Effect of increased free fatty acid supply on glucose metabolism and skeletal muscle glycogen synthase activity in normal man

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1. Experimental elevation of plasma non-esterified fatty acid concentrations has been postulated to decrease insulin-stimulated glucose oxidation and storage rates. Possible mechanisms were examined by measuring skeletal muscle glycogen synthase activity and muscle glycogen content before and during hyperinsulinaemia while fasting plasma non-esterified fatty acid levels were maintained.

2. Fasting plasma non-esterified fatty acid levels were maintained in seven healthy male subjects by infusion of 20% (w/v) Intralipid (1 ml/min) for 120 min before and during a 240 min hyperinsulinaemic euglycaemic clamp (100 m-units h⁻¹ kg⁻¹) combined with indirect calorimetry. On the control day, 0.154 mol/l NaCl was infused. Vastus lateralis muscle biopsy was performed before and at the end of the insulin infusion.

3. On the Intralipid study day serum triacylglycerol (2.24 ± 0.20 versus 0.67 ± 0.10 mmol/l), plasma non-esterified fatty acid (395 ± 13 versus 51 ± 1 μmol/l), blood glycerol (152 ± 2 versus 11 ± 1 μmol/l) and blood 3-hydroxybutyrate clamp levels [mean (95% confidence interval)] [81 (64–104) versus 4 (3–5) μmol/l] were all significantly higher (all P<0.001) than on the control study day. Lipid oxidation rates were also elevated (1.07 ± 0.07 versus 0.27 ± 0.08 mg min⁻¹ kg⁻¹, P<0.001). During the clamp with Intralipid infusion, insulin-stimulated whole-body glucose disposal decreased by 28% (from 8.53 ± 0.77 to 6.17 ± 0.71 mg min⁻¹ kg⁻¹, P<0.005). This was the result of a 48% decrease in glucose oxidation (3.77 ± 0.32 to 1.95 ± 0.21 mg min⁻¹ kg⁻¹, P<0.001), with no significant change in non-oxidative glucose disposal (4.76 ± 0.49 to 4.22 ± 0.57 mg min⁻¹ kg⁻¹, not significant).

4. Basal and insulin-stimulated glycogen synthase activities (13.1 ± 1.9 versus 11.4 ± 2.3 and 30.8 ± 2.3 versus 27.6 ± 4.5%, respectively) were unaffected by the increased plasma non-esterified fatty acid levels. Similarly, basal (36.2 ± 2.7 versus 37.2 ± 1.4 μmol/g) and stimulated (40.0 ± 0.6 versus 37.6 ± 4.4 μmol/g) muscle glycogen levels were unaltered. Insulin-stimulated hexokinase activity was also not affected (0.52 ± 0.08 versus 0.60 ± 0.08 units/g wet weight).

5. Maintenance of plasma non-esterified fatty acid levels at fasting values resulted in an increase in lipid oxidation and was associated with a decrease in insulin-stimulated whole-body glucose uptake and glucose oxidation rates, but no change in non-oxidative glucose disposal. Increased plasma non-esterified fatty acid levels did not appear to have a direct inhibitory effect on glycogen synthase activity or storage of glucose as glycogen at these insulin levels.

INTRODUCTION

It is now well established that peripheral tissue insulin insensitivity is a characteristic feature of type 2 diabetes, and the skeletal muscle is the main site of resistance [1]. Decreased insulin-stimulated glucose uptake, glucose oxidation and glucose storage rates have all been well documented in such patients [2, 3]. The anti-lipolytic action of insulin is also impaired in type 2 diabetes, resulting in elevated plasma non-esterified fatty acid (NEFA) levels and increased lipid oxidation rates [4–6]. Randle et al. [7] suggested that the high plasma NEFA levels were causally related to the abnormalities of carbohydrate metabolism in type 2 diabetes via the glucose–fatty acid cycle. An essential feature of their proposal was that increased NEFA oxidation imposed restrictions upon the metabolism of glucose by heart and skeletal muscle, thereby leading to insulin insensitivity. It is possible therefore that elevated plasma NEFA levels may explain, at least in part, the decreased insulin-stimulated glucose metabolism observed in type 2 diabetic patients.

A number of studies have demonstrated that the glucose–fatty acid cycle operates in normal man, using an infusion of Intralipid and/or heparin to elevate plasma NEFA levels. The high plasma NEFA levels were associated with decreased rates of insulin-stimulated whole-body glucose uptake and glucose oxidation [8–15].

Key words: glucose uptake, glycogen synthase, insulin action, lipid oxidation, skeletal muscle.

