The glucose–fatty acid cycle and human diabetes

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The task we set ourselves was to test as best we could in human subjects the hypothesis of the role of the glucose–fatty acid cycle in the cause of diabetes. In his paper Dr Hales (1966) has reviewed this theory, founded mainly on experimental work on animal tissues in vitro. Human beings, of course, are much less susceptible to the experimental method of approach, and we had so far as possible to choose circumstances where we could expect to obtain useful information, while the subjects were deriving benefit to themselves, admittedly at some inconvenience. The people we studied were fifteen patients who originally sought assistance for the control of their very gross obesity. All but two were women and they varied in age from their early twenties to their late sixties. Most were diabetic, but only one had required treatment with a hypoglycaemic agent to control the primary symptoms of diabetes; individuals who are so obese do not lack endogenous insulin. From the nature of our investigations we had to select subjects who would be willing to remain under continuous observation with strict control of the diet for periods of approximately 2 months. Thus some of our subjects were so obese as to be virtually immobile and found it no hardship to vegetate in the ward rather than at home.

The plan of investigation was as follows. For the initial period of at least 8 days the subjects were given a diet which was usually of 2500 kcal so that initial control values could be obtained on a diet which was thought to approximate to that taken at home. To our surprise, however, weight losses of up to 4 lb in 8 days occurred on intakes of 2500 kcal a day in some subjects. After the initial study period the subjects were given a completely liquid diet consisting of Metercal to provide 600 kcal/day. This diet contained 48 g protein to limit body protein breakdown; the fat content was 14 g and the carbohydrate content 76 g/day. The only additions allowed to this diet were unsweetened tea or coffee. Serial measurements were made of plasma levels of triglycerides, non-esterified fatty acids (NEFA), ketones and cholesterol, all in the fasting state. At intervals the intravenous glucose tolerance test was performed, and also oral glucose tolerance tests, during which blood was taken for plasma insulin immuno-assay by Dr Hales. (Hales & Randle, 1963*a*).

Respiratory quotient determinations were done 2 h after the first meal of the day during initial and low-calorie diet periods. NEFA was determined colorimetrically by Duncombe's (1963) method, triglyceride by Van Handel & Zilversmit's (1957) method and ketones by Reid's (1960) method. The intravenous glucose tolerance results have been expressed as 'increment indices' as described by Duncan (1956).

It was expected that the 600 kcal diet would produce a rise in NEFA, with a corresponding change in glucose tolerance, but other methods had to be employed to produce more drastic rises in plasma NEFA. Furthermore the methods employed needed to be ones which would not be likely to affect the level of blood glucose or plasma insulin. Thus hormones such as adrenaline, glucagon, cortisone, ACTH and growth

hormones were ruled out on this score. The most useful technique used to raise the plasma NEFA was that suggested by Schalch & Kipnis (1964). A large dose of heparin was injected intravenously 3 h after a meal of 60 g fat. This dose acts as an activator of lipoprotein lipase, which converts triglycerides into NEFA. The duration of the effect varies with the dose of heparin used. Intravenous glucose tolerance tests were done during the periods of raised NEFA levels in the plasma. Later this system was used to test various drugs which might lower the plasma level in an attempt to correlate this with changes in the intravenous glucose tolerance. Plasma insulin immuno-assays, done in parallel, served to indicate whether changes in glucose tolerance could easily be ascribed to this factor.

Results

It was confirmed that plasma fasting NEFA levels tended to rise after the diet was changed from 2500 kcal to 600 kcal, although after about 2–3 weeks the NEFA values tended to fall again. An example is shown in Fig. 1. The plasma triglycerides did not fall appreciably on the 600 kcal regime unless they were initially

