Diabetologia (1991) 34: 205–211
0012186X91000547

Diabetologia

Originals

Effects of glycogen stores and non-esterified fatty acid availability on insulin-stimulated glucose metabolism and tissue pyruvate dehydrogenase activity in the rat

Y. T. Kruszynska[1], J. G. McCormack[2] and N. McIntyre[1]

[1] Department of Medicine, Royal Free Hospital School of Medicine, London,
[2] Department of Biochemistry, Leeds University, Leeds, UK

Summary. The effects of increased tissue glycogen stores on insulin sensitivity, and on the response of insulin-stimulated glucose utilisation to an acute elevation in plasma fatty acid levels (~1.5 mmol/l), were investigated in conscious rats using the hyperinsulinaemic euglycaemic clamp. Studies were performed in two groups of rats; (a) fasted 24 h; (b) fasted 4.5 h, but infused with glucose for 4 h (0.5 g/h) of this period before the clamp (fed, glucose infused rats). Clamp glucose requirement and 3-^{3}H-glucose turnover were 20–25% lower in the fed, glucose-infused rats. In these rats, elevation of plasma fatty acid levels resulted in impaired suppression of hepatic glucose output (residual hepatic glucose output: 41 ± 4 vs 8 ± 6 μmol · min^{-1} · kg^{-1}, $p < 0.001$) but did not further decrease 3-^{3}H-glucose turnover. Elevated non-esterified fatty acid levels had no significant effect on glucose kinetics in 24 h fasted rats. In the fed glucose-infused rats, at low plasma fatty acid levels, there was no deposition of glycogen in muscle during the clamp and liver glycogen levels fell. With elevation of non-esterified fatty acid levels muscle glycogen deposition was stimulated in both groups, and there was no fall in liver glycogen during the clamps in the fed glucose-infused rats. Increased non-esterified fatty acid availability during the clamps decreased pyruvate dehydrogenase activity in liver, heart, adipose tissue and quadriceps muscle, in both groups of rats. The findings are consistent with an inhibition of glycolysis in liver, skeletal muscle and heart by increased fatty acid availability. Increased glycogen synthesis, however, compensates for decreased glycolytic flux so that glucose turnover is not decreased. When liver glycogen stores are high, an acute increase in non-esterified fatty acid availability impairs suppression of hepatic glucose output. A chronic increase in non-esteriefid fatty acid availability may lead to insulin resistance by increasing glycogen stores.

Key words: Glucose-fatty acid cycle, non-esterified fatty acids, rat, glucose clamp, glycogen, pyruvate dehydrogenase, glucose turnover.

Increased availability of non-esterified fatty acids (NEFA) has been suggested as an important cause of insulin resistance and glucose intolerance in obesity and diabetes [1]. The underlying mechanism is believed to be the "glucose-fatty acid cycle" [2]; in heart and red skeletal muscle, increased fatty acid and ketone body oxidation inhibits glucose oxidation at the level of pyruvate dehydrogenase (PDH) [3–6], while glycolysis is inhibited at the level of phosphofructokinase [7–9]. Inhibition of flux through phosphofructokinase increases glucose-6-phosphate concentrations and thus inhibits muscle hexokinase and glucose uptake [2].

Since skeletal muscle and liver are quantitatively the most important tissues for disposal of an oral glucose load [10, 11] the glucose-fatty acid cycle must operate in these tissues if it is to explain insulin resistance and glucose intolerance. However, in contrast to the impaired glucose utilisation accompanying a chronic elevation in plasma NEFA, as occurs in starvation [12, 13] and in rats fed a high fat diet [14–17], an acute increase in NEFA availability does not consistently impair glucose utilisation by resting skeletal muscle [18–23]. The effects of an acute increase in NEFA levels on hepatic glucose metabolism are also controversial. Thus, in man and rats insulin-mediated suppression of hepatic glucose production during a glucose clamp has been reported to be unchanged [23, 24] or impaired [22] by increased NEFA availability.

The failure of an acute increase in NEFA levels to decrease skeletal muscle or whole body glucose utilisation during physiological hyperinsulinaemia could be explained by increased deposition of muscle glycogen, as this might compensate for decreased oxidative glucose metabolism [22, 23]. Our previous studies on this subject [23] were performed in rats fasted overnight whose muscle glycogen was partially depleted. Glycogen deposition is inhibited at high tissue glycogen concentrations [25,

Table 1. Plasma insulin and lipid levels in ad libitum fed rats and in 24 h fasted and 4.5 h fasted glucose-infused (4.5 h FGI) rats in the basal state and at the end of a hyperinsulinaemic euglycaemic clamp

	Ad libitum fed	Basal		Clamp		Clamp with Intralipid	
		4.5 h FGI	24 h fasted	4.5 h FGI	24 h fasted	4.5 h FGI	24 h fasted
n	6	12	12	6	6	6	6
Insulin (μg/l)	3.35 ± 0.64	0.62 ± 0.05	0.63 ± 0.04	3.25 ± 0.16	3.08 ± 0.17	3.47 ± 0.22	3.34 ± 0.22
Triglyceride (mmol/l)	0.91 ± 0.09	0.82 ± 0.04	0.80 ± 0.06	0.49 ± 0.07[a]	0.34 ± 0.02[b]	1.13 ± 0.09	0.97 ± 0.09
NEFA (μmol/l)	230 ± 21	625 ± 36[e]	1059 ± 77	123 ± 36[c]	103 ± 36[c]	1558 ± 106	1461 ± 142
Glycerol (μmol/l)	68 ± 8	144 ± 7[e]	225 ± 15	84 ± 11[c]	89 ± 10[c]	197 ± 14	193 ± 16
3-Hydroxybutyrate (μmol/l)	206 ± 31	131 ± 22[e]	730 ± 77	26 ± 5[c]	21 ± 3[c]	184 ± 25	213 ± 18

Mean ± SEM.
Plasma insulin, triglyceride and NEFA concentrations and blood glycerol and 3-hydroxybutyrate values in the basal state before the clamps did not differ between groups and for clarity have been pooled.

