A DEFECT OF GLUCOSE UTILIZATION IN ADIPOSE TISSUE OF ADULT DIABETICS AND IN SOME CONDITIONS WHICH MAY PREDISPOSE TO DIABETES

D. J. GALTON AND J. P. D. WILSON

Endocrine Unit, Department of Medicine, Royal Postgraduate Medical School, London

(Received 27 June 1969)

SUMMARY

1. The effects of glucose, glucose 6-phosphate, and L-glycerol 3-phosphate on the incorporation of [1-14C]palmitate into neutral lipid have been studied in homogenates of adipose tissue obtained from adult diabetics and control patients. In the diabetic, glucose, glucose 6-phosphate and L-glycerol 3-phosphate supported incorporation of 19, 27 and 62% of total counts respectively; whereas in paired non-diabetic controls the respective values were 78, 80 and 84%.

2. Confirmation of these results were provided by finding slower rates of conversion of [U-14C]glucose into phosphate esters in diabetic adipose tissue compared to paired non-diabetic controls. Rates were improved but not restored to normal by treatment of diabetic patients with insulin.

3. These results could not be explained on the basis of differences in isotope dilution in precursor pools or to the presence of raised levels of free fatty acids in diabetic tissue.

4. A group of latent diabetics showed a pattern of substrate utilization more like fully developed diabetes than non-diabetic controls. Rates of glucose phosphorylation were slower in latent diabetics than in paired controls.

5. Studies were also performed with homogenates of adipose tissue prepared from patients with Cushing’s disease, obesity and pregnancy, these groups being predisposed to develop adult diabetes.

6. In Cushing’s disease (eight patients) glucose, glucose 6-phosphate and L-glycerol 3-phosphate supported incorporation of 9±1 (13), 19.2±3.9 (10) and 23.2±3.3 (9) % of total counts at 2 hr respectively; whereas in paired controls respective values were 19.7±3.4 (10), 25.7±2.8 (10) and 26.4±2.9 (10)%. Support for these results was provided by finding slower rates (by about 50%) of conversion of [U-14C]glucose into phosphate esters in tissue from Cushing’s disease compared to paired controls.

7. Ten obese patients with no clinical evidence of diabetes showed a pattern of sub-
strate utilization more like diabetic adipose tissue; whereas another eight obese patients resembled their non-obese controls.

8. Adipose tissue from six pregnant patients (over 10 weeks) showed a diabetic pattern of substrate utilization; and confirmation was provided by rates of $[\text{U}^{-}\text{14C}]$-glucose conversion into phosphate esters (as counts $\times 10 \text{min}^{-1} \text{mg protein}^{-1} \text{hr}^{-1}$) of $929 \pm 75$ (5) in pregnancy versus $1386 \pm 198$ (5) in paired controls.

During a glucose tolerance test, patients with adult diabetes frequently display raised levels of plasma insulin in association with raised concentrations of blood glucose (Yalow & Berson, 1960). The combination of these two factors would be expected to lead to a rapid removal of glucose from the blood stream into insulin-sensitive tissues, mainly adipose tissue and muscle, until normal fasting levels of blood sugar are attained. Since this does not occur it suggests either that the insulin is physiologically defective or that peripheral tissues are resistant to the hormone's action. This paper presents evidence in support of the latter view. A defect in the utilization of glucose has been found in adipose tissue taken from twenty-seven patients with adult diabetes which could possibly be responsible for the development of hyperglycaemia and lead to an excessive secretion of insulin. A preliminary report of this work has been published (Galton & Wilson, 1969). The study has been extended to other conditions which may predispose to adult diabetes; and results from three such groups, namely patients with Cushing's disease, obesity and pregnancy are reported.