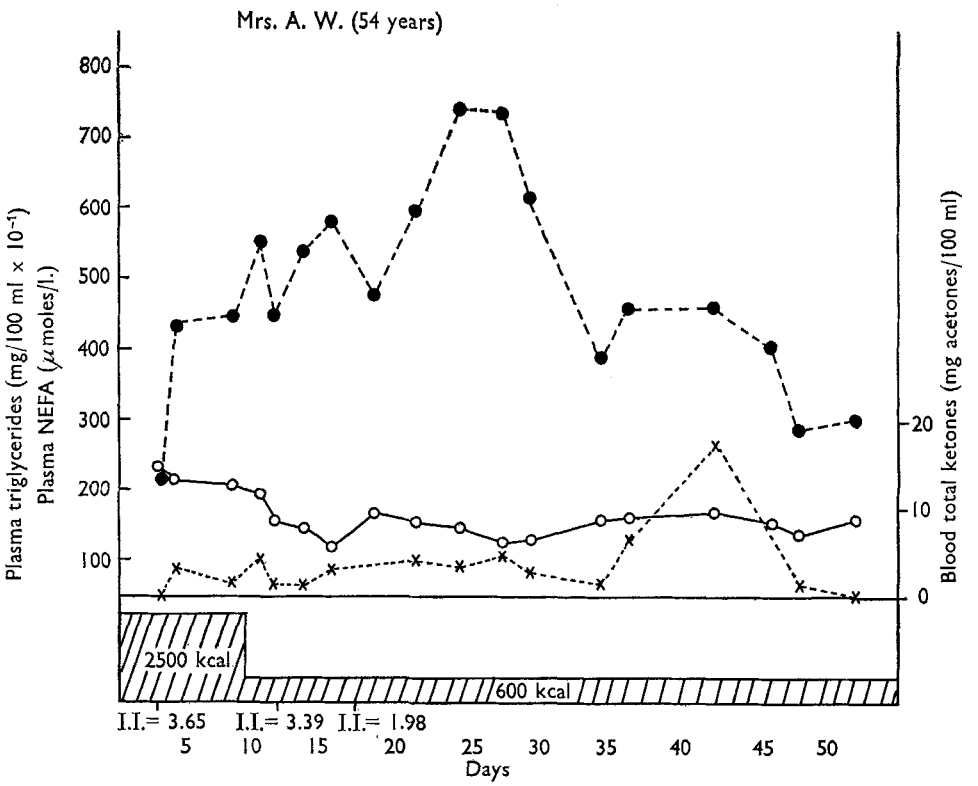

Fig. 1. Course of plasma levels of non-esterified fatty acids, triglycerides and blood ketones on a dietary regime if 2500 kcal for 9 days followed by 600 kcal for 42 days. The triglyceride levels are expressed in terms of glycerol content. These values need to be multiplied by 9·6 to represent approximate concentrations of whole triglyceride molecules. ●--●, NEFA; ○—○, triglycerides; ×--×, ketones. I.I., increment index (see p. 67).

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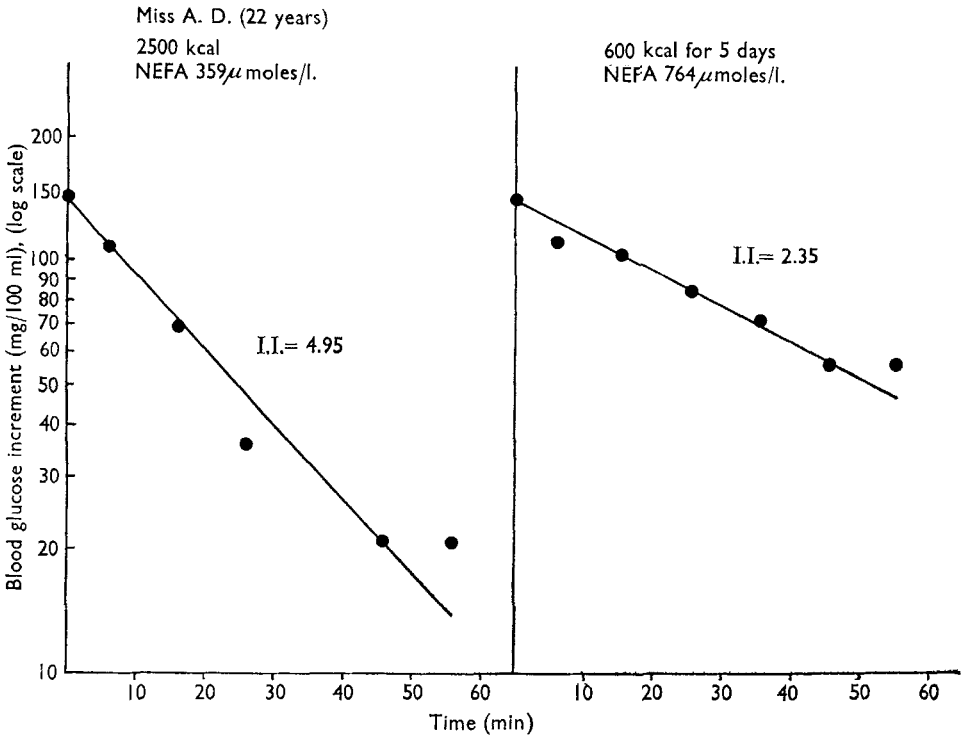

