obesity, since the post-treatment mean body-weight of the thyrotoxic and euthyroid subjects did not differ. It may indicate that those patients who had developed hypothyroidism had been selected by their disease from a different population sample than that from which the thyrotoxic subjects had been drawn.

The prediabetic state of 2 patients was revealed by the development of frankly diabetic glucose-tolerance curves when they were thyrotoxic; the curves reverted to normal with treatment of the thyrotoxicosis. Their insulin release was greatly impaired, and although it improved when they were euthyroid it was still subnormal. Conversely, in 2 hypothyroid subjects a diabetic tendency only became manifest on replacement therapy, and these patients also had subnormal insulin release. This situation is analogous to the experimental "thyroid diabetes" induced in partially pancreatectomised dogs given thyroid extract (Houssay 1944).

Some of the abnormalities of glucose tolerance in thyroid disease could be due to altered absorption within the small bowel. Work is now in progress to measure directly the changes in absorption in thyroid disease and to clarify the abnormalities in insulin release and sensitivity.

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Requests for reprints should be addressed to C. D. H.

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INSULIN-STIMULATED LIPOGENESIS IN ARTERIAL TISSUE IN RELATION TO DIABETES AND ATHEROMA

R. W. STOUT
M.B. Belf., M.R.C.P.
RESEARCH FELLOW, ROYAL VICTORIA HOSPITAL, AND DEPARTMENT OF MEDICINE, QUEEN'S UNIVERSITY, BELFAST

Summary Intravenous injection of rats with insulin and ^{14}C-labelled substrate containing either glucose or acetate led to much greater incorporation of these substances into the aortic lipids than when the substrate alone was injected. Since insulin is known to inhibit tissue-lipase in arterial tissue, excess of circulating insulin could cause accumulation of fat in the arterial wall by both increasing its deposition and inhibiting its removal. It is suggested that insulin thus plays a major role in the pathogenesis of atherosclerosis.

Introduction

THE association of ischæmic vascular disease and diabetes mellitus has been known for many years (Brunton 1907, Warren et al. 1966), and has been confirmed by the International Atherosclerosis Project (Robertson and Strong 1968). It has recently become apparent that abnormalities of carbohydrate metabolism are present in a large proportion of patients with ischæmic heart-disease, without clinical diabetes mellitus. These abnormalities may be summarised as follows:

(*a*) Many patients with premature coronary-artery disease have hyperglycæmia and abnormal glucose-tolerance tests (e.g., Sowton 1962, Cohen and Shafrir 1965, Epstein 1967). That this abnormality is not a result of acute myocardial infarction is shown by its presence long after the acute episode, and also in patients with angiographically proven coronary arteriosclerosis without myocardial infarction (Falsetti et al. 1968, Heinle et al. 1967). Abnormally high levels of circulating insulin have been found in subjects with similar abnormalities of glucose tolerance (Yalow and Berson 1960, Hales et al. 1965).

(*b*) Abnormally high insulin responses to glucose loading have been found in patients with ischæmic heart-disease (Nikkila et al. 1965, Peters and Hales 1965, Tzagournis et al. 1967).

(*c*) Increased synalbumin insulin-antagonism was found in 73% of patients with ischæmic heart-disease compared with 23% of normal controls (Vallance-Owen and Ashton 1963).

(*d*) Abnormal plasma-lipid patterns, similar to those found in diabetics, are common in patients with ischæmic heart-disease (Adlersberg and Eisler 1959, Albrink 1962, Brown et al. 1965). In particular, carbohydrate-induced hyperglyceridæmia is common in these patients (Reaven et al. 1965)—an abnormality which is associated with high plasma-insulin levels (Davidson and Albrink 1966, Reaven et al. 1967, Eden and Phaire 1968).

(*e*) The incidence of ischæmic heart-disease is related to dietary carbohydrate, particularly refined sugars (Cohen et al. 1961, Yudkin and Roddy 1964). Sugar ingestion causes hyperlipæmia (Kuo and Bassett 1965, Lees and Fredrickson 1965) and stimulates insulin secretion (Yalow and Berson 1960).

The common factor in these abnormalities is a high level of circulating insulin. It therefore seemed important to investigate the action of insulin on vascular metabolism.

Method

Male Wistar rats, weighing 90–120 g., fed and unanæsthetised, were investigated by a modification of the method of Rafaelson et al. (1965). In any one experiment animals of the same weight were used. The animals were injected intravenously, using the tail vein, with Gey and Gey buffer (2·5 ml. per 100 g. body-weight) containing 4 μC per ml. of the ^{14}C-labelled substrate alone or with 4000 μ units per ml. of insulin. The substrate was sodium-acetate-1-^{14}C (specific activity 40·0 mC per mmole) in the first series of experiments, and D-glucose-U-^{14}C (4·9 mC per mmole) in the second. In each experiment half of the animals received buffer and substrate, and the remainder buffer, substrate, and insulin.