Abbreviations: G6P, glucose 6-phosphate; GS, glycogen synthase; GS₁, physiologically active glycogen synthase; GS₂, total glycogen synthase activity; NEFA, non-esterified fatty acids.

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Decreased glucose storage was also observed in a number of these studies \[8, 9, 15\]. An effect on glucose storage was not originally proposed by Randle \[7\], who suggested that increased NEFA metabolism might favour the conversion of glucose to glycogen rather than to lactate. This discrepancy could be explained by one of two possible mechanisms. First, a direct effect of fatty acids on liver glycogen synthase (GS, EC 2.4.1.11) has been demonstrated, causing it to dissociate into subunits \[16\]. A similar effect of fatty acids on skeletal muscle GS would be expected to result in decreased GS activity and thus decrease glycogen synthesis. Alternatively, increased lipid oxidation may lead to increased intracellular acetyl-CoA and citrate levels. These metabolites then inhibit earlier steps of glucose metabolism, eventually leading to decreased glucose transport \[17, 18\] into muscle and a reduction in both glucose oxidation and storage.

The present study was undertaken to determine the effect of maintaining plasma NEFA levels at fasting values in healthy man upon insulin-stimulated glucose metabolism, skeletal muscle glycogen synthesis and skeletal muscle GS activity, both basally and after insulin stimulation.

**MATERIALS AND METHODS**

**Subjects**

Seven healthy male subjects were studied. Their age was 42 ± 5 years and their mean body mass index was 25.2 ± 1.5 kg/m² (means ± SEM). No subject had a family history of diabetes mellitus, was taking any medications or undertook regular exercise. All were consuming a weight-maintaining diet before the study that contained at least 200 g of carbohydrate/day. The protocol was approved by the Ethical Committee of the Newcastle upon Tyne Health Authority, and written informed consent was obtained from each subject before the study.

**Experimental protocol (Fig. 1)**

All subjects were admitted to the metabolic unit on the evening before the study, fasted overnight (12-14 h) and were maintained on strict bed rest. Each subject was studied on two occasions in random order. On the morning of each study, two teflon cannulae (Venflon, Viggo, Helsingborg, Sweden) were inserted under local anaesthesia. The first was inserted into an antecubital vein for the infusion of all test substances, and the second into a contralateral dorsal hand vein in a retrograde fashion for intermittent blood sampling. This hand was then placed in a temperature-regulated box. Basal samples were taken at least 30 min after cannulation.

At time -120 min an infusion of either 0.154 mol/l NaCl in water (control study day) or 20% (w/v) Intralipid (Intralipid study day) was begun at a rate of 1 ml/min. Between time -30 min and time 0 min of this equilibration period, a needle biopsy (UCH-type needle) of the vastus lateralis muscle was performed under local anaesthesia in five of the subjects. The muscle tissue obtained by this technique (50-150 mg) was immediately frozen in liquid nitrogen and was stored at -70°C until assay. Once the basal biopsy had been taken, an intravenous insulin infusion (Human Actrapid; Novo, Basingstoke, Hants, U.K.) was commenced at time 0 min at a rate of 100 m-units h⁻¹ kg⁻¹. The blood glucose concentration was allowed to fall to 4.0 mmol/l and was maintained at that level by measuring the blood glucose concentration and adjusting the rate of infusion of 20% (w/v) glucose in water at 5 min intervals. The duration of the insulin infusion was 240 min. A repeat biopsy of the contralateral vastus lateralis muscle was performed during the last 30 min of the insulin infusion.

Hepatic glucose production was not measured, as this has been shown to be completely suppressed in normal subjects during comparable studies \[15\]. Throughout the study blood samples were taken for determination of serum insulin, plasma NEFA and blood intermediary metabolite concentrations. Samples were taken at 30 min intervals, except during the basal measurement period, and from 180 to 210 min, when samples were taken at 15 min intervals. A portion was centrifuged immediately and the plasma was aspirated and frozen for subsequent measurement of NEFA levels.

**Indirect calorimetry**

During the basal (-150 to -120 min) and equilibration (-60 to -30 min) periods and during the insulin infusion (90-120 and 180-210 min), oxygen uptake and carbon dioxide production were measured by using a Datex Deltatrac Metabolic Monitor (Datex, Helsinki, Finland). This uses a computerized open-circuit system to measure gas exchange through a clear plastic 2.5 litre canopy placed over the subject's head. Flow through the hood was calibrated by the gas injection method and quantitative alcohol burning. Carbon dioxide concentration was measured by an infra-red analyser, and oxygen concentration by a fast differential paramagnetic sensor. The Deltatrac was calibrated with a gravimetric carbon
dioxide and oxygen mixture before the start of each measurement period. The monitor has a precision of 3.1% for oxygen consumption and 2.6% for carbon dioxide production. Urine was collected from -120 to 240 min for the determination of total nitrogen excretion.