**MATERIALS**

**Adipose tissue**

Abdominal tissue was obtained from subcutaneous and other extraperitoneal sites from patients undergoing routine abdominal surgery and the tissue was transferred to the laboratory in 0.9% (w/v) saline. Other specimens (200–600 mg) were obtained by needle biopsy from the anterior abdominal wall under local anaesthesia (<2 ml of 1% lignocaine) from a group of diabetic patients. These comprised twenty-seven patients (seven males, twenty females) whose ages ranged from 23 to 66 years (mean: 51 years) and weights from 6 st 9 lb to 15 st 7 lb (mean: 11 st to the nearest stone) with five patients over 12 st in weight. Fifteen of the diabetic patients were on no therapy, five were taking oral hypoglycaemic drugs and a further seven were on insulin. Biopsy specimens were also obtained from patients (one male, seven females) with Cushing's disease whose ages ranged from 28 to 67 years (mean: 48 years). Diagnosis was based on assays of serum cortisol and excretion of urinary steroids. All required treatment by adrenalectomy or pituitary ablation. A further series of specimens were obtained from eighteen obese patients (seventeen females, one male) whose ages ranged from 24 to 66 years (mean: 40 years) and weights from 9 st 9 lb to 20 st 13 lb (all over 14% of ideal weight).

Controls were run as paired experiments under as similar experimental conditions as possible and if no control tissue was available on the day of biopsy the nearest control value to that day was chosen for comparison. Attempts were made to match the control tissue to the age and sex of the biopsy material, but this was not often possible. The controls for the diabetic patients comprised nine males and nineteen females whose ages ranged from 17 to 67 years (mean: 40 years) and weights from 7 st 2 lb to 13 st 2 lb (mean of twenty-seven patients: 10 st to the nearest stone). The controls for patients with Cushing's disease were three males and seven females whose ages ranged from 26 to 61 years (mean: 38 years) and weights from 8 st
Lipogenesis in diabetes

4 lb to 11 st 3 lb. One out of nineteen controls was reported as having a family history of diabetes; and one other control was found to have mild diabetes. Finally tissue was obtained from five patients during Caesarian section (between 36–39 weeks) and from one patient during hysterotomy at 13 weeks. The reasons for Caesarian section were: mild hypertension (one case), previous elective Caesarian section for disproportion (one), failed anterior rupture of membranes (one) and previous big baby (one) whose prednisone glycosuria test was normal. A fifth patient had a Caesarian section because of associated cardiomyopathy, and the hysterotomy was performed for 'social reasons'.

Tissue from all sources were homogenized in an equal volume of buffer containing: KCl: MgCl₂: EDTA: mercaptoethanol (150 mM:5 mM:5 mM:10 mM) and then centrifuged at 700 g for 7–10 min. The upper fat cake and nuclear pellet were discarded and the majority of experiments were performed with the middle aqueous layer (called the fat-free homogenate).

Chemicals

The sources and grades of chemicals were as described in the previous paper (Galton & Wilson, 1970).

Radioactive assays

Fat-free homogenates of adipose tissue (approximately 50μl, 0·2 mg protein) were incubated in a total volume of about 0·2 ml under similar conditions to the preceding paper (Galton & Wilson, 1970). Incubations were carried out at 37° for periods up to 2 hr in test-tubes and the synthesis of neutral lipid was measured by a modification of Goldfine's (1966) method as previously described (Galton & Wilson, 1970).

Assay for glucose phosphorylation

The radiochemical method described by Newsholme et al. (1967) was followed except that 50 μl of fat-free homogenate were incubated in a total volume of 0·2 ml with phosphate buffer (pH 7·4) 15 mM; MgCl₂ 19 mM; ATP 5 mM; glucose 5 mM; and [U-14C]glucose 0·1 μCi/μmole. No attempts were made to stop phosphorylation at glucose 6-phosphate. Aliquots (25 μl) of the incubate were then pipetted onto DEAE-cellulose paper discs at intervals of 15 min for periods up to 1 hr and the reaction stopped by addition of 0·1 ml of methanol (Analar grade) to the disc. The discs were then washed and dried with slight modification of Newsholme, Robinson & Taylor's (1967) method and then counted under similar conditions to the assay for neutral lipid. This assay system was found to incorporate [U-14C]glucose into phosphorylated derivatives in a linear manner with time (up to 90 min), to be linearly dependent on the amount of added homogenate over range of 0·05–0·2 ml and to depend on the presence of ATP with a maximum activity of about 4 mM and no inhibition up to 12 mM. Although this assay suffers from the disadvantage of being open-ended it was found to be of use as an ancillary test to the studies on synthesis of neutral lipid. Protein was assayed by the method of Lowry et al. (1951).

