Reviews in Medicine

New concepts in diabetes mellitus I: treatment, pregnancy and aetiology

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Introduction

The aim of this review is to summarize the clinically relevant studies of the preceding year which advance or challenge current opinion on diabetes mellitus.

What is new in treatment?

(a) Monitoring

Anyone who has looked through sequential outpatient clinic letters will be in no doubt in which area the next major improvement in management of diabetes will transpire. The procrastination of positive action until the HbA_{1c} result is available, often in conjunction with seemingly favourable home blood glucose monitoring data, renders the measurement almost useless unless the patient is seen again with the result. Reports of rapid HbA_{1c} assay methods which could be used in the clinic itself are therefore welcome. An immunologically based assay system which takes 9 minutes per sample (DCA 2000) was found to perform well compared with a Bio-Rad column laboratory method with correlation coefficients between 0.95 and 0.99.¹ The intra- and inter-assay coefficients of variation were less than 5%. This is a step in the right direction, but a turnover time of 9 minutes per sample would be far too long for most UK diabetes clinics. The cost of such assays is currently high, but hopefully new developments and competition will decrease the expense of instant HbA_{1c} assays in the near future.

A further glimpse of the future was provided by a study of self-monitoring of plasma triglyceride levels.² Twelve non-insulin-dependent diabetes mellitus (NIDDM) patients with fasting plasma triglyceride levels >1.7 mmol/l were randomly assigned to self-monitoring or control groups, and

followed-up monthly for 6 months. The self-monitoring group, who effectively had immediate feedback on dietary indiscretions, exhibited a fall in mean plasma triglyceride levels from 2.7 to 1.4 mmol/l. The control group showed no change. The home measurements were carried out using capillary samples, with test strips being read by a Reflotron machine. When the costs of this measurement fall, it could contribute significantly to management of highly selected NIDDM subjects.

(b) Modification of food intake

All doctors and specialist nurses concerned with advising people with diabetes about what they should eat are properly reminded of the extent of the normal day to day variation in intake.³ Life simply is not as regular as dietary sections of textbooks imply it should be.

Most of the work demonstrating that moderately high fat diets are bad for blood glucose control has been carried out in NIDDM. Now anyone doubting the relevance of the conclusions for IDDM can be reassured that the message is the same.⁴ Two weeks on a diet with 53% of energy as fat compared with 16% as fat (with the same carbohydrate but different protein content) resulted in a higher fasting and postprandial blood glucose level in a group of IDDM subjects even though insulin requirements fell in the low fat period. It is likely that at least the short-term effects are mediated through dietary change in insulin sensitivity.

The message that dietary fibre itself is largely irrelevant to practical, everyday blood glucose control is slowly being accepted. The concept had such an intuitive appeal that conclusions from short experimental periods of bean feeding were extrapolated to clinical practice without due consideration. A useful review of the era of fibre feeding has been published.⁵ Inclusion of reasonable amounts of soluble fibre in any diet does have a beneficial effect upon low-density lipo-

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Part II of this review will be published in the July issue.

What is new in treatment?

- Rapid HbA_{1c} assays for use in the clinic will be available soon.
- Obese patients respond less rapidly to subcutaneous injection of soluble insulin due to poorer subcutaneous blood flow.
- A multiple injection regimen allows a degree of flexibility but does not improve overall glycaemic control.
- The annual rate of progression to requiring insulin therapy in NIDDM is 5%, and insulin treatment in poorly controlled patients is associated with a significant improvement in well-being.
- Pre-conception care of IDDM women is essential not only medically but also economically.

protein (LDL)-cholesterol levels. The lack of importance of fibre itself in achieving blood glucose control should not be confused with the proven value of complex as opposed to simple carbohydrate foods.

Caution is required in interpreting the results of a study which compared the glycaemic and hyperlipidaemic effects of two large meals with those of six small meals in an 8 hour period.⁶ The amplitude of blood glucose changes and the mean blood glucose and plasma insulin incremental areas were smaller with the divided meals. However, absolute mean blood glucose levels were not dissimilar and in considering whether or not to endorse the 'grazing' pattern of eating, it has to be considered that in everyday life more calories are likely to be consumed in this way. Perhaps our patients will enjoy lower amplitudes of glycaemic excursion as they get fatter.

One popular food for 'grazers' is pizza, and it may be bad news for pizzerias that this food apparently causes late postprandial hyperglycaemia in IDDM subjects for at least 9 hours.⁷ Before condemning the pizza, it has to be said that the rise in plasma free insulin was substantially lower on the pizza day compared with the control meal day despite standardization of injection technique.

(c) Insulin therapy

It should be regarded as astonishing that current therapy works reasonably well despite dumping insulin into a tissue ill equipped to receive or dispense the hopeful dose. Injection sites bear some consideration. It has been known for some time that insulin is absorbed more rapidly when injected into the abdominal subcutaneous tissue rather than elsewhere, and this clinically important detail has recently been confirmed and extended.⁸ Using combined injections of [¹²⁵I]insulin and [⁹⁹Tc]pertechnetate the early phase of insulin absorption and subcutaneous blood flow was assessed. In non-obese subjects the first 10% of the insulin had been absorbed from the abdomen in 29 minutes and from the thigh in 53 minutes. However, in the

obese subjects (BMI 28–41) the equivalent figures were 62 and 80 minutes. Not only was the absorption half as slow from the obese abdomen but the differential between injection sites appeared to be less. The differences were related to subcutaneous blood flow as assessed by the ⁹⁹Tc washout. The practical implication is that obese patients will not respond to subcutaneous injection of soluble insulin as rapidly as may be expected.

Another approach to speeding the absorption of insulin has been to change the molecule itself. The insulin analogue B28Asp has similar receptor binding properties to insulin itself.⁹ This is important as earlier analogues stimulated growth factor receptors and were carcinogenic in rats. In healthy volunteers, insulin action of the analogue increased to 64% of maximum in 45 minutes compared with 30% for human insulin over the same time after injection. Whether this could confer clinical benefits remains uncertain, as does any commercial decision to market such a new product in the wake of the media hysteria over human insulin.

Conventional wisdom links the occasional inadvertent intramuscular injection of insulin with higher risk of hypoglycaemia. In a study which compared intramuscular thigh, subcutaneous thigh and subcutaneous abdomen injection as part of a basal/bolus type regimen over 3 months, this has been shown not to be so.¹⁰ The blood glucose profile and rates of hypoglycaemia were similar with intramuscular thigh and subcutaneous abdomen injection, but most interesting of all was the demonstration that subcutaneous injection into the thigh before the evening meal was associated with significantly lower blood glucose levels at 3 a.m. despite a lower dose of isophane insulin at bedtime.