[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$ compared to values in the same six rats in the basal state (paired *t*-test); [e] $p < 0.001$ compared to 24 h fasted rats in the basal state

26]; we have therefore used the glucose clamp technique [27], to examine whether, in conscious rats, when tissue glycogen levels are maintained, at or above those found in ad libitum fed rats, an elevation of plasma NEFA results in inhibition of whole body glucose utilisation at physiological insulin levels. In view of the key role of PDH in the glucose fatty acid cycle [3–6, 23, 28, 29], the activity of this enzyme was measured in muscle, heart, liver and adipose tissue, both in the basal state and at the end of the glucose clamps.

Materials and methods

Animals

Syngeneic male Ludwig-Wistar rats were maintained on standard laboratory chow and handled daily for four weeks before study. Indwelling cannulae for metabolic studies were implanted, under ether anaesthesia, in the external jugular and femoral veins 24 h before the experiment. Insulin sensitivity was assessed by the euglycaemic clamp technique [27], in rats weighing 280–335 g, at 4.5 or 24 h after withdrawal of food. To maintain tissue glycogen concentrations at fed levels in the 4.5 h fasted group, a 4 h i.v. infusion of 500 g/l glucose in water (1 ml/h) was started immediately after food withdrawal at 07.00 h (4.5 h fasted, glucose infused (FGI) rats). The glucose infusion was stopped 30–40 min before the start of the glucose clamp studies to allow blood glucose concentration and insulin secretion to fall to basal levels.

Radioisotopic glucose turnover and euglycaemic clamp studies

All blood samples were taken from conscious, unrestrained animals. After basal blood samples were taken, neutral soluble human insulin (Humulin S, Eli Lilly, Basingstoke, UK), diluted in Haemaccel, was infused at 85 mU/h for 2 h through one limb of a double lumen cannula (Miles, Stoke Poges, UK) connected to the jugular venous cannula. The insulin solution also contained 3-^{3}H-glucose and potassium chloride, which were infused at rates of 0.12 μCi/min and 0.15 mmol/h respectively. In half of the insulin-infused rats, plasma NEFA levels were raised during the clamps by a priming bolus of heparin (10 U at 0 min), followed by a constant infusion of heparin (40 U/h) and a 20% weight/volume triglyceride emulsion (Intralipid, Kabivitrum Ltd., Uxbridge, UK) at 0.6 ml/h, delivered through the injection port of the double lumen cannula.

Blood samples for glucose (30 μl) were taken every 5–10 min and blood glucose was maintained by a variable infusion of 500 g/l glucose in water. Blood samples were taken for insulin, triglyceride, NEFA, glycerol and 3-hydroxybutyrate estimations at 0 and 120 min (400 μl), and for glucose specific activity (200 μl) at 105 and 115 min; they were replaced with an equal volume of fresh washed rat erythrocytes in 0.15 mol/l NaCl. At + 120 min, while maintaining the clamp, rats were anaesthetised by i.v. injection of 15 mg amylobarbitone (100 g/l, Eli Lilly) and liver, heart, epididymal adipose tissue and quadriceps muscle were freeze clamped within 15 s, ground under liquid nitrogen and stored at −70 °C until assayed for glycogen content and PDH activity.

Basal glucose turnover was measured in six 24 h fasted rats by a primed constant infusion of 3-^{3}H-glucose (0.03 μCi/min). Blood samples (150 μl) were taken from the femoral venous cannula at + 50 and + 55 min for determination of plasma glucose concentration and specific activity. Tissue was removed from these animals at the end of the basal glucose turnover, and also from a group of 4.5 h fasted rats infused with glucose for 4 h after withdrawal of food (basal 4.5 h FGI rats).

For determination of glucose specific activity, plasma samples were deproteinised with $Ba(OH)_2/ZnSO_4$ as previously described [30]. Glucose turnover was calculated from the formula:

$$\text{Glucose turnover} = \frac{\text{tracer infused (DPM/min)}}{\text{glucose specific activity (DPM/μmol)}}$$

Pyruvate dehydrogenase

PDH was measured spectrophotometrically [31], by coupling the acetyl CoA generated in the assay to p-aminophenylazobenzene sulphonic acid using arylamine acetyl transferase prepared from pigeon liver. Total PDH (PDH_T) was measured after incubation of extracts with purified pig heart PDH phosphate phosphatase [31]. One unit of enzyme is defined as the amount converting 1 μmol/min of pyruvate to acetyl CoA at 30 °C.