Fig. 2. Deterioration of intravenous glucose tolerance found with change from 2500 kcal diet to 600 kcal diet for 5 days with associated rise in plasma non-esterified fatty acids. I.I., increment index (see p. 67).

abnormally high, nor did the levels of blood ketones rise appreciably (with the unaccountable exception of day 43 in Fig. 1). There was a tendency for the intravenous glucose tolerance to fall, as shown by a decrease of the increment index, as the plasma fasting NEFA values rose above 600 μmoles/l. (see Fig. 2). Greater elevations of plasma NEFA, produced for instance by large doses of nicotinic acid by mouth, were associated with more dramatic deterioration of intravenous glucose tolerance (Fig. 3).

The giving of a large dose of heparin intravenously 3 h after a meal of fat produced a transient variable rise of plasma NEFA (Figs. 4 and 5). Again the rise in plasma NEFA value was associated with deterioration of intravenous glucose tolerance to 2·17, compared with 3·39 when Mrs A. W. had received the 600 kcal diet for 3 days (Fig. 4); and to 0·99, compared with 1·72 when Miss D. E. had received the 600 kcal diet for 5 days (Fig. 5).

One male subject had hypertriglyceridaemia even without a preceding fatty meal and in his case there was a dramatic rise in plasma NEFA level when heparin was administered intravenously with the glucose in the fasting state (Fig. 6).

Estimation of plasma insulin suggested that the changes in glucose tolerance were not due to changes in endogenous insulin production and, when there was an apparent

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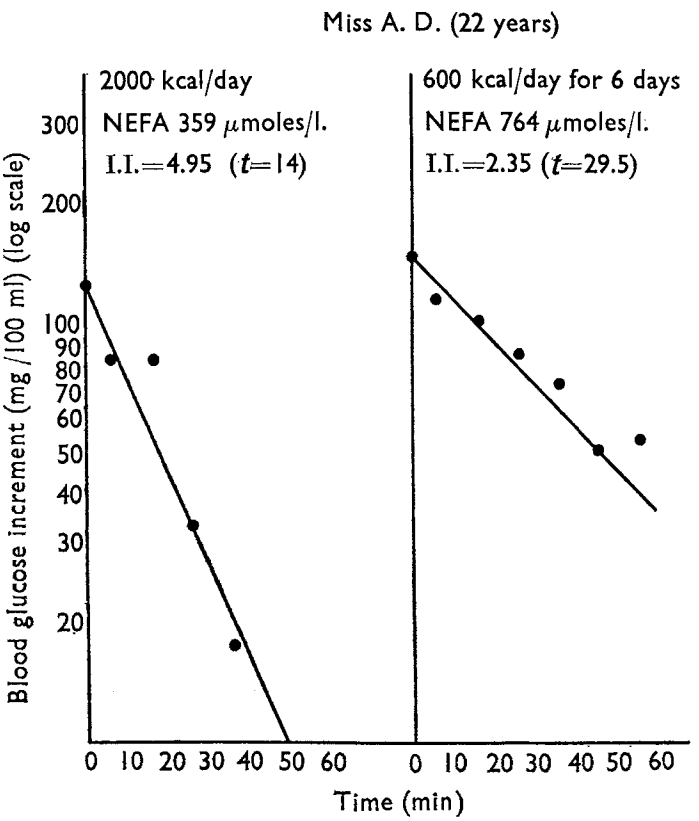