Although lipoatrophy became exceptionally rare after the introduction of highly purified insulins, occasional severe cases still occur. The species of insulin used does not appear important, and another case of lipoatrophy brought about solely by use of human insulin has been reported.¹¹ Within one year of therapy the problem became evident, and in this case the authors report regression over 10 weeks after changing to continuous insulin infusion. They hypothesize that avoidance of the complexed insulin in intermediate acting preparations could be responsible for the reversal of the abdominal lipoatrophy and the stabilization of the condition over the thighs.

There is a considerable danger that the results of the Diabetic Control and Complications Trial (DCCT) (see below) may be interpreted as indicating that a multiple injection regimen, rather than intensive supervision, improved blood glucose control. Whilst multiple injections confer a degree of flexibility it has been shown before that such regimens do not improve control compared with

twice daily combined insulin injections providing that the degree of nurse and doctor input is controlled. These data are extended by a large retrospective study of Danish children.¹² Mean HbA_{1c} levels were almost identical in those treated with twice daily compared with multiple injection regimens. Of concern is the observation that obesity became a greater problem in girls on multiple injections.

Interest continues in the question of treating advanced 'NIDDM' with insulin.¹³ Most studies observe a low incidence of hypoglycaemia, but this apparent difference between IDDM and NIDDM disappears if groups with equivalent duration of insulin therapy are compared.¹⁴ Future studies will have to take careful note of any change in blood pressure after the observation of a 16 mm/9 mm rise in systolic/diastolic pressure in a group of NIDDM subjects who required insulin therapy.¹⁵ The rise was greatest in the most obese. This change was seen in Black and Asian subjects and has not been previously reported in the many studies on Caucasians, thus raising questions about racially determined susceptibility to insulin-induced increments in blood pressure. If once-daily isophane insulin is to be used, a marginal advantage has been shown for administering the dose in the evening rather than the morning.¹⁶ The same was found to be true when patients were given both insulin and sulphonylureas.¹⁷ The latter study was well designed in that it was possible to compare the effect of the combination therapy with insulin alone. As may have been expected, giving both insulin and sulphonylureas conferred no advantage but did push up the cost of treatment.

A multicentre study of four different insulin regimens in NIDDM people with inadequate control on diet and oral agents suggested that treatment with multiple injections of short-acting insulin led to greater weight gain than two or less injections per day (2.9 versus 1.8 kg in 3 months, respectively).¹⁸ If a single daily dose of isophane insulin was to be added to oral agent therapy this was most appropriately given in the evening rather than in the morning. The study also demonstrated that well-being improved in all people given insulin compared with the control group who were maintained on oral agent therapy. The latter point is most important as there remains a bias against use of insulin in the minds of many doctors. This may reflect confusion of the issue of non-compliance with the natural history to NIDDM towards pancreatic failure. Treatment which makes people feel better must be good news.

(d) Oral agents

Sulphonylureas and metformin remain the mainstay of oral therapy. A remarkable personal series

of 1,133 NIDDM patients covering the first 30 years of oral agent therapy has been published.¹⁹ The rate of proceeding to require insulin therapy was observed to be 5% per year. The author concluded that the biggest problem in management is the tendency to continue to use oral agents in the face of hyperglycaemia rather than change to insulin therapy.

Double-blind randomized crossover studies are always required to establish the extent of therapeutic efficacy, and such studies are often lacking for older drugs including metformin. In a group of recently diagnosed overweight NIDDM subjects, 3 months of metformin therapy resulted in mean fasting blood glucose of 6.8 mmol/l compared with 8.3 mmol/l on diet alone.²⁰ The improvement was shown to result from increased insulin sensitivity in both muscle and liver, although there was no direct stimulation of muscle glycogen synthase.

The question of efficacy of acarbose continues to be posed, not least because of the recent UK licensing of this product. All of the earlier controlled studies showed that acarbose had no effect on overall blood glucose control even though the shape of the postprandial blood glucose curve was changed markedly. The latest study, a randomized placebo-controlled double-blind shows the same, with HbA_{1c} levels of 9.7 and 9.9% on acarbose or placebo.²¹ Surprisingly, the paper concludes that the drug is 'an effective new treatment for diet-treated NIDDM patients'. The authors do not highlight the 79% of treated people who complained of side effects. If acarbose does have a therapeutic niche, it has not yet been identified.

The possibility of improving insulin sensitivity and hence blood glucose control by modifying plasma non-esterified fatty acid (NEFA) levels has received much attention recently. Depending upon the experimental conditions, increased peripheral tissue insulin sensitivity²² or suppression of hepatic glucose output²³ may be observed. Acipimox, a nicotinic acid derivative, achieves rapid suppression of plasma NEFA levels and also has an independent effect on increasing glucose uptake by peripheral tissues.²⁴ However, using a long-acting Acipimox preparation in moderately obese NIDDM men, a decrease in gluconeogenesis was observed without any change in overall hepatic glucose output or fasting blood glucose.²⁵ When used as chronic therapy, no effect of Acipimox on insulin sensitivity or plasma glucose could be detected even though the plasma lipid profile was significantly changed.^{26,27} Previous well-designed studies have shown bezafibrate to have beneficial effects upon fasting blood glucose in NIDDM. It is therefore surprising that two studies have shown the closely related agent gemfibrozil to have no effect upon blood glucose control and insulin

action.^{28,29} As both gemfibrozil and bezafibrate exert major beneficial effects upon the plasma lipid profile in diabetes this area requires further carefully designed research.

(e) *Pancreas transplantation*

Now that segmental pancreas transplantation is associated with one year graft survival rates of almost 70%, it has to be considered as a treatment option in any insulin-dependent diabetic undergoing renal transplantation. The major justification for removing the need for strict dietary regimes, frequent blood glucose monitoring and insulin injection is that of the improved quality of life. A recent study has compared the well-being of groups of individuals who have had either successful or unsuccessful pancreatic transplantation.³⁰ The groups did not differ in duration of diabetes or of complications, but the successful group reported significantly better quality of life and perception of health. It is noteworthy that 52% of the successful group were employed, whereas this was so in only 23% of those with failed pancreas transplants.

