The Role of Insulin in Atherosclerosis in Diabetics and Nondiabetics

A Review

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SUMMARY

Disease of coronary, cerebral, and peripheral arteries is associated with exaggerated insulin responses to oral glucose. In three populations, high fasting or post-glucose insulin levels have a predictive value in the incidence of ischemic heart disease and in cardiac mortality. Diabetics who are obese or who have received treatment with insulin have elevated insulin levels and, as a group, have an increased incidence of cardiovascular disease. Insulin, in small concentrations, has effects on arterial tissue including stimulation of smooth muscle cell proliferation and of glucose incorporation into lipid. It is suggested that insulin has a role in the development of atherosclerosis. DIABETES *30* (Suppl. 2):54–57, 1981.

The evidence linking insulin to atherosclerosis can be considered under two headings: clinical and epidemiologic evidence that elevated insulin levels are associated with atherosclerosis and its complications, and evidence that insulin has biologic actions on the arterial wall that may be relevant to the development of atheromatous lesions. A third and crucial type of evidence, that reduction of insulin levels will prevent or reduce the incidence of atherosclerosis, is not available at present.

CLINICAL AND EPIDEMIOLOGIC EVIDENCE

Because of the frequent finding of abnormalities in glucose tolerance in patients with ischemic vascular disease,[1] insulin levels during glucose tolerance tests have been measured in people with atherosclerosis. Subjects with disease of coronary arteries,[2–9] cerebral arteries,[10] and the arteries of the lower limbs[9,11,12] have elevated insulin responses to oral glucose compared with control subjects without evidence of vascular disease. A number of questions arise from these studies.

What is the cause of the abnormal insulin response? The answer to this question remains unknown. However, the insulin responses to i.v. glucose[8] or to i.v. tolbutamide[3,5] have been less clearly elevated in atherosclerotic subjects, suggesting that a gastrointestinal factor might be involved in the hyperinsulinemic response to oral glucose.

Are the abnormal insulin levels associated with ischemia or with arterial disease? The presence or absence of atherosclerosis is difficult to determine except when the disease is advanced and organ damage has occurred. However, two studies have used angiographic evidence of coronary[4] or peripheral[11] arterial disease and have shown that elevated insulin responses are present in the absence of complete occlusion and infarction. In contrast, in the acute phase of myocardial infarction and in congestive cardiac failure, depression of the insulin response to oral glucose is found,[13] perhaps due to over-activity of the sympathetic nervous system.

Is the relationship of insulin to arterial disease mediated by its effect on other risk factors? A number of investigations have included other risk factors for atherosclerosis, including blood pressure and serum cholesterol[4,11] and it has been found that the relationship of elevated insulin levels to arterial disease is independent of the influence of serum lipids or blood pressure. In the three prospective studies to be reviewed later, multivariate analysis showed that the risk associated with elevated insulin levels was independent of other risk factors, including cholesterol, blood pressure, and blood sugar.

Do insulin levels in populations reflect the incidence of atherosclerosis in those populations? In South Africa the white population has a much higher incidence of ischemic heart disease than the Bantu population. Insulin responses to glucose in the white population are almost twice as high as those in the Bantu population.[14] Edinburgh men have a higher incidence of ischemic heart disease than men in Stockholm. Although the glucose responses to oral glucose were identical in Edinburgh and Stockholm, the insulin re-

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sponses to oral glucose were significantly higher in the Scottish men than in the Swedish men.[15] Edinburgh and Stockholm men also differed in other risk factors for atherosclerosis including blood pressure and lipids.

Do abnormal insulin responses precede the development of coronary heart disease? Recently, the results have been published of three prospective studies of the relationship of insulin levels to ischemic heart disease. In the Helsinki Policeman study,[16] elevated fasting and post-glucose plasma insulin levels had a strong predictive value for the development of coronary artery disease over a 5-yr period. Multivariate analysis showed that the predictive value of plasma insulin responses to oral glucose was independent of other risk factors, including plasma cholesterol, blood pressure, and blood glucose. In the Paris study,[17] elevated plasma insulin levels and insulin:glucose ratios were associated with an increased risk of coronary artery disease and the association was independent of other risk factors. In Busselton, Australia, there was positive relationship between insulin responses 1 h after glucose and the incidence of and mortality from coronary heart disease in men aged 60–69 yr.[18] The relationship was also present in all men and was independent of other major risk factors. This association was not found in women. These studies provide the first evidence of the predictive value of raised plasma insulin levels in the development of ischemic heart disease.

