Etiology and Pathogenesis of Non-insulin dependent Diabetes Mellitus (NIDDM): Current concepts

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ABSTRACT

We discuss the etiopathogenesis of NIDDM in the light of new data. The role of insulin deficiency and insulin resistance is discussed. The ultrastructure of amylin in the islet cells is described. The role of abnormalities in hepatic glucose metabolism is discussed. The effect of glucotoxicity on the islet cell funtion is brought out. We have attempted to construct the teleological sequence of pathogenesis of NIDDM describing the role of above discussed etiological factors at various stages. Finally, the role of environmental factors is discussed.

INTRODUCTION

Both genetic and environmental factors play important roles in the genesis of the two cardinal pathophysiologic lesions of type II diabetes, namely "insulin deficency" and "insulin resistance". However, the relative contribution, (primacy and magnitude) and the temporal sequence of these two lesions, in terms of the pathogenesis and clinical manifestations of NIDDM continues to be debated. Increased hepatic glucose output (HGO) and the phenomenon of "glucotoxicity" appear to be secondary lesions in diabetogenesis. (1, 2)

INSULIN DEFICIENCY

Insulin secretory dynamics: In the analysis of insulin secretory dynamics in NIDDM, it is important to distinguish between impaired beta cell secretory function and decreased absolute circulating insulin levels. In NIDDM fasting (basal) insulin levels are normal or “increased” (elevated, but inadequate in reference to prevailing hyperglycaemia i.e., “relative” insulin deficiency). Stimulated insulin levels are low, normal or high, and depend on type of the stimulus and the severity of diabetes. A characteristic pathophysiologic feature of NIDDM is the loss of the first or early phase insulin response to intravenous glucose (fig 1). This lesion is universally found in subjects with fasting plasma glucose greater than 140 mg/dl. This loss of first phase

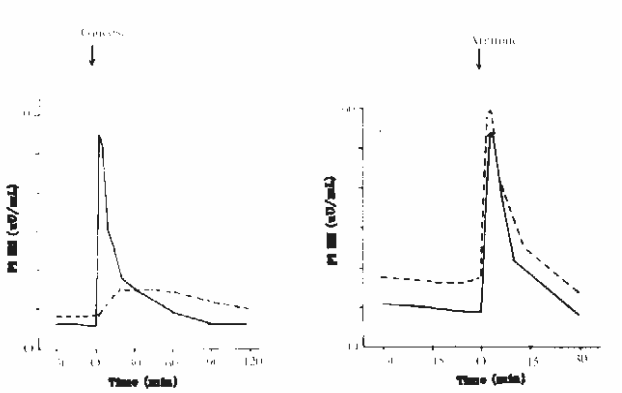

Figure 1 shows loss of first phase insulin response to intravenous glucose infusion in early NIDDM despite no loss of insulin response to intravenous arginine. (Broken line = NIDDM; Continuous line = Controls). (After Ward WK, Beard JC, Halter JB, et al.: Pathophysiology of insulin secretion in non-insulin dependent diabetes mellitus. Diabetes Care 1984; 7: 491-502.)

insulin response to intravenous glucose is “restored” by insulin therapy, salicylates and alpha adrenergic blockers. Second phase insulin response is normal or “low.” On the other hand, insulin responses to other secretogogues like arginine, glucagon, secretin, isoproterenol, and oral glucose is relatively better preserved (but decreased in magnitude compared to that in normal subjects). This “selective” loss of the acute insulin response to intravenous glucose is “non-specific” in that the same lesion is observed in pre-type I diabetes, after pancreatectomy, following high glucose infusions in normal animals, in streptozotocin diabetes; it may thus reflect decreased beta cell mass or over-stimulated beta cells. Glucose augments beta cell response to non-glucose stimuli; and based on this the changes in the acute insulin responses to arginine or isoproterenol can be expressed as a function of increasing plasma glucose, thus yielding a “glucose potentiation slope.” In NIDDM, this glucose potentiation of beta cell functions is reduced.