Intuitively, it may be thought that islet transplantation would be more likely to be satisfactory than the gross procedure of implanting part of an organ most of which is unwanted. However, human islets transplantation has proved to be unsatisfactory to date. If islets from other sources or glucose-sensing, insulin-producing bioengineered cells are to be used, it is likely that some physical barrier to prevent immune attack will be needed.³¹ Work on both diffusion chambers and microencapsulation is proceeding steadily, although clinical application is not yet in sight.

Pregnancy

Hard data on the cost-benefit ratio of any therapy is frequently demanded by purchasers of health care. Such information has been gathered with respect to provision of pre-conception care for women with IDDM.³² Using best available estimates of increased risks of congenital malformations (2.4% versus 8.3% for pre-conception care and pregnancy only care, respectively) and maternal adverse outcomes, there were clear savings associated with pre-conception care. The total medical costs of a pregnancy with pre-conception care totalled \$17,519 compared with \$13,843 for pregnancy care alone, but when maternal and neonatal adverse outcomes were included there was an average saving of \$1,720 per pregnancy by applying good overall management of the individual. The authors did not attempt to assess the emotional cost of congenital malformations.

The need for more emphasis on education of all

women of child-bearing age has been commented on in several small studies.³³ A larger scale perspective has been provided by the state-wide programme of education about the need for good pre-pregnancy control of IDDM which was launched in Maine in 1985.³⁴ The results provide further validation for the data used in the above cost-benefit calculation. The rates of congenital malformation and fetal/neonatal death were 1.6% and 6.4% for those having pre-conception care. The respective rates for those not having such care were 6.5% and 21.1%. Naturally in such a study the two groups were not precisely matched, but the only major difference was in the percentage of smokers (13 versus 31%), with minor differences in age and duration of diabetes. The latter factors may have been expected to bias the data against a more favourable outcome for the pre-conception care group. The message is unequivocal: action at the national level can affect the behaviour of patients and doctors.

In view of data from Italy and the USA suggesting that impairment of glucose tolerance (IGT) (not diabetes) in pregnancy may be associated with poorer obstetric outcome, a large study from Belfast is particularly welcome.³⁵ By comparing groups of women with pregnancy-associated impaired glucose tolerance (120), normal glucose tolerance (826) and IDDM (135), Hadden's group demonstrated the lack of effect on fetal outcome or neonatal morbidity associated with IGT. This was so despite a higher rate of induced labour (43% versus 32%) and Caesarean section (24% versus 14%) in the IGT group compared with the normal group. The expected higher rate of problems in the IDDM group was confirmed with 28% pregnancy induced hypertension, 3% stillbirths and 3.7% major malformations. Urinary tract infections were also commoner in the latter group (20% compared with 5% for normal and IGT groups).

The higher prevalence of gestational diabetes in women from ethnic minorities in the UK has been quantified using data on over 11,000 women screened for diabetes in pregnancy between 1984 and 1988.³⁶ The prevalence rose from 0.4% in Whites to 1.5% in Blacks and 4.4% in Indians. The anticipated risk factors of obesity, parity and age appeared to operate to approximately similar extents in each ethnic group. The assumption that glucose tolerance deteriorates as an inevitable physiological change in pregnancy is challenged by data from Tanzania.³⁷ Two hour post-glucose load levels did not change during or after pregnancy in this group of women who tended to continue physical work up to the onset of labour and who had a mean BMI of 21.5%.

Traditionally, a sudden fall in insulin requirements in late pregnancy is regarded as an indicator of feto-placental compromise. It is not

uncommon to observe a slight decline in insulin requirements from 36 weeks gestation to term, and this was found to average 3.0 units (12% of daily requirement) in IDDM.³⁸ This trend did not occur in those IDDM women within 5 years of diagnosis nor in insulin treated gestational diabetic women.

Is pregnancy bad for you? Textbooks still list parity as an independent risk factor for the later development of NIDDM. Now data from 113,606 nurses consign this to history.³⁹ After correcting for age and obesity, parity had no bearing on the risk of developing NIDDM.

What causes NIDDM?

The pursuit of genes which may carry abnormalities related to the cause of NIDDM was notably unsuccessful until 1992. Until then the gene coding for glucokinase, the enzyme regulating glucose-stimulated insulin release from the beta cell and glucose uptake by the liver, was one of many possible 'candidate genes'. It is now established that this gene is defective in some cases of maturity onset diabetes of youth.^{40,41} This is an autosomal dominant form of NIDDM and is rare, but the excitement lies in that it represents the first definite genetic defect in a disease long thought to have a predominantly genetic basis. The question immediately arises as to whether abnormalities of this gene could underlie the common form of NIDDM. Several studies suggest that this is not the case, with no significant abnormalities being found in 60 Black Americans,⁴² nor in a detailed analysis of 12 Caucasian pedigrees⁴³ and only one out of 209 Japanese subjects.⁴⁴ However, when gestational diabetic subjects who also had a first degree relative with NIDDM were screened, two out of 40 subjects were found to be heterozygous for functionally relevant defects in enzyme structure.⁴⁵ The full picture is yet to emerge, but it is unlikely that this gene defect is common.

Diabetes virtually never occurs in the presence of normal pancreatic islet function. The enigma of the dysfunctional islets in NIDDM continues but further information in emerging about the role of islet amyloid. This substance, which is laid down in close proximity to the beta cell membrane, is very likely to be involved in the decline in insulin secretory capacity in some people with NIDDM. Rhesus monkeys develop a form of diabetes indistinguishable from human NIDDM, and recent data demonstrate that islet amyloid precedes the onset of hyperglycaemia.⁴⁶ All eight animals in the group with overt diabetes exhibited this pathological change. The question should now be amenable to study of whether the amyloid appeared as a consequence of the phase of insulin hypersecretion (amylin is co-secreted with insulin)

or whether it appeared as a primary abnormality. Naturally other pathology may affect insulin secretion. Concern about the prevalence of undiagnosed haemochromatosis in the diabetic clinic prompted a study of the effect of desferrioxamine on NIDDM subjects.⁴⁷ The authors treated nine patients with NIDDM and a raised serum ferritin for up to 8 months but were unable to find any effect on basal or stimulated insulin production despite a decrease in serum ferritin. It is rather more likely that some people initially labelled NIDDM do in fact have autoimmune diabetes, explaining a small part of the presumed heterogeneity in islet pathology in NIDDM. Out of 201 people diagnosed as having NIDDM, 18 were noted to have islet cell autoantibodies.⁴⁸ After 3 years, nine out of the 11 antibody-positive NIDDM subjects were on insulin therapy compared with none of a group of 10 matched but antibody-negative NIDDM subjects. This is of aetiological interest but the findings do not indicate the need for another laboratory test. All therapy in the study was determined upon clinical need.

