# Role of Insulin and Proinsulin in Diabetic Vascular Disease

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**Associations between loss of glucose tolerance, insulin resistance, and ischemic heart disease (IHD) are of great current concern. Considerable controversy and uncertainty relates to the mechanism(s) that underlies these associations. Whilst there is some evidence in prospective studies of an association between hyperinsulinemia and future IHD, it is by no means strong or consistent between different studies. Hypertriglyceridemia is another possible factor involved in the linkage between glucose intolerance and IHD. There is good evidence for an affect of plasma nonesterified fatty acids (NEFA) to increase hepatic output of VLDL. Insulin, contrary to some suggestions, acts to lower plasma VLDL by actions directly on hepatic output and activation of adipose tissue lipoprotein lipase, and indirectly via the hormones affect of lowering plasma NEFA. Glycosylation and oxidation of lipoproteins may enhance their atherogenic potential. It is highly probable that procoagulant changes are also important processes predisposing to IHD. Associations between plasminogen activator inhibitor-1 and insulin, intact and 32,33 split proinsulin hypertriglyceridemia, and insulin resistance have been reported, but a unifying hypothesis explaining these links remains elusive. Epidemiological studies now repeated in a number of centers have shown links between infant mortality and birth weight and risk of IHD, and between birth weight and risk of impaired glucose tolerance and non–insulin-dependent diabetes mellitus (NIDDM). It has been proposed, therefore, that impairment of fetal and infant growth may underlie the associations between loss of glucose tolerance and risk of IHD. Animal models form the basis of much current research to test this concept.**



ASSOCIATIONS BETWEEN abnormal glucose tolerance, glycosuria, and myocardial infarction have been described over the past 70 years. It has also been known for 30 years that plasma insulin concentrations during an oral glucose tolerance test may be elevated in individuals who have had a myocardial infarction approximately 1 year previously.[1] This was interpreted as a sign of insulin resistance in such patients. In recent years, the links between abnormal glucose tolerance, insulin resistance, and ischemic heart disease (IHD) have been matters of much interest and speculation. As will become apparent herein, we are still a considerable distance from a convincing explanation of the basic mechanisms that underlie these links.

One possibility that has been suggested in relation to the frequently observed association with increased plasma insulin concentration is that insulin itself might be directly or indirectly involved through its effects on lipids and supposedly blood pressure.[2] This suggestion remains highly controversial, and equally strong arguments opposed to it have been made.[3] It is the purpose of this review to focus on two aspects of this question: the epidemiological studies that have examined prospectively the associations between plasma insulin concentration and cardiovascular disease and what, if any, mechanisms might explain the statistical associations. The role of proinsulin-like molecules will also be discussed in the light of recent evidence that elevations in their concentrations might also play a role. Finally, a radically different hypothesis will be outlined that attempts to unify our present understanding.

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## EPIDEMIOLOGY

The association of non–insulin-dependent diabetes mellitus (NIDDM) and impaired glucose tolerance (IGT) with the future risk of IHD is strong, independent, and consistent between studies, as briefly reviewed later. Emphasis has been placed in this review on the more uncertain question of the possible underlying mechanisms, particularly the role that may be played by insulin.

### *Are Individuals With Diabetes or IGT at Increased Risk of Cardiovascular Disease?*

A number of different studies have addressed this question, and the results have been broadly similar. In the Whitehall Study of 18,403 male civil servants aged 40 to 65 years, the 10-year mortality rate from IHD was significantly elevated for subjects with diabetes (plasma glucose 2 hours after a 50-g glucose load, $\geq 11.1$ mmol $\cdot$ $L^{-1}$) and also for those with a lesser degree of glucose intolerance (plasma glucose at 2 hours, 5.4 to 11.0).[4] Although it is possible to explain such observations on the basis of clustering of other established cardiovascular risk factors in individuals with glucose intolerance, multivariate analysis suggested that this was not the case. In other studies such as those from Framingham,[5] Tecumseh,[6] and Chicago,[7] the association between diabetes and risk of coronary heart disease is independent of other risk factors such as age, cholesterol, blood pressure, and cigarette smoking. The following sections review the possible role that insulin may play in this relationship.