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Another very early lesion that has recently been described in NIDDM is loss of the normal pulsatile insulin secretory response. Normal individuals in the fasting stage exhibit regular pulses of insulin secretion at a frequency of about 12-15 minutes. But first degree relatives of NIDDM subjects with minimum glucose intolerance exhibit no regular oscillatory activity in insulin secretion (time series analysis of plasma insulin level every one minute for 150 minutes). This lesion is observed even before the impairment of first phase insulin response to intravenous glucose, mentioned above appears (3).

Insulin response to oral glucose and mixed meal (effect of gastrointestinal peptides, and aminoacids) in NIDDM are more variable. In subjects with impaired glucose tolerance (IGT), insulin responses are often increased, in milder NIDDM there may be delay in insulin secretion and in severe NIDDM insulin responses are decreased. Greater the fasting plasma glucose, greater is magnitude of beta cell dysfunction in NIDDM (fig 2).

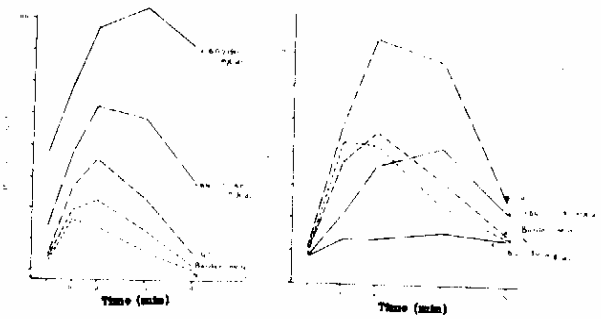


Figure 2 shows Plasma Glucose and Immuno-reactive insulin (IRI) responses to oral glucose in five groups of patients belonging to various 'Stage' of development of NIDDM from normal glucose tolerance to Fasting blood glucose > 150 mg/dL.

In non-diabetic subjects, obesity is associated with increased insulin secretion. Obese subjects with NIDDM also have increased insulin secretion; insulin levels in obese NIDDM are consistently higher than in lean NIDDM.

About 3-25% of subjects with IGT progress to NIDDM subsequently. IGT subjects who are low insulin responders more often progress to NIDDM, compared to IGT subjects who are high insulin responders, thus providing a predictive value for this parameter.

Islet pathology

Islet beta cell mass in NIDDM is reported to be decreased by about 30-50% on the average. Since a loss of 80-90% of the normal insulin secretory reserve is a prerequisite for the development of hyperglycaemia, decrease in the functional capacity of the remaining beta cells in NIDDM is anticipated, along with insulin resistance.

Islet morpological changes in NIDDM are non-specific and non-diagnostic. The most important pathologic lesion is insular hyalinisation (islet amyloidosis). Insular fibrosis manifests as intra and inter-acinar fibrosis, arterio and arteriolo-sclerosis and fatty atrophy of the pancreas. Islet hypertrophy and insular regeneration may be observed in the early stages. Reversible cellular injury to the islet cells include cloudy swelling and hydropic degeneration and glycogen infiltration; whether these lesions progress to irreversible islet cell necrosis is unclear. Margination of granules and degranulation of beta cells are physiologic changes associated with active insulin secretion.

Gross pathological changes in the pancreas in NIDDM include a reduction in weight, up to 50% of normal and accentuation of lobular markings. Insular hyalinisation (amyloidosis) refers to the eosinophilic (amyloid stains positive) meshwork of microfibrils deposited between basement membrane of islet parenchyma and islet capillary network i.e. in the islet interstitium. Ultrastructurally islet amyloid comprises of thin branching microfibrils 75-100 A. Islet amyloid comprises of thin branching microfibrils 75-100 A. Islet amyloid most likely originates in the beta cells, is associated with diabetes of both man and animals (cat, monkey), but is absent in conventional amyloidosis (primary, secondary, or isolated). Its frequency is related both to the presence of diabetes and increased age (staining with metachromatic dyes, Congo red, (a) Age less than 50 Y: DM=15-20%, non-DM 0%; (b) Age greater than 50 Y: DM=50% (4+) in some 90% of islet volume, non-DM=10-15% (1+). Studied by thioflavin fluorescence/electron microscopy, islet amyloidosis is present in greater than 95% of subjects with chemical diabetes of 10-15 Y duration. Islet amyloidosis results in decrease islet volume and may interfere with islet function (7).