The proclivity of diabetic women to produce large babies and the familial nature of NIDDM may lead one to suppose that people developing NIDDM may have been on the large size at birth. This supposition has now been exploded. A population based study of men and women for whom complete birth records exist has shown convincingly that the reverse is true.⁴⁹ The prevalence of impaired glucose tolerance or undiagnosed diabetes rose in a stepwise fashion from 6% in those who weighed more than 3.4 kg at birth to 27% in those who weighed less than 2.5 kg at birth. This was not the first study to observe this trend, but it does provide data on both men and women and it was able to eliminate possible confounding effects of length of gestation and social class. The effect appeared to be much more pronounced in women than in men (NIDDM prevalence of 5, 12, 25, 43% and 7, 10, 13, 13%, respectively, for each quartile of birthweight). The same effect was apparent when a larger but overlapping data set was examined with respect to the syndrome of IGT, hypertension and above average triglyceride levels (loosely claimed to be syndrome X).⁵⁰ Perhaps not surprisingly in view of the foregoing, adults who were of low birth weight

What causes NIDDM?

- The glucokinase gene has been shown to be defective in some cases of the rare autosomal dominant form of NIDDM.
- The deposition of islet amyloid is likely to be involved in the decline in insulin secretory capacity in some cases of NIDDM.
- In a population-based study low birthweight was associated with impaired glucose tolerance and the development of NIDDM.

have a subnormal beta cell response to glucose.⁵¹ The group responsible for all these studies believe that the phenomenon is related to maternal nutrition or the intra-uterine environment. It must also be considered that genetically determined insulin resistance or impaired capacity to produce insulin could cause both the fetal and adult outcomes. This is so because insulin is a growth factor *in utero*, and resistance to normal fetal insulin levels or inability to secrete adequate insulin could cause firstly subnormal antenatal growth and secondly abnormal metabolism later in life.

Although lack of physical activity is not strictly an aetiological factor in NIDDM, it has been regarded as a potential modulator of the time at which symptomatic disease may present. A study of 7,611 British men has now established that both occasional and frequent physical activity is associated with decreased chances of having a fasting blood glucose above 7.8 mmol/l (86% and 62% chances, respectively).⁵² Similar findings were reported for Pima Indian men, although in this population the level of physical activity was so low in women that sensible analysis was not possible.⁵³

Pathophysiology of NIDDM

(a) *Insulin secretion*

Attempts to fit observations into rigid hypotheses have led to confusion about the presence or otherwise of hyperinsulinaemia or decreased insulin secretory capacity in NIDDM. Raised fasting plasma levels of immunoreactive insulin in NIDDM have been reported over many years, and recently it became fashionable to suppose that this reflected solely an assay problem. The situation is now clear. In a group of newly presenting Caucasian NIDDM subjects, true fasting insulin levels were about 12 pmol/l (25%) higher than control values, and the potentially cross-reacting split proinsulin and intact proinsulin were each about 6 pmol/l (three-fold) raised.⁵⁴ After oral glucose, true insulin levels rose more briskly in the control subjects. These findings reproduce those of many previous studies but their importance lies in the fact that specific immunoradiometric assays were used, hence ending the confusion about potential assay problems. Thus true basal hyperinsulinaemia co-exists with a markedly diminished dynamic response to acute secretory stimuli.

The same group demonstrated the impairment of acute insulin secretory response in subjects with impaired glucose tolerance.⁵⁵ More information is accumulating on the change in beta cell function with time and the progression of beta cell defect in a group with impaired glucose tolerance was documented by Cook and colleagues.⁵⁶ Beta cell

function, expressed as percentage of normal, had deteriorated significantly over 2 years from a median of 90% to 75%.

Indirect evidence for the biological action of islet-associated polypeptide was obtained by study of a patient with an endocrine pancreatic tumour secreting the polypeptide.⁵⁷ Intravenous glucose tolerance tests showed that insulin secretion was markedly impaired in the presence of a 400-fold excess of islet-associated polypeptide and within one year fasting blood glucose had risen to over 20 mmol/l. Whilst the possibility of coincidental and unrelated diabetes cannot be ruled out, this would appear unlikely. Indeed the authors were able to show that the patient's serum could inhibit insulin release *in vitro* from cultured rat islets. The importance of the case report lies in the probable relationship of islet-associated polypeptide to decreased insulin secretion but unchanged insulin sensitivity.

(b) *Insulin sensitivity*

The familial nature of NIDDM has prompted research into the possible genetic nature of defects in insulin sensitivity. Observations of impaired insulin sensitivity in first-degree relatives of NIDDM subjects have been made before, but the report of such changes in offspring of two NIDDM parents differed in several respects.⁵⁸ The group of subjects, average age 39 years, were shown to have normal glucose tolerance by WHO criteria. Rates of insulin-stimulated glycogen synthesis were decreased by around 40% compared with weight-matched controls who did not have a family history of diabetes. Both fasting and stimulated rates of insulin production were elevated, this being established by C-peptide measurement to circumvent any problem in measurement of true plasma insulin. Thus these people who were at high risk of developing NIDDM exhibited decreased insulin sensitivity before decreased insulin secretion.

If the problem of decreased stimulation of the key enzyme glycogen synthase was genetically determined, it may be expected that it would be detectable in cells cultured from NIDDM subjects. In order to test this hypothesis, skin fibroblasts from NIDDM subjects with a strong family history of the disease were grown in culture.⁵⁹ After many passages in culture the cells were found to exhibit decreased rates of glycogen synthesis both basally and in response to insulin when compared with cells grown from people without diabetes and without a family history of diabetes. Not all actions of insulin were impaired, and this reinforces the concept that specific aspects of insulin sensitivity can be impaired, implying that the defect in NIDDM may lie in the control of the pathway for stimulation of glycogen synthase.

Pathophysiology of NIDDM?

- Specific immunoradiometric assays have confirmed that basal hyperinsulinaemia coexists with a markedly diminished dynamic response to acute secretory stimuli in NIDDM.
- People at high risk of developing NIDDM exhibit decreased insulin sensitivity before decreased insulin secretion.
- The primary target for the insulin receptor tyrosine kinase activity (IRS-1) has been demonstrated.

