Insulin and Atherosclerosis*

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The introduction of insulin therapy revolutionised the treatment of diabetes. However, not all the problems of diabetes were solved. Although deaths from ketoacidosis declined, deaths from atherosclerosis have correspondingly increased. Atherosclerosis is now the major cause of death in both insulin dependent and non-insulin dependent diabetes.

The exact relationship between diabetes and atherosclerosis remains unclear. Although risk factors for atherosclerosis such as abnormal blood lipids and hypertension are common in diabetes they do not appear to entirely account for the increased incidence of cardiovascular disease[1]. For the past twenty years there has been accumulating evidence of a relationship between insulin and atherosclerosis. Although this evidence has mainly been gathered in studies of non-diabetic patients there is reason to believe that it might be relevant to diabetics as well.

Clinical and Epidemiological Studies

Insulin and Cardiovascular Disease

The first studies relating insulin to cardiovascular disease showed elevated insulin responses to oral glucose tolerance tests in subjects who had recovered from myocardial infarction[2]. Later studies[3] indicated that cardiac disease is not necessary for the abnormal insulin response as it was also found in patients with cerebrovascular[4] and peripheral arterial disease[5] as well as in those whose coronary artery disease was angiographically demonstrated in the absence of myocardial damage[6]. The abnormal insulin levels only occurred in response to oral glucose and were not present when intravenous glucose or tolbutamide[7] were the challenge.

Population studies are an important investigative technique in atherosclerosis research. Two studies have indicated that populations with a high incidence of cardiovascular disease have greater insulin responses to oral glucose than populations with lower rates of atherosclerosis. In South Africa the white population, which has a Western type incidence of cardiovascular disease, has a response to oral glucose considerably higher than that of the black population which has relatively little ischaemic heart disease[8]. A study of 40 year old healthy men in Edinburgh and Stockholm showed that Scottish men have higher insulin responses to oral glucose than Swedish men[9]. The incidence of ischaemic heart disease in Scotland is one of the highest in the world whereas that in Sweden is lower.

Three prospective epidemiological studies have investigated the relationship between insulin and the subsequent development of cardiovascular disease. In the Helsinki policemen study[10] men aged 30–59 years were given a 75–90 g oral glucose tolerance test with glucose and insulin sampling in the fasting state and one and two hours after glucose. It was found that the five year incidence of myocardial infarction and death from coronary heart disease was highest in those with highest plasma insulin levels at all times of sampling. The Paris civil servants study[11] was a study of men aged 43–54 years with glucose and insulin sampled in the fasting state and two hours after a 75 g oral glucose load. The $5\frac{1}{4}$ year incidence of myocardial infarction and coronary death was related to insulin levels but most closely related to the

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insulin to glucose ratio both fasting and two hours after glucose. The Busselton, Western Australia study[12] differed somewhat from the other two in that it included both men and women in an age range of 21 to over 70 years. A 50 g oral glucose load was given in the non fasting state and glucose and insulin were measured one hour afterwards. The six year coronary heart disease incidence and 12 year coronary heart disease mortality rate were related to the baseline insulin levels in men aged 60 years and over. No significant relationship was found in men of any other age or in women. Thus, the Busselton study differs from the other two in a number of respects including the fact that women studied, the wider age range of the subjects and the fact that fasting insulin levels were not measured. In all three studies multivariate analysis showed that the relationship of insulin to cardiovascular disease was independent of the other risk factors measured including plasma lipids, blood pressure and blood sugar.

The three prospective studies have all shown that plasma insulin levels have a predictive value in the development of ischaemic heart disease, at least in men. Because the study subjects differed in age and sex it is difficult to find a consensus on the exact role of insulin as a risk factor for ischaemic heart disease. Further prospective epidemiological studies are essential.

Insulin Levels in Diabetes

Although the glucose intolerance of diabetes is due to relative or absolute deficiency of insulin secretion, circulating insulin levels in diabetics may be higher than those in non-diabetics. The reasons differ in the two major types of diabetes.