INSULIN, DIABETES, AND ATHEROSCLEROSIS

Diabetes is an important risk factor for atherosclerosis and the risk is independent of abnormalities in plasma lipids and blood pressure.[19] Although relative or absolute insulin deficiency is the cause of glucose intolerance, it does not necessarily follow that the tissues of the diabetic are exposed to low concentrations of insulin. Insulin regulation is different in non-insulin-dependent and insulin-dependent diabetics. Non-insulin-dependent diabetics are frequently obese. As obesity is associated with elevations of both fasting and post-glucose insulin levels irrespective of the presence of diabetes,[20] non-insulin-dependent diabetics who are obese will have higher insulin levels than nondiabetics who are thin. The majority of studies of atherosclerosis in diabetes have not taken into account the weight of the diabetics and the nondiabetic controls. However, two studies have shown that the large vessel complications of diabetes are associated with excessive weight gain or obesity.[21,22] In addition, mild diabetes of the type that is commonly associated with an increased incidence of atherosclerosis is also associated with high insulin levels.[23]

Insulin-dependent diabetics with fasting hyperglycemia, a tendency to ketoacidosis, and absolute insulin deficiency are treated with insulin. The dose of insulin administered to diabetics is considerably greater than the normal daily output of the pancreatic islets and, hence, insulin levels in insulin-treated diabetics are higher than those in nondiabetics.[24,25] In insulin-treated diabetics, insulin levels are not related to meals and to blood sugar levels. Hence, between meals and at night, insulin levels in diabetics are higher than in nondiabetics.[25] Another difference between nondiabetics and diabetics is the route of insulin delivery into the circulation. Normally, insulin is secreted into the portal system and reaches the liver in high concentrations. The liver is the major site of action of insulin and also degrades about 50% of insulin on its first passage. Thus in the normal person, insulin concentrations in the portal vein are much higher than those in the systemic circulation.[26] In the diabetic treated with s.c. insulin the reverse situation will occur. Long-term treatment with insulin induces the formation of insulin antibodies, which bind and probably inactivate some of the insulin, resulting in an increase in the patient's insulin requirements.[24] As a result, very high total insulin levels are found in the circulation of many insulin-treated diabetics.

There have been only two studies of the relationship of the macrovascular complications of diabetes to circulating insulin levels. One study, in non-insulin-dependent diabetics, indicated that those with atherosclerosis had higher insulin responses to glucose than diabetics without vascular disease.[8] Another study found little difference in the fasting insulin levels but the insulin:glucose ratio was higher in the diabetics with atherosclerosis.[22]

INSULIN AND THE ARTERIAL WALL

THE EFFECT OF INSULIN ON ARTERIAL LESIONS

Insulin excess. Chickens fed a normal diet and injected with insulin for 19 wk developed lipid-containing lesions of the aorta.[27] The vascular lesions were not associated with weight gain or changes in lipid levels.[28] Insulin administration to chickens can also prevent the regression of experimental atherosclerosis that occurs when a cholesterol-rich diet is removed, and can overcome the inhibitory effect of estrogens on experimental atherosclerosis.[29] Infusion of insulin into one femoral artery of alloxan-diabetic dogs resulted in lipid infiltration and medial proliferation that was not seen in the other saline-treated artery.[30]

Insulin deficiency. In cholesterol-fed rabbits, ablation of insulin-secreting tissue with alloxan reduced the incidence of experimental atherosclerosis compared with nondiabetic controls.[31–33] Treatment with insulin restored the incidence of atherosclerosis to that of the controls.[34] In pancreatectomized, diabetic, cholesterol-fed rats, treatment with insulin reduced serum cholesterol levels but had no effect on arterial lesions, in contrast to the reduction in arterial lesions that occurred when a similar fall in serum cholesterol was induced by adjustment of the diet.[35]