Experimental design

To exclude the possibility that the differences between diabetic and non-diabetic tissue to be reported were simply due to differences in anaesthesia, three sets of comparisons have been...
made: (a) the oxygen uptakes of adipose tissue obtained under general anaesthesia versus tissue taken under spinal block, (b) the pattern of substrate utilization has been compared in specimens from the same patients (three in number) obtained separately using general and local anaesthesia, and (c) measuring the pattern of substrate utilization in the absence and presence of lignocaine (150 μg/ml) added to the incubation system. No evidence for an effect of anaesthetic agent on the preparation was observed in this series of experiments. To eliminate the possibility that glassware might interfere with the metabolic activity of homogenates the assay system has been conducted in two different types of glass tubes (Pyrex 7·5 × 1 cm; Soda glass 10 × 1·3 cm), and no differences in activity were observed.

Expression of results

Rates of incorporation are usually expressed as counts/min retained on filter discs from 25 μl of incubation system. Alternatively, results are presented as counts/min incorporation into product per mg of protein homogenates.

Statistics

Comparisons are based on means of paired observations where possible and the significance of the difference (P) was calculated by a non-parametric sign test (Siegel, 1956), since this makes no assumption about the distribution of the data.

RESULTS

Adult diabetes

The evidence for a defect in glucose utilization in adipose tissue from diabetic patients is illustrated in Fig. 1(a), which shows that glucose or glucose 6-phosphate does not effectively support the incorporation of [1-14C]palmitate into neutral lipid when compared to L-glycerol 3-phosphate. Multiple sampling was chosen for this system since the rates of reactions under the present conditions were non-linear. In the paired non-diabetic controls (Fig. 1b) both glucose and glucose 6-phosphate supported rates of lipogenesis more equivalent to that of L-glycerol 3-phosphate. The per cent of total counts present incorporated into neutral lipids (as counts/min per mg protein at 2 hr incubation) using glucose, glucose 6-phosphate or L-glycerol 3-phosphate as initial substrate were for diabetic tissue: 19±3±2±7 (9); 27±3±3±4 (9) and 62±4±13±1 (8) respectively; and for non-diabetic controls were: 77±9±16±4 (7); 80±12±0 (9) and 84±0±12±7 (8) respectively. Additional evidence for the presence of a defect in phosphorylation of glucose in diabetic tissue is presented in Figs. 2(a) and 2(b). This experiment measures the rate of transfer of [U-14C]glucose into the phosphate esters of glycolysis (mainly glucose 6-phosphate, fructose 6-phosphate and fructose 1,6-diphosphate) and shows that homogenates prepared from diabetic tissue were less effective in hexose phosphorylation than tissue from non-diabetic controls. This difference (Fig. 2b) still appeared when patients with adult diabetes were treated with insulin. Since the comparison between diabetic and non-diabetic tissue in Figs. 1(a) and 1(b) could possibly be including differences in isotope dilution of [1-14C]palmitate in precursor pools, we measured the radioactivity appearing in the pool of long-chain acyl-CoA after 10 min of incubation using each of the initial substrates. The results are presented in Table 1 and no differences in pool labelling between normal and diabetic
Lipogenesis in diabetes

665
tissue were detected. The results of Figs. 1 and 2 might be due to the effects of raised concentrations of intracellular fatty acids in diabetic tissue since it is well established that lipolysis is accelerated in the diabetic state; and free-fatty acids are known to inhibit hepatic hexokinase (Lea & Weber, 1968). This possibility was made unlikely by the data in Fig. 3 where rates of

