baseline and 10 years follow-up were calculated as 1984/1994 - date of birth as appropriate.

Beta-cell function in part depends on the insulin sensitivity and the CIGMA results were adjusted either for BMI or for %S by fitting a regression model in the group of siblings and offspring studied in 1994:

I) $Log_e(\%\beta_{BMI\,adj})=Log_e(\%\beta)+0.019*(27.2-BMI)$, corrected to mean BMI of 27.2 kg/m^2

ii) $Log_e(\%\beta_{\%S\,adj})=Log_e(\%\beta)-0.3041*(4.21-Log_e(\%S))$, corrected to mean $log_e(\%S)$ of 4.21 (%S = 67.4)

1st phase insulin response in 1984 was expressed as the % rise in 1st phase insulin concentration over the fasting value (Bagdade *et al*,1967).

The two sample t-test and χ^2 test were used to compare baseline variables for those subjects who progressed to Type 2 diabetes with those who remained non-diabetic throughout the follow-up period. Univariate odds ratios for risk factors for progression to diabetes were calculated using logistic regression, having divided continuous variables into 2 groups about their median, with the exception of FPG, which was categorised as normoglycaemic or IFG, and age >40 years. Analyses were repeated for diabetes defined by the ADA criterion of FPG ≥ 7.0 mmol/l. Multivariate analyses were not, in general, appropriate given the numbers of transitions to diabetes, but a model was fitted with both beta cell function and insulin sensitivity as explanatory variables. Analyses were performed using SPSS and SAS.

IV. Results

Development of Type 2 diabetes.

Siblings: 62 siblings (mean (SD) age 50 (9) y) were first studied in 1984.

Eleven (18%) had already been diagnosed as diabetic and were aged 61 (5) y

(Table 1, p84), compared with 48(10) y in those without known diabetes. 42

out of a possible 47 living non-diabetic siblings were tested; 34 (81%) were

normoglycaemic and 8 (19%) had IFG.

Ten years later, in 1994, none of the 42 siblings were known to have had

diabetes or had any symptoms suggesting hyperglycaemia. Three had died, 2

had moved and 37 (88%), mean age 58 (10) y, were re-studied. On retesting,

7 (19%) were found to have developed diabetes and 5 (14%) had IFG. The

outcome of follow-up of the siblings is shown as a flow diagram in Figure 1

(p82). In 1994, of the 62 siblings ascertained in 1984, a cumulative total of 18

(29%) had developed diabetes with an additional 5 (8%) having IFG.

The baseline characteristics for the siblings who progressed to diabetes by

1994 compared with those who remained non-diabetic are shown in Table 2

(p85) . Significant differences were found for fasting and 1h glucose levels,

BMI, fasting insulin and C-peptide and the 1st phase insulin response.

Offspring: 93 offspring (mean (SD) age 28 (8) y) were first studied in 1984.

Five (5%) had already been diagnosed as diabetic and were aged 35 (5) y,

compared with 28 (8) y in those without known diabetes. 83 out of the 88 non-

diabetic offspring were tested; 78 (94%) were normoglycaemic and 5 (6%) had

IFG.

Ten years later, in 1994, all 83 offspring were contacted and none were known

to have had diabetes or had any symptoms suggesting hyperglycaemia. 59

(71%), mean age 40 (8) y, were re-studied. On retesting, 4 (7%) were found to

have developed diabetes and 5 (8%) had IFG. The outcome of follow-up of the

offspring is shown as a flow diagram in Figure 2 (p83). In 1994, of the 93

siblings ascertained in 1984, a cumulative total of 9 (10%) had developed

diabetes with an additional 5 (5%) having IFG (Table 1,p84).

The baseline characteristics for the offspring who had developed Type 2

diabetes in 1994 compared with those who had remained non-diabetic are

shown in Table 2 (p85). Age, fasting glucose, BMI, fasting C-peptide and 1st

phase insulin response were significantly different.

Sensitivity, specificity and positive predictive values for the development of diabetes over 10 years. The sensitivity, specificity and positive predictive values of having IFG in 1984 for developing diabetes ten years later in siblings and offspring combined were 45%, 92% and 42% respectively. The specificities in the siblings and offspring considered separately were 87% and 95% but the numbers were too small to compare sensitivities and positive predictive values.

Risk factors for progression to Type 2 diabetes and abnormal glucose tolerance. For siblings and offspring combined, the difference in baseline variables between those who progressed to Type 2 diabetes and those who did not are shown in Table 3 (p86). Univariate logistic regression analyses (Table 4,p87) indicate that fasting glucose, male gender, age and BMI were significant risk factors. Fasting insulin and C-peptide values and insulin sensitivity were also of prognostic significance on univariate analysis. Beta cell function was not significant (adjusted for BMI or for %S), even though the incremental 1st phase insulin response was significant. In a multivariate model containing both %B$_{BMI adj}$ and %S, only %S was significant (P<0.05).

Analysing progression to diabetes as defined by the revised 1997 ADA criterion of FPG≥7.0 mmol/l, in the 92 subjects who had FPG<7.0 mmol/l at baseline, only male gender and BMI were significant univariate predictors with odds ratios of 8.6 and 9.9 respectively. In a multivariate models containing both %B $_{BMI\,adj}$ and %S, neither term was significant.

V.   Discussion

The data show a high risk of developing diabetes in the siblings of white Caucasian subjects, particularly in those who had raised fasting glucose levels ≥6.1 mmol/l, now termed impaired fasting glucose (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (1997)). At baseline, at mean age 50(9) y, 11/62 (18%) siblings had already developed diabetes, and as expected the diabetic subjects were older than the non-diabetic subjects, their age being 61(5) y. When restudied 10 years later, the cumulative prevalence of diabetes was 29% and an additional 8% had IFG. The study demonstrates that to assess the prevalence and cumulative incidence, it is necessary to take into account both unavailable, including dead, siblings and prospective follow-up. Population studies that assess only the current

prevalence of diabetes in relatives may provide an under-estimate.

The incidence of diabetes (FPG 7.8 mmol/l) over 10 years in the nondiabetic siblings, who were aged 48 (10) years in 1984, was 19%. In those with increased fasting glucose values ≥ 6.1 mmol/l, the risk was greater, at 50% per decade, similar to that reported in the white Caucasians in the Baltimore longitudinal study of ageing with FPG 6.1 - 7.7 mmol/l (Edelstein *et al*,1997). Conversely, in siblings with FPG<6.1 mmol/l, the rate of progression was relatively low at 10% per decade. A family history of diabetes has little effect on progression to diabetes in those already identified as having raised glucose levels (Edelstein *et al*,1997). These rates of progression are approximately half those reported in Hispanics and in American-indians (Edelstein *et al*,1997).

We found the 10 year incidence in the siblings was considerably greater than in the offspring, in whom only 7% of the group aged 28 (8) years progressed to diabetes, in keeping with the known greater transition rate to diabetes with age. On the other hand, Koberling *et al*,1985, reported, based on the known history of diabetes in families, that there might be a lower incidence of diabetes in offspring than siblings .

In the combined group of relatives, the major risk factor for progression to diabetes was the fasting glucose level, in keeping with other studies (Edelstein et al,1997). Assessing tolerance to intravenous glucose, rather than fasting glucose levels, did not improve predictive power (data not shown). The fasting glucose was more informative than either beta cell function or insulin sensitivity on their own. Thus it is the combination of these two pathophysiologies that leads to the raised glucose levels that is the main predictor. When glycaemia was excluded from the analysis, impaired insulin sensitivity rather than reduced beta cell function was a risk factor for developing diabetes with fasting glucose 7.8 mmol/l, although in the subgroup in which it was measured, the 1st phase insulin response was significantly impaired in the subjects who developed diabetes in 1994 ($p<0.001$). These results may have a helpful practical connotation, in that assessing solely the degree of glycaemia provided most information and little was gained from assessing the pathophysiology that induced the increased fasting glucose levels.

Impaired insulin sensitivity has been reported in normoglycaemic 1st degree relatives of Type 2 diabetic subjects in a number of cross-sectional studies (Eriksson et al,1989; Groop et al,1996; Humphriss et al,1997; Vaag et

al,1995). Martin *et al*,1992, reported that, in their 32(10) y old offspring of two diabetic parents, insulin resistance was a major predictor of subsequent progression to diabetes, but they used the glucose disappearance rate following an intravenous glucose tolerance test as an index of insulin resistance, whereas impaired beta cell function and insulin sensitivity can affect glucose uptake. They found no indication of reduced beta cell function being a predictor, based on first and second phase insulin responses. It is possible that, at mean age 32 years, insulin resistance was detectable and that impaired beta cell function had yet to develop. Other studies, e.g. Kadowaki *et al*,1984, in a non-obese Japanese population, have found impaired beta cell function is a predictor. Although we did not find that impaired beta cell function was a baseline risk factor for the development of diabetes, Cook *et al*,1993, in a study of 1st degree relatives of white Caucasian subjects, reported that progression to diabetes was associated with deterioration of beta cell function, similar to that reported by studies in other ethnic groups (Lillioja *et al*,1988). Progressive deterioration of beta cell function is also the major cause of the progressive hyperglycaemia of patients with Type 2 diabetes (UKPDS Group (1995) UK Prospective Diabetes Study 16). It is possible that the raised glucose levels induced by insulin resistance, beta cell dysfunction or both, contribute to

greater beta cell dysfunction by a glucose toxicity effect (Weir *et al*,1986; Yki-Jarvinen,1992) although there is no direct evidence that glucose levels have a direct, short-term effect on insulin secretion in man (Flax *et al*,1991).

Obesity was a significant risk factor for progression to diabetes, in accordance with the findings of some previous studies (Keene *et al*,1982; Modan *et al*,1986), though in others the difference in body weight between subjects who did and did not progress was small or non-significant (Jarrett *et al*,1984; Martin *et al*,1992; Shimokata *et al*,1991). We found that male gender was also a risk factor for development of diabetes, as also suggested by more male than female newly diagnosed patients recruited to the UK Prospective Diabetes Study (UKPDS Group (1988) UK Prospective Diabetes Study IV) and a male excess in Type 2 diabetic subjects in Australia (Welborn *et al*,1989). A similar but non-significant trend for progression from IGT to diabetes was found in the Rancho Barnado Study (Wingard *et al*,1993).

In summary, the study shows a high rate of progression to diabetes in 1st degree relatives of Type 2 diabetes. Fasting glucose levels ≥ 6.1 mmol/l

provided a means of identifying those most at risk, as also reported by

Edelstein *et. al*,1997, in several high risk populations. Identifying the

pathophysiology contributing to hyperglycaemia did not provide additional

useful information. Obesity and male gender were also important risk factors

in these first degree relatives.