### *Do Individuals With Diabetes or IGT Have Hyperinsulinemia?*

The validity of the observation of elevated fasting and postload insulin levels in individuals with glucose intolerance has been questioned by the demonstration of cross-reactivity of standard assays for insulin with proinsulin-like molecules[8] and the disproportionate secretion of these insulin-precursor molecules in IGT and NIDDM.[9] The Isle of Ely Diabetes Project in which we are presently engaged is

the largest study using specific assays for insulin, proinsulin, and 32-33 split proinsulin in a population-based cohort of people aged 40 to 65 years. This study has demonstrated that there is a small but significant degree of hyperinsulinemia in people with newly diagnosed NIDDM and IGT when a specific insulin assay is used and the data are adjusted for confounding by age, gender, and obesity (Wareham, Byrne, and Hales, unpublished observation, June 1995).

*Is Hyperinsulinemia Associated With a Cluster of Cardiovascular Risk Factors in Subjects With Normal and Abnormal Glucose Tolerance?*

This question is complicated by the problem of specificity of the insulin assay, since the elevation of proinsulin-like molecules in glucose intolerance makes it possible for observed associations between nonspecific insulin and cardiovascular risk factors to be exaggerated by measurement error.

Numerous studies have demonstrated cross-sectional associations between fasting hyperinsulinemia measured using nonspecific assays and the components of syndrome X.[2] In longitudinal studies, fasting hyperinsulinemia is predictive of the development of a cluster of metabolic abnormalities: hypertension, low high-density lipoprotein (HDL) cholesterol, elevated triglyceride, and NIDDM.[10]

In a small study of diabetic subjects, Nagi et al[11] demonstrated slightly different associations between the cluster of cardiovascular risk factors and each of the insulin-like molecules measured using specific assays, but these may have arisen by chance alone. In an analysis from the San Antonio Heart Study, Haffner et al[12] suggested that the pattern of associations may be different between proinsulin and insulin, and a similar idea was raised by Davies et al.[13]

The observation that insulin is associated with a cluster of established cardiovascular risk factors makes it vital to examine the prospective studies that suggest an association between hyperinsulinemia and coronary heart disease for evidence that this relationship is independent.

*Is Hyperinsulinemia Independently Associated With Future Development of Cardiovascular Disease in Nondiabetic Individuals?*

Although initial studies of the association between IHD and hyperinsulinemia were cross-sectional, the major studies that have examined this relationship have been longitudinal. In the Helsinki Study, a cohort of 982 policemen aged 35 to 64 years were evaluated for 9.5 years. Members of the cohort were free of symptoms of IHD at baseline and had no electrocardiographic changes suggestive of previous myocardial infarction. Follow-up data on the incidence of myocardial infarction were collected by postal questionnaire, and cause of death from death certificates. The age-adjusted 9.5-year incidence of IHD was not significantly associated with fasting plasma insulin concentration, but was associated with both 1-hour plasma insulin ($P = .002$) and 2-hour insulin ($P = .016$). In three separate multivariate analyses, risk of IHD was associated with 1-hour and 2-hour insulin but not with fasting insulin after adjustment for age, body mass index, cholesterol, physical activity, smoking, diastolic blood pressure, and plasma triglyceride.[14] However, the glucose load in this study was not standardized, and varied between 75 and 90 g according to surface area. If fasting insulin is taken as the best measure of insulin resistance in this type of study, then this study presents little to suggest a strong relationship between hyperinsulinemia and IHD.