\[ \text{FIG. 1. Effects of glycolytic intermediates on the incorporation of } [1-^{14}\text{C}]\text{palmitate into neutral lipid in diabetic and control tissue. Glucose (6 mm), glucose 6-phosphate (6 mm) and } L\text{-glycerol 3-phosphate (8.5 mm)} \text{ were incubated with fat-free homogenates (approximately 50 } \mu\text{L) of human adipose tissue in the assay system described in Methods. Points are means of twenty-five experiments (twenty-five diabetic patients) in A; and twenty-eight experiments (twenty-eight non-diabetic patients) in B. G} = \text{glucose, G6P} = \text{glucose 6-phosphate; GP} = L\text{-glycerol 3-phosphate.}} \]

\[ \text{FIG. 2. Utilization of glucose in diabetic tissue from patients treated with or without insulin, compared to normal tissue. Fat-free homogenates (about 50 } \mu\text{L) of adipose tissue were incubated with } [U-^{14}\text{C}]\text{glucose (5 mm, 0.5 } \mu\text{Ci)} \text{ in the assay system described in Methods. Aliquots (25 } \mu\text{L) were removed at intervals and the radioactivity appearing in phosphate esters was measured by the method of Newsholme et al. (1967). Results are means of seven experiments (seven patients) in (a); and eight experiments (eight patients) in (b). (a): curve 1 – non-diabetic controls; curve 2 – diabetics not on insulin. (b): curve 1 – non-diabetic controls; curve 2 – diabetics treated with insulin.} \]
Table 1. To assess extent of dilution of [1-14C]palmitate in endogenous fatty acid pools in diabetic and non-diabetic adipose tissue

<table>
<thead>
<tr>
<th>Substrate addition</th>
<th>Incorporation of [1-14C]palmitate into acyl-CoA (counts min⁻¹ mg protein⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetic</td>
</tr>
<tr>
<td></td>
<td>Non-diabetic</td>
</tr>
<tr>
<td>Glucose</td>
<td>1830±479 (7)</td>
</tr>
<tr>
<td>Glucose 6-phosphate</td>
<td>1576±500 (6)</td>
</tr>
<tr>
<td>L-Glycerol 3-phosphate</td>
<td>1032±202 (7)</td>
</tr>
<tr>
<td></td>
<td>2152±525 (7)</td>
</tr>
<tr>
<td></td>
<td>1468±326 (6)</td>
</tr>
<tr>
<td></td>
<td>1207±200 (7)</td>
</tr>
</tbody>
</table>

Homogenates of human adipose tissue (50 µl) were incubated at 37°C in test-tubes with the system described in Methods. Aliquots (25 µl) were removed after 10 min incubation and extracted for long chain acyl-CoA as previously described (Galton & Fraser, 1969). Results are means ± SEM (number of experiments).

Fig. 3. The effects of palmitate on glucose utilization by homogenates of human adipose tissue. Fat-free homogenates of adipose tissue were incubated with [U-14C]glucose (5 mM; 0.5 µCi) in the same system as in Fig. 2 with increasing concentrations of palmitate. Radioactivity appearing in phosphate esters was measured as described in Methods. Points are means of four experiments. Curve 1: No added palmitate; curves 2, 3, 4: with palmitate at 0.25 mM, 0.5 mM and 1.0 mM respectively.
hexose phosphorylation were measured in the presence of increasing concentrations of palmitate, which was used as a representative fatty acid. Palmitate at 1 mM produced no inhibition of glucose phosphorylation.

Since it has been reported that citrate accumulates in heart tissue in diabetic rats (Garland, Randle & Newsholme, 1963) and since citrate is known to inhibit phosphofructokinase in adipose tissue (Denton & Randle, 1966), the results in Fig. 1 might be explained on this basis. However, 3'-5'-AMP overcomes the inhibition of phosphofructokinase by citrate in rat adipose tissue (Denton & Randle, 1966) and when 3'-5'-AMP was added to homogenates prepared from diabetic patients using glucose 6-phosphate as initial substrate no stimulation of lipogenesis was observed (Table 2). It has previously been shown that 3'-5'-AMP does stimulate lipogenesis which has been inhibited by citrate in normal human adipose tissue when glucose 6-phosphate is the initial substrate.