Figure 1. Schematic representation of the results of sibling follow-up 1984-1994

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                          ┌─────────────┐
                          │ 62 siblings │────────── (8 deceased-4/8DM)
                          └─────────────┘
                                 │
                     ┌────────────────────────┐
                     │ 54 siblings alive in 1984│──── (7 known DM)
                     └────────────────────────┘
                                 ↓
          ┌──────────────────────────────────────────────┐
          │ 42/47 nondiabetic siblings tested in 1984     │
          └──────────────────────────────────────────────┘
```

| 34 normal FPG | | | | 8 IFG | | | **1984** |

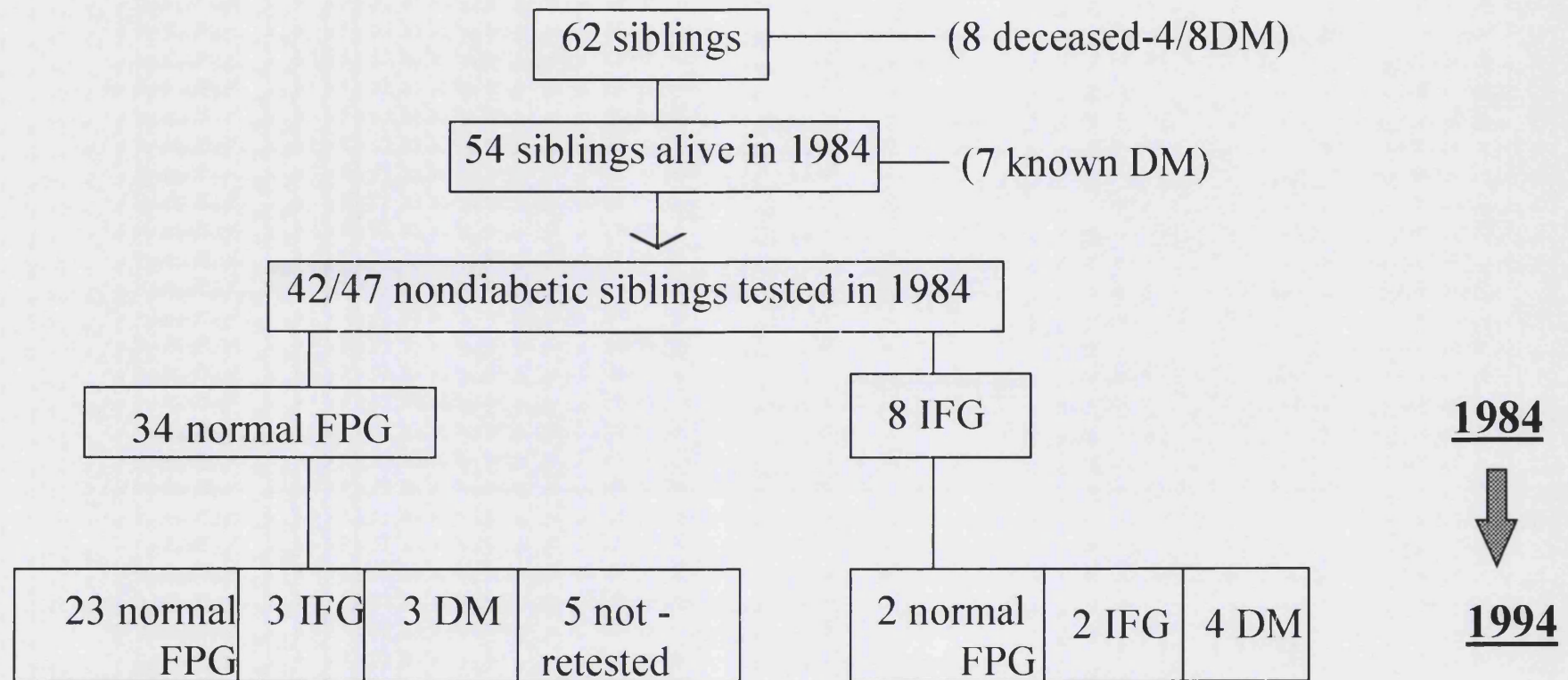| 23 normal FPG | 3 IFG | 3 DM | 5 not-retested | 2 normal FPG | 2 IFG | 4 DM | **1994** |

Figure 2. Schematic representation of the results of offspring follow-up-1984-1994.
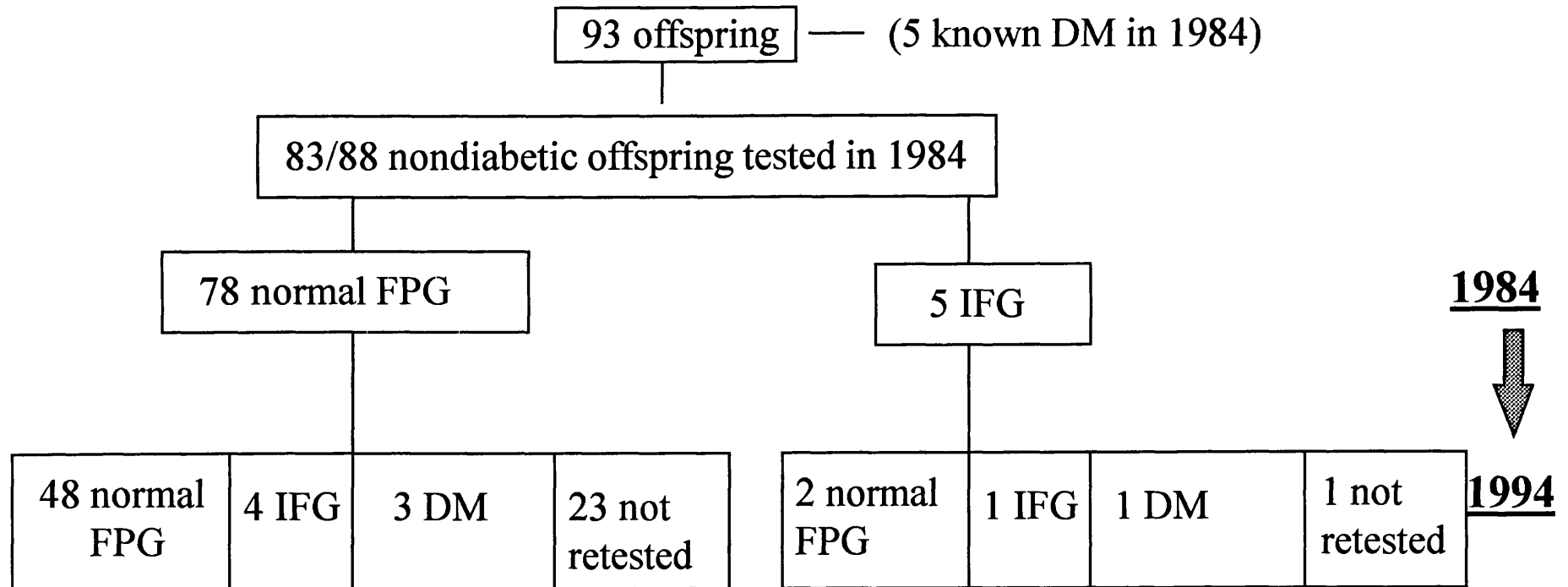
Table 1. Cumulative prevalence of diabetes and IFG in 1984 and 1994

| Year | Siblings (n=62) | | | Diabetic n (%) |
	Diabetic n (%)	IFG n (%)	Age of cohort in 1984 (y)	
1984	11 (18%)	8 (13%)	50 (9)	5 (5%)
1994	18 (29%)	5 (8%)		9 (10%)

Table 2. Baseline characteristics in 1984 of the siblings and offspring who had progressed to DM in 1994 compared with the those who had remained nondiabetic.

	Siblings			Offspring		
	Diabetic N=7	**Non-diabetic** N=30	*p*-value	**Diabetic** N=4	**Non-diabetic** N=55	*p*-value
Age (years)	52 (11)	47 (10)	ns	40 (7)	30 (8)	<0.05
Gender (male:female)	6:1	10:20	ns	3:1	31:24	ns
BMI (kg/m^2)	33 (7)	26 (4)	<0.002	33 (7)	26 (5)	<0.05
Fasting glucose (mmol/l)	6.4 (0.8)	5.3 (0.6)	<0.0002	5.6 (1.2)	4.9 (0.5)	<0.05
1 hr glucose (mmol/l)	10.3 (0.8)	9.3 (1.0)	<0.05	9.5 (2.8)	8.5 (1.2)	ns
Fasting insulin (pmol/l)	113 (77-166)	69 (39-122)	<0.05	115 (76-175)	80 (54-117)	ns
Fasting C-peptide (nmol/l)	0.8 (0.6-1.1)	0.5 (0.4-0.8)	<0.01	0.7 (0.6-0.9)	0.5 (0.4-0.7)	<0.05
Incremental 1st phase insulin response (%)	10 (5-20)	64 (23-177)	<0.0001	30 (15-60)	73 (34-159)	<0.05

Table 3. Baseline characteristics and metabolic variables for the 11 siblings and offspring who had progressed to type 2 diabetes by 1994 compared with 85 relatives who had remained non-diabetic

	Diabetic N=11	Non-diabetic N=85	p-value
Age (years)	45 (11)	36 (12)	0.02
Gender (male:female)	9:2	41:44	0.04
BMI (kg/m^2)	32.9 (6.3)	26.5 (4.6)	0.0001
Fasting glucose (mmol/l)	6.1 (1.0)	5.1 (0.6)	0.006
1 hr glucose (mmol/l)	10.6 (2.4)	8.8 (1.3)	0.03
β-cell function (BMI adj) (%β)[a]	85 (60-120)	93 (68-127)	0.4
β-cell function (%S adj) (%β)[a]	83 (59-118)	93 (69-125)	0.3
Insulin sensitivity (%S)	58 (46-74)	76 (51-113)	0.06
Fasting insulin (pmol/l)	122 (88-169)	74 (47-117)	0.0008
Incremental 1st - phase insulin response (%)	17 (7-41)	70 (29-167)	0.0001
1 hr.insulin (pmol/l)	222 (159-310)	194 (134-280)	0.3
Fasting C-peptide (nmol/l)	0.8 (0.6-1.1)	0.5 (0.4-0.7)	0.0001
1st phase C-peptide (nmol/l)	0.9 (0.7-1.2)	0.7 (0.5-0.9)	0.005
1 hr. C-peptide (nmol/l)	1.4 (1.1-1.7)	1.0 (0.7-1.5)	0.02

[a] using CIGMA

Table 4. Univariate odds ratios for progression to type 2 diabetes (FPG ≥ 7.8 mmol/l) in 1994 by 1984 values. Continuous variables are divided into 2 categories about the median, except for FPG which is divided about 6.0 mmol/l and age about 40 years.

Variable	n	No. Diabetic in 1994	Odds Ratio (95% CI)	p
Age (>40 years)	96	11	4.9 (1.2, 20)	**0.03**
Sex (male)	96	11	4.8(1.0, 24)	0.05
BMI (> 26kg/m^2)	96	11	12.4(1.5, 101)	**0.02**
FPG (≥ 6.0 mmol/l)	96	11	9.3 (2.3, 38)	**0.002**
β-cell function (>95%)[a][b]	95	10	0.4 (0.1, 1.7)	0.2
Insulin sensitivity (>78%)[a]	94	9	0.1 (0.01, 0.89)	**0.04**
Fasting Insulin (> 83pmol/l)	96	11	5.6 (1.1, 27)	**0.03**
Incremental 1st -phase insulin response (> 74%)	90	7	*	**0.01[c]**
Basal C-peptide (> 0.54nmol/l)	96	11	12.4 (1.5, 101)	**0.02**

[a] using CIGMA
[b] β-cell function adjusted for BMI
[c] all 7 subjects who progressed to diabetes and had data were below the median 1st phase insulin response, Fisher's Exact test.

Chapter 7

The cross-sectional study:

No evidence for disproportionately raised proinsulin or IAPP, or raised NEFA, in glucose intolerant first degree relatives of Type 2 diabetic subjects.

Introduction

Pathophysiological features of Type 2 diabetes include:

i.   Islet amyloid formation.

ii.   Raised proinsulin secretion

iii.   Raised NEFA

Islet amyloid is found in over 90% of subjects with Type 2 diabetes at post-mortem (Clark,1988; Johnson et al,1989). The deposition of islet amyloid is associated with loss of beta cells and reduction of insulin secretion; the degree of amyloidosis is increased in subjects who have progressed to insulin therapy (Schneider *et al*,1980; Westermark *et al*,1994). The β-cell mass is reduced in amyloid-containing islets of diabetic man (Clark *et al*,1988), cats (O'Brien *et al*,1986) and in transgenic mice expressing the gene for human islet amyloid polypeptide (Verchere *et al*,1996). These data suggest that the deposits contribute to the final decline of β-cell function in

the course of Type 2 diabetes. In monkeys and cats, longitudinal studies have shown that islet amyloid is present before the onset of hyperglycaemia (De Koning *et al*,1993; O'Brien *et al*,1986). However, the relationship of amyloid to progression of diabetes in man is unclear. Islet amyloid is formed from islet amyloid polypeptide (IAPP) (Cooper *et al*,1987; Westermark *et al*,1987) by a process of oligomerisation of IAPP molecules as a result of diabetes-related conditions which are yet to be determined. IAPP, and insulin (and their respective precursor peptides, proIAPP and proinsulin) are co-localised in β-cell secretory granules and proportionally co-secreted in response to β-cell secretagogues (Kahn *et al*,1990; Sanke *et al*,1991). Circulating IAPP concentrations are raised to a similar degree to that of insulin in obese and glucose intolerant subjects (Kautzky-Willer *et al*,1994) and are not elevated in diabetic subjects beyond that expected from their obesity (Enoki *et al*,1992); diabetic subjects who have progressed to insulin therapy have lower IAPP levels than diabetic subjects treated with sulphonylureas (Lindstrom *et al*,1997; Van Jaarsveld *et al*,1993).

If amyloid deposition were associated with increased production of IAPP, elevated circulating IAPP concentrations would be expected at some stage

of the disease. Disproportionate hyperproinsulinaemia relative to insulin occurs in established Type 2 diabetes (Clark *et al*,1992; Davis *et al*,1993; Saad *et al*,1990; Temple *et al*,1989; Ward *et al*,1987; Yoshioka *et al*,1988). The chronology of the onset of this phenomenon relative to the development of diabetes has been examined in a number of cross-sectional and prospective studies. Elevated absolute proinsulin concentrations occur in glucose intolerance (Davies *et al*,1993; Reaven *et al*,1993; Shiraishi *et al*,1991; Williams *et al*,1991). Disproportionate hyperproinsulinaemia is found in glucose intolerance (Davies *et al*,1993; Reaven *et al*,1993) and has been shown to be a marker for progression to diabetes in some prospective studies (Haffner *et al*,1997; Kahn *et al*,1995; Mykkanen *et al*,1995). However, in a cohort of Dutch IGT subjects, fasting proinsulin but not the proinsulin:insulin ratio was found to be a risk factor for progression to diabetes (Nijpels *et al*,1996). In addition, increased proportion of split proinsulin was detected in normoglycaemic first degree relatives of diabetic subjects but not in Asians (Gelding *et al*,1995). The underlying pathology resulting in increased secretion of proinsulin in Type 2 diabetes is unknown but may represent a β-cell pathology which could also affect IAPP secretion