Other analyses

Glycogen was assayed by the amyloglucosidase method [32]. Blood for estimation of intermediary metabolites was deproteinised with perchloric acid (0.6 mol/l), and the extract was assayed for glycerol

Table 2. Glucose kinetics in the 24 h fasted and 4.5 h fasted glucose-infused (4.5 h FGI) rats

	Ad libitum fed	Basal	Clamp		Clamp with Intralipid[c]	
		24 h fasted	4.5 h FGI	24 h fasted	4.5 h FGI	24 h fasted
n	6	6	6	6	6	6
Blood glucose (mmol/l)	6.2 ± 0.2	3.9 ± 0.1	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.1	4.0 ± 0.0
Plasma glucose (mmol/l)	–	6.2 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.5 ± 0.1
^{3}H-Glucose turnover ($\mu mol \cdot min^{-1} \cdot kg^{-1}$)	–	57 ± 3	114 ± 9[a]	139 ± 6	108 ± 4[a]	134 ± 8
Clamp glucose requirement ($\mu mol \cdot min^{-1} \cdot kg^{-1}$)	–	–	107 ± 10[b]	141 ± 6	67 ± 5[b]	129 ± 7
Residual hepatic glucose output ($\mu mol \cdot min^{-1} \cdot kg^{-1}$)	–	–	8 ± 6[b]	−3 ± 3	41 ± 4[b]	5 ± 4

Mean ± SEM.
[a] $p < 0.005$; [b] $p < 0.001$ compared to 24 h fasted clamped rats by analysis of variance; [c] by analysis of variance there was a significant effect of Intralipid on clamp glucose requirement ($p < 0.005$) and residual hepatic glucose production ($p < 0.001$)

and 3-hydroxybutyrate by enzymic fluorimetric methods [33] using an LS50 luminescence spectrometer (Perkin Elmer, Beaconsfield, UK). Plasma insulin was determined by radioimmunoassay [34], using a rat or human insulin standard (Novo Industri, Bagsvaerd, Denmark) as appropriate. Blood glucose was measured by a glucose oxidase method (Yellow Springs Glucose Analyser; Clandon Scientific, London, UK). Plasma NEFA were determined with an acyl-CoA oxidase based colorimetric kit (WAKO NEFA-C; Wako Chemicals GmbH, Neuss, FRG) and plasma triglyceride with a GPO-PAP kit (Boehringer, Mannheim, FRG).

Statistical analysis

Results are presented as mean ± SEM. Significant differences between groups were assessed by Student's paired or unpaired *t*-test or by analysis of variance as appropriate.

Results

Plasma insulin, lipid and metabolite concentrations

Steady-state plasma insulin concentrations during the glucose clamps (human insulin standard) were similar to those found in six ad libitum fed rats (rat insulin standard) and were not affected by infusion of Intralipid and heparin (Table 1).

Thirty minutes after stopping the glucose infusion at the start of the glucose clamps, plasma NEFA, blood glycerol and 3-hydroxybutyrate concentrations were lower in the 4.5 h FGI rats than in 24 h fasted rats (all $p < 0.001$, Table 1). Whilst plasma NEFA and blood glycerol levels were higher in the 4.5 h FGI rats than in ad libitum fed rats (both $p < 0.001$), 3-hydroxybutyrate levels were not different. Plasma triglyceride, NEFA, glycerol and 3-hydroxybutyrate concentrations were suppressed during the glucose clamps and were then not different in the 4.5 h FGI and 24 h fasted rats (Table 1). Infusion of Intralipid and heparin during the glucose clamps resulted in an increase in plasma NEFA and triglyceride to levels which were higher than basal (Table 1); blood glycerol concentrations were similar to those found in rats after a 24 h fast. During the clamps with Intralipid there was incomplete suppression of 3-hydroxybutyrate concentrations in both groups of rats.

Glucose kinetics

Blood glucose concentrations during the last 30 min of the euglycaemic clamp were identical in the four groups of rats (Table 2). The coefficient of variation of blood glucose calculated for each animal was 3.3 ± 0.2% (± SD). During the glucose clamp, 3-^{3}H-glucose turnover and glucose requirement to maintain the desired blood glucose concentration were both lower in 4.5 h FGI rats than in rats fasted for 24 h (Table 2). Hepatic glucose production during the clamp, calculated as the difference between glucose turnover and the rate of glucose infusion at steady-state, was completely suppressed and not different between the two groups (Table 2). When plasma lipid levels were raised during the clamps by infusion of Intralipid and heparin, there was a further 38% decrease in clamp glucose requirement in the 4.5 h FGI group (Table 2, $p < 0.01$), but not in rats fasted for 24 h. Increased NEFA availability had no effect on 3-^{3}H-glucose turnover in either 24 h fasted or 4.5 h FGI rats. Thus, in the 4.5 h FGI rats, increased lipid fuel availability resulted in a marked impairment of suppression of hepatic glucose output during the clamps ($p < 0.001$, Table 2).

Tissue glycogen concentrations

Quadriceps muscle glycogen concentration was partially depleted in 24 h fasted rats (Table 3, $p < 0.001$). In 4.5 h FGI rats, muscle glycogen concentration was higher than in ad libitum fed rats (49.7 ± 3.6 vs 38.1 ± 1.7 μmol/g wet weight, $p < 0.05$). Muscle glycogen deposition was stimulated by the glucose clamp in 24 h fasted rats (Table 3, $p < 0.001$) but not in 4.5 h FGI rats. Intralipid enhanced muscle glycogen deposition during the clamps in both groups of rats (Table 3, $F = 12.98$, $p < 0.005$ by analysis of variance [ANOV]).