Latent diabetes

We then examined a group of patients who are predisposed to develop the diabetic syndrome, namely latent diabetes (defined as a mild abnormality in the glucose tolerance test or prednisone glycosuria test (Joplin, Fraser & Keeley, 1961) with no clinical evidence of diabetes). The rates of isotope incorporation into neutral lipid in seven latent diabetics are presented in Fig. 4(a) and 4(b). The pattern of incorporation using either [1-14C]palmitate or [U-14C]glucose resembles that seen in fully developed adult diabetes rather than normal tissue. The first patient studied in this group (L.O.) has been left out of the means in Fig. 4 because a 1-glycerol 3-
phosphate time-course was not performed at the same time as the other substrates. However, she showed a flat curve for glucose utilization (in triplicate), but a more active rate of utilization of glucose 6-phosphate.

**Fig. 4.** Lipogenesis and utilization of glucose in adipose tissue from latent diabetics. Fat-free homogenates of adipose tissue were incubated under the conditions of Fig. 1 for panel (a) and under the conditions of Fig. 2 for panel (b). Points are means of eight experiments (seven patients) in (a), and three experiments (three patients) in (b). Abbreviations are the same as in Figs. 1 and 2. Curve 1: diabetic patients; curve 2: controls.

**Cushing's disease**

Homogenates prepared from five patients with Cushing's disease showed a reduction in incorporation of [1-14C]palmitate into neutral lipid when glucose was used as initial substrate, but this was not so marked when glucose was replaced by glucose 6-phosphate or L-glycerol 3-phosphate (Fig. 5a). Of the five patients in Fig. 5(a) only one had a markedly abnormal glucose tolerance test and two others were mildly abnormal. The percentage of total counts in the medium incorporated into neutral lipid using glucose, glucose 6-phosphate and L-glycerol 3-phosphate as initial substrate for eight patients with Cushing's disease and their paired controls, are presented in Table 3. Further confirmation of these findings were obtained by measuring the rates of conversion of [U14-C]glucose into phosphate esters in homogenates of Cushing's tissue and normal controls (Table 4). A 50% reduction in phosphorylation of glucose was observed in four patients with Cushing's disease after 1 hr incubation. However not all patients with Cushing's disease showed an impaired ability to phosphorylate glucose and some were found whose tissue utilized glucose at rates exceeding the control. We thought this variability may be due to possible differences in types of adrenal steroids secreted in this disease.

**Obesity**

Fig. 6 illustrates the pattern of substrate utilization in eighteen patients selected for obesity who had no glycosuria or clinical evidence of diabetes. They have been divided into two groups
Lipogenesis in diabetes

Fig. 5. Effects of glycolytic intermediates on the incorporation of \([1-^{14}C]\)palmitate into neutral lipid in adipose tissue from patients with Cushing's disease and paired controls. Glucose (6 mm), glucose 6-phosphate (6 mM) and \(L\)-glycerol 3-phosphate (8.5 mM) were incubated with fat-free homogenates (about 50 μl) of adipose tissue in the assay system described in Methods. Points are means of seven experiments (five patients) in (a) and five experiments (five patients) in (b). (a): Cushing's tissue; (b): paired controls; G: glucose; G6P: glucose 6-phosphate; GP: \(L\)-glycerol 3-phosphate. (Initial experiments with \(L\)-glycerol 3-phosphate are not exactly similar to those with other substrates due to a period of pre-incubation before the final incubation of 1 hr.)

Table 3. Effect of glycolytic intermediates on lipogenesis in Cushing's disease

<table>
<thead>
<tr>
<th>Substrate addition</th>
<th>Cushing's tissue</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>9.0±1.0 (13)</td>
<td>19.7±3.4 (10)</td>
</tr>
<tr>
<td>Glucose 6-phosphate</td>
<td>19.2±3.9 (10)</td>
<td>25.7±2.8 (10)</td>
</tr>
<tr>
<td>(L)-Glycerol 3-phosphate</td>
<td>23.2±3.3 (9)</td>
<td>26.4±2.9 (10)</td>
</tr>
</tbody>
</table>

Fat-free homogenates (50 μl) of adipose tissue from patients with Cushing's disease and normal controls were incubated with glucose (6 mm), glucose 6-phosphate (6 mM) and \(L\)-glycerol 3-phosphate (8.5 mM) and the incorporation of \([1-^{14}C]\)palmitate into neutral lipid was measured as described in Methods. Results are means±SEM (number of experiments on eight patients)
depending on whether they demonstrate normal or 'diabetic' types of isotope incorporation. Ten out of eighteen obese patients resemble the diabetic pattern (Fig. 6a).