and amyloid formation. It is not clear whether one abnormality occurs before the other or whether they occur in tandem.

Raised NEFA are found in Type 2 diabetic patients and may play a role in the pathogenesis of the disease. Randle *et al*,1963, first suggested a link between glucose and NEFA metabolism when they described the glucose-fatty acid cycle and the idea of substrate competition. Increased NEFA inhibit glucose oxidation in muscle, although the exact mechanism is still unclear. In vivo studies in humans have demonstrated reduced glucose utilization under situations of raised circulating NEFA -resulting in the hypothesis that raised NEFA possibly induce a state of insulin resistance (Bonadonna *et al*,1989; Lillioja *et al*,1985). Recently two prospective studies have demonstrated that a high NEFA concentration is a risk marker for the development of Type 2 diabetes, independent of percentage body fat, fat distribution, and insulin mediated glucose uptake (Charles *et al*, 1997; Paolisso *et al*,1995). Studies in 1st degree relatives of diabetic subjects have failed to correlate the apparent defects in non-oxidative glucose metabolism with raised NEFA concentrations (Eriksson *et al*,1989; Capaldo *et al*,1991; Groop *et al*,1991; Vaag *et al*,1992).

Aims of the cross-sectional study.

The degree to which abnormal proinsulin and IAPP concentrations occur in relation to worsening glucose tolerance and β-cell dysfunction was examined in unrelated and first degree relatives of diabetic subjects at different stages of the development of diabetes, according to fasting glucose. These were subjects with normal, intermediate and impaired fasting glucose (IFG). Observations were also made in diabetic subjects on different treatment regimes related to the severity of their symptoms. In addition fasting and stimulated NEFA concentrations were examined in relation to degree of glucose intolerance and measures of insulin sensitivity and beta-cell function.

Subjects and methods

Subjects

The relatives and diabetic subjects formed part of a 10 year longitudinal study on the progression of diabetes in families which was started in 1984 (Chapter 6)(O'Rahilly *et al*,1986; O'Rahilly *et al*,1988).

The characteristics of the 116 subjects studied are shown in Table 1. These comprised fifty eight non-diabetic 1st degree relatives of Type 2 diabetic patients, 25 males, mean age, 42y (±10) and mean BMI 28(±4) and 33 females aged 50y (±12), mean BMI 27 (±5). In addition, 19 non-diabetic spouses were included: 2M:17F, mean age 64y (±16), mean BMI 29 (±4).

Thirty-nine Type 2 diabetic patients were examined: 28 males, mean age 62y (±11), mean BMI 30 (±5) and mean duration of diabetes 10y (±6), 11 females mean age 66y (±14), mean BMI 33.5 (±6) and mean duration of diabetes 10y (±5). Nine of the diabetic subjects were diet treated, 19 were treated with oral hypoglycaemic agents and 11 were insulin treated. All of the diabetics had been diagnosed ≥30 years and were non-ketotic at

diagnosis and maintained on diet or tablets for at least 6 months. In addition

ICA and anti-GAD antibodies were checked and were all in the range

consistent with Type 2 diabetes (ICA<4 and anti-GAD <20).

Study procedure

This is as described in Chapter 6. In addition to insulin, C-peptide and

glucose measurements, IAPP, proinsulin and NEFA were measured at all

time-points. Diabetic subjects were asked not to take their treatment on the

morning of the test.

Biochemical methods.

See chapter 6 for details on the glucose and C-peptide assays. IAPP was

measured by sandwich ELISA (Percy *et al*,1996) (Amylin Pharmaceuticals

Inc. Sand Diego, USA). Two assays were performed in parallel on the

samples to estimate amylin-like peptides (ALP) and amylin. The minimum

detectable concentrations were 2.3 pM and 3.2 pM respectively; interassay

CVs of 13.7% (8.7 pM) and 9.6% (80 pM) for amylin and 13.6% (4.1 pM)

and 5.8% (63 pM) for ALP. Proinsulin was measured in heparinised

plasma using a two site sandwich ELISA (NovoNordisk A/S, Bagsvaerd,

Denmark.) with a human recombinant proinsulin standard (Sigma Chemical

Company Ltd., Poole, Dorset, UK.) The ELISA had and cross reacted 100%

with intact proinsulin and to a lesser degree with split 32-33 proinsulin

(74%) and split 65-66 proinsulin (59%). The assay range was 0-200pmol/l

(sensitivity, 0.2pmol/l) with interassay CV of 15.5 at 5.4pmol/l and 6.0 at

21.0 pmol/l.

NEFA was measured by an enzyme calorimetric method-endpoint, using

Wako NEFA-C kit (Alpha Laboratories, Eastleigh, Hants UK) on a Cobas

MIRA discrete analyser (Roche Diagnostic Systems, Welwyn Garden City,

Herts, UK). A Precipath U standard was used (Boehringer Mannheim,

Lewes, Sussex, UK).

Statistical methods

Data are shown as mean ± SD or as geometric mean with SD range.

Proinsulin, C-peptide ,IAPP and NEFA are expressed as pmol/l and molar

ratios as %. There was strong correlation between the IAPP assays for

amylin and amylin-like-peptides (ALP)- (r=0.8, p<0.0001) and data are

presented for the ALP assay only. Within group differences were analysed

by ANOVA and non-diabetics compared with diabetics using non-paired

student t-test. Proinsulin, C-peptide and IAPP data were log transformed

prior to analyses. An estimate of beta-cell function is based on the

incremental rise in 1-hr C-peptide concentration over baseline value (%)-

similar to the 1st phase insulin response described by Bagdade *et al*, 1967.

This correlated with %B(CIGMA) (p<0.05). Fasting C-peptide was used

as a crude measure of insulin sensitivity. The correlation coefficient for

fasting C-peptide values and %S(CIGMA) was 0.53 (p<0.0001). A *p*- value

of <0.05 was assumed to be significant.

Results:

Glucose tolerance: Characteristics of the study subjects are shown in Table

1(p110). The non-diabetic subjects were divided according to glucose

tolerance into three groups incorporating the new diagnostic criteria that

have been adopted by the ADA (1997) (Table 2, p111);

Group 1; Normal glucose tolerance- (fpg <5.5mmol/l),which included 38

relatives and 14 spouses;

Group 2, Intermediate glucose tolerance- (fpg≥5.5 <6.1mmol/l). Thirteen of the relatives and one spouse had intermediate glucose tolerance.

Group 3, Impaired fasting glucose- (IFG) (fpg≥ 6.1 <7.0mmol/l). Seven relatives and four spouses had impaired fasting glucose. Following the glucose infusion, there was a significant increase in the 1-hr glucose concentration compared to the other non-diabetic groups ($p<0.0001$).

Diabetic subjects Of the diabetic subjects, nine were diet controlled with mean duration of diabetes 2y (±3), 19 were treated with tablets, mean duration of diabetes 12.5y (±5) and 11 subjects were treated with insulin, mean duration of diabetes 10y (±6). Diabetes had been diagnosed in all subjects over the age of 40y.

Age, Gender and BMI.

The relatives with IFG were approximately ten years older than subjects in Groups 1 and 2 ($p<0.05$)(Table 2, p111). There was no difference in distribution of gender in the non-diabetic groups but there was a larger percentage of males in the diabetic group ($p<0.05$). There was no significant