Molecular nature of the protein comprising the islet amyloid has been recently characteristed in detail (islet amyloid polypetide, IAPP). IAPP is a novel 37 amino acid polypeptide, (?) putative hormone, with molecular weight of 3850 daltons. IAPP exhibits greater than 40% amino acid sequence identity with the neuropeptide calcitonin gene related peptide (CGRP) and weaker homology with insulin A chain. IAPP is quantitatively the major protein content of islet amloidosis. By immunohistochemical analysis

with anti-IAPP antisera, it is present in all (100%) islet beta cells, but not in alpha cells.

Immunoelectron microscopy reveals a subcellular localisation in the outer electron-lucent compartment of the beta cell granule-10 particles/granule cross section (compared with insulin in the electron dense core-20-50 particles/granule cross section). IAPP possibly has a role in normal endocrine regulatory function (synthesis, storage and cosecretion in beta cell), and may also play a role in the pathogenesis of islet beta cell dysfunction in NIDDM. Study of IAPP like immunoreactivity in the islet beta cells in NIDDM reveals: intracellular beta cell IAPP deficiency, associated with its deposition in the interstitium as islet amyloid (polymerisation, beta pleated) (1) intracellular beta cell IAPP immunoreactivity: (a) non-DM=11/11, (b) NIDDM (very few cells IAPP positive); (2) interstitial amyloid deposits: (a) non-DM=6/11 (3-11% of islets), (b) NIDDM 12/13 (20-99% of islets).

Next to amyloidosis, the most frequent lesion found in NIDDM is insular fibrosis, often seen in older diabetics, in association with atrophy and fibrosis of the exocrine pancreas; aging and vascular lesions both appear to play a role.

Analysis of quantitative changes in islet cell mass has been technically more difficult with problems related to sampling (fragment vs whole pancreas), lack of reported distinction based on type (type I, type II) and duration of diabetes. Older reports have mentioned “decrease in the number of islets and the area occupied by them”; “islet volume markedly decreased in 60%” etc. A study of older diabetics (all ages greater than 50 Y) revealed a significant decrease in total islet and beta cell weights (diabetics vs non-diabetics: (1) total islet weight =0.765 Vs 1.358 g: beta cell weight =0.301 vs .754 g: alpha cell weight =0.319 vs 0.341 g). Studies in NIDDM of shorter duration (age 31-68 y) reveal a normal or increased islet cell mass and evidence of islet hyperplasia and neoformation, with normal beta cell proportions (9,10).

INSULIN RESISTANCE

Insulin resistance is a metabolic state in which a normal concentration of insulin produces a less than normal biological response. It can involve any of the multiple metabolic effects of insulin. However, resistance to the effect on glucose metabolism is the most extensively studied.

Table 1
Causes of insulin resistance

Abnormal beta cell product
- Abnormal insulin molecule
- Incomplete conversion of proinsulin molecule

Circulating insulin antagonists
- Elevated levels of counter regulatory hormones eg. growth hormone, cortisol, glucagon, catecholamines
- Antiinsulin antibodies
- Antiinsulin receptor antibodies

Target tissue defects
- Insulin receptor defects
- Post-receptor defects

Abnormal beta cell secretory product: Mutations of the islet structural gene can result in insulin molecules with abnormal structure and defective biological function. Mutation at the pro-insulin to insulin cleavage sites results in (familial) hyperproinsulinemia. Such molecular defects are extremely rare and have no relevance to the pathogenesis of NIDDM. These subjects are not truly insulin resistant, since they are sensitive to exogenous insulin.

Circulating insulin antagonists: Hormonal insulin antagonists include growth hormone, cortisol, glucagon, nor-epinephrine and epinephrine. In the usual case of obesity or NIDDM excessive levels of counterregulatory hormones are not an important contributory factor to peripheral insulin resistance.