Fig. 3. Deterioration of intravenous glucose tolerance associated with marked rise in plasma non-esterified fatty acid level produced by administration of nicotinic acid by mouth (3·5 g/day in divided doses). I.I., increment index (see p. 67); *t* time taken for blood glucose increment to fall by 50%.

slight improvement in oral glucose tolerance on a prolonged 600 kcal dietary regime, there was a notable reduction in the levels of plasma insulin (Fig. 7). One of our subjects had extraordinarily high levels of plasma insulin, confirmed by biological assay, and yet oral glucose tolerance was perfectly normal (Fig. 8) until her plasma NEFA level was much raised by oral nicotinic acid (cf. Fig. 3). Not surprisingly Dr Vallance-Owen found that the plasma of this patient contained a relatively high concentration of synalbumin insulin antagonist. Another of the fifteen subjects in this study had abnormally high plasma insulin values in the initial normal oral glucose tolerance, but they did not approach the values shown in Fig. 8.

Conclusions

Under the conditions of a prolonged 600 kcal diet, plasma ketone levels never rose above about 20 mg/100 ml and were usually much lower. Thus the ketone concentrations never approached those shown by Randle, Garland, Hales & Newsholme (1963) to impair glucose metabolism in rat heart and diaphragm in vitro. Such levels would, however, be reached in diabetic ketoacidosis due to insulin

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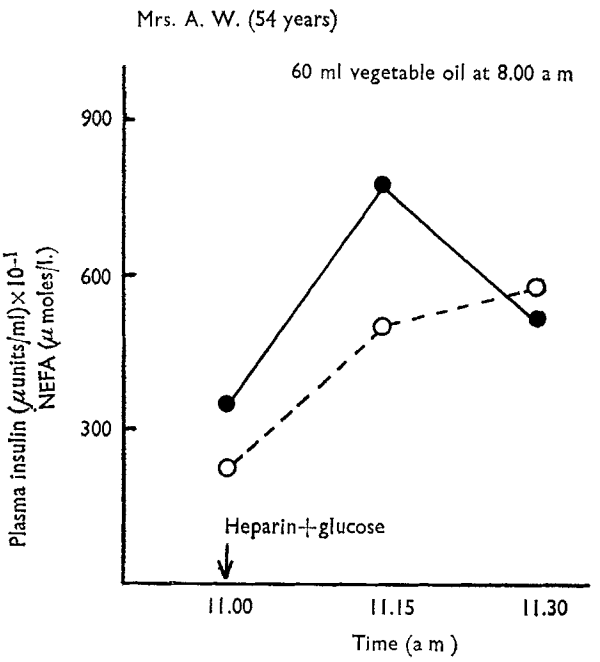

Fig. 4. Effect of heparin (25 000 units) given with 25 g glucose intravenously 3 h after oral administration of 60 ml vegetable oil. Modest rise in plasma non-esterified fatty acids and normal rise in plasma insulin following the injection. ●—●, NEFA; ○ - - ○, plasma insulin.