In the Paris Prospective Study, 6,903 men free of diabetes and with no evidence of cardiovascular disease underwent a standard 75-g oral glucose tolerance test.[15] An analysis of the risk of death from IHD during a 15-year follow-up study of this population plus a group of 125 subjects with known NIDDM showed that mortality was greatest in subjects in the highest quintile for both fasting and 2-hour plasma insulin. In multivariate analysis, IHD mortality risk was not independently associated with either fasting or 2-hour plasma insulin when these were treated as continuous variables. However, it was associated with the 2-hour insulin when this was treated as a binary variable, ie, comparing the highest quintile with the other four. Since the top quintile for 2-hour plasma insulin is likely to contain a disproportionate number of the individuals with diabetes in this study, it would have been preferable to examine these same associations stratified for the World Health Organization category of glucose intolerance.

Although an initial report from the Busselton Study suggested an association between hyperinsulinemia and risk of death from IHD,[16] a further analysis of 1,564 subjects suggested that the 1-hour postload insulin concentration was not predictive of cardiovascular mortality at 13 years.[17] In a recent report from the Rancho Bernado Study,[18] the 5-year risk of mortality from IHD was measured in a population of subjects without diabetes. Baseline fasting insulin concentration had no association with mortality in either men or women, and in men the 2-hour insulin was significantly inversely associated with cardiovascular risk. Finally, in a nested case-control study of the Multiple Risk Factor Intervention Trial, fasting insulin was not associated with mortality from IHD, but further analysis suggested that an association was found in a subgroup with the apolipoprotein (apo) E phenotype 3/2.[19]

Thus, although there is some evidence of an association between hyperinsulinemia and future risk of IHD, we would agree with other reviewers that it is not strong or consistent.[3]

*Are Individuals With a Cluster of Cardiovascular Risk Factors at Increased Risk of Diabetes?*

The direction of the relationship between the cluster of cardiovascular risk factors and diabetes is not clear, since many studies have demonstrated that individuals with a cluster of established cardiovascular risk factors are at increased risk of developing diabetes.[20-22]

*In Diabetic Populations, Does Hyperinsulinemia Predict the Incidence of Cardiovascular Disease or IHD Mortality?*

Evidence of an association between IHD and hyperinsulinemia in diabetic populations is limited and obviously

affected to a considerable extent by therapeutic intervention.[23,24] In the Pima Indian population, there is no association between either fasting or 2-hour postload insulin concentrations and the subsequent development of ischemic electrocardiographic abnormalities.[25] Since Pima Indians have the highest reported prevalence of NIDDM in the world[26] and are as a population relatively hyperinsulinemic, it is important to find an explanation for the discordant observation of low risk of IHD in this population.[27] The absence of a satisfactory theory to explain this finding must cause one to question the suggestion that hyperinsulinemia has a causal relationship with the development of IHD.

## MECHANISMS OF VASCULAR DISEASE ASSOCIATED WITH INSULIN, PROINSULIN, INSULIN RESISTANCE, AND DIABETES

As reviewed earlier, epidemiological data suggest that the association between hyperinsulinemia and vascular disease is weak, and no major studies have examined prospectively the associations between hyperproinsulinemia and vascular disease. It is therefore appropriate to consider other potential mechanisms contributing to vascular disease that are associated with hyperinsulinemia, hyperproinsulinemia, insulin resistance, and diabetes. In subjects with diabetes, there is a twofold to threefold increase in the relative risk of IHD, although the mechanisms by which insulin resistance and diabetes contribute to vascular disease are not fully understood. The rate of development of vascular disease is influenced by many factors, and it is likely that the complex interaction between factors such as smoking and distribution of body fat will adversely affect many of the metabolic processes known to be altered in insulin resistance and diabetes. Many metabolic processes associated with vascular disease are directly affected by diabetes, but it is not the purpose of this review to be fully comprehensive. It is our intention to focus on mechanisms involving triglyceride-rich lipoproteins (including modified lipoproteins) and disorders of components of the hemostatic system involving coagulation and fibrinolysis associated with diabetes and the insulin resistance syndrome.