Cardiac muscle glycogen concentration was higher after a 24 h fast than in ad libitum fed rats (Table 3, $p < 0.01$). No stimulation of glycogen deposition by the clamp was observed in either 24 h fasted or 4.5 h FGI rats. In both groups, however, Intralipid resulted in significant stimulation of glycogen deposition during the clamp (Table 3, $p < 0.001$ by ANOV).

Table 3. Tissue glycogen concentrations in ad libitum fed rats and in 24 h fasted and 4.5 h fasted glucose-infused (4.5 h FGI) rats in the basal state and at the end of a hyperinsulinaemic glucose clamp

Glycogen (μmol/g wet weight)	Ad libitum fed rats	Basal		Clamp	
		4.5 h FGI	24 h fasted	4.5 h FGI	24 h fasted
Heart					
– Intralipid	11.7 ± 0.9	20.8 ± 3.7[a]	26.2 ± 3.2[b]	19.5 ± 3.1	26.4 ± 1.9
+ Intralipid	–	–	–	33.0 ± 2.7[e]	43.2 ± 2.3[e]
Muscle					
– Intralipid	38.1 ± 1.7	49.7 ± 3.6[a]	18.6 ± 1.1[c]	46.2 ± 2.1	28.0 ± 0.7
+ Intralipid	–	–	–	63.6 ± 5.6[d]	33.6 ± 2.1[d]
Liver					
– Intralipid	375 ± 31	347 ± 38	11.8 ± 3.7[c]	234.7 ± 9.9	28.0 ± 1.7
+ Intralipid	–	–	–	332.8 ± 26.0[e]	53.5 ± 2.7[e]

Mean ± SEM, $n = 6$ in each group.
[a] $p < 0.05$; [b] $p < 0.002$; [c] $p < 0.001$ compared with ad libitum fed rats. [d] $p < 0.005$; [e] $p < 0.001$ by analysis of variance for the effect of Intralipid on glycogen deposition during the clamp

Table 4. Tissue pyruvate dehydrogenase (PDH) activity in rats in the fasting and ad libitum fed states and at the end of a 2 h hyperinsulinaemic glucose clamp

PDH (U/g wet weight)	Ad libitum fed rats	Basal		Clamp[c]		Clamp with Intralipid[e]	
		4.5 h FGI	24 h fasted	4.5 h FGI	24 h fasted	4.5 h FGI	24 h fasted
Muscle							
PDH_T	1.41 ± 0.12	1.60 ± 0.17	1.68 ± 0.25	1.12 ± 0.18	1.26 ± 0.22	1.49 ± 0.31	1.54 ± 0.19
% Active	17.2 ± 2.3	11.2 ± 2.1	3.1 ± 0.7[b]	10.6 ± 3.4	4.6 ± 0.7	2.6 ± 0.9	1.8 ± 0.4
Heart							
PDH_T	7.38 ± 0.67	7.20 ± 0.52	8.22 ± 0.69	7.93 ± 0.35	8.35 ± 0.38	8.89 ± 0.17	8.40 ± 0.47
% Active	35.8 ± 2.5	34.7 ± 5.6	8.3 ± 2.4[b]	47.8 ± 7.0	27.8 ± 3.6	18.5 ± 3.6	5.6 ± 1.2
Adipose Tissue							
PDH_T	0.26 ± 0.03	0.19 ± 0.00	0.24 ± 0.02	0.23 ± 0.01	0.22 ± 0.02	0.22 ± 0.01	0.20 ± 0.01
% Active	50.6 ± 6.8	21.0 ± 2.8[b]	6.6 ± 1.2[b]	31.7 ± 2.6[b]	18.4 ± 2.1	18.5 ± 3.0	11.1 ± 1.3
Liver							
PDH_T	1.47 ± 0.09	1.36 ± 0.07	2.02 ± 0.08	1.64 ± 0.05	1.81 ± 0.11	1.44 ± 0.07	1.79 ± 0.06
% Active	15.7 ± 3.0	15.5 ± 3.1	4.0 ± 0.5[a]	15.8 ± 2.8	14.0 ± 2.9[d]	2.5 ± 0.3	2.2 ± 0.2

4.5 h FGI = 4.5 h fasted rats infused with glucose for 4 h after food withdrawal; PDH_T = Total PDH; PDH_a% = percent of PDH in the active form. Mean ± SEM, $n = 6$ in each group
[a] $p < 0.005$; [b] $p < 0.001$ compared with ad libitum fed rats;
[c] by analysis of variance the increase in PDH^a% from "basal" to "clamp" was significant in heart ($p < 0.01$) and adipose tissue ($p < 0.001$)
[d] $p < 0.01$ compared with 24 h fasted rats in the basal state;
[e] by analysis of variance the decrease in clamp PDH^a% in the presence of Intralipid was significant in all tissues: muscle ($p < 0.01$); heart, liver and adipose tissue ($p < 0.001$)

Liver glycogen was almost completely depleted after a 24 h fast (Table 3, $p < 0.001$). In 24 h fasted rats there was a small increase in liver glycogen content at the end of the glucose clamp (28.0 ± 1.7 vs 11.8 ± 3.7 μmol/g wet weight, $p < 0.01$) and a further small increase during the clamps with Intralipid (Table 3, $p < 0.001$). In 4.5 h FGI rats, liver glycogen at the start of the glucose clamps was not different from that in ad libitum fed rats (Table 3). In this group liver glycogen fell during the clamp (Table 3, $p < 0.05$) but was maintained at basal levels by concomitant infusion of Intralipid and heparin (Table 3).