Pregnancy

Adipose tissue was also obtained from six pregnant patients during Caesarian section or hysterotomy. Rates of isotope incorporation were similar to that of diabetic tissue (Fig. 7) and this was supported by measuring rates of [U-14C]glucose utilization (Table 5). In addition, tissue was obtained from a pregnant patient at 10 weeks and it showed a normal pattern of substrate utilization.

<table>
<thead>
<tr>
<th>TABLE 4. Utilization of [U-14C]glucose by homogenates of adipose tissue from patients with Cushing's disease and paired controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Radioactivity recovered in phosphate esters (counts x 10 min⁻¹ mg protein⁻¹)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>0 min</td>
</tr>
<tr>
<td>Cushing's disease</td>
</tr>
<tr>
<td>Paired controls</td>
</tr>
<tr>
<td>P value</td>
</tr>
</tbody>
</table>

Fat-free homogenates (50 µl) of adipose tissue were incubated with [U-14C]glucose (5 mm, 0·5 µC) in phosphate buffer (pH 7·4) 15 mM; MgCl₂ 19 mM; and ATP 5 mM. Radioactivity in phosphate esters was measured by the method of Newsholme, Robinson & Taylor (1967). Results are expressed as means ± SEM (number of experiments on four patients).

Fig. 6. Effects of glycolytic intermediates on the incorporation of [1-14C]palmitate into neutral lipid in adipose tissue from obese patients. Fat-free homogenates of adipose tissue were incubated under the conditions described in Fig. 1. (a): points are means of eleven experiments (ten obese patients). (b): points are means of eight experiments (eight obese patients). G: glucose; G6P: glucose 6-phosphate; GP: L-glycerol 3-phosphate.
Lipogenesis in diabetes

Fig. 7. Effects of glycolytic intermediates on the incorporation of [1-14C]palmitate into neutral lipid in adipose tissue from pregnant patients. Glucose (6 mM), glucose 6-phosphate (6 mM) and L-glycerol 3-phosphate (8.5 mM) were incubated with fat-free homogenates of adipose tissue. Incubation conditions were the same as in Fig. 1 and points are means of six experiments (six patients).

Table 5. Utilization of [U-14C]glucose by adipose tissue from pregnant patients

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pregnancy</strong></td>
<td>255±31 (4)</td>
<td>473±60 (5)</td>
<td>664±72 (5)</td>
<td>874±121 (5)</td>
<td>929±75 (5)</td>
</tr>
<tr>
<td><strong>Paired controls</strong></td>
<td>239±64 (5)</td>
<td>627±112 (5)</td>
<td>886±160 (5)</td>
<td>1167±192 (5)</td>
<td>1386±198 (5)</td>
</tr>
<tr>
<td><strong>P values</strong></td>
<td>—</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Fat-free homogenates (50 μl) of adipose tissue were incubated with [U-14C]glucose (5 mM, 0.5 μCi) in phosphate buffer as described in Table 3. Results are expressed as means±SEM (number of experiments on five patients).