Decreased insulin sensitivity could result from defects at any point of the pathway of intracellular insulin signalling. The first step in the pathway is binding of insulin to its cell surface receptor. The complete sequence of the receptor has been known for about 6 years, and increasing numbers of naturally occurring mutations have been reported. A recent review listed 40 documented mutations resulting in varying degrees of insulin resistance.⁶⁰ Although it is unlikely that any one mutation accounts for a substantial number of cases of NIDDM, it has to be acknowledged that 0.1–1.0% of the general population are heterozygous for insulin receptor gene mutations, and this could represent another susceptibility factor for low insulin sensitivity.

Just as the sequencing of the insulin receptor opened up the possibility of identifying mutations, identification and sequencing of the molecules involved in passing on the insulin signal will allow testing of normality in NIDDM. Recently the putative main target for the insulin receptor's kinase activity, insulin receptor substrate 1 (IRS1), has been identified and characterized.^{61,62} IRS1 appears to act as a docking protein, allowing interaction with several different proteins and thus possibly providing the main branch point on the pathways of insulin action. Up to 20 possible association sites are present on IRS1, and each may be specific for another protein containing characteristic sequences. The complete characterization of the insulin signalling pathway might be in sight.

Although there is a major defect of cellular insulin action in NIDDM, this does not preclude the presence of other defects which prevent optimum muscle glucose uptake. Uptake depends upon delivery of substrate by the vascular tree, and insulin has a modest action at physiological concentrations to increase muscle blood flow. Baron and colleagues have now demonstrated that this action is decreased in NIDDM, with a maximum increase of 1.3-fold compared to 1.6-fold in weight-matched normal controls.⁶³ The insulin concentrations which brought about a half-maximal effect were 266 and 957 pmol/l for lean and obese normal controls, respectively, but could not be estimated for the NIDDM group because of the low response. Although this reflects a further facet of decreased insulin action—on vascular smooth muscle—the

significance of the observation lies in explaining the lack of precise relationship between overall *in vivo* glucose uptake and myocyte response to insulin.

The close interrelationship of carbohydrate and lipid metabolism has therapeutic implications in that therapy to decrease plasma fatty acids would be expected to increase muscle glucose oxidation and suppress hepatic glucose uptake. The magnitude of these effects has been the subject of many studies. Two recent studies have examined the action of acipimox, a nicotinic acid analogue. Walker and colleagues found that acute doses of acipimox lowered hepatic glucose output more than placebo during a subsequent low-dose insulin infusion during which no drug effect upon forearm glucose uptake was evident.²³ The second study attempted to identify the effects of suppressing plasma fatty acid levels overnight, and used under these circumstances the drug decreased fasting hepatic glucose output by 21% and insulin-stimulated glucose oxidation rates by 26%.²⁷

Several drugs are known to change insulin sensitivity. Oral contraceptive agents have been investigated particularly because these agents are taken by large numbers of healthy women. The effect of the progestogen component to decrease insulin sensitivity has been recognized for some time, with some agents such as levonorgestrel being most potent. Continuous subdermal levonorgestrel was observed to cause slight hyperinsulinaemia and decreased glucose tolerance within one month.⁶⁴ A large-scale epidemiological study has estimated that past oral contraceptive use brought about a 10% increase in risk of developing NIDDM.⁶⁵ Although the authors regard this as a small risk, it clearly is relevant to consider it in those individuals most likely to develop NIDDM in later life. Godsland and colleagues examined the effect of hormone replacement therapy in postmenopausal women by comparing one of the newer progestogens, norethindrone, delivered percutaneously in combination with oestrogen with a standard oestrogen + levonorgestrel oral preparation.⁶⁶ Although the former preparation caused no deterioration in insulin sensitivity, further information on plasma lipid changes will be required before this approach can be regarded as optimal in women prone to NIDDM. This is so because of the major protective effect of standard hormone

What causes IDDM?

- Current genetic markers alone or in combination have a low predictive value in identifying those individuals who will develop IDDM.
- Independent relationships exist between both early exposure to cow's milk and the presence of beta-lactoglobulin antibodies with the development of IDDM.
- Future studies will determine whether nicotinamide or related agents will prevent the onset of IDDM.

replacement therapy on the development of ischaemic heart disease.

Amongst the environmental factors which can impair insulin action, ethanol has received little attention. In elderly men, elevation of plasma ethanol levels to around 15 mmol/l (about 70 mg/dl) brought about a 63% decrease in rates of insulin-mediated glucose storage.⁶⁷ This was largely an effect of decreased glucose oxidation, as there was a 100-fold increase in plasma acetate, an eight-fold increase in muscle lactate and no change in muscle glycogen synthase activity. Ethanol did not change muscle blood flow. The authors point out that this effect would be clinically insignificant in lean healthy individuals, but if they happen to be in the subclinical phase of NIDDM, then it would be deleterious. Perhaps if such individuals walk to the pub the effect of alcohol will be cancelled out by the physical exercise.

What causes IDDM?

Both genetic and environmental influences are important in the aetiology of IDDM. Between different populations the incidence varies widely within Europe alone, for instance between 4.6 per 100,000 person years in Northern Greece to 42.9 per 100,000 person years in two regions in Finland.⁶⁸ Furthermore, genetically similar populations exhibit widely differing rates as illustrated by the six-fold difference between Estonia and Finland.⁶⁹

The search continues for predictive markers which would identify those individuals destined to develop IDDM. Use of islet cell antibodies (ICA) alone (>4 JDF units) is not of practical use. In a recent study 2.8% of British schoolchildren were found to be positive.⁷⁰ Interest in the combined use of several tests led to an 8 year follow-up study of sibs of IDDM subjects.⁷¹ Overall, 4.4% of the sibs developed IDDM and the risk for an HLA-identical sib was between 10 and 30%. The risk of developing IDDM was found to be 11.8% for DR3- and DR4-positive individuals, and 41% for ICA positivity > 5 JDF units. The relatively low predictive value of the genetic markers alone reflects previous observations that many suscepti-

ble subjects never develop the disease. Combining ICA and DR3 + DR4 positivity, the risk of developing IDDM was 58%. This is too low to be of practical clinical significance but does indicate that, as more precise markers are identified, detecting very early disease is likely to become a possibility. Such predictive combinations will only be valid for specific populations, as certain combinations of DQ A1 and DQ B1 genes were found to be associated with susceptibility or resistance to IDDM to varying degrees in Caucasian, Black and Japanese IDDM subjects.⁷² It must be remembered that 10–20% of IDDM patients do not exhibit known susceptibility genes.