Pyruvate dehydrogenase

Total PDH in muscle, heart and adipose tissue was not affected by nutritional state and did not change during the glucose clamps (Table 4). In liver, PDH_T was lower in 4.5 h FGI rats compared to 24 h fasted rats ($p < 0.001$). However, liver PDH_T activity relative to mitochondrial glutamate dehydrogenase was not statistically significantly different between groups (results not shown), suggesting that the lower PDH_T (per g wet weight) in livers from fed rats was due to increased liver glycogen and water content in these animals.

In all four tissues the percent of PDH in the active form (PDH_a%) was lower in 24 h fasted rats than in ad libitum fed rats (Table 4). In adipose tissue PDH_a% was also lower in the 4.5 h FGI rats than in ad libitum fed rats (21.0 ± 2.8 vs 50.6 ± 6.8%, $p < 0.005$). PDH_a% was increased by the hyperinsulinaemic glucose clamp in heart and adipose tissue (Table 4, both $p < 0.001$ by ANOV), but not in quadriceps skeletal muscle (Table 4). In liver, significant stimulation of PDH_a% was seen only in 24 h fasted rats in which basal PDH_a% was low. PDH_a% was lower at the end of the glucose clamp in 24 h fasted rats than in the 4.5 h FGI rats in heart ($F = 16.02$, $p < 0.002$) and adipose tissue ($F = 21.28$, $p < 0.001$), but not in liver or quadriceps muscle.

When plasma NEFA and triglyceride levels were raised by concomitant infusion of Intralipid and heparin, end of clamp PDH_a% was decreased markedly in all four tissues (Table 4, all $p < 0.001$ by ANOV). The inhibition of PDH activation was independent of nutritional state.

Discussion

During a hyperinsulinaemic glucose clamp, skeletal muscle glycogen deposition is a major determinant of whole body glucose utilisation [27, 35]. However, high muscle glycogen levels inhibit glycogen deposition [25, 26]; one might therefore expect insulin sensitivity to be lower in fed rats than in fasted rats. In vitro studies support this idea. The insulin sensitivity of glucose uptake by mouse soleus [36], or perfused rat hind limb [37], is less in the fed state. Even a short period of fasting results in depletion of tissue glycogen stores [38] and it is difficult to measure in vivo insulin sensitivity in fed animals, because a valid glucose clamp study cannot be performed if glucose is still entering the circulation from the intestine.

In the current study, one group of rats was infused with glucose for 4 h after removal of food (i. e. 4.5 h FGI rats) to maintain tissue glycogen stores whilst allowing sufficient time to complete intestinal glucose absorption before the start of the clamps. In these rats liver glycogen concentrations at the start of the clamps were the same as those of ad libitum fed animals, while their muscle glycogen concentrations were actually higher (Table 3). Our finding of decreased whole body insulin-stimulated glucose utilisation in these 4.5 h FGI rats, compared to 24 h fasted rats (Table 2), is in keeping with the in vitro studies mentioned above [36, 37], but contrasts with our previous demonstration of normal rates of insulin mediated glucose utilisation in rats fasted for a similar period (3.5 h) but without glucose infusion [12]. This difference is probably explained by the higher muscle glycogen concentrations in the rats infused with glucose after removal of food. Despite high glycogen stores, the infusion of Intralipid was not associated with a further decrease in insulin-stimulated whole body glucose utilisation. These findings are in keeping with the studies of Jenkins et al. [22], who used a similar clamp protocol in 5 h fasted rats, and with studies of Felber et al. in man [39]. The latter authors [39] employing indirect calorimetry during glucose clamp studies found lower insulin-stimulated whole body glucose utilisation and decreased glucose storage when Intralipid was infused prior to the start of a euglycaemic clamp but not when an infusion of Intralipid was started simultaneously with an infusion of insulin. They suggested that increased lipid fuel oxidation prior to the clamp prevented muscle glycogen breakdown and that the resulting higher glycogen stores inhibited insulin-mediated glycogen deposition. They did not, however, measure muscle glycogen content or rates of synthesis directly.

Assuming quadriceps muscle to be representative of most skeletal muscle, and a rat to be 40% muscle by mass [40], it can be estimated that about one-third of the glucose infused during the clamp in 24 h fasted rats was deposited as muscle glycogen. Thus, the reduced muscle glycogen deposition during the glucose clamps in the 4.5 h FGI rats (Table 3) could readily explain the 20% decrease in glucose turnover observed. Contrary to expectations, the elevated basal muscle glycogen concentrations in the 4.5 h FGI rats did not prevent further muscle glycogen deposition during the clamps with Intralipid (Table 3); and glycogen deposition also occurred in heart; this increase in glycogen in muscle and heart when NEFA levels were elevated is consistent with the operation of the glucose-fatty acid cycle in these tissues [7, 8, 23].