differences in BMI between the non-diabetic groups but the diabetics, as a whole, were significantly more obese than the non-diabetic subjects, ($p<0.05$)

C-peptide secretion.

Non-diabetics: Patients with IFG had significantly higher fasting C-peptide (CP) than subjects in Groups 1 and 2 ($p<0.02$). There was no difference in the 1-hr C-peptide concentrations following the glucose infusion between the non-diabetic groups (Table 2, p111). However, the glucose stimulated change in C-peptide secretion (delta C-peptide) expressed as a percent incremental rise over baseline, (Table 2) was significantly reduced in the subjects with IFG compared to groups 1 and 2 ($p<0.02$).

Diabetic patients: The fasting and 1hr C-peptide concentrations were similar for the diet and tablet treated groups. However, in the insulin-treated group, basal and 1hr C-peptide concentrations were significantly lower ($p<0.0001$ and $p<0.005$ respectively).

Proinsulin secretion.

Non-diabetic subjects: Fasting proinsulin levels (PI) were significantly elevated in the IFG group compared with other non-diabetic groups (p=0.002), Table 2 (p111). The difference in proinsulin remained significant after correction for BMI (p<0.005). The fasting and stimulated PI/C-peptide ratios were in the order of 1-2% in each of the non-diabetic groups and not significantly different between groups (Table2). Fasting PI correlated with fasting C-peptide in the non-diabetic subjects (r^2=0.65, p<0.0001) (Figure 1a, p113). Proinsulin concentrations had a weak correlation (r^2=0.1) with age, fpg and BMI.

Diabetic subjects: Fasting PI were similar in each of the diabetic groups but significantly elevated in the diabetic group as a whole compared to IFG (p<0.001). Proinsulin levels had a weak correlation with BMI (r=0.13, p<0.05) but not with FPG or age. The increased PI in the diabetic group remained significant after correction for BMI. In contrast, 1-hr PI concentrations were not significantly higher compared with the non-diabetic groups (Table 2, p111 and Table3, p112). The fasting and 1hr PI/C-peptide ratios were significantly elevated in the diabetics compared to the non-

diabetic subjects as a whole - 3.0 (1.2-7.7) *vs* 1.5 (0.8-2.5)(p<0.0001).

Fasting and 1hr PI/C-peptide were similar for diet and tablet treated groups

but significantly elevated in the insulin treated group compared to the diet

group (p<0.02) (Figure 2, p115).

IAPP secretion:

Non-diabetic subjects: Fasting IAPP concentrations were significantly

elevated in the IFG group compared to the other non-diabetic groups

(p<0.05). The absence of significant differences in the 1hr IAPP

concentrations were similar to that observed with C-peptide data (Table 2,

p111). Fasting and 1-hr IAPP concentrations correlated with fasting and 1

hr C-peptide secretion respectively in the 77 non-diabetic subjects - (r^2=0.4,

p<0.0001) (Figure 1b, p114). There was no significant correlation of IAPP

concentrations with fasting glucose, BMI or age in the non-diabetic subjects.

Fasting IAPP/C-peptide ratios (%) in the non-diabetic subjects were of the

order of 1.0% and not significantly different between the groups.

Diabetic subjects: Fasting IAPP concentrations in the diet and tablet treated

groups were similar to those in the non-diabetic group with IFG. However,

fasting IAPP was significantly lower in the insulin treated subjects compared to other diabetics (p<0.0001) and 1 hr IAPP was similarly reduced (Table 3, p112). Fasting and 1-hr IAPP/C-peptide ratios (%) were similar for diet and tablet treated diabetic groups and IFG subjects (Figure 2, p115). In contrast, this ratio was significantly lower in the insulin treated diabetic subjects (p<0.01).

NEFA secretion

Non-diabetic subjects:

There was no significant difference in fasting or 1-hr NEFA values amongst the non-diabetics (Table 2, p111). 1 hr NEFA values were not significantly correlated with age, gender, FPG, BMI, W:H ratio, fasting C-peptide or 1-hr incremental change in C-peptide.

Diabetic subjects:

1hr NEFA concentrations were significantly higher in the diabetics compared with the non-diabetics (p<0.0001) (Table 3, p112). There was no significant correlation with FPG, BMI,W:H ratio, fasting C-peptide or 1hr incremental change in C-peptide secretion. 1hr NEFA values were

equivalent for all three treatment groups.

Discussion

Most patients with Type 2 diabetes have a combination of increased insulin resistance and reduced beta-cell function which is evident before the onset of hyperglycaemia from many measurements made in susceptible subjects. First degree relatives of patients with diabetes are estimated to be at a three fold increased risk for developing diabetes compared with the general population (Kobberling *et al*,1985) and provide a suitable group for longitudinal and cross-sectional studies of the pathophysiologies at different stages of the development of diabetes. Previous studies have indicated that an early predictor for progression to diabetes is an increase in fasting glucose to levels defined as impaired fasting glucose (Edelstein *et al*,1997). In this study, 18% of the first degree relatives had IFG with fasting glucose >6.1mmol/l and 21% were in the high normal range, 5.5 to 6.1mmol/l. IFG was associated with significantly higher fasting C-peptide concentrations as well as a significant reduction in incremental C-peptide secretion at 1hour. These data suggest that both increased insulin

resistance and beta cell dysfunction are associated with the development of impaired fasting glucose.

Elevated levels of intact proinsulin and split products of biosynthesis (des-31,32 split proinsulin in particular) have been considered to be markers for beta-cell dysfunction in patients with overt Type 2 diabetes(Saad *et al*,1990; Temple *et al*,1989; Ward *et al*,1987; Yoshioka *et al*,1988) and the PI/insulin ratio can be as high as 50% in diabetes (Ward *et al*,1987). However, since the half lives of PI and C-peptide are similar (30-40mins) (Watanabe *et al*,1989), the PI/C-peptide ratio may be a more accurate description of β-cell secretion in non-fasting conditions. In our study, PI/C-peptide was 1.5% in non-diabetic groups and approximately double this in the diabetics- with levels up to 20% in some of the insulin treated diabetics. Nagi *et al*, 1998, have recently compared secretion of specific insulin and proinsulin in three ethnic groups at varying risk for the development of Type 2 diabetes. PI/C-pep ratios were equivalent to those described in our study. However one discrepancy was the reduced proportion of proinsulin molecules seen in insulin treated Pima Indians compared with sulphonylurea and diet treated subjects. This finding is contrary to our results which show a significantly

higher PI/CP(%) in the diabetics treated with insulin. This could reflect the relative hypoinsulinaemia of longstanding Type 2 diabetes in Caucasian subjects compared with persistent hyperinsulinaemia in Pima Indians. In a comprehensive analysis of basal and arginine stimulated secretion, PI was found to be approximately 4% of IRI released from the beta cells in non-diabetic subjects and up to 15% in diabetics (Kahn and Halban,1997); however, since PI and the proportion of split PI products were unchanged following acute β-cell stimulation in both diabetic and non-diabetics, this suggested that acute increased secretory demand on the beta cell does not lead to any further increase in unprocessed proinsulin secretion.