### *Triglyceride-Rich Lipoproteins and Vascular Disease*

Epidemiological studies show an association between hyperinsulinemia, insulin resistance, diabetes, and triglyceride concentrations. Plasma triglyceride concentrations are determined by the amounts of very–low-density lipoprotein (VLDL) and chylomicrons (and their remnants) and to a lesser extent by the amounts of intermediate-density lipoprotein, HDL, and low-density lipoprotein (LDL) in the circulation. Triglyceride-rich lipoproteins originate from hydrolysis of VLDL or chylomicrons by lipoprotein lipase. In the fasting state, only VLDL particles circulate in the plasma, whereas chylomicrons and their remnants are produced 30 minutes to 8 hours after a meal. VLDL particles are produced in various sizes depending on the amount of triglyceride present in the particle.

An increased plasma triglyceride level is the most frequently observed lipid disorder associated with diabetes mellitus, occurring in 25% to 30% of subjects with NIDDM. Plasma triglyceride concentrations are usually increased 50% to 100% above the normal range, and increases in triglyceride concentration above this are usually attributable to genetic defects of lipoprotein metabolism exacerbated by diabetes. The mechanism by which hypertriglyceridemia occurs in diabetes is uncertain, and there is debate as to whether the causes of hypertriglyceridemia in insulin-dependent diabetes involve the same mechanisms as those involved in NIDDM. There is general agreement that VLDL production is increased, although the mechanism by which this arises is uncertain. Increased nonesterified fatty acids (NEFA) may modulate the effects of insulin on VLDL production, since we have shown in vitro that increased NEFA may decrease the inhibitory effect of insulin to reduce hepatic triglyceride secretion.[28] We have also shown recently that plasma NEFA concentrations are increased in both subjects with newly diagnosed diabetes and those with IGT, and the most important determinants of plasma triglyceride concentration were NEFA suppression and waist to hip ratio.[29] In the physiological state, insulin inactivates hormone-sensitive lipase, and therefore plasma NEFA concentrations increase with fasting and are reduced after a meal. Increased NEFA concentrations may exacerbate insulin resistance through the Randle cycle[30] and be a marker of deteriorating glucose tolerance. It has been shown that increased NEFA concentrations are associated with increased triglyceride concentrations in white and Asian subjects with normal glucose tolerance,[31] and we have shown in a subgroup of subjects with normal glucose tolerance that decreased NEFA suppression was associated with many of the features of the insulin resistance syndrome (Byrne et al, Diabetologia 1995, in press). Decreased NEFA suppression may be linked to the development of vascular disease by increasing the concentration of triglyceride-rich lipoproteins in the circulation, and in vitro data support the notion that NEFA are an important stimulus and substrate for hepatic VLDL production.[28,32,33] Mechanisms that may link control of plasma NEFA concentration to some aspects of insulin action and triglyceride production are shown in Fig 1.

The involvement of triglyceride-rich lipoproteins in the formation of atheromatous plaques is a complex process. In subjects with hypertriglyceridemia, large VLDL particles tend to predominate in the plasma, and it has been shown that these particles bind with high affinity to LDL receptors and suppress hepatic hydroxymethyl glutaryl coenzyme A reductase (the rate-limiting step in cholesterol synthesis). The ligand for binding of these particles is apo E and not apo B, and only a certain conformation of apo E present in large VLDL particles is able to be proteolytically cleaved, allowing binding to the receptor (for review, see Bradley and Gianturco[34]). Monocyte-macrophage foam cells occur in all atherosclerotic lesions, and VLDLs from hypertriglyceridemic individuals have been shown to be avidly taken up by macrophages, creating foam cells, although there is some uncertainty concerning the mechanism of uptake. Many macrophages secrete lipases, and it has been shown that in the presence of cholesterol, macrophages express apo E. It