In 24 h fasted rats, less than 10% of the glucose infused during the 2 h clamp was deposited as liver glycogen. With elevated plasma NEFA levels liver glycogen deposition increased approximately two-fold (Table 3). As little net glucose uptake by liver would occur at a blood glucose concentration of 4.0 mmol/l, enhanced liver glycogen deposition was probably due to stimulation of gluconeogenesis by increased fatty acid availability [41, 42]. By contrast with 24 h fasted rats, liver glycogen concentrations in 4.5 h FGI rats decreased by approximately 100 μmol/g during the clamp. Since hepatic glucose output was almost completely suppressed in these 4.5 h FGI rats, one must assume that the glucose-6-phosphate resulting from liver glycogen breakdown was metabolised by glycolysis to pyruvate which would then be converted to acetyl CoA by PDH. As was found by Jenkins et al. [22] in 5 h fasted rats, increasing the plasma NEFA in the 4.5 h FGI rats caused a marked impairment of suppression of hepatic glucose production (Table 2). Since there was no decrease in liver glycogen concentration during the clamps in these 4.5 h FGI rats given Intralipid (Table 3) the glucose released by the liver must have been synthesised by gluconeogenesis, implying a reversal of the flux through glycolysis/gluconeogenesis in liver because of increased lipid fuel availability. These findings are consistent with the known ability of fatty acids to stimulate gluconeogenesis [41, 42] and to inhibit glycolysis in rat hepatocytes [43].

The stimulation of gluconeogenesis by fatty acids is generally attributed to an increase in mitochondrial acetyl CoA, and to a more reduced cytosolic redox state resulting from the generation of reducing equivalents in the mitochondria during B-oxidation of fatty acids. Acetyl CoA is an allosteric activator of the key gluconeogenic enzyme, pyruvate carboxylase [44] while NADH is required for the glyceraldehyde-3-phosphate-dehydrogenase reaction. A further mechanism involves a decrease in the concentration of hepatocyte fructose-2,6-bisphosphate (F-2,6-P_2) [29, 43], an important positive effector of phosphofructokinase-1 (PFK-1). In the current study, increased provision of glycerol (present in the Intralipid) would be expected to raise hepatocyte glycerol-3-phosphate levels. As well as representing a source of carbon for glucose synthesis, glycerol-3-phosphate inhibits PFK-2 and stimulates fructose-2,6-bisphosphatase, leading to a fall in F-2,6-P_2 levels which would favour gluconeogenic flux.

The marked inhibition of hepatocyte PDH_a% during the glucose clamps when circulating NEFA levels were increased, irrespective of nutritional state, is in keeping with previous studies [45]. Inactivation of PDH would be ex-

pected to act in concert with the above mechanisms to promote carbon flux through gluconeogenesis. Our findings contrast with those of French et al. [29] who found no inhibition of liver PDH in ad libitum fed rats when NEFA levels were raised by administration of corn oil and heparin; these authors did, however, find an increase in PDH activity when lipolysis was inhibited in stressed animals, supporting a role for lipid oxidation in the regulation of this enzyme in liver.

It is noteworthy that insulin sensitivity was lower in the 4.5 h FGI rats than in 24 h fasted rats despite significantly increased PDH activity in heart, adipose tissue and quadriceps muscle (Table 4). As in liver, elevated NEFA levels during the clamps resulted in significant inhibition of PDH activity in heart, adipose tissue and quadriceps muscle, the degree of inhibition being similar in the 24 h fasted and 4.5 h FGI rats (Table 4). Inhibition of PDH was not associated with any impairment of tissue glucose disposal as indicated by unchanged 3-^{3}H-glucose turnover. Glucose metabolism was, however, qualitatively different in the presence of Intralipid. Thus, increased glycogen synthesis in muscle and heart, with unchanged glucose turnover, implies a decrease in flux through glycolysis. This is consistent with previous studies showing decreased glycolysis and pyruvate oxidation when lipid oxidation is increased [3, 6].

Although the effects of Intralipid on glucose metabolism are generally ascribed to the direct effects of increased tissue NEFA oxidation, it remains possible that alterations in circulating levels of hormones important in the regulation of carbohydrate metabolism could contribute to the observed effects. Plasma insulin levels were not affected by Intralipid during the clamps but its influence on other hormones is unknown.

In summary, an acute elevation of plasma NEFA does not impair whole body glucose utilisation in rats during physiological hyperinsulinaemia, as increased glycogen synthesis compensates for decreased glycolytic flux. However, when liver glycogen stores are high, an acute elevation in plasma NEFA impairs insulin-mediated suppression of hepatic glucose production. This probably explains the intolerance to oral or i.v. glucose induced by concomitant lipid administration [39, 46–48]. Our findings support the idea [39, 49, 50] that a chronic increase in lipid availability may lead to insulin resistance by increasing tissue glycogen stores.

Acknowledgements. The study was supported by the British Diabetic Association, the British Heart Foundation, the Medical Research Council and the Lister Institute. JG McCormack is a Lister Institute Research Fellow.