Non-hormonal antagonists include free fatty acids (FFA). Increased circulating FFA and increased intracellular FFA oxidation are associated with decreased peripheral glucose utilization and insulin resistance. However, the pathophysiological role of FFA in the insulin resistance of NIDDM is unclear.

Anti-insulin antibodies develop in almost all subjects receiving exogenous insulin, particularly animal (bovine, porcine, or less purer forms of insulin.) Spontaneous insulin autoantibodies also develop in pre-type I diabetes, and in the spontaneous insulin autoimmune (hyperglycaemia and hypoglycaemia) syndrome. Insulin antibodies bind/trap insulin in the plasma compartment and alter the usual time course of insulin action. However, only in unusual cases do these antibodies cause a true insulin resistance state, and they have no significance in the pathogenesis of NIDDM.

Insulin receptor autoantibodies are present in the very rare type B syndrome of insulin resistance associated with acanthosis nigricans. These receptor antibodies bind to insulin receptors in vivo and cause severe

insulin resistance. They also do not have any role in NIDDM.

Cellular defects in insulin action: Available evidence indicates that target tissue defect(s) in insulin action is a major cause of insulin resistance in NIDDM. This tissue insulin resistance can be due to receptor defects or post-receptor defects.

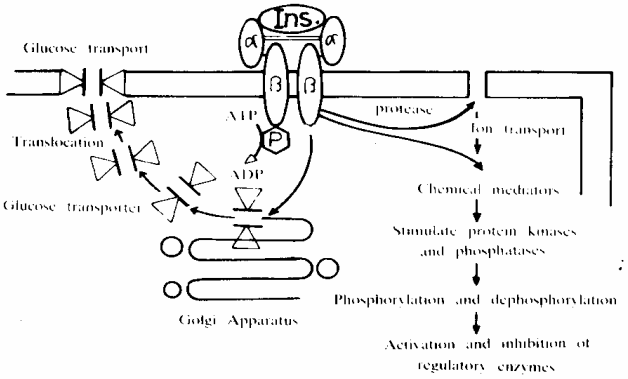


Figure 3 shows proposed model of insulin receptor and post receptor mechanisms involved in the action of insulin action.

Insulin exerts its biological effects by initially binding to its specific cell surface receptor (fig 3). After this binding event, the insulin receptor complex is formed and one or more signals of insulin action are generated. The signal, or second messenger may involve the production of a chemical mediator, a conformational change within the plasma membrane, phosphorylation, alterations in ion flux, or other information transfers. Regardless of its precise physicochemical nature, this signal (or signals) interacts with a variety of effector units, which mediate the entire host of biological actions attributable to insulin. In many instances the effector unit consists of a series of steps such as a sequentially linked enzyme system (i.e., the glycogen synthase/phosphorlase system) or series of enzymes involved in the degradation of a particular substrate (glucose). Another important effector unit in the intracellular mechanism of insulin action leads to ("coupled") translocation of intracellular glucose transporter molecules to the surface of plasma membrane, where they augment glucose influx. Clearly, insulin action involves a cascade of events, and abnormality anywhere along this sequence can lead to insulin resistance. For any convenience, tissue abnormalities in insulin can be categorized under the headings of receptor and post-receptor (or post-binding) defects.

Decreased cellular insulin receptors have been described in a variety of pathophysiological situations including obesity and NIDDM. Decreased insulin receptors have also been described in acromegaly, following glucocorticoid treatment, and after oral contraceptive therapy. The relationship between decreased insulin receptors and insulin action is influenced by the "spare receptor concept" (maximal insulin effect is achieved at a concentration of insulin at which less than the total number of cellular receptor are occupied). For example, in adipocytes 10% insulin receptor occupancy results in maximal stimulation of glucose transport, the remaining 90% of the receptors being "spare"; once the maximal response is attained steps distal to the receptor are rate limiting. In an insulin dose response curve, decrease in the number of insulin receptors results in a rightward shift, with a normal maximum response. Only when the receptor number is severely decreased (i.e. to less than 10% in adipocytes), is there a decrease in the maximal response. Percentage of the spare receptor varies depending on the cell type and particular insulin action measured.