Proinsulin and split products and/or increased PI/IRI ratio have been considered to be markers or "risk factors" in subjects with impaired glucose tolerance and in "at risk" non-diabetic subjects for progression to diabetes (Haffner et al,1997; Kahn et al,1995; Mykkanen et al,1995). However, there was no evidence for disproportionately increased proinsulin in the IFG group in the present study. This corresponds with the findings of the Hoorn study (Nijpels et al,1996) and may reflect the fact that subjects with IFG represent a very early stage in the pathophysiology of diabetes compared to

patients with IGT. The causal factors for increased proinsulin production

which was most apparent in insulin treated diabetic subjects are unclear.

Mutations in the endopeptidases PC2 and PC3 which are involved in the

processing of proinsulin in the beta cell granule are not found in the majority

of subjects with Type 2 diabetes (Ohagi *et al*,1996). However, an individual

with mutations in PC3 was shown to have severe hyperproinsulinaemia

(O'Rahilly *et al*,1995). MODY2 subjects with diabetes caused by

mutations in glucokinase do not have disproportionate hyperproinsulinaemia

(Hattersley *et al*,1997). Chronic increased secretory demand on the β-cell

by sustained increased levels of blood glucose could result in defective

proinsulin conversion associated with release of premature β-cell granules.

Alternatively, an intrinsic defect in the post-translational processing system

could be unmasked by such a sustained demand for insulin. Constitutive

secretion of proinsulin might also occur as a result of mis-targeting of β-cell

granule contents as occurs in insulinomas (Halban,1990). Disproportionate

changes in IAPP secretion was not demonstrated in this study and there was

no dissociation of IAPP with C-peptide production at any stage. Subjects

with IFG had higher basal and stimulated IAPP concentrations than the other

non-diabetic groups but this increase was in parallel with changes in C-peptide as was seen in the diabetic subjects. Formation of islet amyloid in vitro is promoted by increased concentration of IAPP (Chargé et al,1995), and increased production of IAPP has been proposed as a causative factor for amyloidosis in vivo (Johnson et al,1991); renal failure results in dramatically raised circulating concentrations of IAPP (Ludvik et al,1991) and there is increased prevalence of islet amyloidosis in non-diabetic subjects with end-stage renal failure (de Koning et al,1995). If increased IAPP production is associated with amyloid deposition in prediabetic and/or diabetic subjects, this would have to be achieved by either a sustained increase in beta-cell granule products (both C-peptide and IAPP) in response to increased insulin resistance or by increased granule content of IAPP. However, from this cross-sectional study, there does not appear to be any stage in the diabetes syndrome which is associated with dramatically elevated IAPP secretion over that which would be expected from release of β-cell granules with the usual complement of peptide which could be associated with islet amyloid formation. These findings of IAPP secretion corroborates those made by Kahn et al,1998, in non-diabetic and diabetic

Japanese American subjects where the molar ratio of amylin-like peptides

to insulin was not different under different degrees of glucose tolerance .

Serum NEFA concentrations are not significantly raised in the non-diabetics.

Chronic elevation in NEFA has been shown to inhibit glucose stimulated

insulin secretion. This has been termed the lipotoxic effect (Unger,1995).

Prediabetic rats have been shown to have higher NEFA concentrations than

non-predabetic rats. Similarly prospective studies in Pima Indians and in

Caucasians have shown that elevated NEFA values are a risk factor for

conversion to diabetes independent of obesity, ambient glucose

concentration or insulin level. In the case of the Pima Indians, raised NEFA

remained significant even when insulin stimulated glucose disposal was

added to the regression model. However when acute insulin response was

added, NEFA values were no longer predictive (Paolisso *et al*,1995). In the

case of the Paris Prospective Study no measure of %S or %B has been made

so that the conclusions regarding the impact of NEFA on these parameters

in the development of Type 2 diabetes in Caucasians remains to be

confirmed (Charles *et al*, 1997). Although subjects with IFG had evidence

for both decreased beta-cell function and reduced insulin sensitivity, there

was no evidence for elevation in NEFA concentrations. Raised fasting NEFA concentrations are thought to augment hepatic gluconeogenesis in Type 2 diabetics and contribute to the fasting hyperglycaemia which characterises the condition (Boden,1997). However basal NEFA values were not significantly elevated in the diabetics in our study and thus cast doubt on this hypothesis. 1-hr NEFA concentrations were significantly raised in all of the diabetics regardless of treatment type and are likely to reflect relative insulopaenia.

Conclusions. Increased proinsulin production appears to be unrelated to abnormal IAPP secretion in established Type 2 diabetes even though severe islet amyloidosis and raised proinsulin have been described as concurrent findings in a type 2 diabetic patient(Clark *et al*,1987). Since both pro- IAPP and proinsulin are processed by β-cell granule endopeptidases (Badman *et al*,1996; Hutton *et al*,1994), it is possible that measurement of proIAPP may provide clues to an underlying defect of this β-cell pathophysiology. However, the present data suggests that increased secretion of proinsulin is likely to reflect a different pathological process from the diminished C-

peptide in the later stages of severe beta cell dysfunction in diabetes although both could be linked to islet amyloid deposition and β-cell destruction.

Elevated NEFA values are not seen in 1st degree relatives with IFG suggesting that they do not play a significant role in the pathogenesis of Type 2 diabetes. Basal NEFA values are not significantly elevated in the Type 2 diabetic subjects, indicating that they do not have an impact on fasting or post-prandial hyperglycaemia.

Table 1: Characteristics of the study subjects.

	Control subjects N=19	1st degree relatives N=58		Diabetic subjects N=39	
Gender	2M:17F	25M	33F	28M	11F
Age	64 (16)	42 (10)	50 (12)	62 (11)	66 (14)
BMI (1994)	29(4)	28 (4)	27 (5)	30 (5)	33.5 (6)

Table 2 Data from Nondiabetic subjects

	FPG<5.5 N=52	Intermediate glucose tolerance N=14	IFG N=11	Significance p
Gender	13M:39F	10M:4F	5M:6F	ns
Age (years)	48 (12)	46 (15)	59 (14)	p<0.05
BMI (kg/m2)	27 (5)	28 (5)	29 (4)	ns
Fasting glucose (mmol/l)	4.9 (0.3)	5.7 (0.1)	6.3 (0.3)	p<0.0001
1 hr glucose (mmol/l)	8.7 (1.2)	9.5 (0.8)	11.3 (1.5)	p<0.0001
Fasting CP (nmol/l)	0.6 (04-0.9)	0.8 (0.6-1.0)	1.1 (0.7-1.6)	p<0.02
1 hr CP (nmol/l)	1.5 (1.1-2.1)	1.6 (1.3-2.0)	1.5 (1.1-2.3)	ns
delta CP %	135(98-187)	102 (72-145)	79 (47-134)	p<0.002
Fasting PI (pmol/l)	7.0 (4-12)	11 (6-19)	15 (9-26)	p=0.002
1 hr PI (pmol/l)	23 (13-39)	26 (16-42)	35 (18-55)	ns
Fasting PI/CP (%)	1.1 (0.7-1.8)	1.5 (1.1-2.2)	1.4 (0.9-2.3)	ns
1 hr PI/CP (%)	1.6 (0.9-2.6)	1.7 (1.2-2.5)	1.6 (1.1-2.7)	ns
Fasting IAPP (pmol/l)	5.5 (3-10)	7 (4-12)	8 (3-17)	p<0.05
1 hr IAPP (pmol/l)	12.5 (8-20)	13 (8-24)	13 (5-33)	ns
F. IAPP/CP (%)	0.8 (0.5-1.3)	1.1 (0.7-1.6)	1.0 (0.7-1.6)	ns
1 hr IAPP (pmol/l)	12.5 (8-20)	13 (8-24)	13 (5-33)	ns