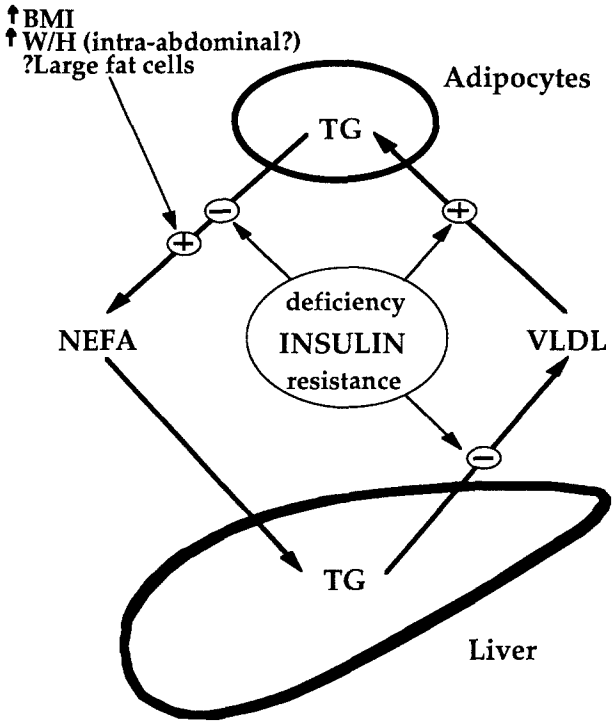


**Fig 1. Diagram highlighting some key insulin actions that control plasma triglyceride (TG) concentration. Physiologically, these actions would reduce plasma TG. Elevation of plasma TG therefore may result from an absolute deficiency of insulin or resistance to insulin at some or all of these regulatory steps. BMI, body mass index; W/H, waist to hip ratio; ↑, increase.**

is likely that several mechanisms of uptake could be involved. These mechanisms may involve lipolysis of triglyceride-rich particles, thus producing an LDL-like particle that is subsequently modified by oxidation. An apo E receptor–mediated mechanism could also be involved, and finally, direct uptake of triglyceride-rich particles may occur.

*Modified Lipoproteins and Vascular Disease*

Modifications of lipoproteins associated with diabetes may enhance the atherosclerotic vascular process by altering the biological properties of the particle, although HDL particles may protect LDL particles from peroxidation. Peroxidation of fatty acids within the triglyceride molecule releases free radicals that are an extremely reactive species capable of initiating reactions that damage organic molecules of biological importance. Lipid hydroperoxides have been shown to be extremely toxic in a number of studies, and their decomposition products are even more toxic. Loss of an OH radical by cleavage of the relatively weak O—O bond gives rise to another radical capable of breaking C—C bonds. Other decomposition aldehydes are reactive toward proteins that are in close proximity to lipids in cell membranes. Reactions that occur with proteins are capable of causing cross-linking of the proteins, resulting in loss of biological function.

It has been shown that peroxidated LDL particles are toxic to endothelial cells in vitro and that lipid peroxides inhibit antithrombin III, producing a procoagulant state and enhancing platelet activation by increasing thrombin concentrations. In vitro, it has been shown that glucose increases LDL oxidation, and in vivo, a strong association has been noted between LDL glycosylation and oxidation in subjects with or without NIDDM. Hypertriglyceridemia may also increase lipid peroxidation, since it has been shown that marked increases in stimulated superoxide production are present in mononuclear cells obtained from diabetic subjects with hypertriglyceridemia.[35]

It is likely that the processes of glycation and oxidation are intimately linked through the processes of autoxidative glycosylation and glycoxidation. Glycosylation affects all lipoproteins, and these modifications may affect the biological function of the lipoproteins. VLDLs from normolipidemic subjects with insulin-dependent diabetes mellitus or NIDDM were found to interact abnormally with macrophages in vitro, stimulating increased cholesterol ester synthesis and accumulation.[36] It has also been shown that glycosylated HDL is cleared more rapidly from the circulation and is less effective at removing cholesterol from the circulation. It is likely that the association between increased lipid oxidation and diabetes is a complex one, since diabetes does not always result in elevated levels of oxidized lipids, and despite the higher levels of oxidative stress associated with diabetes, individuals often have lower levels of antioxidants such as ascorbic acid and vitamin E.