In non-insulin dependent (type 2 diabetes) insulin secretion in response to glucose is deficient compared to weight matched non-diabetics[13]. However, basal insulin levels are unchanged and therefore obese people will have higher basal insulin levels than thin people irrespective of the presence of diabetes. Because obesity is much more common in diabetes of this type than in the population as a whole, many of these patients will have higher circulating insulin levels than many non-diabetics. The relationship of body weight to the development of vascular complications of diabetes has not been widely studied. However, in a number of studies it has been shown that diabetics who are overweight[14] or who have gained weight[15] are more prone to vascular disease than those whose weight has remained normal.

Insulin dependent (type 1) diabetes is a condition of severe insulin deficiency. However, these patients are not allowed to remain insulin deficient but are treated with exogenous insulin, usually given as one or two subcutaneous injections each day. The insulin is thus given by an abnormal route, into the systemic rather than the portal circulation, and the regulation of insulin delivery into the blood stream is not related to nutrient needs. At many time of the day, particularly between meals and overnight, insulin treated diabetics have higher insulin levels than non diabetics[16]. The use of exogenous insulin induces the development of anti-insulin antibodies. As well as inhibiting the action of insulin and hence producing the need for higher insulin doses, these reversibly complex with insulin to form a reservoir from which insulin can be released. For this reason insulin levels are high in patients who have had long term treatment with insulin[17].

The relationship of insulin levels to cardiovascular disease in diabetics has not been studied in a prospective fashion. There are two studies of small numbers of diabetics which have found higher insulin levels[18], or insulin/glucose ratios[14], in the diabetics with cardiovascular disease than in the controls. Prospective studies of the relationship of insulin levels to cardiovascular disease in diabetes mellitus are urgently needed.

Experimental Studies

Experimental Atherosclerosis

Animal experiments allow study of the effect of changes in diet or treatment on the development of vascular lesions. The exact relevance of these experiments to spontaneous human disease can be questioned. Nevertheless, they provide supporting evidence for epidemiological and clinical studies.

Cholesterol fed rabbits are a classical model for experimental atherosclerosis. Two groups of workers in the late 1940s studied the effect of alloxan diabetes on dietary cholesterol induced vascular lesions in rabbits. The untreated diabetic animals had alower incidence of vascular disease than the controls[19,20]. Later experiments showed that treatment with insulin reversed the apparent protective effect of diabetes on experimental atherosclerosis[21].

Another animal model of atherosclerosis is the cholesterol fed chicken. The regression of vascular disease which occurred when the chickens were changed from a high cholesterol diet to a normal diet could be prevented by the treatment with insulin at that time[22]. Insulin also overcame the protective effect of oestrogens on experimental atherosclerosis in chickens[22]. More recently the effect of insulin on chickens fed a normal diet has been studied. Nineteen weeks treatment with insulin zinc suspension in a mean dose of 3 units per day resulted in increased lipid containing lesions in the aorta compared with control animals not treated with insulin[23].

The artery is not an inert tube but consists of active metabolic tissue and the regulation of arterial metabolism is relevant to the development of vascular disease. When incubated in vitro insulin has no effect on aortic metabolism[24]. However, if the aorta is perfused at a physiological intraluminal pressure insulin stimulates lipid metabolism[25]. This is consistent with the findings that in vitro metabolism of the aorta is directly related to insulin levels in the animal at the time the aorta is removed[24,26]. Insulin levels were manipulated by streptozotocin[24], by dietary change or by insulin injections[26], the relationship of insulin levels to aortic metabolism being the same in all cases indicating that the aorta responds to exogenous and endogenous insulin in a similar fashion.

Insulin and Arterial Cells

Atherosclerosis is a disease of the intima and inner media of artery. There are two cells in this part of the artery—endothelial cells and smooth muscle cells. A current theory of the pathogenesis of atherosclerosis suggests that one of the earliest lesions is an alteration or injury to the endothelium, removing its protective effect and allowing exposure of the adjacent tissue to harmful constituents in the plasma, including platelets, lipoproteins and hormones[27]. An early response is proliferation of smooth muscle cells in the intima and inner media. Later the cells incorporate lipoproteins and together with monocyte-macrophages from the circulation become the foam cells of the lesion. Atherosclerosis thus involves four cell types, two arterial cells—endothelial and smooth muscle cells, and two circulating cells—platelets and monocytes[27]. The effect of insulin has been studied on the arterial cells.