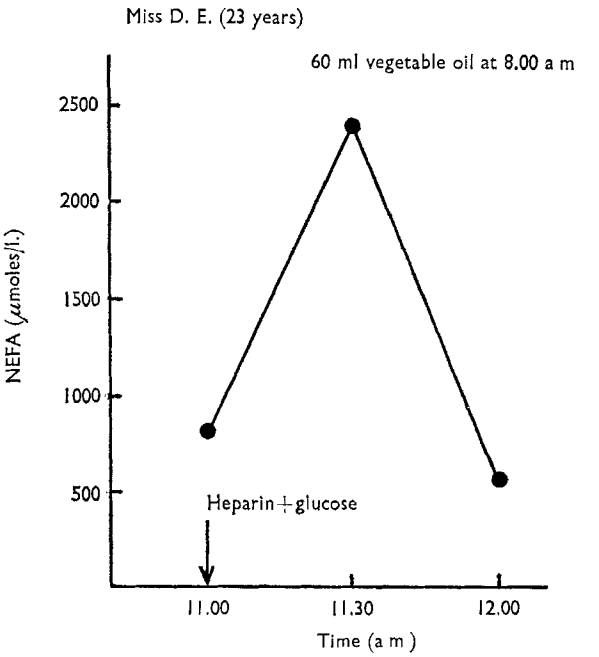

Fig. 5. Similar test to that adopted in Fig. 4, but with more dramatic transient rise in plasma non-esterified fatty acids.

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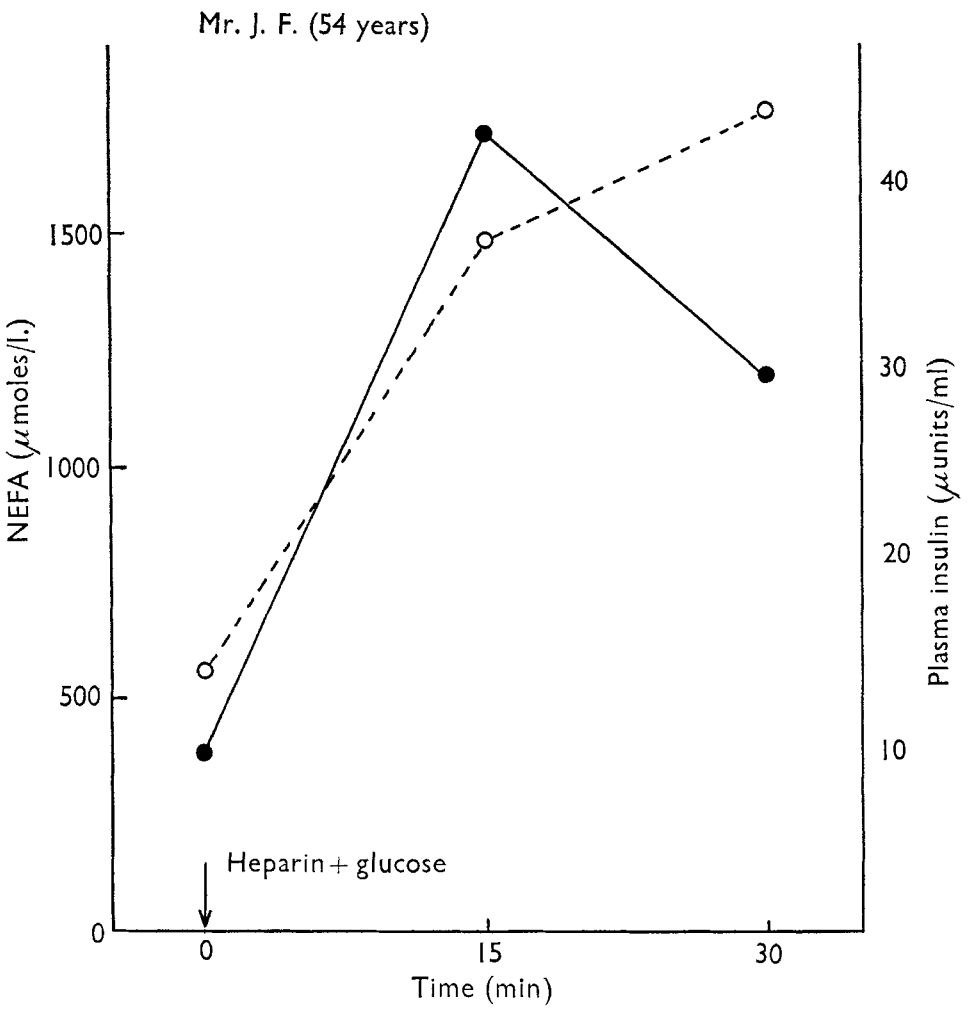