References

1. Randle PJ, Garland PB, Hales CN, Newsholme EA (1963) The glucose fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet I: 785–789
2. Randle PJ, Newsholme EA, Garland PB (1964) Regulation of glucose uptake by muscle. Effects of fatty acids, ketone bodies, pyruvate, and of alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles. Biochem J 93: 652–665
3. Garland PB, Newsholme EA, Randle PJ (1964) Regulation of glucose uptake by muscle. Effects of fatty acids and ketone bodies, and of alloxan-diabetes and starvation, on pyruvate metabolism and on lactate/pyruvate and L-glycerol 3-phosphate/dihydroxyacetone phosphate concentration ratios in rat heart and rat diaphragm muscles. Biochem J 93: 665–678
4. Wieland O, Funcke HV, Loffler G (1971) Interconversion of pyruvate dehydrogenase in rat heart muscle upon perfusion with fatty acids or ketone bodies. FEBS Lett 15: 295–298
5. Randle PJ (1986) Fuel selection in animals. Biochem Soc Trans 14: 799–806
6. Maizels EZ, Ruderman NB, Goodman MN, Lau D (1977) Effect of acetoacetate on glucose metabolism in the soleus and extensor digitorum longus muscles of the rat. Biochem J 162: 557–568
7. Rennie MJ, Holloszy JO (1977) Inhibition of glucose uptake and glycogenolysis by availability of oleate in well-oxygenated perfused skeletal muscle. Biochem J 168: 161–170
8. Newsholme EA, Randle PJ (1964) Regulation of glucose uptake by muscle. Effects of fatty acids, ketone bodies and pyruvate, and of alloxan-diabetes, starvation, hypophysectomy and adrenalectomy, on the concentrations of hexose phosphates, nucleotides and inorganic phosphate in perfused rat heart. Biochem J 93: 641–651
9. Zorzano A, Balon TW, Brady LJ, Rivera P, Garetto LP, Young JC, Goodman MN, Ruderman NB (1985) Effects of starvation and exercise on concentrations of citrate, hexose phosphates and glycogen in skeletal muscle and heart. Evidence for selective operation of the glucose-fatty acid cycle. Biochem J 232: 585–591
10. Katz LD, Glickman MG, Rapopart S, Ferrannini E, DeFronzo RA (1983) Splanchnic and peripheral disposal of oral glucose in man. Diabetes 32: 675–679
11. Ferrannini E, Bjorkman O, Reichard GA, Pilo A, Olsson M, Wahren J, DeFronzo RA (1985) The disposal of an oral glucose load in healthy subjects. A quantitative study. Diabetes 34: 580–588
12. Kruszynska YT, McCormack JG (1989) Effect of nutritional status on insulin sensitivity in vivo and tissue enzyme activities in the rat. Biochem J 258: 699–707
13. Issad T, Penicaud L, Ferre P, Kande J, Baudon MA, Girard J (1987) Effects of fasting on tissue glucose utilisation in conscious resting rats. Major glucose-sparing effect in working muscles. Biochem J 246: 241–244
14. Kraegen EW, James DE, Storlien LH, Burleigh KM, Chisholm DJ (1986) In vivo insulin resistance in individual peripheral tissues of the high fat fed rat: assessment by euglycaemic clamp plus deoxyglucose administration. Diabetologia 29: 192–198
15. Storlien LH, James DE, Burleigh KM, Chisholm DJ, Kraegen EW (1986) Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats. Am J Physiol 251: E576–E583
16. Christophe J, Mayer J (1959) Influence of diet on utilisation of glucose and incorporation of acetate-1-^{14}C into liver fatty acids and cholesterol in rats. Am J Physiol 197: 55–59
17. Bringolf M, Zaragoza N, Rivier D, Felber JP (1972) Studies on the metabolic effects induced in the rat by a high fat diet. Inhibition of pyruvate metabolism in diaphragm in vitro and its relation to the oxidation of fatty acids. Eur J Biochem 26: 360–367
18. Schonfeld G, Kipnis D (1968) Effects of fatty acids on carbohydrate and fatty acid metabolism of rat diaphragm. Am J Physiol 215: 513–522
19. Goodman MN, Berger M, Ruderman NB (1974) Glucose metabolism in rat skeletal muscle at rest. Effect of starvation, diabetes, ketone bodies and free fatty acids. Diabetes 23: 881–888
20. Berger M, Hagg SA, Goodman MN, Ruderman NB (1976) Glucose metabolism in perfused skeletal muscle. Effects of starvation, diabetes, fatty acids, acetoacetate, insulin and exercise on glucose uptake and disposition. Biochem J 158: 191–202
21. Ruderman NB, Goodman MN, Conover CA, Berger M (1976) Substrate utilisation in perfused skeletal muscle. Diabetes 28 [Suppl 1]: 13–17