*Abnormalities of Hemostasis and Fibrinolysis Associated With Vascular Disease*

Oxidized LDL induces tissue factor expression on monocytes and endothelial cells and provides a link between coagulation and atherosclerosis. Tissue factor forms a complex with factor VII, initiating the coagulation cascade leading to thrombin generation and platelet activation and aggregation. Recently, thrombin receptors have been identified,[37] and mRNA encoding for these receptors is present in atherosclerotic plaques.[38] It is therefore possible that thrombin activates platelets and acts as a mitogen for smooth muscle cells.

Plasminogen activator inhibitor-1 (PAI-1) is a potent inhibitor of fibrinolysis by binding to and rapidly inactivating both tissue-type and urokinase-type plasminogen activator. Increased concentrations of PAI-1 have been demonstrated in survivors of myocardial infarction and in patients with coronary artery stenosis. A positive association with plasma insulin and 32/33 split proinsulin has been observed and increased PAI-1 concentrations have been shown to be present in association with many features of the insulin resistance syndrome. It is uncertain whether hyperinsulinemia and hyperproinsulinemia cause increased plasma PAI-1 concentrations, although a recent study involving sequential hyperinsulinemia-euglycemic clamps has shown that PAI-1 concentrations are strongly associated with peripheral glucose uptake as a measure of insulin resistance.[39] The mechanism causing increased PAI-1 is uncertain. Using a hepatoma cell line, it has been shown that insulin increases PAI-1 mRNA, and using endothelial cells, VLDL increases PAI-1 secretion. At present, one can only

speculate how increased PAI-1 concentrations cause or exacerbate development of atheromatous plaques. PAI-1 gene expression has also been identified in the intima of atherosclerotic human arteries, and smooth muscle cells contained in atherosclerotic plaques overexpress PAI-1. It is possible that high levels of PAI-1 in the plaque inhibit plasmin formation, decreasing metalloproteinase activation and resulting in less extracellular matrix degradation. Decreased matrix degradation may result in a larger, more stable early atherosclerotic lesion. Thus, the adverse effect on coagulation and fibrinolysis may be associated with an accelerated atherosclerotic process.

Coagulation is usually initiated by activation of factor VII by tissue factor. Activity of the factor VII/tissue factor complex is regulated by a serine protease inhibitor called tissue factor pathway inhibitor (TFPI). TFPI binds to factor Xa and induces feedback inhibition of initiation of the coagulation pathway by inhibiting activation of factors IX and X. TFPI is present in platelets and endothelial cells and is associated with lipoproteins (usually small, dense LDL and lipoprotein(a).[40] At present, it is not known if free TFPI and lipoprotein-associated TFPI have the same inhibitory activity. It has been shown that TFPI activity and factor VII levels are increased in subjects with hypertriglyceridemia, although it remains to be established if TFPI is involved in thrombosis and atherosclerosis.

Hypertriglyceridemia is associated with a procoagulant state. Activation of factor VII may occur because liberated fatty acids contain sufficient negative charge to activate factor XII (which in turn leads to activated factor VII). This hypothesis is supported by in vivo data showing that in subjects consuming different diets, factor VII coagulant activity is strongly associated with plasma concentrations of the saturated fatty acid, stearic acid.[41] Alternatively, the association between increased factor VII activity and increased plasma triglyceride concentrations may be explained by binding of factor VII to triglyceride-rich lipoproteins, with subsequent reduction in catabolism of factor VII,[42] although binding between these two molecules has not been shown in vivo.

A unifying explanation linking abnormalities of hemostasis, hypertriglyceridemia, insulin resistance, and vascular disease is still elusive. It is known, for example, that many genetic and environmental factors contribute to PAI-1 activity, and it seems likely that both genetic and environmental factors contribute to the hemostatic abnormalities associated with hypertriglyceridemia, insulin resistance, and other features of the insulin resistance syndrome. Although it still remains to be proven, poor fetal nutrition and growth may be a unifying factor linking many of the abnormalities associated with vascular disease.