1 hour after injection the animals were killed and the radioactivity in the aortic lipids determined. The whole aorta from its origin to the bifurcation was removed and carefully dissected free of adventitious tissue. It was then weighed on a torsion balance and placed in 1 ml. of a 2/1 solution of chloroform/methanol. Total lipids were isolated and purified by a modification of the method of Folch et al. (1957). The aortas were refluxed in chloroform/methanol (2/1) at 70°C for 3 hours (Rao and Rao 1968). The solution was centrifuged and the supernatant washed with calcium chloride (0·05%) to remove water-soluble radioactive compounds. The purified solution was made up to 2·5 ml. 0·5 ml. samples were pipetted into 5 ml. scintillation fluid and counted for 10 minutes in a Packard 'Tri-carb.' scintillation spectrometer. Quench correction was performed using the automatic external standard (A.E.S.) in the acetate experiments. In the glucose experiments the A.E.S. was inoperative and channels-ratio quench correction was used.

Results

The mean uptake of sodium-acetate-1-^{14}C, in counts per mg. per minute ±S.E.M., was 0·0365±0·0062 (100%) in 16 controls, and 0·0638±0·0068 (174·8%) in 17 rats which received insulin. The mean uptake of D-glucose-U-^{14}C, in counts per mg. per minute ±S.E.M., was 0·0139±0·0018 (100%) in 19 controls, and 0·0241±0·0026 (173·7%) in 20 rats which received insulin. The values are extremely small, in keeping with the well-known slow metabolism of vascular tissue (Briggs et al. 1949). The values obtained in each experiment were similar. Application of Student's *t* test shows that the difference between the control and insulin-treated groups in each case is highly significant ($P<0.01$). These results show that insulin greatly enhances the incorporation of both glucose and acetate into the lipids of the rat aorta.

The ^{14}C-labelled lipid in the aortic wall is probably triglyceride synthesised from fatty acid and α-glycerophosphate, both of these having been metabolised from the injected substrates (Chernick et al. 1949). Further work on this is in progress.

Discussion

The results reported here show that insulin stimulates the synthesis of lipid in the arterial wall. The fact that such a striking effect was found in a short time suggests that sustained elevation of circulating insulin would result in the accumulation of important amounts of lipid. It is well known that maturity-onset diabetics, who have high serum-insulin levels, lay down fat in their adipose tissue. It is suggested that, by the same mechanism, they can also accumulate fat in their arterial walls. This process would act for many years, long before the diabetes mellitus became clinically apparent. Indeed, a vascular catastrophe may well precede the appearance of clinical diabetes, and appropriate dietary treatment at that time may prevent it ever appearing.

Mahler (1966) has shown that insulin inhibits tissue-lipase in arterial tissue, and suggests that this results in the accumulation of lipids and hence the formation of atherosclerosis. The present work provides evidence that insulin also stimulates lipogenesis in vascular tissue, an action similar to its effect on adipose tissue (Renold et al. 1965). Excess of circulating insulin would thus cause an accumulation of fat in the vessel-wall by both increasing its deposition and inhibiting its removal.

The published reports contain no consistent evidence for a direct and rapid action of insulin on vascular tissue. Wertheimer and Ben-Tor (1961, 1962) reported that the oxygen consumption and glucose uptake of rat aortas were depressed by alloxan diabetes and increased by insulin in vivo and in vitro. Other workers have confirmed the effect of alloxan diabetes (Foster and Siperstein 1960, Urrutia et al. 1962, Yalcin and Winegrad 1963), but were unable to demonstrate any insulin effect in vitro on either normal or diabetic aortas (Mulcahy and Winegrad 1962, Urrutia et al. 1962). Treatment of diabetic animals with insulin for more than 18 hours was required to return arterial metabolism towards normal. It seems, therefore, that the in-vitro preparation is unsuitable for demonstrating insulin action on vascular tissue. The reason is not clear.

There has been no reported attempt to induce experimental atherosclerosis with insulin. However, the opposite situation has been found to occur. Rabbits rendered insulin-deficient with alloxan developed less striking vascular lesions on an atherogenic diet than normal controls (Duff and McMillan 1949, McGill and Holman 1949) This effect was reversed by treatment with insulin (Duff et al. 1954). Stamler et al. (1960) found that administration of insulin inhibited the regression of atherosclerotic lesions in chickens when the atherogenic diet was replaced by a normal diet. Insulin also had an inhibitory effect on the atheroma-sparing action of exogenous œstrogens in chickens.

The evidence presented here, together with that in the published reports, support the hypothesis that insulin plays a major role in the pathogenesis of atherosclerosis (e.g., Vallance-Owen 1967). Further work is in progress.

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Requests for reprints should be addressed to the Department of Medicine, Institute of Clinical Science, Grosvenor Road, Belfast BT12 6BJ.

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