1hrIAPP/CP (%)	0.8 (0.5-1.3)	1.1 (0.7-1.7)	1.0 (0.8-1.4)	ns
Fasting NEFA	655 (300)	600 (245)	700 (250)	ns
1hr NEFA	350 (150)	245 (100)	320 (135)	ns

Table 3: Data from 39 NIDDM subjects

Characteristic (1994)	Diet controlled N=9	Tablet tx. N=19	Insulin tx N=11	Significance p
Gender	8M:1F	13M:6F	7M:4F	ns
Duration of diabetes (years)	2 (3)	10 (6)	13 (5)	p<0.05
Age(years)	54 (11)	64 (10)	68 (11)	p<0.05
BMI(kg/m2)	32 (6)	30 (5)	31 (6)	ns
Fasting glucose (mmol/l)	8.3 (1.4)	9.5 (2.5)	9.3(3.4)	P<0.05
1hr glucose (mmol/l)	13 (1.5)	15 (3)	15 (4)	p<0.05
Fasting CP (nmol/l)	1.0 (0.8-1.2)	0.9 (0.6-1.5)	0.3 (0.2-0.6)	p<0.0001
1hr CP (nmol/l	1.3 (1.0-1.7)	1.2 (0.8-1.8)	0.5 (0.3-1.0)	p<0.005
delta CP	32 (20-51)	23 (11-45)	26 (13-52)	ns
Fasting PI (pmol/l)	24 (12-49)	21 (8-53)	23(9-58)	ns
1hr PI (pmol/l)	36 (15-83)	28 (13-62)	19 (5-67)	ns
Fasting PI/CP (%)	2.5 (1.4-4.4)	2.3 (1.2-4.6)	5.8 (1.7-19.2)	p<0.02
1hr PI/CP (%)	2.8 (1.5-5.2)	2.5 (1.4-4.6)	5.0 (1.0-26)	p<0.02
Fasting IAPP (pmol/l)	11 (5-23)	8.0 (4-16)	2.0 (0.5-4.0)	p<0.0001
1hr IAPP	16 (7-35)	12 (7-22)	3.0 (0.5-6)	p<0.0001
Fasting IAPP/CP (%)	1.2 (0.7-2.0)	0.9 (0.6-1.5)	0.5 (0.3-0.8)	p<0.01
1hr IAPP/CP (%)	1.2 (0.7-2.2)	1.0 (0.6-1.7)	0.5 (0.3-0.8)	p<0.01
Fasting NEFA	600 (250)	750 (300)	750(400)	ns
1-hr NEFA	500 (150)	550 (250)	600(275)	ns

Figure 1a. Plot of fasting C-peptide (nmol/l) against fasting proinsulin (pmol/l) in 77 nondiabetics.
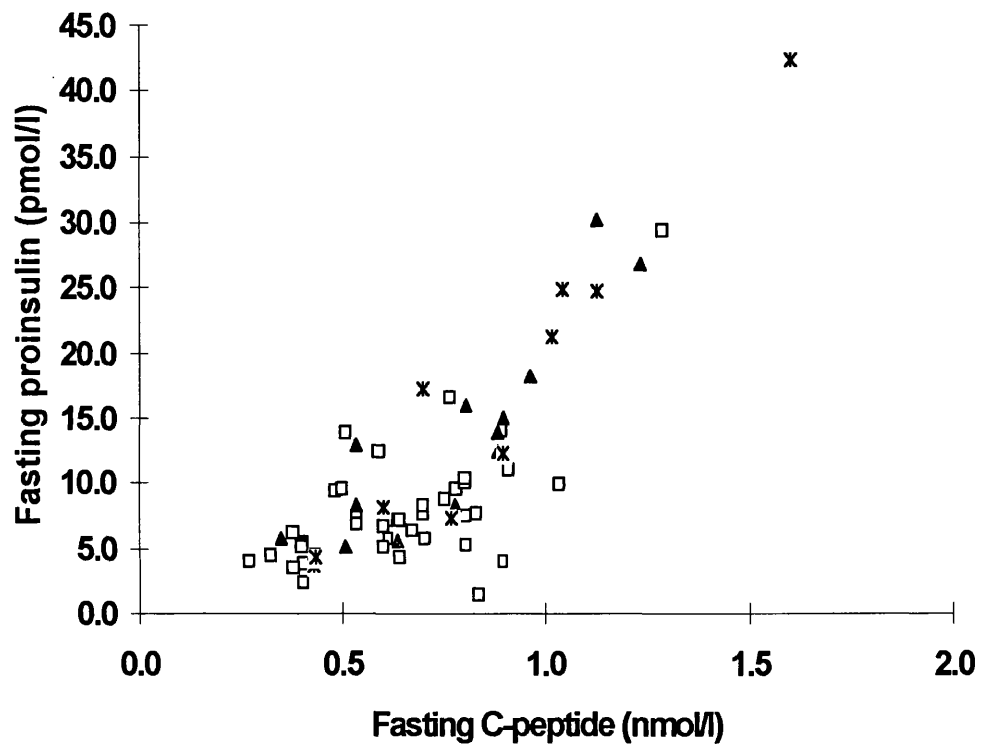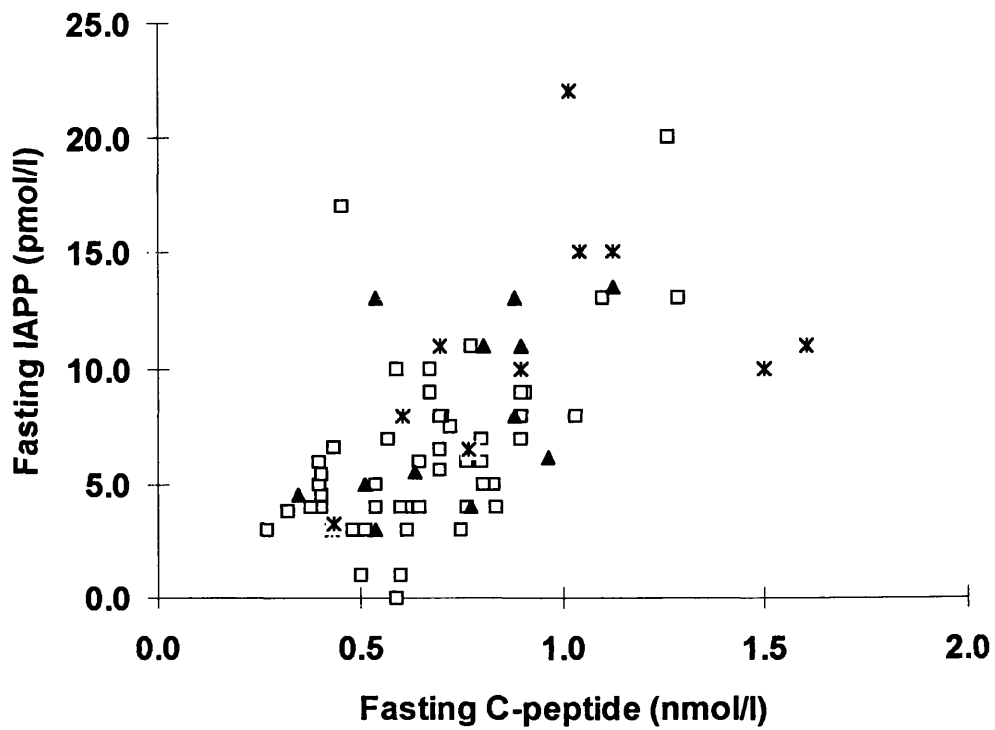

☐ =non-diabetics with FPG<5.5mmol/l

▲= non-diabetics with intermediate glucose tolerance

✗=non-diabetics with IFG (FPG≥6.1<7.0).

## Figure 1b. Plot of fasting C-peptide against fasting IAPP in 77 nondiabetic subjects.

□ =non-diabetics with FPG<5.5mmol/l

△= non-diabetics with intermediate glucose tolerance (FPG>5.5<6.1).

✗=non-diabetics with IFG (FPG≥6.1<7.0).

Figure 2. Proinsulin, C-peptide and IAPP secretion in different groups of non-diabetic and diabetic subjects

Groups	Normal	Inter	IFG	Diet	Oral	Insulin
fpg	4.9	5.7	6.3	8.3	9.5	9.3
fast PI/CP	1.1	1.5	1.4	2.5	2.3	5.8
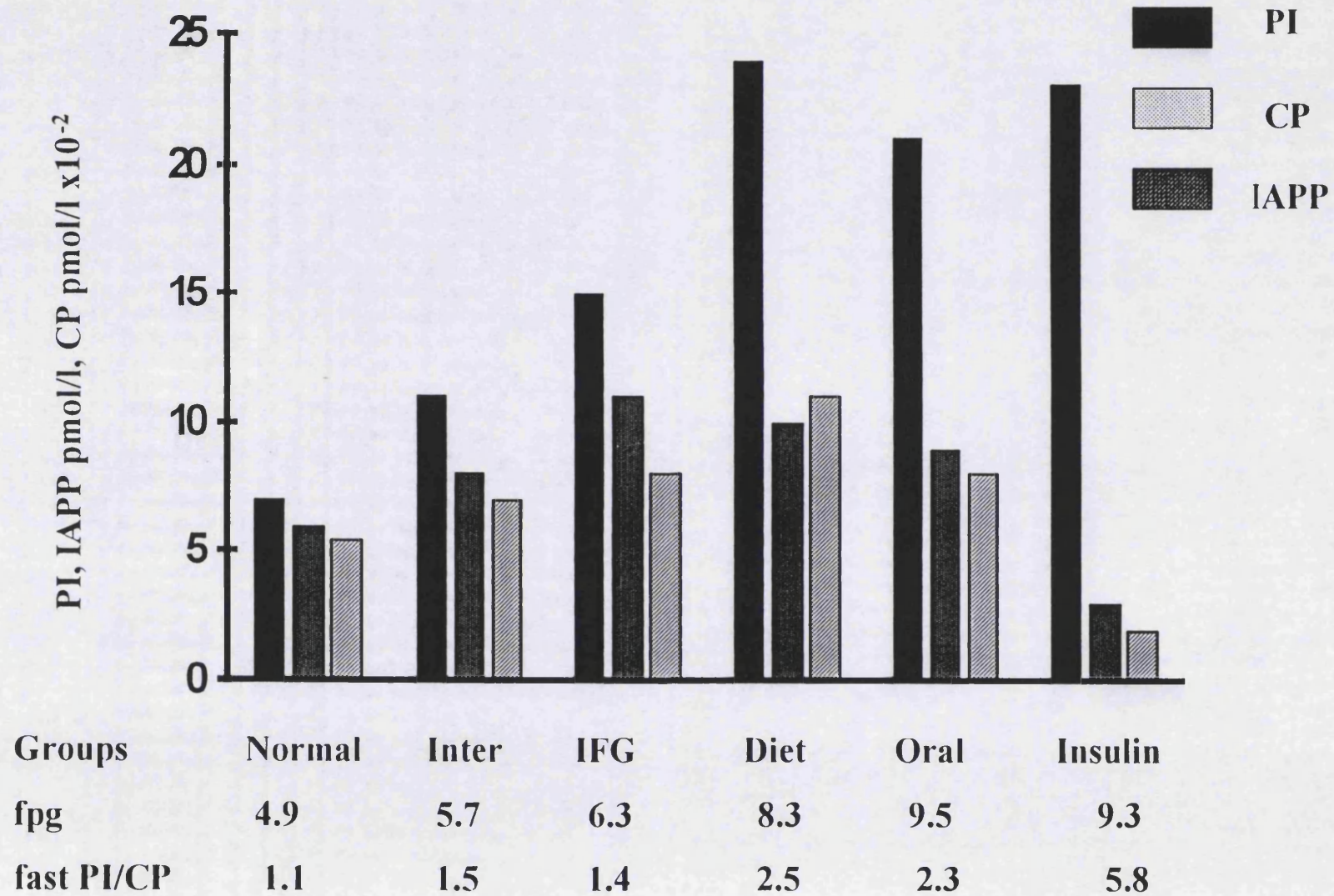
Chapter 8

Sib-ship BA: (cross-sectional and longitudinal physiology with pancreatic histology)

Is islet amyloid formation initiated by hypersecretion of IAPP and associated with progressive beta-cell failure in Type 2 diabetes?

Introduction

The final study outlined in this thesis is a retrospective analysis of prospective and cross-sectional physiological data in a particular male sib-ship (BA), which was studied as part of the original cohort of relatives in 1984.

The proband and his identical twin died during the course of the study providing us with the unique opportunity to relate physiological parameters associated with progression to diabetes with pancreatic histology.

As already described, islet amyloid is one of the histopathological features of Type 2 diabetes. It is not known whether the deposition of this fibrillar material occurs as a secondary phenomenon once diabetes is established or whether the process has a primary role in the development of the disease. Islet amyloid is deposited adjacent to the beta-cell and results in disruption of the

beta-cell membrane caused by invaginations (Westermark,1994). In addition

accumulation of amyloid is associated with loss of beta-cell mass

(Westermark,1978). The number of beta-cells required to maintain

normoglycaemia in humans is unknown. Some subjects who have undergone

almost total pancreatectomy are able to maintain normal glucose homeostasis.

It is estimated that up to 30% of beta-cells have been replaced by islet amyloid

in established Type 2 diabetes-(Figure 1, p128). It is therefore possible that

in insulin resistant states with limited beta-cell capacity, a loss of 30% of cells

will 'unmask' the diabetic state.

The causative factors for islet amyloid deposition are unknown but seem

partly dependent on species-specific variations in the amino acid sequence

between positions 20-29 in the amino acid chain (Westermark *et al*,1990).

However this cannot be the only factor since islet amyloid is infrequently

found in non-diabetic individuals and is not found in canine islets, although the

amyloidogenic sequence is expressed in dogs with insulinomas (Jordan *et al*,

1990). The overproduction of IAPP is an important factor for islet amyloid

deposition and is supported by *in-vitro* studies using transgenic mouse islets

expressing human IAPP (Chargé *et al*, 1995). Elevated IAPP concentrations

occur in parallel with insulin in insulin resistant states including obesity, IGT

and in Type 2 diabetes (Kautzky-Willer *et al*, 1991; Enoki *et al*,1992). Lower

IAPP levels occur in Type 2 diabetics treated with insulin than in those treated

with sulphonylureas (Lindstrom *et al*,1997; Hartter *et al*,1991; Van Jaarsveld

et al,1993). Prospective *in vivo* studies in animals indicate that amyloid

formation precedes the development of hyperglycaemia (O'Brien *et al*,1986;

Howard *et al*,1986; de Koning *et al*, 1993). However the relationship of islet

amyloid formation to the onset and progression of Type 2 diabetes in man is

difficult to assess, since damaging pancreatic biopsies would be required.

Aims

The aims of this study were to determine the physiological markers for

progression to diabetes within the particular male sibship -BA and try and

correlate the findings with the post-mortem pancreatic histology available in

the proband and his identical twin.

Subjects and methods

Sib-ship BA. This comprised 5 brothers aged 30-46y in 1984. Figure

2(p130), illustrates sib-ship BA in 1984-1994. The eldest two siblings were

identical twins and one of them, the proband- DY, had been diagnosed

diabetic in 1982. In 1984 and 1994, the 4 youngest siblings underwent physiological study using CIGMA (O'Rahilly *et al*,1986, Chapters 6 and 7). Mean BMI in 1984 was 35(4).

Control group.

14 BMI-matched relatives who participated in the 10-yr follow-up study and remained non-diabetic and without IFG in 1994 were selected as a control group for this study. The gender ratio of the subjects was 4M:10F, mean age in 1984 was 35(15) and mean BMI 33(3) (Table 1,p128).

Study procedures. These have been described in the preceding chapters-6 and 7. Conversion to diabetes at follow-up was determined according to the new diagnostic criteria adopted by the ADA .

Biochemical methods.

Assay methods have been described in Chapters 6 and 7.

Histological methods.

The proband DY died in a road traffic accident in 1985 and pancreas preserved following post-mortem examination. EY, his identical twin, died in 1995 of myocardial infarction, post-mortem examination was also performed. Both siblings had been diabetic for less than 5 years duration at the time of death and were relatively well-controlled on low-dose sulphonylurea treatment. Pancreatic tissue sections were labelled for islet amyloid using primary antibodies raised against IAPP and immunoperoxidase conjugated secondary antibodies. This shows up as brown staining at sites of antibody binding and particularly at sites of amyloid deposition. Sections were also immuno-labelled for insulin.

Statistical methods

See cross-sectional study in Chapter 7 for details. %B and %S were calculated using the CIGMA model using 1-hr glucose and C-peptide values (Levy *et al*,1991)). At follow-up(1994), the proband's twin (EY), was taking glibenclamide 5mg od. This was omitted on the morning of the test. No other medications known to interfere with glucose metabolism were being taken by any of the study participants. Plasma creatinine values for the group lay within

