Insulin Sensitivity and Vascular Disease in Insulin-dependent Diabetics

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The presence of atherosclerosis in non-diabetic individuals has been shown to be associated with a decrease in the hypoglycaemic effect of insulin (Waddell and Field, 1960; Wahlberg, 1966). Similar studies have not been reported in patients with proved diabetes mellitus. We describe here a positive relation between insulin insensitivity and the severity of clinical vascular disease, both atherosclerotic and microangiopathic, in insulin-dependent diabetics of long duration.

Materials and Methods

Subjects were selected for study and insulin tolerance tests were performed and analysed as previously described (Martin and Stocks, 1967). Insulin tolerance tests, with 0.1 i.u./kg. of glucagon-free beef insulin intravenously, were performed in 51 patients (26 men and 25 women) aged 22 to 72 years. None were obese, all had had diabetes mellitus for 15 to 40 years, with insulin doses of between 28 and 88 units per day. All were known to be insulin-dependent ketosis-prone diabetics and were ambulant without evidence of severe renal failure at the time of study. The tests were performed fasting at least 14 hours after the injection of short-acting insulin and 26 hours after long-acting insulin. Blood samples for glucose estimations were obtained 5 minutes before injection, at the time of injection, and 5, 10, 15 20, 30, and 60 minutes after injection of insulin by use of an indwelling needle and were analysed in duplicate with a Technicon Autoanalyzer.

Insulin sensitivity was expressed as the glucose assimilation index, an expression of the maximum percentage fall of blood glucose per minute after insulin (Norgaard and Thaysen, 1929). As discussed elsewhere this was preferred to an exponential rate constant expression because in some cases the glucose fall after insulin injection was not linear (Martin and Stocks, 1967).

The vascular state was assessed by a full clinical examination which was performed at the time of the insulin sensitivity test. This included detailed examination of the optic fundi after dilatation by two observers and a 12-lead electrocardiogram and urinalysis.

The presence of atherosclerotic disease was indicated by a definite history of myocardial infarction, angina pectoris, stroke, intermittent claudication, or gangrene from arterial occlusion and by the appearance of typical changes in the retinal vessels and absence of peripheral pulses in the legs or the presence of typical ischaemic changes in the electrocardiogram. Six patients had widespread atherosclerotic disease and the others ischaemic changes in the electrocardiogram and/or absence of arterial pulses in the feet. Retinal vessel changes were regarded as confirmatory only. Diabetic angiopathy was characterized by the appearance of typical retinal changes, persistent proteinuria in the absence of any symptomatic or microscopical evidence of urinary infection, and objective evidence of peripheral neuritis. No patient had any other condition known to cause peripheral neuritis, and in juvenile type diabetics of long duration we regard it as a manifestation of diabetic angiopathy. In each case an attempt was made to classify the features of both atherosclerosis and microangiopathy as minimal, moderate, or severe, despite the relative imprecision of any clinical estimate of vascular disease.

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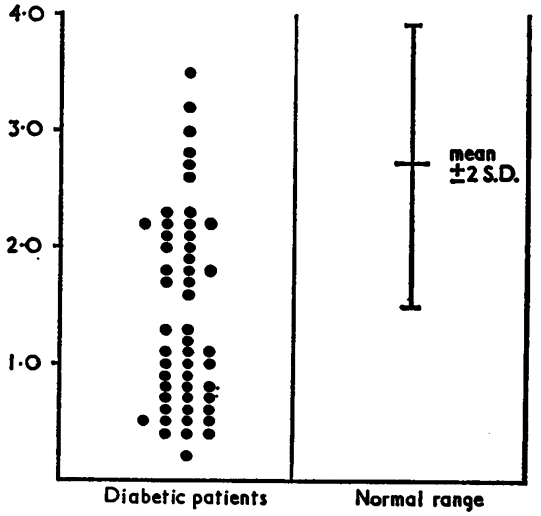
In 47 patients the fasting plasma cholesterol was measured by an autoanalyser technique, and in 43 the fasting plasma triglycerides were determined also by the method of Van Handel and Zilversmit (1957).

Results

The glucose assimilation index of the 51 diabetic patients in this series is shown in the Chart. The lower limit of this expression in normal subjects in this laboratory is a fall of 1.5% per minute. Twenty-eight of the diabetic patients investigated were insensitive to insulin by this criterion. There was no correlation between acute insulin sensitivity and age, sex, duration of diabetes, body build, or daily insulin requirements.