The long-established observations of increased risk of IDDM if cows' milk is introduced early in infancy continue to be confirmed. Analysis of 164 subjects from the Colorado IDDM register allowed comparison of those exposed to cows' milk before the age of 3 months.⁷³ The odds ratio of developing IDDM after early exposure (after appropriate correction for ethnicity, birth order and family income) was 4.5. However, this relationship was restricted to individuals with at-risk HLA markers in whom the odds ratio soared to 11.3 (confidence limits 1.2–102). Independently of early exposure to cows' milk, a relationship between the presence of beta-lactoglobulin antibodies and development of IDDM has been demonstrated in a case-control study.⁷⁴

Remarkably, attempts to hit the immune system with the sledgehammer of cyclosporin A have continued in the hope that the specific immune attack on the beta cells will be halted. In combination with a programme of vigorous exercise and strict diet, a 7 year period without insulin therapy was observed in a single subject.⁷⁵ In 14 IDDM patients aged 2–9 years a honeymoon phase was observed in four patients for the unremarkable times of 4, 12, 15 and 30 months.⁷⁶ The cyclosporin side effects of anorexia, stomach pains, poor weight gain, hypertrichosis, gum hyperplasia, anaemia and elevated creatinine were listed. The authors conclude that nicotinamide may be a better bet than cyclosporin. A multi-centre, prospective placebo-controlled trial of this substance is planned.⁷⁷

References

1. Guthrie, R., Hellmean, R., Kilo, A. *et al.* A multisite physicians office laboratory evaluation of an immunological method for the measurement of HbA1c. *Diabetes Care* 1992, **15**: 1494–1498.
2. Stewart, M.W., Laker, M.F. & Alberti, K.G.M.M. Effects of self monitoring of triglyceride concentrations in non-insulin dependent diabetes. *Br Med J* 1993, **306**: 493.
3. Close, E.J., Wiles, P.G., Lockton, J.A., Walmsley, D., Oldham, J. & Wales, J.K. The degree of day-to-day variation in food intake in diabetic patients. *Diabet Med* 1993, **10**: 514–520.
4. Ireland, P., Odea, K. & Nankervis, A. How important is a high carbohydrate, high fibre diet in the management of insulin dependent diabetes mellitus. *Diabet Nutr Metab* 1992, **5**: 113–120.
5. Nuttall, F.W. Dietary fiber in the management of diabetes. *Diabetes* 1993, **42**: 503–508.
6. Bertelsen, J., Christiansen, C., Thomsen, C. *et al.* Effect of meal frequency on blood glucose, insulin, and free fatty acids in NIDDM. *Diabetes Care* 1993, **16**: 4–7.