Insulin in concentrations within the physiological range causes proliferation of smooth muscle cells cultured from monkey[28], rat[29] and human[30] sources. Serum from which insulin has been removed is less effective in stimulating arterial smooth muscle cell proliferation than whole serum[28], indicating again that native insulin and exogenous insulin have similar effects. Receptors for the growth function of insulin differ from those for its metabolic function, the latter being affected in insulin resistance[31]. Thus, it might be expected that arterial smooth muscle cells will respond to high concentrations of insulin even in states of insulin resistance. Insulin also stimulates the migration of smooth muscle cells[32], another important mechanism in the early stage of atherosclerosis. A further action of insulin of relevance to atherogenesis is the promotion of the interaction of low density lipoproteins with their receptor on the cell surface[33].

Although endothelial cells contain receptors for insulin[34], insulin has no effect on the proliferation or metabolism of these cells[35,36]. In this way endothelial cells may act as a barrier protecting the inner parts of the artery from the effects of circulating insulin. However, endothelial cells from different sources may have different responses to stimuli. Thus, large vessel endothelial cells do not respond to insulin whereas those derived from retinal capillaries do[37]. Whether this reflects a difference in the cells themselves or in their environment remains unclear.

Although endothelial cells do not respond to insulin glucose inhibits the proliferation of endothelial cells[38]. Thus, high glucose concentrations may cause or potentiate endothelial injury. On the other hand, glucose has no effect on smooth muscle cell proliferation[39].

Conclusions

Insulin has a number of effects on the vessel which may be relevant to atherosclerosis (Table). Using the clinical and epidemiological evidence a hypothesis can be formulated showing how the coincidence of high glucose and high insulin concentrations may affect the arterial wall in a way which may predispose to atherosclerosis. Glucose may act on endothelial cells causing or potentiating endothelial injury. This allows the smooth muscle cells of the intima and inner media to be exposed to plasma constituents including insulin as well

Table Actions of insulin which may be related to atherogenesis

1.	Arterial smooth muscle cell proliferation
2.	Migration of arterial smooth muscle cells
3.	Sterol synthesis in arterial smooth muscle cells
4.	Proteoglycan synthesis (in chondrocytes)
5.	Suppression of prostacyclin production by arterial tissue
6.	Lipid synthesis in arterial tissue
7.	Increased formation and decreased regression of arterial lipid lesions

as platelets and lipoproteins. Insulin will cause proliferation of smooth muscle cells and induce their migration into the intima. Lipid will accumulate in the smooth muscle cells as well as in monocyte macrophages and eventually a complicated lesion may occur.

If this hypothesis is true then atherosclerosis might be prevented by methods which reduce glucose levels without the need for high concentrations of insulin. Such methods would include weight reduction in those who are obese, physical exercise and the development of new ways of delivering to those diabetics who require it. It is interesting that in a large study of diabetics in Denmark it was found that among the factors associated with prolonged survival were good control, low insulin dose and low body weight[40]. These would all be consistent with the hypothesis.