Fig. 6. Effect of heparin (25 000 units) given with 25 g glucose intravenously to fasted obese man with hypertriglyceridaemia; blood triglycerides were approx. 40 mg/100 ml, as glycerol, at time of test. ●—●, NEFA; ○ - - ○, plasma insulin.

insufficiency. There was a fairly consistent deterioration in intravenous glucose tolerance when the plasma NEFA levels exceeded approximately 600 μmoles/l. Greater rises in plasma NEFA were associated with further deterioration in glucose tolerance. Drugs such as salicylate and its derivative *o*-cresotinic acid, which have some hypoglycaemic action, did not appear significantly to depress the NEFA rise following a fatty meal and subsequent intravenous injection of heparin. It is still possible that the improved glucose tolerance produced by these drugs is exerted by a blockage of the inhibiting effect of raised NEFA levels on glucose metabolism.

Bierman, Dole & Roberts (1957) and Werk & Knowles (1961) reported that there were raised fasting NEFA levels in diabetic as compared with normal subjects. Both groups found that the difference was significant, although Bierman and his

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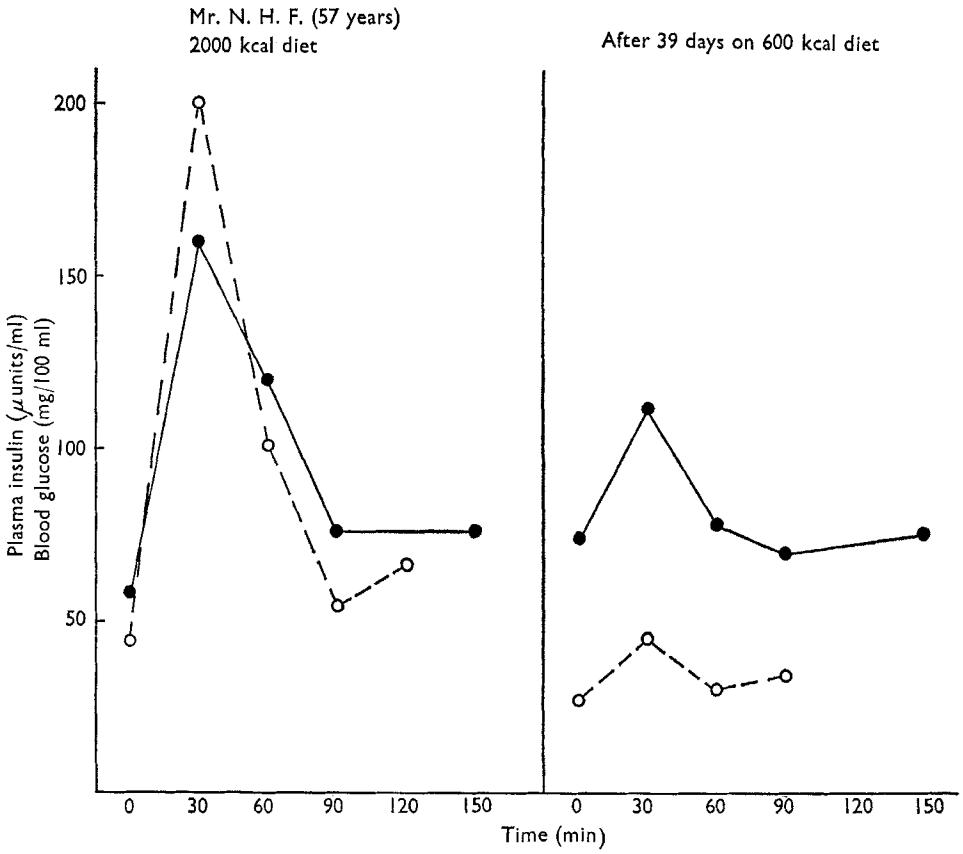