The relation between insulin sensitivity and diabetic angiopathy is shown in Tables I and II. In Table I the degree of angiopathy is classified as minimal, moderate, or severe, and the insulin sensitivity as normal or insensitive. There is a significant tendency for insulin insensitivity to be associated with increasingly severe angiopathy (χ^2 for linear trend = 10.67 $P<0.005$). In order to avoid possible bias in the classification



Glucose assimilation index of 51 insulin-dependent diabetic patients after intravenous insulin compared with the range in normal subjects.

TABLE I.—*Relation Between Range of Glucose Assimilation Index and Microangiopathy*

Degree of Angiopathy	Glucose Assimilation Index	
	< 1·5	> 1·5
Minimal	9	17
Moderate	6	4
Severe	13	2

$\chi^2 = 10.67$. $P < 0.005$.

TABLE II.—*Relation Between Range of Glucose Assimilation Index and Microangiopathy*

Range of G.A.I.	Angiopathy	
	Minimal	Established
> 2·0	12	4
1·0–1·9	8	8
0–0·9	6	13

$\chi^2 = 6.14$. $P < 0.02$.

of microangiopathy, in Table II insulin sensitivity is graded, and angiopathy is classified as minimal or established. There is a significant tendency for patients with clinical microangiopathy to have a greater degree of insulin insensitivity than those without ($\chi^2=6.14$, $P<0.02$).

Only 16 of the 51 patients had definite clinical evidence of atherosclerotic disease, and it was only possible to analyse the degree of insulin sensitivity in relation to the presence or absence of atherosclerosis. As shown in Table III there was a significant trend for atherosclerosis to be associated with a low glucose assimilation index (χ^2 for linear trend$=9.87$, $P<0.005$).

TABLE III.—*Relation Between Range of Glucose Assimilation Index and Atherosclerosis*

Range of G.A.I.	Atherosclerosis	
	Nil	Present
>2·0	16	0
1·0–1·9	10	6
0–0·9	9	10

$\chi^2=9·87$. $P<0·005$.

Clinically detectable atherosclerosis and diabetic angiopathy were closely related, so that if the indices of each were combined the relation with insulin insensitivity was significant at less than one in a thousand. There was no relation between the initial fasting blood glucose and the vascular state. The mean fasting blood glucose of the three groups—minimal, moderate, and severe angiopathy—was 220 mg./100 ml. (range 496–48 mg.), 280 mg. (range 440–144 mg.), and 226 mg. (range 466–70 mg.) respectively. The mean fasting blood glucose of the patients with atherosclerosis was 240 mg. compared with 231 mg. for those without clinical disease. There is no significant difference between any of these groups.

No relation was found between either the presence of angiopathy or atherosclerosis and the plasma cholesterol or triglycerides. The fasting plasma cholesterol varied from 144 to 420 mg./100 ml. Fourteen patients, 11 of whom were women, had a cholesterol of 300 mg./100 ml. or more. The fasting plasma triglycerides were from 27 to 288 mg./100 ml., but only three patients, all females, had levels above 150 mg./100 ml. The triglycerides and cholesterol values correlated closely, but insulin sensitivity was not significantly related to either.

Discussion

An increased incidence of insulin antagonists as measured by the in vitro rat diaphragm technique has been described in juvenile-onset diabetics of long duration with angiopathic vascular disease (Bornstein and Hyde, 1959 ; Downie and Martin, 1959), and Alivisatos and McCullagh (1956) reported that vascular disease was much more common in stable than in brittle diabetics on insulin. The present results show that in insulin-dependent diabetics the presence of both clinical diabetic angiopathy and atherosclerosis is associated with a reduction in the hypoglycaemic effect of intravenous insulin.

Previous investigations have shown that the glucose assimilation index is reproducible, and that there is a significant but low order of correlation ($r=0.38$, $0.02>P>0.01$) between insulin insensitivity and increased plasma protein binding of ^{131}I insulin (Martin and Stocks, 1967). Though it has been suggested that ^{131}I insulin may not be a valid index of physiological insulin activity (Izzo *et al.*, 1964) it is generally accepted that this binding is due to antibodies produced by the injection of heterologous insulin (Berson and Yalow, 1959). Attempts to measure the disappearance rate of immunoreactive insulin and relate it to the degree of hypoglycaemia produced, in similar fashion to normal subjects (Martin *et al.*, 1967 ; Stimmler, 1967), have so far been unsuccessful in these patients because of the high plasma binding of insulin.