Discussion

Diabetes

Homogenates prepared from adipose tissue obtained from patients with adult diabetes, not requiring insulin, show impaired metabolism of glucose and glucose 6-phosphate whether for lipogenesis or conversion to hexose and triose phosphates. The majority of experiments were performed with adipose tissue from the abdominal wall although tissue from other sites, such as perinephric fat, was examined and showed a similar defect in diabetes. This alteration probably represents an early change in the development of diabetes since it is readily detected in a group of eight latent diabetics. In addition, treatment of adult diabetes with insulin did not
D. J. Galton and J. P. D. Wilson

restore rates of glucose phosphorylation to normal. Previous investigators have examined the metabolic activity of intact pieces of adipose tissue from adult diabetes to utilize glucose and convert \(\text{[L-}^{14}\text{C}]\text{palmitate into neutral lipid.} \) Ostman (1965) demonstrated that adipose tissue from patients with adult diabetes (ages from 38 to 75 years) tended to take up less glucose (by about 25%) than a non-diabetic lean group (age range of 17-35 years). He noticed no difference in glucose uptake between a group of adult diabetics and an obese group of similar age range which he took as controls. Field, Johnson & Herring (1961) noticed a similar tendency in isolated human adipose tissue, in which a group of four non-diabetic patients consumed 2.16 ± 0.13 mg glucose/g tissue, compared to four obese diabetics utilizing 1.69 ± 0.12 mg glucose/g tissue. Kahlenberg & Kalant (1964) employing a similar experimental design, confirmed these results and showed that omental tissue from eight diabetics took up less glucose from the incubation medium than their non-diabetic controls whether in the presence or absence of insulin. They concluded that impaired glucose transport was responsible for this effect in diabetic tissue.

As regards incorporation of \(\text{[L-}^{14}\text{C}]\text{palmitate into neutral lipid,} \) Ostman (1965) observed a small decrease of about 23% in adult diabetics compared to a control group of young non-diabetic males. He thought this difference was not due to dilution of \(\text{[L-}^{14}\text{C}]\text{palmitate in an enlarged endogenous pool secondary to accelerated lipolysis,} \) but represented a real difference in metabolic activity between the tissues.

Possible explanations for the magnitude of the effects observed in this paper compared to previous studies are (i) the lack of sensitivity in measuring a small uptake of unlabelled glucose from a large pool in the incubation medium compared to measuring the transfer of \([^{14}\text{C}]\text{substrate into its labelled product,} \) (ii) the difficulty of knowing if incubation conditions are optimum for whole tissue versus the ease of doing so with homogenates (by varying buffer, co-factor and substrate concentrations) and (iii) the multiple sampling technique of our studies allows the time-course of each reaction to be followed, which becomes important if the behaviour, as shown, is non-linear.

The cause of the changes in tissue metabolism described in the present experiments is not clear. Attempts have been made to eliminate possible effects of isotope dilution, raised levels of intracellular fatty acids or raised concentrations of citrate. It is tempting to suppose that alterations in adipose cell metabolism might be an important factor during the development of the diabetic state. The condition might be somewhat analogous to the metabolic defects of glycolysis found in the red cell (pyruvate kinase and hexokinase deficiencies), which, like the adipose cell, is a highly differentiated carrier cell. The consequence of such changes in the adipose cell would be a delay in the clearance of blood glucose during a glucose tolerance test and might cause excessive release of insulin from the pancreas to correct the hyperglycaemia. However, this assumes that human adipose tissue is a quantitatively important site of disposal of a systemic glucose load. Although it is now well established that insulin stimulates the uptake of glucose by human adipose tissue there is very little information regarding the quantities involved \textit{in vivo}. Jansen, Hutchison & Zanetti (1966) have shown that of an oral glucose load (250 mg) given to the mouse, 48% is metabolized to \(\text{CO}_2\), 1.0% and 1.9% is converted into hepatic glycogen and fatty acids respectively, and about 12% is converted to carcass fat presumably by adipose tissue (their experiments took into account the transport of labelled lipid from liver to adipose tissue). It is doubtful whether such data can be applied to man; but in conditions where there is a generalized atrophy of adipose tissue, such as the lipo-
Lipogenesis in diabetes