the normal range. A p-value of <0.05 was assumed to be significant.

Results

BMI

All 5 siblings were obese at baseline(1984) with a mean BMI of 35(4) (Table 1, p128). Subject EY remained the same weight at 10 year follow-up. RY had increased his BMI by 12%. The two younger siblings JY and SY had each reduced their BMIs by 13% and 6% respectively. There was no significant change in BMI for the control group.

Glucose tolerance 1984-1994

The proband DY was diabetic at baseline in 1984, diagnosed two years earlier. He was treated with chlorpropamide 250mg od. His identical twin and younger siblings were all non-diabetic in 1984 (Table 1, p128). Mean FPG in 1984, 4.7 (0.2); mean 1hr glucose 8.1 (0.9)mmol/l. There was no significant difference in fasting and stimulated glucose values for the siblings and controls.

Sibling EY was diagnosed diabetic in 1990, then aged 52y. At the time of follow-up he was treated with glibenclamide 5mg od.

RY was not previously known to be diabetic but had fasting plasma glucose in 1994 of 9.0mmol/l and 1hr value of 13.6mmol/l and was thus newly diagnosed as a Type 2 diabetic.

Siblings JY and SY both remained non-diabetic, with fasting and 1-hr glucose values similar to the control group. None of the non-diabetics had fasting glucose values ≥ 6.1mmol/l.

C-peptide, %S and %B. in 1984-1994

The middle two siblings RY and JY, were significantly more insulin resistant in 1984 than the controls($p<0.05$) (Table 1(p128) and Figure 3(p131)). This is in keeping with their significantly higher BMI at baseline (Table 1). At follow-up, %B was significantly reduced in EY and RY who had both converted to Type 2 diabetes ($p<0.0005$) (Figure 3). Despite significant fall in %B for the two diabetics, %S was not significantly changed. Fasting C-peptide concentrations were not significantly reduced in the siblings who had converted to diabetes, whilst 1-hr values were markedly lower ($p<0.001$). In contrast, JY had evidence of improved %B and %S, possibly in keeping with substantial weight loss during the 10 year follow-up period. C-peptide values, %B and %S were not significantly changed in SY and in the control

subjects.

Proinsulin concentrations in 1994

Fasting and 1-hr proinsulin concentrations were markedly elevated in the two

diabetic siblings EY and RY ($p<0.001$)(Table 1 and Figure 4(p132)). Fasting

and 1-hr PI/CP ratios were significantly raised in the diabetic siblings EY and

RY-13% and 8.5 % respectively, compared with 1-2.5% in the non-diabetic

siblings and the control group ($p<0.0001,p<0.0002$) (Table 1).

IAPP concentrations in 1994

Fasting IAPP levels were significantly higher in the newly diagnosed diabetic

RY ($p<0.02$)(Table 1 and Figure 5(p133)). Similarly, fasting and 1-hr

IAPP/CP ratios were higher -approximately 3% compared with less than 1.5%

in the non-diabetics ($p<0.005$, $p<0.05$). IAPP levels for sibling EY (DM

4years), appeared subnormal, but did not reach statistical significance when

compared with the controls.

Pancreatic histology

All islets examined in both the proband and his twin (EY) - showed significant islet amyloid deposition (Figure 6, p134).

Discussion.

The progression to Type 2 diabetes in sib-ship BA is characterised by marked deterioration in %B (CIGMA) but no change in %S.

Neither sibling had evidence of impaired fasting glucose at baseline in 1984 but all were insulin resistant with evidence of one or more feature of the Metabolic Syndrome (data not shown). As has been discussed earlier, the features of the Metabolic Syndrome can occur up to a decade before onset of Type 2 diabetes and may be related to hyperinsulinaemia.

The marked decline in %B in the siblings who converted to diabetes, may be associated with a glucotoxic effect. However, the twin EY, appeared moderately well controlled, with fasting glucose within the normal range. Therefore beta-cell dysfunction is likely to reflect pathophysiological processes other than those due to glucotoxicity. Examination of pancreas from the twin showed extensive deposits of islet amyloid, as was the case for the proband (data not shown).

As has already been discussed in Chapter 7, islet amyloid formation *in vitro* is favoured by high IAPP concentrations (Chargé *et al*, 1995). The cross-sectional study(Chapter 7)- showed that IAPP concentrations were elevated in a proportional manner to C-peptide in mild glucose intolerance (IFG), and in diet and tablet treated diabetics. At no stage was disproportionate or very increased IAPP secretion demonstrated. In contrast, proinsulin levels were disproportionately raised following onset of diabetes but not before, suggesting that disordered processing of proinsulin occurs as a secondary phenomenon-perhaps associated with premature release from immature insulin granules. In sib-ship BA, both of the diabetics have elevated basal and stimulated proinsulin levels.

Basal IAPP levels at fasting glucose, are disproportionately elevated in the case of the newly diagnosed sibling RY, with IAPP/CP ratio approximately three-fold higher than in the controls or in the diabetic sibling EY. It is not clear whether this increased basal IAPP represents a transient process of increased IAPP output which occurs only over a short period of time. This would explain the absence of any increased IAPP production in the cross-sectional study since few newly diagnosed diabetics were examined. If this disproportionate rise in IAPP compared to CP and PI, is a real phenomenon,

this could 'signal' the onset of fibril formation and subsequent islet amyloid deposition.

So far only one mutation in the amylin gene has been identified in association with Type 2 diabetics in Japanese subjects (Sakagashira *et al*,1996). However whether or not the mutation is associated with excessive deposition of islet amyloid or other pathological process, has not been elucidated.

The postulated mechanisms for disturbed beta-cell function in association with islet amyloid formation include:

i.   Disrupted beta-cell sensing and secretory mechanisms due to mechanical distortion of the basement membrane with invaginations filled with amyloid fibrils (Westermark,1994)

ii.   Loss of beta-cells due to toxicity of amyloid fibrils (Westermark, 1978)

The first mechanism provides a possible primary role for amyloid in the pathogenesis of Type 2 diabetes, whereas the second mechanism suggests that amyloid formation has a secondary role causing progressive beta-cell loss after the establishment of diabetes.

The association of substantial amyloid deposition in the diabetic identical twins examined here, together with marked reduction in beta-cell function is in favour of a primary pathogenic role for islet amyloid. Furthermore the

disproportionate elevation of IAPP levels in the newly diagnosed middle sibling suggests that raised levels of IAPP may be a transient occurrence coinciding with onset of the diabetic state. To further elucidate the situation, more studies of IAPP secretion are required in glucose intolerant and newly diagnosed diabetic subjects. In addition, noninvasive methods for assessing islet amyloid deposition in humans are required to enable appropriate therapy directed towards reduction or prevention of amyloid deposition..

Table 1. Characteristics of Sib-ship BA and 14 controls