7. Ahern, J.A., Gatcomb, P.M., Held, N.A., Petit, W.A. & Tamborlane, W.V. Exaggerated hyperglycemia after a pizza meal in well-controlled diabetes. *Diabetes Care* 1993, **16**: 578–580.
8. Vora, J.P., Burch, A., Peters, J.R. & Owens, D.R. Relationship between adsorptions of radiolabeled soluble insulin, subcutaneous blood flow, and anthropometry. *Diabetes Care* 1992, **15**: 1484–1493.
9. Heinemann, L., Heise, T., Jorgensen, L.N. & Starke, A.A.R. Action profile of the rapid acting insulin analogue: human insulin B28Asp. *Diabet Med* 1993, **10**: 535–539.
10. Henriksen, J.E., Djurhuus, M.S., Vaag, A., Thyse-Ronn, P., Hother-Nielsen, O. & Beck-Nielsen, H. Impact of injection sites for soluble insulin on glycaemic control in Type 1 (insulin-dependent) diabetic patients treated with a multiple insulin injection regimen. *Diabetologia* 1993, **36**: 752–758.
11. Chantelau, E., Reuter, M., Schotes, S. & Starke, A.A.R. Severe lipotrophy with human insulin: successfully treated by CSII. *Diabet Med* 1993, **10**: 580.
12. Mortensen, H.B., Villumsen, J., Volund, A., Petersen, K.E. & Nerup, J. The relationship between insulin injection regimen and metabolic control in young Danish type I diabetic patients. *Diabet Med* 1992, **9**: 834–839.
13. Henry, R.R., Gumbiner, B., Ditzler, T., Wallace, P., Lyon, R. & Glauber, H.S. Intensive conventional insulin therapy for type II diabetes: metabolic effects during a 6-month outpatient trial. *Diabetes Care* 1993, **16**: 21–31.
14. Hepburn, D.A., MacLeod, K.M., Pell, A.C.H., Scougal, I.J. & Frier, B.M. Frequency and symptoms of hypoglycaemia experienced by patients with Type 2 diabetes treated with insulin. *Diabet Med* 1993, **10**: 231–237.
15. Randeree, H.A., Omar, M.A.K., Motala, A.A. & Seedat, M.A. Effect of insulin therapy on blood pressure in NIDDM patients with secondary failure. *Diabetes Care* 1992, **15**: 1258–1263.
16. Seigler, D.E., Olsson, G.M. & Skyler, J.S. Morning versus bedtime isophane insulin in Type 2 (non-insulin dependent) diabetes mellitus. *Diabet Med* 1992, **9**: 826–833.
17. Soneru, I.L., Agrawal, L., Murphy, J.C., Lawrence, A.M. & Abrait, C. Comparison of morning or bedtime insulin with and without glyburide in secondary sulfonylurea failure. *Diabetes Care* 1993, **16**: 896–901.
18. Yki-Jarvinen, H., Kauppila, M., Kujansyuu, E. *et al.* Comparison of insulin regimens in patients with non-insulin dependent diabetes mellitus. *N Eng J Med* 1992, **327**: 1426–1433.
19. Nabarro, J.D.N. Oral hypoglycaemic agents: the first thirty years. *J R Coll Phys Lond* 1992, **26**: 50–55.
20. Johnson, A.B., Webster, J.M., Sum, C.F. *et al.* The impact of metformin therapy on hepatic glucose production and skeletal muscle glycogen synthase activity in overweight type II diabetic patients. *Metabolism* 1993, **42**: 1217–1222.
21. Hotta, N., Kakuta, H., Sano, T. *et al.* Long-term effect of acarbose on glycaemic control in non-insulin-dependent diabetes mellitus: a placebo-controlled double-blind study. *Diabet Med* 1993, **10**: 134–138.
22. Johnson, A.B., Argyraki, M., Thow, J.C., Cooper, B.G., Fulcher, G. & Taylor, R. The effect of increased fatty acid supply upon glucose metabolism and skeletal muscle glycogen synthase activity in normal man. *Clin Sci* 1992, **82**: 219–226.
23. Walker, M., Agius, L., Orskov, H. & Alberti, K.G.M.M. Peripheral and hepatic insulin sensitivity in non-insulin-dependent diabetes mellitus: effect of nonesterified fatty acids. *Metabolism* 1993, **42**: 601–608.
24. Fulcher, G.R., Walker, M., Farrer, M., Johnson, A.B. & Alberti, K.G.M.M. Acipimox increases glucose disposal in normal man independent of changes in plasma non-esterified fatty acid concentration and whole-body lipid oxidation rate. *Metabolism* 1993, **42**: 308–314.
25. Puhakainen, I. & Yki-Jarvinen, H. Inhibition of lipolysis decreases lipid oxidation and gluconeogenesis from lactate but not fasting hyperglycemia or total hepatic glucose production in NIDDM. *Diabetes* 1993, **42**: 1694–1699.
26. Fulcher, G.R., Catalano, C., Walker, M. *et al.* A double blind study of the effect of acipimox on serum lipids, blood glucose control and insulin action in non-obese patients with Type 2 diabetes mellitus. *Diabet Med* 1992, **9**: 908–914.
27. Fulcher, G.R., Walker, M., Catalano, C., Agius, L. & Alberti, K.G.M.M. Metabolic effects of suppression of non-esterified fatty acid levels with acipimox in obese NIDDM subjects. *Diabetes* 1992, **41**: 1400–1408.
28. Vuorinen-Markkola, H., Yki-Jarvinen, H. & Taskinen, M.-R. Lowering of triglycerides by gemfibrozil affects neither the glucoregulatory nor antilipolytic effect of insulin in Type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 1993, **36**: 161–169.
29. Vinik, A.I. & Colwell, J.A. Effects of gemfibrozil on triglyceride levels in patients with NIDDM. *Diabetes Care* 1993, **16**: 37–44.
30. Milde, F.K., Hart, L.K. & Zehr, P.S. Quality of life of pancreatic transplant recipients. *Diabetes Care* 1992, **15**: 1459–1463.
31. Lanza, R.P., Sullivan, S.J. & Chick, W.L. Islet transplantation with immunoisolation. *Diabetes* 1992, **41**: 1503–1510.
32. Elixhauser, A., Weschler, J.M., Kitzmiller, J.L. *et al.* Cost-benefit analysis of preconception care for women with established diabetes mellitus. *Diabetes Care* 1993, **16**: 1146–1157.
33. Njenga, E., Lind, T. & Taylor, R. Five year audit of blood glucose control during labour in insulin dependent diabetes. *Diabet Med* 1992, **9**: 567–570.
34. Willhoite, M.B., Bennert, H.W., Palomaki, G.E. *et al.* The impact of preconception counseling on pregnancy outcomes: the experience of the Maine Diabetes in Pregnancy Program. *Diabetes Care* 1993, **16**: 450–455.
35. Roberts, R.N., Moohan, J.M., Foo, R.L.K., Harley, J.M.G., Traub, A.I. & Hadden, D.R. Fetal outcome in mothers with impaired glucose tolerance in pregnancy. *Diabet Med* 1993, **10**: 438–443.
36. Dornhorst, A., Paterson, C.M., Nicholls, J.S.D. *et al.* High prevalence of gestational diabetes in women from ethnic minority groups. *Diabet Med* 1992, **9**: 820–825.
37. Lutale, J.K., Justeen, A., Swai, A.B.M., Alberti, K.G.M.M. & McLarty, D.F. Glucose tolerance during the after pregnancy in nondiabetic women in an urban population in Tanzania. *Diabetes Care* 1993, **16**: 575–577.
38. McManus, R.M. & Ryan, E.A. Insulin requirements in insulin-dependent and insulin-requiring GDM women during final month of pregnancy. *Diabetes Care* 1992, **15**: 1323–1327.
39. Manson, J.E., Rimm, E.A., Colditz, G.A. *et al.* Parity and incidence of non-insulin dependent diabetes mellitus. *Am J Med* 1992, **93**: 13–18.
40. Zouali, H., Vaxillaire, M., Lesage, S. *et al.* Linkage analysis and molecular scanning of glucokinase gene in NIDDM families. *Diabetes* 1993, **42**: 1238–1245.
41. Hattersley, A.T., Christopher, P., Brown, E. *et al.* Linkage analysis of glucokinase gene with NIDDM in Caucasian pedigrees. *Diabetes* 1992, **41**: 1496–1500.
42. Chiu, K.C., Tanizawa, Y. & Permutt, M.A. Glucokinase gene variants in the common form of NIDDM. *Diabetes* 1993, **42**: 579–582.
43. Cook, J.T.E., Page, R.C.L., O'Rahilly, S. *et al.* Availability of Type II diabetic families for detection of diabetes susceptibility genes. *Diabetes* 1993, **42**: 1536–1543.
44. Eto, K., Sakura, H., Shimokawa, K. *et al.* Sequence variation of the glucokinase gene in Japanese subjects with NIDDM. *Diabetes* 1993, **42**: 1133–1137.
45. Stoffel, M., Bell, K.L., Blackburn, C.L. *et al.* Identification of glucokinase mutations in subjects with gestational diabetes mellitus. *Diabetes* 1993, **42**: 937–940.
46. de Koning, E.J.P., Bodkin, N.L., Hansen, B.C. & Clark, A. Diabetes mellitus in Macaca mulatta monkeys is characterised by islet amyloidosis and reduction in beta-cell population. *Diabetologia* 1993, **36**: 378–384.