THE EFFECT OF INSULIN ON LIPID METABOLISM OF THE ARTERIAL WALL

Aortas removed from rats that had been rendered insulin-deficient with streptozotocin incorporated less glucose into lipid than those removed from untreated animals (Figure 1).[36] The insulin levels in the animals at the time the aortas were removed correlated with the uptake of glucose into the aortic lipids. In vivo injection of insulin into rats enhanced the incorporation of labeled glucose or acetate into aortic lipids.[37,38] Insulin levels in rats fasted or injected with insulin correlates with glucose incorporation into the aorta.[39] Insulin also inhibits lipolysis in the arterial wall.[40]

THE EFFECT OF INSULIN ON CULTURED ARTERIAL CELLS

Cellular proliferation and DNA synthesis. In cultured monkey aortic smooth muscle cells, the addition of insulin to the medium in successively greater concentrations resulted in a progressive stimulation of cell proliferation (Figure 2).[41] The lowest concentrations of insulin used (10 μU/ml) are

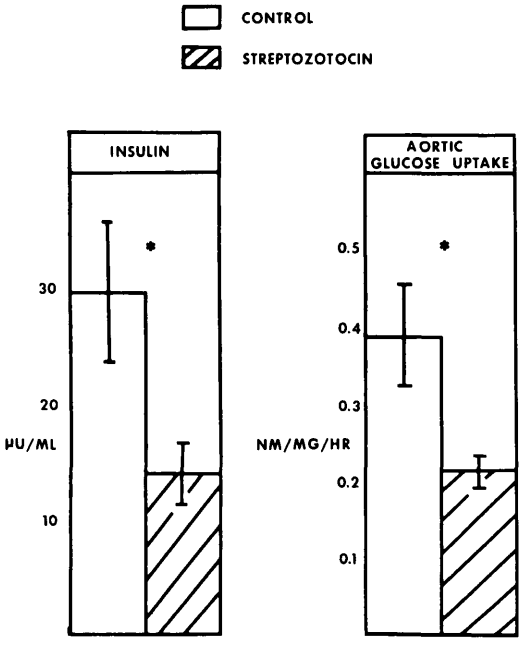
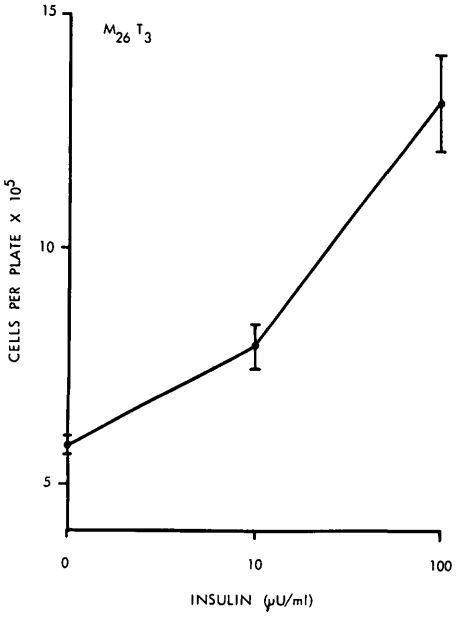


FIGURE 1. The effect of streptozotocin diabetes on serum insulin levels and incorporation of glucose into aortic lipid. Male Wistar rats were injected with streptozotocin (65 mg/kg by i.p. injection). One week later the rats were killed, the aortas removed, and glucose uptake into lipid in isolated intima-media preparations of aorta was measured. The figure shows insulin levels at the time of killing and uptake of glucose into aortic lipid in rats injected with streptozotocin and normal controls (experimental details in ref. 36).

similar to those found in the normal human circulation. When insulin was removed from serum by passage through an affinity column coated with anti-insulin antibody, cell proliferation was attenuated compared with the same concentration of whole serum, although the difference in insulin

FIGURE 2. The effect of two concentrations of insulin on the proliferation of cultured primate aortic smooth muscle cells. The cells were grown in culture dishes in Dulbecco-Vogt's medium supplemented with 1% homologous serum for 1 wk. Insulin (10 μU/ml and 100 μU/ml) was then added to two groups of dishes and the cells were counted on alternate days. The figure shows the cumulative number of cells exposed to two concentrations of insulin. ($M_{26}T_3$ = cells of the third trypsinsalien from monkey 26; experimental details in ref. 41).