22. Jenkins AB, Storlien LH, Chisholm DJ, Kraegen EW (1988) Effects of nonesterified fatty acid availability on tissue-specific glucose utilisation in rats in vivo. J Clin Invest 82: 293–299
23. Kruszynska YT, McCormack JG, McIntyre N (1990) Effects of non-esterified fatty acid availability on insulin-stimulated glucose utilisation and tissue pyruvate dehydrogenase activity in the rat. Diabetologia 33: 396–402
24. Ferrannini E, Barrett EJ, Bevilacqua S, DeFronzo RA (1983) Effect of fatty acids on glucose production and utilisation in man. J Clin Invest 72: 1737–1747
25. Stalmans W, Bollen M, Mvumbi L (1987) Control of glycogen synthesis in health and disease. Diabetes Metab Rev 3: 127–161
26. Danforth WH (1965) Glycogen synthase activity in skeletal muscle. Interconversion of two forms and control of glycogen synthesis. J Biol Chem 240: 588–593
27. Kruszynska YT, Home PD, Alberti KGMM (1986) In vivo regulation of liver and skeletal muscle glycogen synthase activity by glucose and insulin. Diabetes 35: 662–667
28. Holness MJ, Lin Y-L, Sugden MC (1989) Time courses of the responses of pyruvate dehydrogenase activities to short-term starvation in diaphragm and selected muscles of the rat Biochem J 264: 771–776
29. French TJ, Goode AW, Holness MJ, MacLennan PA, Sugden MC (1988) The relationship between changes in lipid fuel availability and tissue fructose 2,6-bisphosphate concentrations and pyruvate dehydrogenase complex activities in the fed state. Biochem J 256: 935–939
30. Kruszynska YT, Home PD, Alberti KGMM (1985) Comparison of portal and peripheral insulin delivery on carbohydrate metabolism in streptozotocin-diabetic rats. Diabetologia 28: 167–171
31. McCormack JG, Denton RM (1989) Influence of Ca^{2+} ions on mammalian intramitochondrial dehydrogenases. Meths Enzymol 174: 95–118
32. Keppler D, Decker K (1974) Glycogen determination with amyloglucoidase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic Press, New York, pp 1127–1131
33. Lloyd B, Burrin J, Smythe P, Alberti KGMM (1978) Enzymatic fluorimetric continuous flow assays for blood glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate. Clin Chem 24: 1724–1729
34. Heding LG (1972) Determination of total serum insulin (IRI) in insulin treated diabetic patients. Diabetologia 8: 260–266
35. Kraegen EW, James DE, Jenkins AB, Chisholm DJ (1985) Dose response curves for in vivo insulin sensitivity in individual tissues in rats. Am J Physiol 248: E353–E362
36. Le-Marchand-Brustel Y, Freychet P (1979) Effect of fasting and streptozotocin diabetes on insulin binding and action in the isolated mouse soleus muscle. J Clin Invest 64: 1505–1515
37. Goodman MN, Ruderman NB (1979) Insulin sensitivity of rat skeletal muscle: effects of starvation and aging. Am J Physiol 236: E519–E523
38. Holness MJ, Sugden MC (1989) Pyruvate dehydrogenase activities during the fed-to-starved transition and on re-feeding after acute or prolonged starvation. Biochem J 258: 529–533
39. Felber JP, Golay A, Felley C, Jequier E (1988) Regulation of glucose storage in obesity and diabetes: metabolic aspects. Diabetes Metab Rev 4: 691–700
40. Curtis-Prior PB, Trethewey J, Steward GW, Hanley T (1969) The contribution of different organs and tissues of the rat to assimilation of glucose. Diabetologia 5: 384–391
41. Sherratt HSA et al. (1981) Inhibition of gluconeogenesis by non-hormonal hypoglycaemic compounds. In: Hue L, Werve G van der (eds) Short-term regulation of liver metabolism. Elsevier Amsterdam, North Holland Biomedical Press, pp 199–227
42. Ross BD, Hems R, Krebs HA (1967) The rate of gluconeogenesis from various precursors in the perfused rat liver. Biochem J 102: 942–951
43. Hue L, Maisin L, Rider MH (1988) Palmitate inhibits liver glycolysis. Involvement of fructose 2,6-bisphosphatase in the glucose/fatty acid cycle. Biochem J 251: 541–545
44. Scrutton MC, Utter MF (1967) Pyruvate carboxylase. Some properties of the activation by certain acyl derivatives of coenzyme A. J Biol Chem 242: 1723–1735
45. Wieland OH, Patzelt C, Loffler G (1972) Active and inactive forms of pyruvate dehydrogenase in rat liver. Effect of starvation and refeeding and of insulin treatment on pyruvate dehydrogenase interconversion. Eur J Biochem 26: 426–433
46. Felber JP, Vannotti A (1964) Effect of fat infusion on glucose tolerance and insulin plasma levels. Med Exp 10: 153–156
47. Gomez F, Jequier E, Chabot V, Buber V, Felber JP (1972) Carbohydrate and lipid oxidation in normal human subjects: its influence on glucose tolerance and insulin response to glucose. Metabolism 21: 381–391
48. Kipnis DM, Schalch DS (1964) The impairment of carbohydrate tolerance by elevated plasma free fatty acids. J Clin Invest 434: 1283–1286
49. Crettaz M, Horton ES, Wardzala LJ, Horton ED, Jeanrenaud B (1983) Physical training of Zucker rats: lack of alleviation of muscle insulin resistance. Am J Physiol 244: E414–E420
50. Mott DM, Lillioja S, Bogardus C (1986) Over-nutrition induced decrease in insulin action for glucose storage: in vivo and in vitro in man. Metabolism 35: 160–165

Received: 27 August 1990
and in revised form: 6 November 1990

Dr. Y. Kruszynska
Academic Department of Medicine
Royal Free Hospital
Pond Street
London NW3 2QG
UK