Dystrophies, a diabetic syndrome is frequently seen. This perhaps can be taken as some evidence to suggest that adipose tissue in man is an important site of disposal of glucose and a block in this process, of the sort described in this paper, might be expected to cause hyperglycaemia. Several recent reviews on the cause of adult diabetes (Kipnis, 1968; Luft, 1968) have laid emphasis on a primary failure of the pancreatic \( \beta \)-cell to secrete insulin. This need not necessarily be a primary defect, since, whatever the pancreatic reserve, an impaired glucose tolerance can be induced in almost all patients by steroid therapy. However, patients with adult diabetes possibly have an inherited reduction in pancreatic reserve to secrete insulin which would be made more quickly manifest by the presence of the peripheral changes described in this paper. The degree of pancreatic reserve before failure occurs might then determine how long a diabetic of the maturity-onset type can be treated with oral anti-diabetic therapy before he requires insulin. An unexplained problem which the present studies raise is why adult diabetics tend to become and remain overweight if they cannot use glucose efficiently for lipogenesis. This question is currently being explored in terms of alternative sources of substrate, such as pyruvate or aminoacids, for lipogenesis.

Conditions which may predispose to diabetes

Cushing's disease. Corticosteroids at physiological concentrations inhibit the uptake of glucose by adipose tissue from the rat (Munck, 1962) although it was not possible to decide whether steroids interfered with membrane transport of glucose or its phosphorylation (Munck, 1968). Our experiments (Fig. 5) show that glucose utilization can be impaired in homogenates of adipose tissue prepared from patients with raised levels of plasma cortisol. A similar effect was observed in two patients receiving corticosteroid therapy for asthma and disseminated lupus erythematosus. The block in human adipose tissue probably occurs at the level of glucose phosphorylation since membrane transport is avoided by the use of homogenates. Of the patients with Cushing's disease two had normal glucose tolerance tests but reduced rates of glucose utilization by tissue homogenates of \( 8 \pm 0.6 \) (6) \%. Four other patients had mildly abnormal tolerance tests and impaired rates of glucose metabolism by adipose tissue. This suggests that the changes in tissue metabolism occur early in relation to the appearance of an abnormal glucose tolerance test and therefore, in combination with increased rates of hepatic gluconeogenesis, could possibly contribute to its onset.

Obesity. This is another condition which predisposes to the development of diabetes. About 60\% of obese patients have abnormal glucose tolerance tests (John, 1929) and conversely about 50\% of patients presenting with adult diabetes are overweight (Joslin, Dublin & Marks, 1936). Two related points are that almost all obese patients have raised levels of plasma insulin in response to a glucose load (Karam, Grodsky & Forsham, 1963) and that adipose cells obtained from obese patients are less sensitive to the action of insulin than adipose cells from lean controls (Salans, Knittle & Hirsch, 1968). If the reduced rates of glucose phosphorylation in adipose tissue from obese patients become less than the rate of entry of glucose into the cell under conditions of hyperglycaemia and raised levels of plasma insulin, then a delay in clearance of glucose from the blood stream would be expected and might result in excessive pancreatic secretion of insulin. Over a period of time this could eventually lead to pancreatic exhaustion and the appearance of clinical diabetes.

Pregnancy. The interesting observation that the tissue changes described in this paper are also detected in pregnancy suggest that they are produced secondary to an alteration in endo-
crine balance although this does not exclude the possibility that they might stand in causal relation to the impaired tolerance to glucose which occurs in pregnant patients (Kalkhoff et al., 1964). Pregnancy is associated with a threefold increase in plasma cortisol (bound plus unbound fractions) and raised levels of placental lactogen after the ninth week (Grumbach et al., 1968). Human placental lactogen has been shown to stimulate lipolysis in isolated adipose cells (Turtle, Littleton & Kipnis, 1966) and part of its function during pregnancy may be to promote release of fatty acids from lipid stores as a source of fuel for peripheral tissue which would probably depress maternal glucose utilization. Since the secretion of placental lactogen does not occur till after the tenth week of pregnancy (Grumbach et al., 1968), it might possibly explain why adipose tissue obtained from a hysterotomy at 10 weeks showed a normal pattern of substrate utilization.

ACKNOWLEDGMENTS
Grateful thanks are due to the Surgical Staff of the Hammersmith Hospital for providing fat specimens; and to Professor Russell Fraser for permission to study his patients and his continuing encouragement. D.J.G. is grateful to the M.R.C. for an instrument grant.

REFERENCES
Lipogenesis in diabetes