Post receptor defects refer to any abnormality in the insulin action sequence following the insulin receptor binding step [insulin receptor defects: binding normal, but transmembrane signalling defective; abnormal coupling between insulin receptor complexes and glucose transporter system; intracellular enzymatic defects located in various pathways of glucose metabolism].

Post receptor defects result in proportionate decrease in insulin action at all insulin concentrations, including maximally effective hormone levels.

Conventionally, based on insulin dose response curves, receptor defects (with rightward shift) are described to be associated with decreased insulin "sensitivity"; and post-receptor defects (with decreases in the maximal response) with decreased insulin "responsiveness"; combined defects produce a decrease in both "sensitivity and responsiveness" (both rightward shift and decrease in maximum response) (fig 4).

IGT is associated with mild insulin resistance and NIDDM with severe insulin resistance. As the degree of carbohydrate intolerance worsens, frequency of insulin

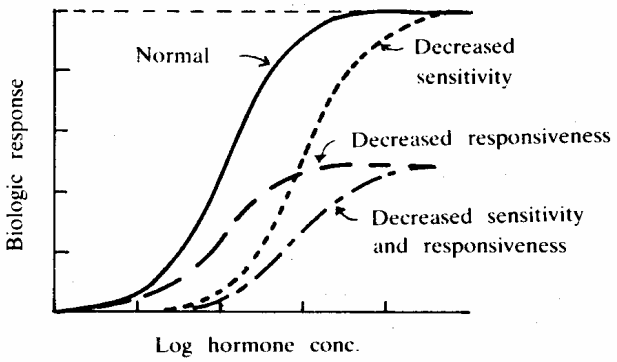


Figure 4 shows the normal dose response curve, and the responses in receptor defect (decreased sensitivity), post receptor defect (decreased responsiveness) and both defects (decreased sensitivity and decreased responsiveness).

resistance increases. Obesity leads to insulin resistance, but does not account for all insulin resistance in NIDDM. Non-obese NIDDM patients are also insulin resistant. In terms of the cellular defects in insulin action, IGT is associated, with decreased insulin receptors (i.e. decrease insulin sensitivity), and NIDDM (with significantly increased plasma glucose) is associated with both decreased insulin receptors and post receptor defect (i.e. decreased insulin sensitivity + responsiveness). Both obese and non-obese NIDDM exhibit post receptor defects. Though the precise biochemical nature of the post-receptor defect(s) in NIDDM remains to be elucidated, it appears to be predominantly a result of a defect in the activity of the plasma membrane glucose transport system (transport governs disposal, for glucose). Additional post-glucose transport intracellular defects in glucose metabolism may exist.

HEPATIC GLUCOSE METABOLISM

Hepatic glucose uptake (not stimulated by insulin) is normal in NIDDM. Hepatic glucose output is however increased, and correlates significantly to insulin deficiency/resistance. The machanisms for increased hepatic glucose output (HGO) in NIDDM include: (1) intra islet insulin deficiency/resistance at the alpha cell level, leading to hyperglucogonemia and increased HGO; in NIDDM, there is also insensitivity to glucose suppression of alpha cell function; (2) hepatic insulin resistance: insulin normally suppresses HGO, and resistance to this effect exists in NIDDM, (3) loss of pulsatile insulin secretory bursts (pulsatile insulin more effective in comparison with continuous insulin delivery); and (4) increased flux of gluconeogenic precursors from peripheral tissues to liver (substrate induced increase in HGO).

GLUCOTOXICITY

Hyperglycemia per se may lead to further functional impairment of the islet beta cells or peripheral tissues, with exacerbation of insulin secretory defects or insulin resistance (hyperglycemia begets more hyperglycemia). Some evidences for these phenomenon include: (1) chronic hyperglycemia desensitises beta cell to glucose stimulation, and exogenously induced hyperglycemia in normal animals can result in loss of beta cell function; (2) control of hyperglycemia by insulin treatment, weight loss, oral hypoglycemic agents leads to improved insulin secretion; and precise mechanisms involved are unclear, but non-enzymatic and down regulation/functional impairment of beta cell glucose recognition system have been postulated; (3) chronic hyperglycemia is associated with mild to severe insulin resistance; and (4) in vitro, increased glucose levels augment the cellular effects of other agents to induce cellular insulin resistance.