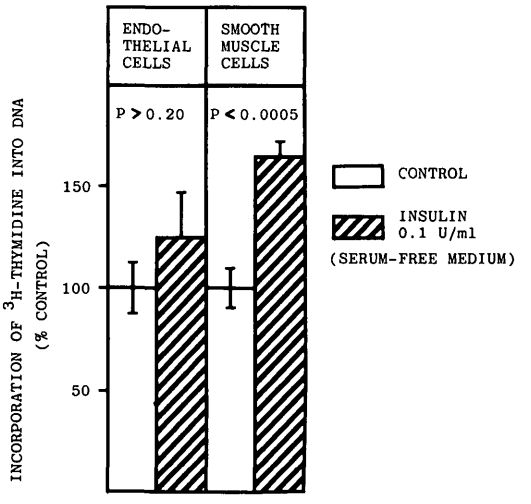
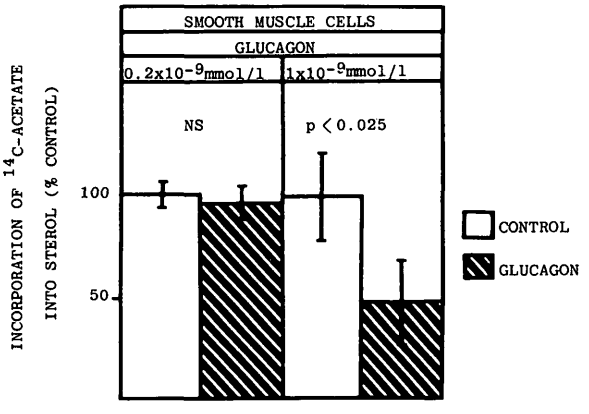


FIGURE 3. The effect of insulin on DNA synthesis in human endothelial cells and rat aortic smooth muscle cells. Cells were grown from human umbilical vein or rat aorta in appropriate media. After exposure to insulin for 24 h in a serum-free medium, incorporation of ^{3}H-thymidine into DNA was measured and the results expressed in terms of cell protein (experimental details in ref. 44).

concentration was only 2–3 μU/ml. The proliferative effects of insulin were only found in cells early in culture life; in later culture life no insulin effect was seen. Thus, the smooth muscle cell of the primate artery is sensitive to the proliferative effects of very small concentrations of insulin. Similar results have been reported in experiments using rat aortic smooth muscle cells.[42] Increasing concentrations of glucose have no effect on the proliferation of arterial smooth muscle cells despite the fact that they stimulate the proliferation of fibroblasts.[43] Cultured human endothelial cells are resistant to the action of insulin (Figure 3)[44] and thus may act as a barrier protecting the arterial media from the effects of circulating insulin.

Lipid metabolism. Insulin enhanced incorporation of labeled acetate into sterols in cultured rat arterial smooth muscle cells.[45] The absence of an effect when labeled mevalonate was the precursor suggests that insulin stimulated the activity of the enzyme HMG-CoA reductase. Glucose had no effect on sterol synthesis in cultured arterial smooth muscle cells, and glucagon only inhibited sterol synthesis

FIGURE 4. The effect of glucagon on sterol synthesis in cultured rat aortic smooth muscle cells. After exposure of the cells to glucagon for 24 h in the concentrations stated, incorporation of sodium ^{14}C-acetate into nonsaponifiable lipids and digitonin-precipitable sterols was measured and the results expressed in terms of cell protein (experimental details in ref. 46).



when present in very high concentrations (Figure 4).[46] There have been no published reports of the effects of insulin on the uptake of lipoproteins by arterial tissue. However, in fibroblasts, insulin increased cellular low density lipoprotein binding and degradation.[47] The exact role of insulin in relation to other factors that affect the arterial wall, including lipoproteins and the platelet-derived growth factor, remains unknown.

CONCLUSIONS

There is both direct and circumstantial evidence that elevated insulin levels are associated with the clinical manifestations of atherosclerosis. The evidence has recently been strengthened by the results of three prospective studies from different parts of the world. The experimental evidence is consistent with a direct relationship of insulin with the arterial lesions. If insulin has a role in the pathogenesis of atherosclerosis, measures to lower insulin levels might prevent the disease. These measures would include avoidance of obesity, regular physical exercise, and in addition, for insulin-dependent diabetics, the development of methods of delivering exogenous insulin in a way that is more nearly physiologic.

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