References

1) Stout, R. W.: Blood glucose and atherosclerosis. Arteriosclerosis, **1**: 227–234 (1981).
2) Peters, N. and Hales, C. N.: Plasma insulin concentrations after myocardial infarction. Lancet, **1**: 1144–1145 (1965).
3) Stout, R. W.: The role of insulin in atherosclerosis in diabetics and nondiabetics. Diabetes, Suppl. **2**: 54–57 (1981).
4) Gertler, M. M., Leetma, H. E., Saluste, E., Covalt, D. A. and Rosenberger, J. L.: Covert diabetes mellitus in ischemic heart and cerebrovascular disease. Geriatrics, **27**: 105–120 (1972).
5) Sloan, J. M., Mackay, J. S. and Sheridan, B.: The incidence of plasma insulin, blood sugar and serum lipid abnormalities in patients with atherosclerotic disease. Diabetologia, **7**: 431–433 (1971).
6) Tzagournis, M., Chiles, R., Ryan, J. M. and Skillman, T. G.: Interrelationships of hyperinsulinism and hypertriglyceridemia in young patients with coronary heart disease. Circulation, **38**: 1156–1163 (1968).
7) Nikkila, E. A., Miettinen, T. A., Vesenne, M. R. and Pelkonen, R.: Plasma insulin in coronary heart disease. Lancet, **2**: 508–511 (1965).
8) Rubenstein, A. H., Seftel, H. C., Miller, K., Bersohn, I. and Wright, A. D.: Metabolic responses to oral glucose in healthy South African white, Indian and African subjects. Br. Med. J., **1**: 748–751 (1969).
9) Logan, R. L., Thomson, M., Riemersma, R. A., et al.: Risk factors for ischaemic heart-disease in normal men aged 40 (Edinburgh-Stockholm Study). Lancet, **1**: 949–955 (1978).
10) Pyorala, K.: Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. Diabetes Care, **2**: 131–141 (1979).
11) Ducimetiere, P., Eschwege, E., Papoz, L., Richard, J. L., Claude, J. R. and Rosselin, G.: Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary heart disease mortality in a middle-aged population. Diabetologia, **19**: 205–210 (1980).
12) Welborn, T. A. and Wearne, K.: Coronary heart disease incidence and cardiovascular mortality in Busselton with reference to glucose and insulin concentrations. Diabetes Care, **2**: 154–160 (1979).
13) Bagdade, J. D., Bierman, E. L. and Porte, D., Jr.: The significance of basal insulin levels in evaluation of the insulin response to glucose in diabetic and non-diabetic subjects. J. Clin. Invest., **46**: 1549–1557 (1967).
14) Santen, R. J., Willis, P. W. and Fajans, S. S.: Atherosclerosis in diabetes mellitus. Arch. Intern. Med., **130**: 833–843 (1972).
15) Reinheimer, W., Bliffen, G., McCoy, J., Wallace, D. and Albrink, M. J.: Weight gain, serum lipids, and vascular disease in diabetics. Am. J. Clin. Nutr., **20**: 986–996 (1967).
16) Rizza, R. A., Gerich, J. E., Haymond, M. W., Westland, R. E., Hall, L. D., Clemens, A. H. and Service, F. J.: Control of blood sugar in insulin dependent diabetes: comparison of an artificial endocrine pancreas continuous subcutaneous insulin infusion, and intensified conventional insulin therapy. New Eng. J. Med., **303**: 1312–1318 (1980).
17) Rasmussen, S. M., Heding, L. G. and Parbst, E.: Serum IRI in insulin-treated diabetics during a 24-hour period. Diabetologia, **11**: 151–158 (1975).
18) Kashyap, L., Magill, F., Rojas, L. and Hoffman, M. M.: Insulin and non-esterified fatty acid metabolism in asymptomatic diabetics and atherosclerotic subjects. Canad. Med. Assoc. J., **102**: 1165–1169 (1970).
19) Duff, G. L. and McMillan, G. C.: The effect of alloxan diabetes on experimental atherosclerosis in the rabbit. I. The inhibition of experimental