Though hyperglycemia can induce reversible cellular functional impairment (both at the islet beta cell and target tissue levels), whether this can progress to irreversible structural damage/cell loss is unclear.

NIDDM: TELEOLOGICAL SEQUENCE

Longitudinal studies of prediabetic Pima Indians, a population with a very high incidence of NIDDM show that they pass through a phase of a decreased insulin stimulated glucose uptake during the transition from normal to impaired glucose tolerance (IGT) state. During IGT their insulin secretory capacity was found to be normal. However when frankly diabetic their insulin secretory capacity was diminished. However, in a study of nondiabetic first degree relatives of NIDDM patients it was found that the earliest defect was at the level of hepatic glucose output (12, 13).

In a metabolic study of five discordant twins, out of 53, it was found that the “unaffected” twins had a higher mean plasma glucose, and a poorer insulin secretion in addition to the abnormal metabolic state (14).

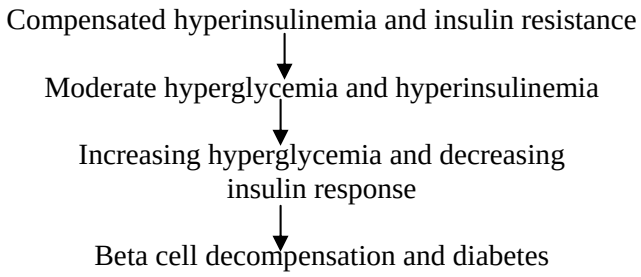
In a study of 3-6 years follow up of 42 rhesus monkeys (Macaca mulata) it was postulated that they pass through 8 phases to become frankly diabetic. In phase 2 the weight increases; in 3, there is a first detectable increase in insulin levels; 4 has significantly high insulin levels; in phase 5 insulin continues to rise with a reduction of disposal rate (Kd) of glucose on IVGTT. Phase 6 is associated with increased fasting plasma glucose and decreased insulin, and a markedly reduced Kd. The last phase has insulinopenic hyperglycemia with reduced weight and body fat proportions. Obesity is necessary but not sufficient trait for development of NIDDM in this group. The primary defect seems to be insulin resistance as shown by euglycemic clamp studies (15).

In the studies of ob/ob and db/db mice it has been shown that initially these animals develop obesity and

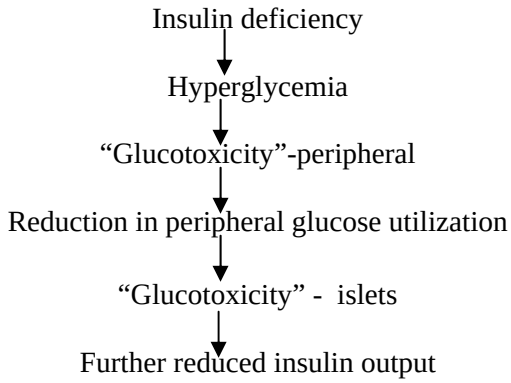
hyperinsulinemia with normal blood glucose, latter increasing over a period of time. Then, the animal may become hyperglycemic and insulinopenic, or, may become normoglycemic with normal insulin concentration-depending on the "genetic background." Sequential histopathology shows B cell hyperplasia, B cell degranulation with increased DNA synthesis and total degranulation, or regranulation, respectively in those developing diabetes or recovering completely. No evidence of insulitis has been found. The insulin receptor number is decreased. Despite hyperinsulinemia gluconeogenic enzymes are increased and insulin dependent enzymes are decreased (16).

Despite decades of intense investigation and research, the precise teleological sequence in the pathogenesis of NIDDM has not yet been conclusively clarified.