Fig. 7. Oral glucose tolerance and plasma insulin levels in obese man before and after 39-day period on 600 kcal diet. ●—●, blood glucose; ○ - - ○, plasma insulin.

colleagues found that in obese, non-diabetic subjects NEFA levels were about as high as in control diabetics. Hales & Randle (1963*b*) reported similar findings. Thus the mean fasting NEFA level was found to be 849 μmoles/l. in twenty-seven diabetic subjects by Bierman *et al.* and 775 in fifty-four diabetics by Werk & Knowles. The corresponding values for the normal control subjects were 572 and 554 μmoles/l. These workers used Dole's method for NEFA determination and this is probably less specific, as then performed, than Duncombe's method which we used. This may explain in part the lower fasting mean NEFA values which we obtained in the fifteen subjects in this series during the initial control period. These were 498 in the eight diabetics and 479 in the seven non-diabetic obese subjects. We cannot claim that our findings obtained in a group of very obese diabetic and non-diabetic subjects apply to diabetic or normal subjects in general, but we failed to find high enough fasting plasma NEFA values to produce a measurable effect on intravenous glucose tolerance, although increases of NEFA values above about 600 μmoles/l. did appear predictably to impair glucose tolerance. We have no evidence that

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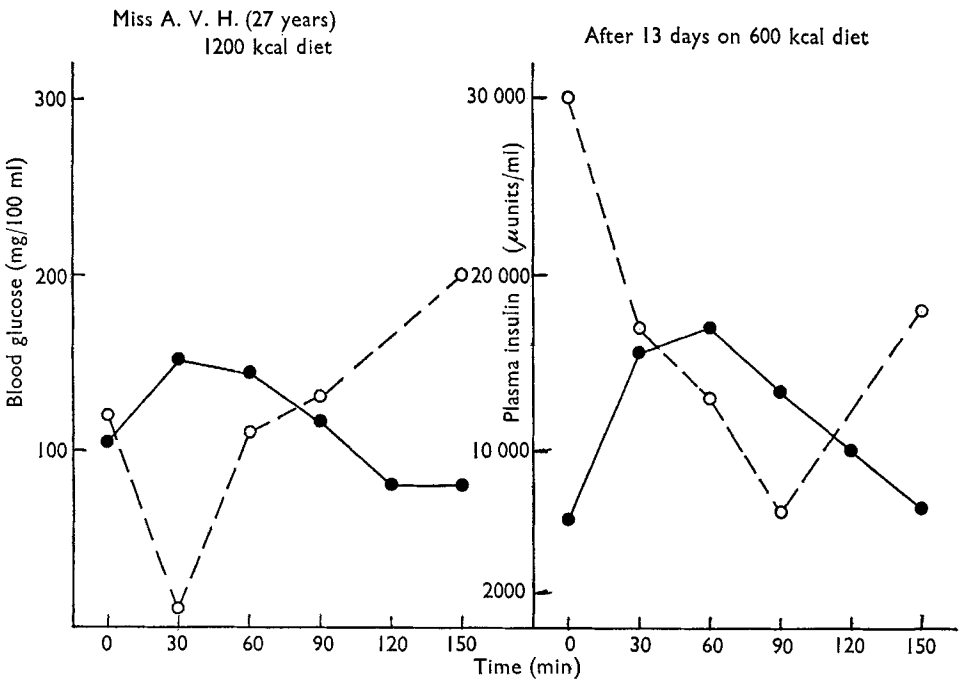

Fig. 8. Oral glucose tolerance and plasma insulin levels in obese woman before and after 39-day period on 600 kcal diet. Note extreme hyperinsulinism with normal glucose tolerance. ●—●, blood glucose; ○ - - ○, plasma insulin.

changes in plasma insulin level were concerned with these changes in glucose tolerance.

Acknowledgement is gratefully made for support given to this work by the Medical Research Council.

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Control of dietary fat in relation to diabetic complications in children

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The aetiology and prevention of vascular complications is now one of the outstanding problems in the management of childhood diabetes mellitus. Most of the work related to the prevention of the vascular lesions in the juvenile diabetic has