## A UNIFYING HYPOTHESIS

In 1977 in Norway, it was reported that there was a correlation between the then-current death rates from ischemic heart disease in different areas of the country and the infant mortality rate pertaining at the time when those presently dying of IHD were themselves infants. No such correlation was seen with the current rates of infant mortality. It was suggested that the childhood and adolescent environments could be important in determining the risk of IHD.[43] In 1979, this observation was confirmed in England and Wales, but correlations were observed between both past and present infant mortality.[44] In 1986, again in England and Wales, the correlation was observed, but it was suggested that mechanisms that linked infant mortality and IHD could be operating during fetal life.[45]

Infant mortality has been known to be strongly related to birth weight for many years. It was discovered that in certain places in the United Kingdom, including Hertfordshire, Preston, and Sheffield, records existed of early anthropometry for people who were currently in the age range of 50 to 80 years.[45] Thus, it was possible to show that in both men and women, those of low birth weight were at considerably higher risk of dying of IHD. These studies were extended to hypertension, and it was observed that adult blood pressure increased in relation to decreasing birth weight. The strongest predictor of adult blood pressure was the ratio of placental weight to birth weight. The highest blood pressure was seen in adults who at birth had a large placenta in relation to birth weight.

The known common coexistence of IHD, hypertension, and NIDDM suggested that a possible link between birth weight and glucose tolerance should be investigated. It was found that in men of mean age 64 in Hertfordshire, glucose tolerance, judged as either the 2-hour glucose level at the end of the test or the prevalence of IGT or newly discovered NIDDM, was strongly related to birth weight and to weight at the age of 1.[46] In either case, the smallest infants had the worst glucose tolerance, the effect being graded through the range of weights with no obvious threshold. A similar finding in relation to birth weight was made in studies of men and women in Preston of mean age 50.[47]

The possibility therefore arose that the combination of reduced glucose tolerance, hypertension, and hypertriglyceridemia, components of what is now referred to as the insulin resistance syndrome, could be linked to birth weight. This proved to be the case both in men in Hertfordshire and in men and women in Preston. Indeed, this relationship expressed as an odds ratio between the risk in the smallest compared with the largest infants proved (in the range of 14- to 18-fold) to be the strongest of all.[48]

It was then shown that adult insulin resistance, as judged from the initial rate of decrease of plasma glucose after a bolus injection of insulin, was related to an index of thinness at birth. Thus, infants of low ponderal index (weight divided by length cubed) were the most insulin-resistant as adults. Adult obesity added to their insulin resistance such that infants who were thin at birth and obese (as judged by body mass index) as adults were approximately twice as insulin-resistant as those who were most plump at birth but thin as adults.[49]

These observations have been independently confirmed by others in populations as divergent as Pima Indians[50] and Mexican-Americans,[51] and also in Sweden.[52] It can be concluded that these relationships are both strong and reproducible. The explanation for the relationships is not yet clear. It is widely believed that NIDDM and insulin

resistance are genetically determined. It is certainly possible that the relationships observed between birth weight and adult disease are also genetically determined, although it is far from clear what the candidate genes would be. However, the observations put forward to support the genetic hypothesis of the origins of NIDDM can all be questioned.[53] There are also epidemiological observations of rapid changes in the incidence of NIDDM that are hard to explain genetically.[54] It has been well recognized for many years in both animals and humans that nutrition before and during pregnancy is a major determinant of birth weight. Therefore, it has been proposed that the link between birth weight and adult disease is determined by some aspects of fetal nutrition (reviewed in Hales and Barker[55] and Barker[56]). The dominant determinant of fetal nutrition is maternal nutrition. Studies on experimental animals have emphasized the detrimental effects of maternal protein/calorie and particularly protein malnutrition on the outcome of pregnancy. Thus, protein malnutrition may play an important but not exclusive role in restricting the growth of the human fetus and in turn determining susceptibility to adult disease. These concepts are clearly amenable to being tested by experiment and are the subject of much current research.

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