Our finding that in diabetic patients of long duration insulin insensitivity is associated with vascular disease could be due to small blood vessel disease impeding the transfer of insulin from the vascular compartment. Previous reports that there is a decreased loss of ^{131}I insulin (Butterfield *et al.*, 1963) and of ^{131}I albumin (Ismail *et al.*, 1965) from the circulation of diabetics as compared with non-diabetics support this hypothesis. However, there appears to have been no attempt to relate these observations to vascular disease. The lack of any relation between the serum lipids and either angiopathy or atherosclerosis is in keeping with previous reports on similar groups of diabetics (Traisman *et al.*, 1960 ; Sterky *et al.*, 1963 ; Knowles *et al.*, 1965).

In this group of insulin-dependent diabetics clinical microangiopathy and atherosclerotic disease were associated with insulin insensitivity and probably with increased protein binding of insulin and the formation of heterologous antibodies, mainly to beef insulin. These findings are in keeping with the histological and experimental investigations summarized by Blumenthal *et al.* (1965), which have suggested that diabetic angiopathy is due to an immune reaction to insulin. It is possible that the capacity of an individual diabetic to form such antibodies may be associated with the development of vascular disease.

A prospective study of insulin-dependent diabetics will be required to define whether acute insulin insensitivity precedes or follows the clinical manifestations of microangiopathy and atherosclerosis and whether it is consistently associated with changes in the binding of insulin to plasma proteins. However, as the present investigation did not show any tendency for insulin sensitivity to decrease with the duration of diabetes, and a previous investigation (Martin and Stocks, 1967) did not show any increase in plasma binding of ^{131}I insulin with duration, we think it likely that insulin insensitivity precedes the clinical manifestations of vascular disease.

Summary

In 51 insulin-dependent diabetics of long duration there was a significant tendency for both microangiopathy and atherosclerosis to be associated with a reduction in the acute hypoglycaemic effect of intravenous beef insulin. These results suggest either that vascular disease in insulin-treated diabetics is related to increased plasma binding of insulin or that insulin insensitivity is a consequence of both angiopathic and atherosclerotic disease.

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REFERENCES

Alivisatos, J. G., and McCullagh, E. P. (1956). *Amer. J. Med.*, **21**, 344.
Berson, S. A., and Yalow, R. S. (1959). *J. clin. Invest.*, **38**, 1996.
Blumenthal, H. T., Goldenberg, S., and Berns, A. W. (1965). In *The Nature and Treatment of Diabetes*, edited by B. S. Leibel and G. A. Wrenshall, p. 397. Amsterdam.
Bornstein, J., and Hyde, D. (1959). *Diabetes*, **8**, 92.
Butterfield, W. J., Garratt, C. J., and Whichelow, M. J. (1963). *Clin. Sci.*, **24**, 331.
Downie, E., and Martin, F. I. (1959). *Diabetes*, **8**, 383.
Ismail, A. A., Khalifa, K., and Madwar, K. R. (1965). *Lancet*, **2**, 810.
Izzo, J. L., Bale, W. F., Izzo, M. J., and Roncone, A. (1964). *J. biol. Chem.*, **239**, 3743.
Knowles, H. C., Guest, G. M., Lampe, J., Kessler, M., and Skillman, T. G. (1965). *Diabetes*, **14**, 239.
Martin, F. I. R., and Stocks, A. E. (1967). *Aust. Ann. Med.*, **16**, 289.
Martin, F. I. R., Stocks, A. E., and Pearson, M. J. (1967). *Lancet*, **1**, 619.
Norgaard, A., and Thaysen, T. E. H. (1929). *Acta med. scand.*, **72**, 492.
Sterky, G., Larsson, Y., and Persson, B. (1963). *Acta paediat. (Uppsala)*, **52**, 11.
Stimmler, L. (1967). *Diabetes*, **16**, 652.
Traisman, H. S., Newcomb, A. L., Sever, J. L., and Hammes, R. (1960). *Diabetes*, **9**, 481.
Van Handel, E., and Zilversmit, D. B. (1957). *J. Lab. clin. Med.*, **50**, 152.
Waddell, W. R., and Field, R. A. (1960). *Metabolism*, **9**, 800.
Wahlberg, F. (1966). *Acta med. scand.*, Suppl. No. 453, p. 51.