A: Sequence one:



B: Sequence two:



GENES

NIDDM has a very strong genetic basis. In monozygotic twins there is almost 100% concordance for the development of NIDDM. Among siblings of NIDDM, about 30% have abnormal glucose tolerance. Incidence of NIDDM in the off-springs (Caucasoid) is 40% when both parents are diabetic 6% if one parent is diabetic and 0% if none of the parents are diabetic; similar figures in a higher risk population (micronesians) are 79%, 4% and 5% respectively. Very high prevalence of NIDDM has been reported in Pima Indians and Naurans (25%, age > 20 years), where OGTT glucose responses show a definite bimodality; single gene defect and gene dose effect (homozygotes: early diabetes onset, heterozygotes: late onset). High prevalence of NIDDM has also been described in Mexican Americans and Asian Indians (including those migrant to different continents). Despite these indirect evidences for the genetic etiology of NIDDM the specific gene(s) leading/contributing to NIDDM remain unrecognised.

The objectives of current genetic studies of NIDDM include; (1) to identify and characterise gene(s) responsible for NIDDM (2) to determine the precise metabolic functions of those genes in healthy individuals; (3) to identify the specific DNA sequences in defective genes; and (4) to consider feasibility of gene replacement therapy.

Table 2 NIDDM: genetics

Insulin Deficiency	Insulin Resistance
--Insulin gene* coding/regulatory	--Insulin receptor gene**
--Islet 'dystrophy' genes	--Glucose transporter
Growth and Differentiation genes (eg.: 'reg.')	--Obesity related genes db, ob, fa

* Insulinopathies
** extreme insulin resistance

Several candidate genes have been examined for their possible association with NIDDM (table 2).

1. Insulin gene is located on chromosome 11; both population and family studies involving RFLP (s) of the 5 flanking region and direct sequencing have not revealed any association with NIDDM.
2. Insulin receptor gene is located on chromosome 19; no association with NIDDM by RFLP (s) studies have been observed (specific mutations in the insulin receptor gene have been identified in the very rare syndromes of extreme insulin resistance)
3. Erythrocyte type glucose transporter gene is located on chromosome 1; no association with NIDDM has been demonstrated by RFLP (s).

Since the currently assessed candidate genes for NIDDM have not yeilded any positive association it is important to examine new paradigms, like the role

of putative genes controlling growth and differentiation of the islet cells. Are mutations and molecular defects in functionally significant islet cell 'differentiation molecules' responsible for the islet beta cell dysfunction/dystrophy of NIDDM (8, 11)

Recently a novel gene ("reg") which is specifically activated in regenerating islets has been identified. Utilizing islet mRNA from remnant pancreases of 90% pancreatectomised rats (treated with nicotinamide for three months) and differential screening, a preferentially hybridizing clone was selected. With a relative abundance of 0.7% in regenerating islets this gene codes for a 165 amino acid protein. This is expressed only in regenerating pancreatic islets, but not in normal islets (trace 1%), insulinomas or regenerating liver. It is also expressed in hyperplastic islets from aurothioglucose treated NOD mice (impaired glucose tolerance, followed by decreased blood glucose). In the 90% pancreatectomised and nicotinamide treated rats, expression of the 'reg' gene parallels islet regeneration and decrease in blood glucose (expression maximum at three months, but negative at 1 year). A human 'reg' homologue has been identified in the human pancreas cDNA library. Expression of 'reg' in both regenerating and hyperplastic islets suggests possible roles in replication, growth and maturation of islet beta cells. Its role in the pathogenesis and potential novel treatment(s) of human diabetes remains to be explored.

ENVIRONMENT

The important environmental factors contributing to the development of NIDDM include obesity (excess caloric intake), diet (altered composition), and physical activity (level of physical training). Stress hormone excess/deficiency drugs/toxins and aging also contribute to diabetogenesis. Most of these factors result in insulin resistance, whereas some of the latter (stress hormones, drugs, aging) can also impair beta cell function. (6).

In obesity, it is the enlarged adipose cell size (rather than increase in cell number), and central obesity (increased waist: hip ratio), that are important in the genesis of insulin resistance and diabetes. Body weight reduction improves glucose tolerance and decreases insulin resistance.