Characteristic	EY	RY	JY	SY	Controls N=14	p
Age in 1984	46y	45y	40y	30y	35 (15)	ns
FPG in 1984	4.5	4.8	4.9	4.8	4.9(0.4)	ns
FPG in 1994	4.8*	9.0*	5.3	5.1	5.0(0.4)	*=DM
1hr glucose 1984	7.5	7.8	9.3	7.6	9.0(1.4)	ns
1hr glucose 1994	14.2*	13.6*	8.6	7.6	8.9(1.0)	*=DM
BMI 1984	33.6	36.4*	38.6*	30.4	33(3)	*p<0.05
BMI 1994	33.4	40.7*	33.6	28.7	33(5)	*p<0.05
Fasting CP 1984	0.7	1.0	0.9	0.6	0.6(0.5-0.9)	ns
Fasting CP 1994	0.5	0.9	0.6	0.5	0.7(0.4-1.1)	ns
1hr CP 1984	1.3	2.1*	2.2*	1.4	1.2(0.9-1.6)	*p<0.05
1hr CP 1994	0.7*	1.2	1.8	1.4	1.5(1.0-2.3)	*p<0.05
%B 1984	99	129	95	125	80(53-119)	ns
%B 1994	23*	28*	109	135	94(62-142)	*p<0.0005
%S 1984	65	36*	32*	73	64(43-96)	*p<0.05
%S1994	66	50	85	99	58(30-116)	ns
Fasting PI 1994	66*	72*	12.5	8.7	10(5-20)	*p<0.001
1hr PI 1994	75*	93*	42	20	27(15-48)	*p<0.02
Fasting IAPP 94	4	23*	5	6	9(5-16)	*p<0.02
1hr IAPP 94	5	27	9	11	17(8-33)	ns
Fasting PI/CP(%)	13.0*	8.4*	2.1	1.6	1.5(1.0-2.2)	*p<0.0001
1hr PI/CP(%)	11*	8.0*	2.3	1.4	1.9(1.2-2.8)	*p<0.0002
Fasting IAPP/CP(%)	0.8	2.7*	0.8	1.1	0.9(0.5-1.5)	*p<0.005
1hr IAPP/CP(%)	0.7	2.2*	0.5	0.8	0.9(0.6-1.3)	*p<0.05

Figure 1. Pancreatic islet from subject with Type 2 diabetes showing loss of beta cell cells (immunoperoxidase stained for insulin -*brown area*); with replacement with islet amyloid (stained with Congo Red -*pink area*).
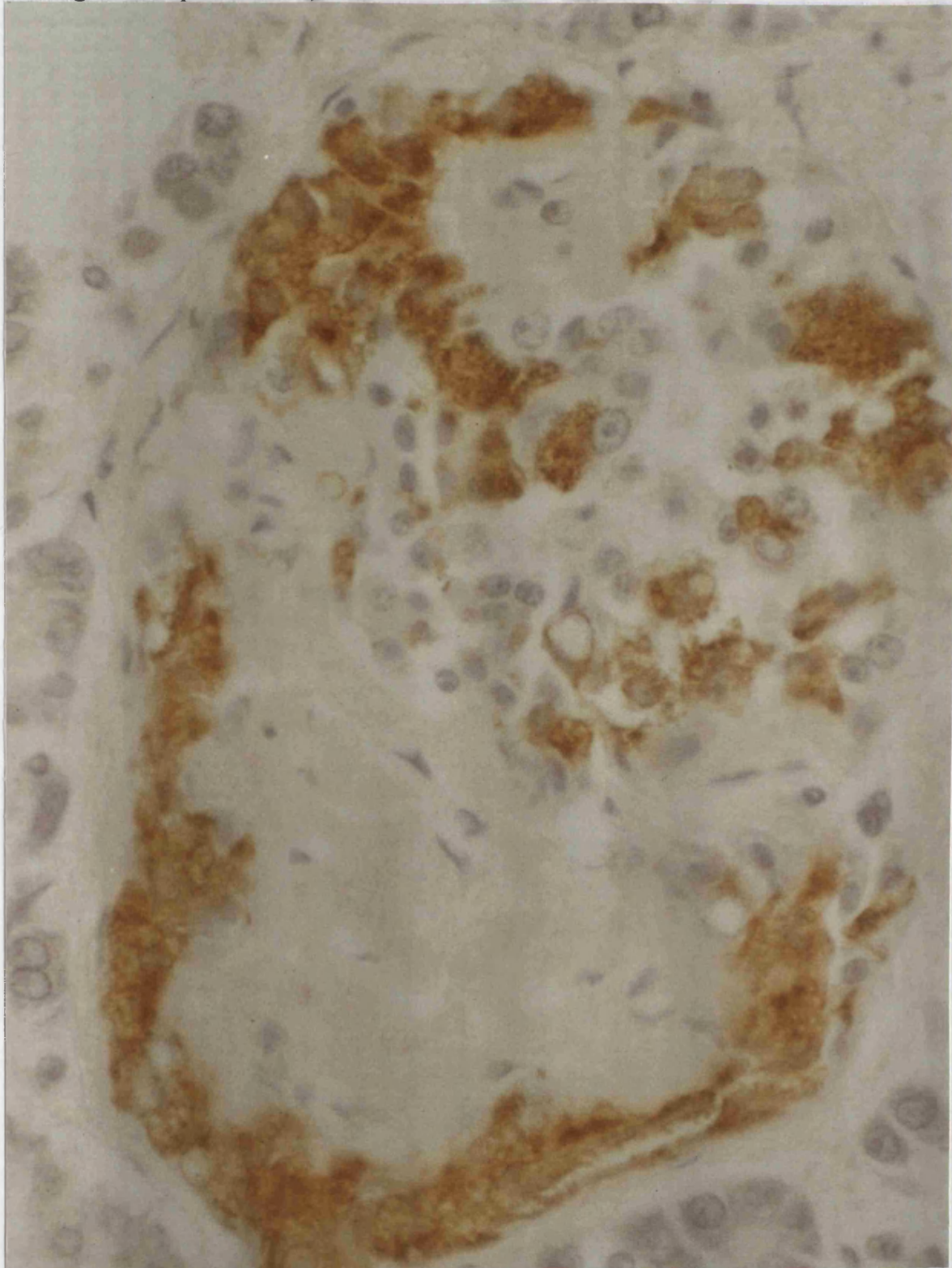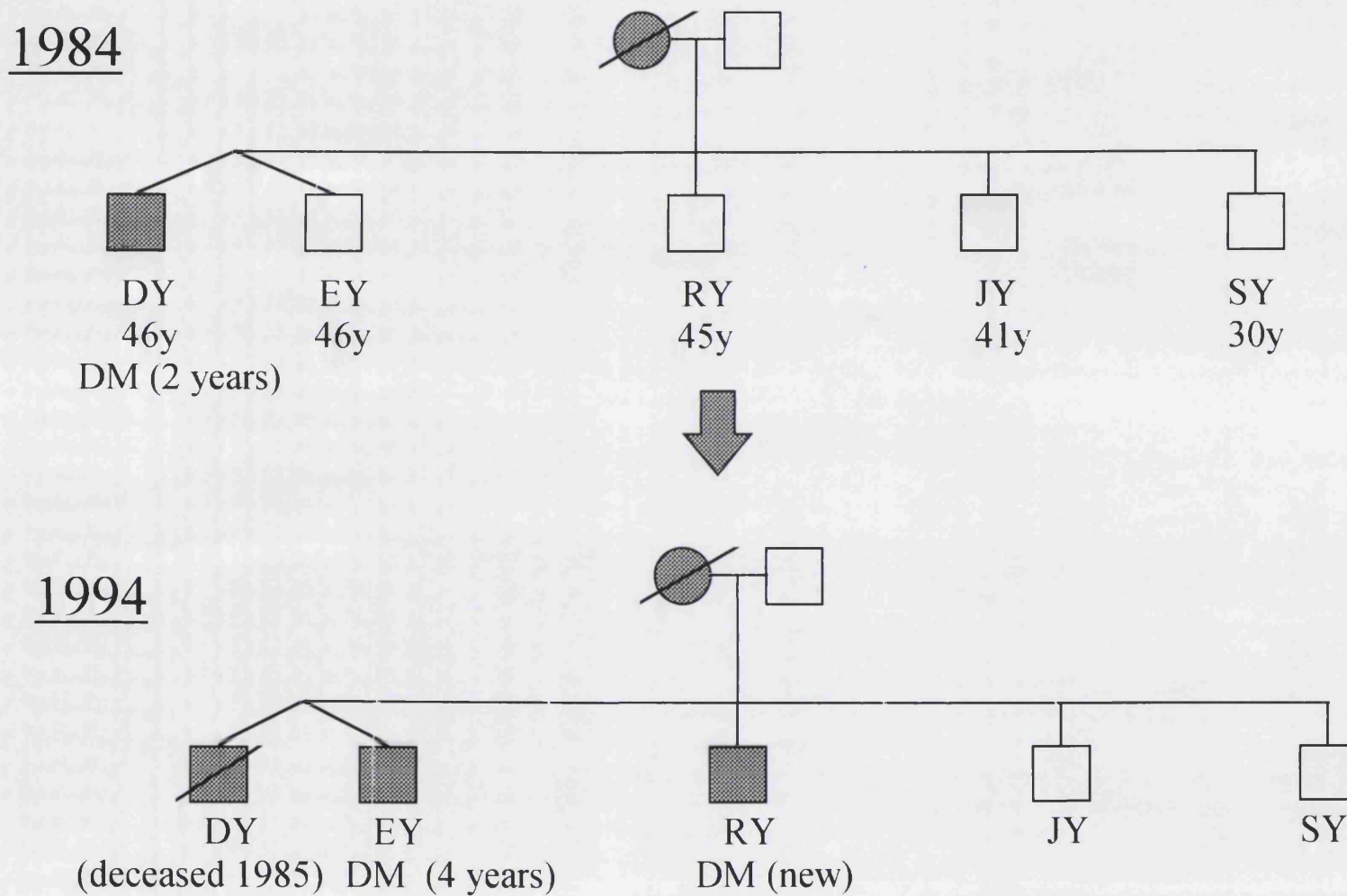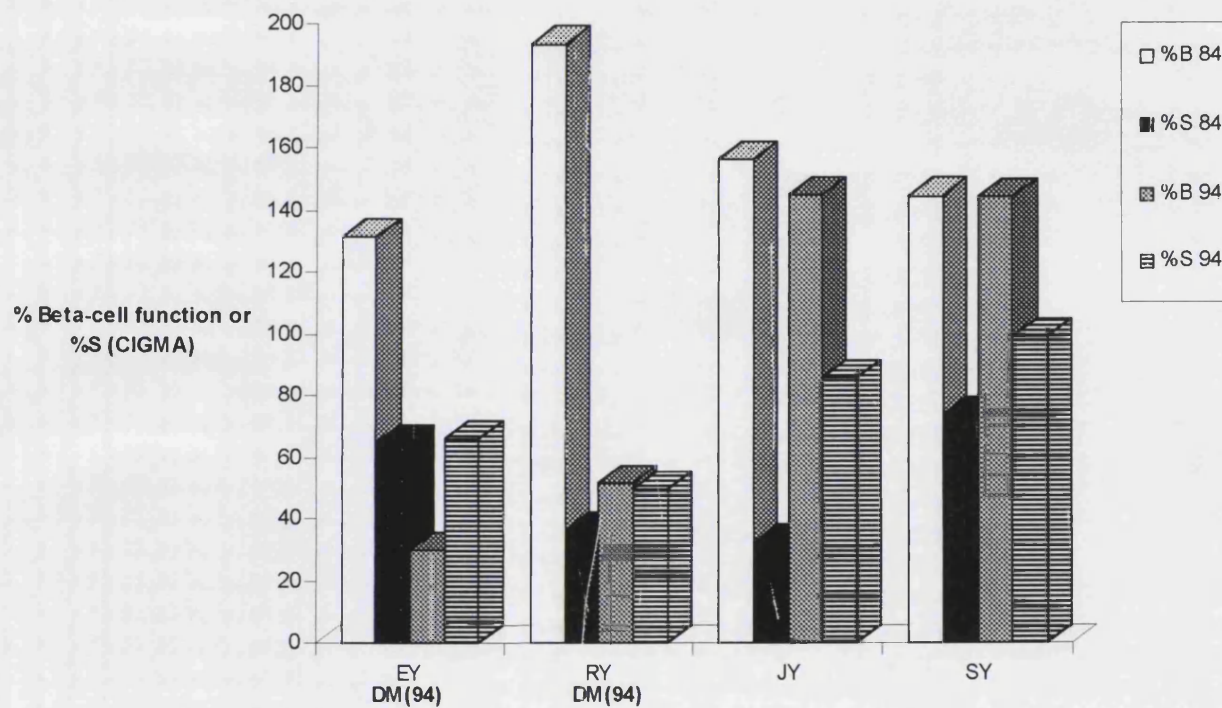
Figure 2. Sibship BA (1984-1994)

Figure 3. %B and %S for sib-ship BA in 1984-1994.
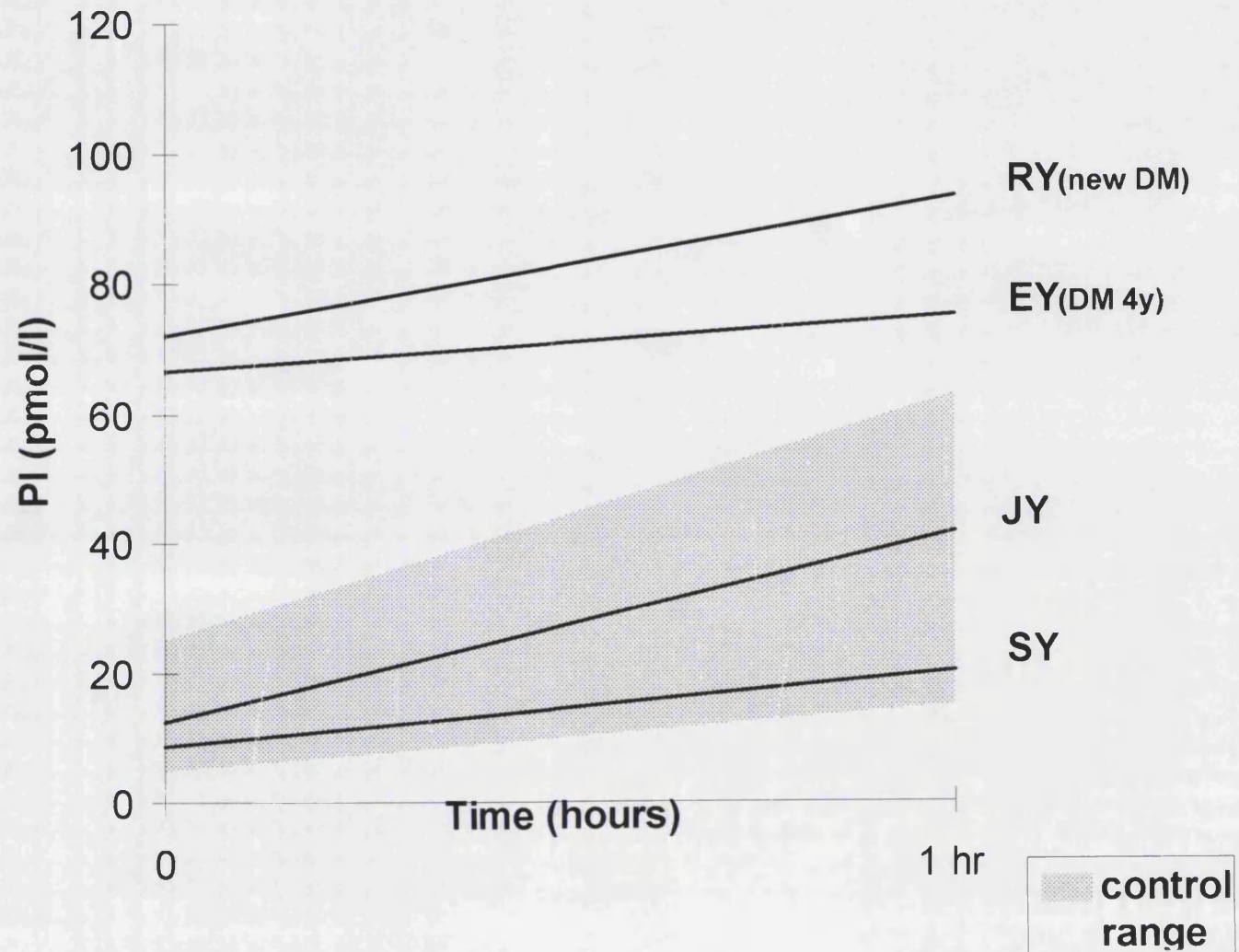
Figure 4. Plot showing PI values in sib-ship BA

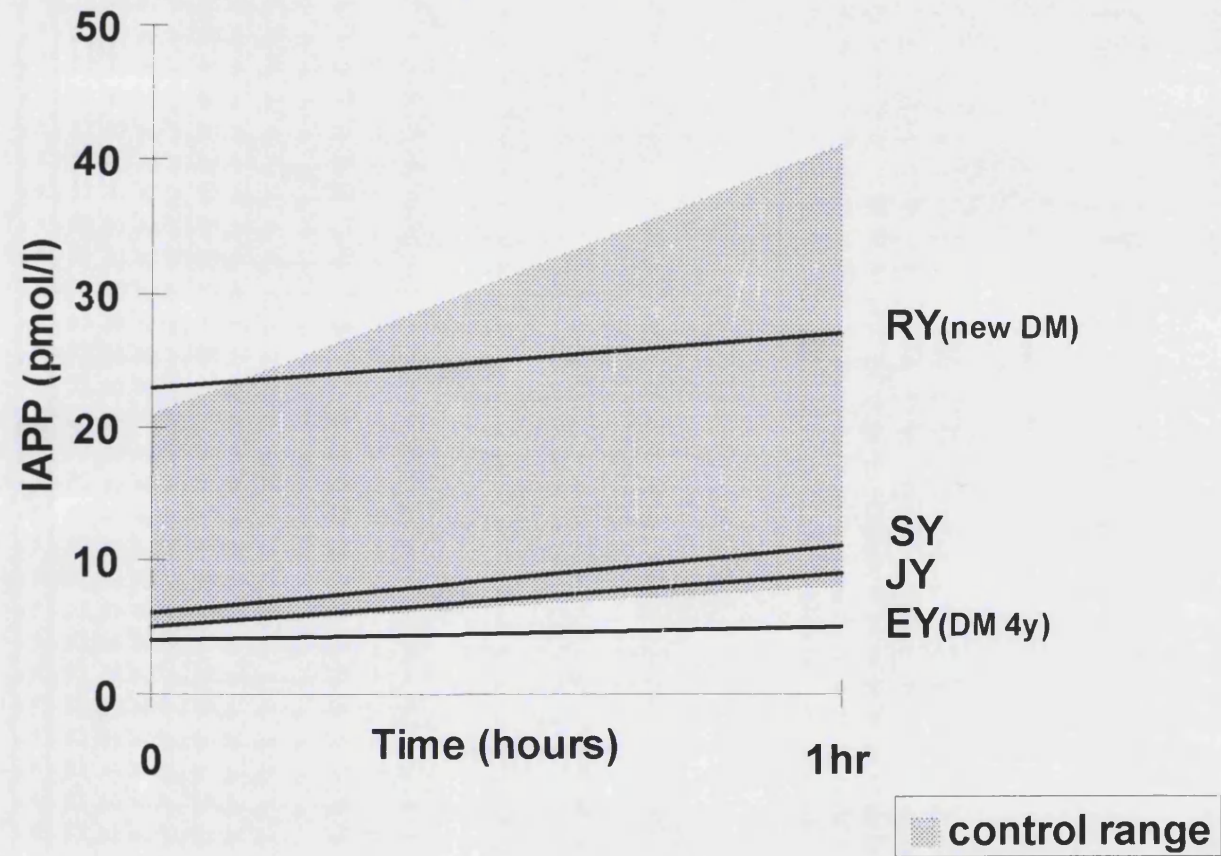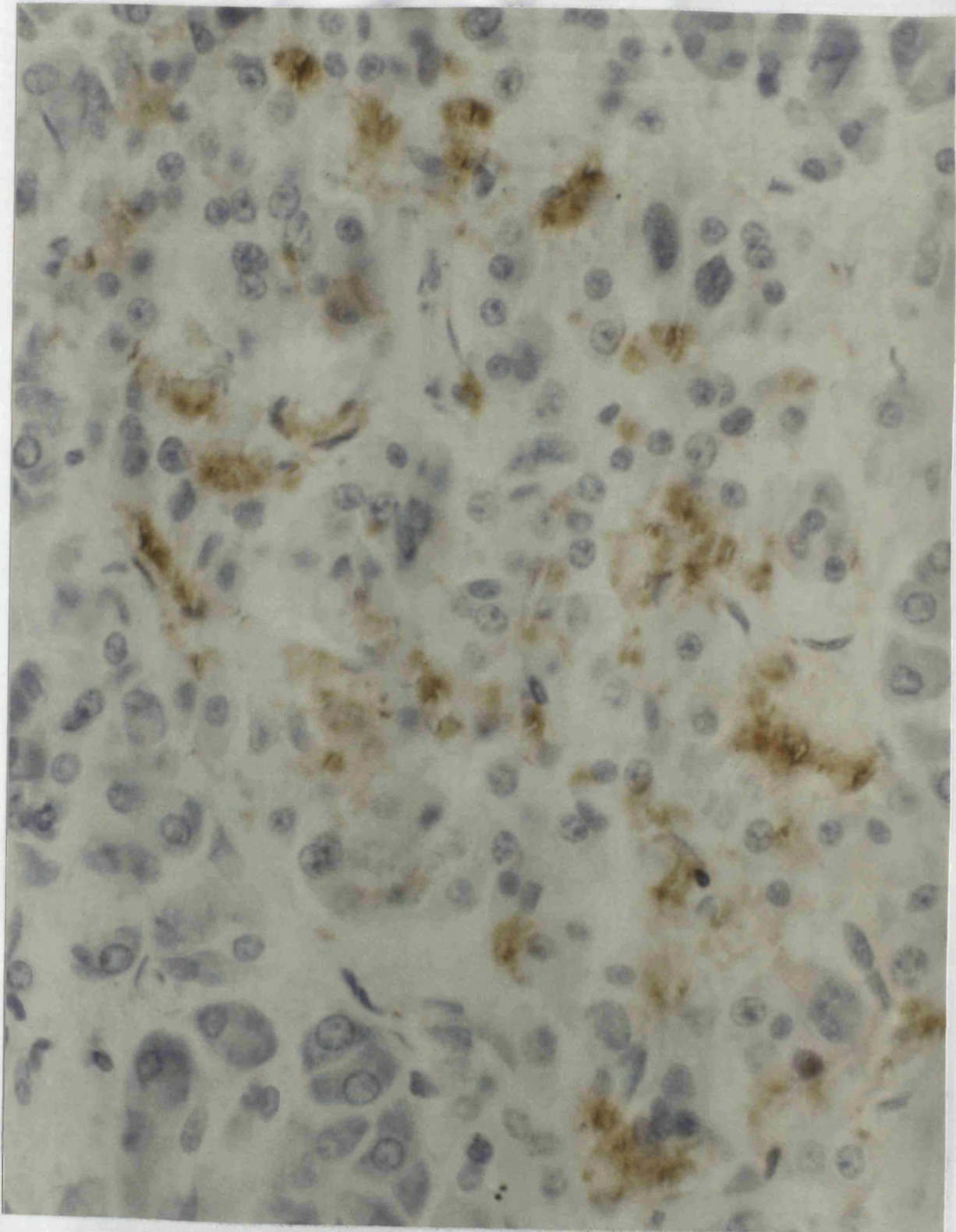**Figure 5.** Plot showing IAPP levels in sib-ship BA

Figure 6. Islet from sibling EY showing islet amyloid stained with immunoperoxidase labelled with antibodies against IAPP *-light brown areas*.

Chapter 9

General overview and discussion.

The 10-year follow-up study outlined in Chapter 6, illustrates some of the practical difficulties in carrying out large-scale prospective studies to determine risk factors for conversion to Type 2 diabetes. Despite detailed records of original study participants; deaths, family rifts and geographical re-location, made recruitment difficult. Generally the response was encouraging, however knowledge in the family members concerning the familial risks and complications of the disease was poor.

Public awareness about Type 2 diabetes in the UK has been shown to be very limited by a recent survey commissioned by the BDA(OPCS Omnibus Survey, 1996). There is a general view that Type 2 diabetes represents a 'mild' form of the disease and it has not been the subject of campaigns by the Health Education Authority or by the various charities devoted to the interests of older people.

In the recruitment process a lot of consideration was given to excluding relatives of probands with probable LADA. This was helped by access to

antibody titres for anti-GAD and ICA. Another problem encountered was the question regarding 'cut-offs' for the diagnosis of IGT and diabetes. A number of subjects had baseline levels of glycaemia which fulfilled the ADA's new criteria for IFG and diabetes. The lack of predictive value of %B and %S in the 10-year follow-up study probably reflects the small number of subjects that developed diabetes and also the wide age discrepancy between the sibling and offspring groups. In the case of the siblings, fifty percent of those who developed diabetes, had done so already at baseline. In the case of the offspring, mean age at follow-up was still only 40y so that the cumulative total with diabetes was only 10%. The offspring proved most difficult to recruit. Many of the women were now mothers and unable to participate in the study because of family commitments. Inability to take time off work was the other major cause for lack of follow-up. The limited additional information provided by the 1-hr physiological data in this study, raises questions regarding the feasibility of carrying out further studies of this kind.

The cross-sectional study provided the opportunity to study proinsulin, IAPP and NEFA in relation to glucose tolerance. Proinsulin had been studied in 1984 but unfortunately the original data had been lost. The recent study clearly shows that PI and IAPP secretion are raised in a colinear fashion to

C-peptide in subjects with IFG. Once diabetes is established, disproportionately raised proinsulin secretion is present and persists in diabetics with severe beta-cell failure despite dwindling C-peptide concentrations. A number of investigators have previously suggested that disproportionate hyperproinsulinaemia occurs in IGT. However this discrepancy may be due to differences in diagnostic criteria for IGT as well as ethnic variations in pathophysiology. In this respect, IFG may represent a very early stage of IGT. Although the cross-sectional study provides little evidence in favour of IAPP and islet amyloid as a primary aetiological factor in the pathogenesis of Type 2 diabetes, the sib-ship BA study illustrates that islet amyloid is present in significant quantities after relatively short duration of diabetes. The disproportionately raised IAPP levels in the newly diagnosed diabetic is a unique observation and requires further substantiation. Although certain animal models are in favour of the islet amyloid hypotheses, lack of human pancreatic tissue is likely to impede progress unless noninvasive techniques for measuring islet amyloid can be developed. The future development of assays to measure pro-IAPP may also provide helpful clues in disease pathogenesis.

Sib-ship BA illustrates the typical phenotypic characteristics of Type 2 diabetes. All of the brothers are markedly obese and have one or more associated feature of the Metabolic Syndrome. Three out of four maternal uncles in this pedigree were also affected with Type 2 diabetes. Unlike sib-ship BA however, the uncles had much lower BMI but were hypertensive. The marked obesity in sib-ship BA could reflect the life-style changes which have taken place in the farming community in Britain over the last twenty years,with much greater reliance on machinery.

The inhibitory effects of glucotoxicity on long-term beta-cell function has led investigators to adopt intensive treatment regimens aimed at conserving secretory capacity. The impact of delayed diagnosis of sub-clinical cases of Type 2 diabetes in relation to 'recoverable' beta-cell function is unknown. Intensive treatment requires that post-prandial and basal glucose levels are maintained below 7mmol/l and frequently involves multiple boluses or continuous infusion of insulin. Such intensive regimens are difficult to enforce due to the associated problems of needle phobia, weight gain and hypoglycaemia. The future development of inhaled insulin may provide a more acceptable adjunct to oral hypoglycaemic therapy-aimed at controlling post-prandial glucose excursions and ultimately slowing progression of the disease.

Whilst the search for major genes involved in the pathogenesis of Type 2 diabetes continues, the need for further physiological studies remains important. Perhaps future physiological studies should place greater emphasis on assessing the 'modulating' effects of various therapies on disease expression and outcome. This may help to determine which particular pathological mechanisms are of primary significance.

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