atherosclerosis in alloxan diabetes. II. The effect of alloxan diabetes on the retrogression of experimental cholesterol atherosclerosis. J. Exper. Med., **89**: 611–630 (1949).
20) McGill, H. C. and Holman, R. L.: The influence of alloxan diabetes on cholesterol atheromatosis in the rabbit. Proc. Soc. Exp. Biol. Med., **72**: 72–75 (1949).
21) Duff, G. L., Brechin, D. J. H. and Findelstein, W. E.: The effect of alloxan diabetes on experimental cholesterol atherosclerosis in the rabbit. IV. The effect of insulin therapy on the inhibition of atherosclerosis in the alloxan-diabetic rabbit. J. Exper. Med., **100**: 371–380 (1954).
22) Stamler, J., Pick, R. and Katz, L. N.: Effect of insulin in the induction and regression of atherosclerosis in the chick. Circulation Res., **8**: 572–576 (1960).
23) Stout, R. W.: Development of vascular lesions in insulin-treated animals fed a normal diet. Br. Med. J., **3**: 685–687 (1970).
24) Stout, R. W., Buchanan, K. D. and Vallance-Owen, J.: Arterial lipid metabolism in relation to blood glucose and plasma insulin in rats with streptozotocin-induced diabetes. Diabetologia, **8**: 398–401 (1972).
25) Capron, L., Philippe, M., Guilmot, J.-L., Fiessinger, J.-N. and Housset, E.: Effects of insulin exposure upon the metabolism of rat aortic media: influence of hydrostatic forces. Arteriosclerosis, **1**: 345–352 (1981).
26) Capron, L., Housset, E. and Hartmann, L.: Effects of in vitro and in vivo exposure to insulin upon glucose carbon accumulation in rat aorta: different patterns of response for intima-media and adventitia. Metabolism, **29**: 859-866 (1980).
27) Ross, R.: Atherosclerosis: a problem of the biology of arterial wall cells and their interactions with blood components. Arteriosclerosis, **1**: 293–311 (1981).
28) Stout, R. W., Beirman, E. L. and Ross, R.: Effect of insulin on the proliferation of cultured primate arterial smooth muscle cells. Circulation Res., **36**: 319–327 (1975).
29) Pfeifle, B., Ditschuneit, H. H. and Ditschuneit, H.: Insulin as a cellular growth regulator of rat arterial smooth muscle cells in vitro. Horm. Metab. Res., **12**: 381–385 (1980).
30) Pfeifle, B. and Ditschuneit, H.: Effect of insulin on growth of cultured human arterial smooth muscle cells. Diabetologia, **20**: 155–158 (1981).
31) King, G. L., Kahn, C. R., Rechler, M. M. and Nissley, S. P.: Direct demonstration of separate receptors for growth and metabolic activities of insulin and multiplication-stimulating activity (an insulin like growth factor) using antibodies to the insulin receptor. J. Clin. Invest., **66**: 130–140 (1980).
32) Nakao, J., Ito, H., Kanaysu, T. and Murota, S.-I.: Stimulatory effect of insulin on aortic smooth muscle cell migration induced by 12-L-hydroxy-5, 8, 10, 14-eicosatetraenoic acid and its modulation by elevated extracellular glucose levels. Diabetes, **30**: 185–192 (1985).
33) Chait, A., Bierman, E. L. and Albers, J. J.: Regulatory role of insulin in the degradation of low density lipoprotein by cultured human skin fibroblasts. Biochim. Biophys. Acta, **529**: 292–299 (1978).
34) Bar, R. S., Hoak, J. C. and Peacock, M. L.: Insulin receptors in human endothelial cells: identification and characterization. J. Clin. Endocr. Metab., **47**: 699–707 (1978).
35) Taggart, H. and Stout, R. W.: Control of DNA synthesis in cultured vascular endothelial and smooth muscle cells. Atherosclerosis, **37**: 549–557 (1980).
36) Schwartz, S. M., Gajdusek, C. M. and Selden, S. C.: III. Vascular wall growth control: the role of the endothelium. Arteriosclerosis, **1**: 107–126 (1981).
37) King, G. L., Buzney, S. M., Kahn, C. R., Hetu, N., Buchwald, S., MacDonald, S. G. and Rand, L. I.: Differential responsiveness to insulin of endothelial and support cells from micro- and macrovessels. J. Clin. Invest., **71**: 974–980 (1983).
38) Stout, R. W.: Glucose inhibits replication of cultured human endothelial cells. Diabetologia, **23**: 436–439 (1982).
39) Turner, J. L. and Bierman, E. L.: Effects of glucose and sorbitol on proliferation of cultured human skin fibroblasts and arterial smooth muscle cells. Diabetes, **27**: 583–588 (1978).
40) Deckert, T., Poulsen, J. E. and Larsen, M.: Prognosis of diabetics with diabetes onset before the age of thirty-one. II. Factors influencing the prognosis. Diabetologia, **14**: 371–377 (1978).