Diet influences the development of NIDDM not only through its effect on overall energy balance (obesity), but also through the effects of altered dietary composition. With over feeding, excess calorie intake and gain in weight, but not the specific composition of the diet that are important in the production of hyperinsulinemia and insulin resistance. However, in subjects on weight maintaining diets, carbohydrate and fat content of the diet effect glucose tolerance and plasma insulin responses; with isocaloric diets, high carbohydrates result in improved oral glucose tolerance and decreased insulin responses, compared to low carbohydrate diets. Besides altered dietary composition, complex (vs refined) carbohydrates and dietary fiber also influence glucose tolerance.

Physical training increases insulin sensitivity and improves glucose tolerance by several possible mechanisms. In Muscle, insulin stimulated glucose disposal increases by 30-35% with physical training. In monocytes, there is increased binding of 125-I insulin; sustained exercise (untrained individuals) increases receptor affinity, where as physical training increases receptor numbers. In muscle and adipose tissue, no clearcut increase in insulin binding is demonstrable with physical training, whereas increased glucose transport and changes in intracellular glucose metabolism are a major cause of increased insulin effect. Thus physical training increases glucose transport and intracellular glucose metabolism by post-insulin receptor effect.

REFERENCES

1. Olefsky JM. Pathogenesis of non-insulin dependent (type II) diabetes. In: Endocrinology, Second edition, LJ. De-Groot et al., Eds; WB Saunders and Co., Philadephia, USA 1989, 1369-89.

2. Cahill GF. Beta cell deficiency, insulin resistance, or both? New Engl. J. Med 1988 318: 1268-70.

3. O'Rahill S, Turnea RC, Mathews DR. Impaired pulsatile secretion of insulin in relatives of patients with non-insulin dependent diabetes. New Engl. J Med 1988; 318: 1225-30.

4. Lillioga S, Mott DM, Howord BV, Bennett PH, Yki-jarvinen H, Freymond D et al. Impaired glucose tolerance as a disorder of insulin action: longitudinal and cross-sectional studies in the Pima Indians. N. Engl. J Med 1988; 318: 1217-25.

5. Howard CF. Longitudinal studies on the development of diabetes in individual Macaca nigra Diabetologia 1986; 129: 301-6.

6. Horton ES. Role of environmental factors in the development of non-insulin-dependent diabetes mellitus. Amer J Med (suppl; Glipizide symposium) 1983; 32-40.

7. Clark A. Islet amyloid and type II diabetes. Diabetes Medicine 1989; 6: 561-7.

8. Renold AE. Animal models: an aid to the understanding of the etiology and pathogenesis of diabetes mellitus. In: The Diabetes Annual/4, KGMM Alberti, LP Krall Eds; Elsevier, Amsterdam, 1988 p 592-608.

9. Secondary diabetes: the spectrum of the diabetic syndromes. S. Podolsky, M Viswanathan, Eds; Raven Press, New York, 1980 p.33-46.

10. Textbook of endocrine pathology. Bloodworth, Ed; 1982 p 576-631.

11. Terazono K, Yamamoto H, Takasawa S, Shiga K, Yonemura Y, Tochino Y et at. A novel gene activated in regenerating islets. J Biol Chem 1988; 263:2111-4.

12. Lilliioja S, Mott DM, Howard BV et al. Impaired glucose tolerance as a disorder of insulin action: longitudinal and cross sectional studies in Pima Indians. N Engl J Med 1988; 318: 1217-25.

13. Osei K. Increased basal glucose production and utilisation in non-diabetic first degree relatives of patients with NIDDM. Diabetes 1990; 39:597-601.

14. Barnett AH, Spiliopoulos AJ, Pyke DA, Stubbs WA, Burrin J, Alberti KGMM. Metabolic studies in unaffected cotwins of non-insulin-dependent diabetes. British Medical Journal 1981; 282: 1656-8.

15. Hansen BC. Hetrogeneity of insulin responses: phases leading to type II (non insulin dependent) diabetes in the rhesus monkey. Diabetologia 1986; 29: 713-19.

16. Bell RH. Animal models of diabetes mellitus: physiology and pathology. J Surg Res 1983; 35: 433-60.