47. Redmon, J.B., Pyzdrowski, K.L. & Robertson, R.P. No effect of desferrioxamine therapy on glucose homeostasis and insulin secretion in individuals with NIDDM and elevated serum ferritin. *Diabetes* 1993, **42**: 544–549.
48. Gottsater, A., Landin-Olsson, M., Fernlund, P., Lernmark, A. & Sundkvist, G. B-cell function in relation to islet cell antibodies during the first 3 years after clinical diagnosis of diabetes in type II diabetic patients. *Diabetes Care* 1993, **16**: 902–910.
49. Phipps, K., Barker, D.J.P., Hales, C.N., Fall, C.H.D., Osmond, C. & Clark, P.M.S. Fetal growth and impaired glucose tolerance in men and women. *Diabetologia* 1993, **36**: 225–228.
50. Barker, D.J.P., Hales, C.N., Fall, C.H.D., Osmond, C., Phipps, K. & Clark, P.M.S. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X); relation to reduced fetal growth. *Diabetologia* 1993, **36**: 62–67.
51. Cook, T.J.E., Levy, J.C., Page, R.C.L., Shaw, J.A.G., Hattersley, A.T. & Turner, R.C. Association of low birth weight with beta cell function in the adult first degree relatives of non-insulin diabetic subjects. *Br Med J* 1993, **306**: 302–306.
52. Perry, I.J., Wannamethee, S.G., Walker, M.K. & Shaper, A.G. Sporting activity and hyperglycemia in middle-aged men. *Diabetes Care* 1993, **16**: 581–583.
53. Kriska, A.M., LaPorte, R.E., Pettitt, D.J. *et al.* The association of physical activity with obesity, fat distribution and glucose intolerance in Pima Indians. *Diabetologia* 1993, **36**: 863–869.
54. Davies, M.J., Metcalfe, J., Gray, I.P., Day, J.L. & Hales, C.N. Insulin deficiency rather than hyperinsulinaemia in newly diagnosed Type 2 diabetes mellitus. *Diabet Med* 1993, **10**: 305–312.
55. Davies, M.J., Rayman, G., Gray, I.P., Day, J.L. & Hales, C.N. Insulin deficiency and increased plasma concentration and intact and 32/33 split proinsulin in subjects with impaired glucose tolerance. *Diabet Med* 1993, **10**: 313–320.
56. Cook, J.T.E., Page, R.C.L., Levy, J.C., Hammersley, M.S., Walravens, E.K.N. & Turner, R.C. Hyperglycaemic progression in subjects with impaired glucose tolerance: association with decline in beta cell function. *Diabet Med* 1993, **10**: 321–326.
57. Stridsberg, M., Berne, C., Sandler, S., Wilander, E. & Oberg, K. Inhibition of insulin secretion, but normal peripheral insulin sensitivity, in a patient with a malignant endocrine pancreatic tumour producing high amounts of an islet amyloid polypeptide-like molecule. *Diabetologia* 1993, **36**: 843–849.
58. Gulli, G., Ferrannini, E., Sern, M., Haffner, S. & Defronzo, R.A. The metabolic profile of NIDDM is fully established in glucose tolerant offspring of 2 Mexican-American NIDDM parents. *Diabetes* 1992, **41**: 1575–1586.
59. Wells, A.M., Sutcliffe, I.C., Johnson, A.B. & Taylor, R. Abnormal activation of fibroblast glycogen synthesis in familial NIDDM: evidence for an abnormality specific to glucose metabolism. *Diabetes* 1993, **42**: 583–589.
60. Taylor, S.I. Lilly Lecture: molecular mechanisms of insulin resistance: lessons from patients with mutations in the insulin-receptor gene. *Diabetes* 1992, **41**: 1473–1490.
61. Myers, M.G. & White, M.F. The new elements of insulin signaling—insulin receptor substrate 1 and proteins with SH2 domains. *Diabetes* 1993, **42**: 643–650.
62. Araki, E., Sun, X.J., Haag, B.L. *et al.* Human skeletal muscle insulin receptor substrate—characterisation of the cDNA, gene, and chromosomal localisation. *Diabetes* 1993, **42**: 1041–1054.
63. Laakso, M., Edelman, S.V., Brechtel, G. & Baron, A.D. Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes* 1992, **41**: 1076–1083.
64. Konje, J.C., Otolurin, E.O. & Lapido, O.A. The effect of continuous subdermal levonorgestrel (norplant) on carbohydrate metabolism. *Am J Obstet Gynecol* 1992, **166**: 15–19.
65. Rimm, E.B., Manson, J.E., Stampfer, M.J. *et al.* Oral contraceptive use and the risk of Type 2 (non-insulin-dependent) diabetes mellitus in a large prospective study of women. *Diabetologia* 1992, **35**: 967–972.
66. Goddard, I.F., Ganger, K., Walton, C. *et al.* Insulin resistance, secretion, and elimination in postmenopausal women receiving oral or transdermal hormone replacement therapy. *Metabolism* 1993, **42**: 846–853.
67. Boden, G., Chen, D., Desantis, R., White, J. & Mozzoli, M. Effects of ethanol on carbohydrate metabolism in the elderly. *Diabetes* 1993, **42**: 28–34.
68. Green, A., Gale, E.A.M. & Patterson, C.C. Incidence of childhood-onset insulin dependent diabetes mellitus. *Lancet* 1992, **339**: 905–909.
69. Tuomilehto, J., Podar, T., Brigs, G. *et al.* Comparison of the incidence of insulin dependent diabetes mellitus during childhood among 5 Baltic populations during 1983–1988. *Internat J Epidemiol* 1992, **21**: 51.
70. Bingley, P.J., Bonifacio, E., Shattock, M. *et al.* Can islet cell antibodies predict IDDM in the general population? *Diabetes Care* 1993, **16**: 45–50.
71. Deschamps, I., Boitard, C., Hors, J., Busson, M., Marcel-liberge, A. & Robert, J.J. Life table analysis of the risk of type 1 (insulin dependent) diabetes mellitus in siblings according to islet cell antibodies and HLA markers—an 8 year prospective study. *Diabetologia* 1992, **35**: 951–957.
72. Thorsby, E. & Ronningen, K.S. Particular HLA-DQ molecules play a dominant role in determining susceptibility or resistance to type 1 diabetes mellitus. *Diabetologia* 1993, **36**: 371–377.
73. Kostraba, J.N., Cruickshanks, K.J., Lawler-Heavner, J. *et al.* Early exposure to cow's milk and solid foods in infancy, genetic predisposition, and risk of IDDM. *Diabetes* 1993, **42**: 288–295.
74. Dahlquist, G., Savilahti, E. & Landin-Olsson, M. An increased level of antibodies to B-lactoglobulin is a risk determinant for early-onset Type 1 (insulin-dependent) diabetes mellitus independent of islet cell antibodies and early introduction of cow's milk. *Diabetologia* 1992, **35**: 980–984.
75. Koivisto, V.A., Leirisalo-Repo, M., Ebeling, P. *et al.* Seven years of remission in a Type 1 diabetic patient. *Diabetes Care* 1993, **16**: 990–995.
76. Jenner, M., Bradish, G., Stiller, C. & Atkinson, P. Cyclosporin A treatment of young children with newly-diagnosed Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1992, **35**: 884–888.
77. Pociot, F., Reimers, J.L. & Anderson, H.U. Nicotinamide-biological actions and therapeutic potential in diabetes prevention. IDIG Workshop, Copenhagen, Denmark, 4–5th December 1992. *Diabetologia* 1993, **36**: 574–576.