Calculations

The whole-body glucose disposal rate was measured as the glucose infusion rate (mg min⁻¹ kg⁻¹) from 90 to 120 and 180 to 210 min of the insulin infusion. Substrate oxidation rates were calculated over the same periods from the respiratory exchange measurements and urinary nitrogen excretion according to standard stoichiometric equations [19]. Non-oxidative glucose metabolism was calculated by subtracting the glucose oxidation rate from the total body glucose uptake rate during the clamp.

Muscle GS and glycogen assay

Muscle samples were weighed while still frozen and were homogenized with a Polytron homogenizer (Polytron Kinematica, Lucerne, Switzerland) in an ice-cold Tris/HCl buffer (1 ml of buffer/50 mg of frozen muscle), pH 7.3 containing 100 mmol/l KCl, 2 mmol/l EDTA, 2 mmol/l dithiothreitol, 25 mmol/l KF and 0.1% (v/v) Triton X-100. Rabbit liver glycogen (2.0 g/l; Boehringer Mannheim, Germany) was added to 800 µl of the homogenate and mixed well. This was then centrifuged at 10 000 g for 30 s in a microcentrifuge. The supernatant was used for the assay of GS activity. The rest of the homogenate was refrozen in liquid nitrogen and was stored at -70°C for the later assay of hexokinase activity and glycogen content.

GS activity was assayed by measuring the incorporation of UDP-d-[U¹⁴C]glucose into glycogen at 30°C using the modified method of Golden et al. [20]. Physiologically active GS (GS₃) was assayed in the absence of glucose 6-phosphate (G6P), whereas total GS activity (GS₄) was measured at a saturating concentration of G6P (6.7 mmol/l). Enzyme activity is expressed in units/g wet weight, where 1 unit is the amount of enzyme activity required to convert 1 µmol of substrate into glycogen/min at 30°C. The percentage fractional activity is calculated as the activity GS, divided by GS₄ multiplied by 100.

For the glycogen assay, 50 µl of muscle homogenate was incubated with 100 µl of 0.2 mmol/l acetate buffer containing amyloglucosidase (0.1 mg/ml; Sigma) at 42°C for 90 min. Appropriate sample blanks were also run. The tubes were immediately spun for 30 s at 10 000 g and the supernatant glucose content was assayed by the hexokinase method using a centrifugal analyser [21]. Glycogen content is expressed as µmol of glucose/g muscle wet weight.

Hexokinase activity was assayed using a modification of the method of Easterby & Quadri [21]. The G6P produced in the reaction was coupled to G6P dehydrogenase. The reaction was followed by measuring the increase in absorbance at 340 nm due to NADPH formation.

Other analyses

All blood glucose concentrations were measured by the glucose oxidase method (Yellow Springs Glucose Analyser; Clandon Scientific, London, U.K.). Serum insulin concentration was measured by a double-antibody radioimmunoassay [22]. Serum C-peptide concentration was measured by a radioimmunoassay kit [23] (Novo Industri, Bagsvaerd, Denmark). Blood for intermediary metabolites was immediately deproteinized in 0.5 mol/l perchloric acid, and the extract was stored at -20°C until analysis by automated fluorimetric methods [24]. Plasma NEFA level was measured enzymically by using a centrifugal analyser [25]. Serum triacylglycerol concentration was measured using standard enzymic methods. Urinary nitrogen was measured by the Kjeldahl's method [26].

Statistics

All results are expressed as means ± SEM unless otherwise indicated. The 3-hydroxybutyrate data was log-transformed for statistical analysis. Statistical analyses were performed by using Student’s paired t-tests and analysis of variance as appropriate. The sample size and power calculations were performed by the methods of Armitage & Berry [27].

RESULTS

Blood glucose and serum insulin concentrations

Fasting blood glucose concentrations were comparable on both Intralipid and control study days, and were unaltered by the infusion of Intralipid (4.8 ± 0.1 versus 4.8 ± 0.1 mmol/l). Fasting serum insulin concentrations were also comparable on both study days and were similarly unaffected by the Intralipid infusion (4.5 ± 0.9 versus 4.4 ± 1.0 m-units/l). The blood glucose concentrations (means ± SD) for the periods 90–120 min and 180–210 min were 4.0 ± 0.1 and 4.0 ± 0.1 mmol/l on both study days, respectively. The coefficient of variation of the blood glucose concentration was 3.6 ± 0.5% and 2.9 ± 0.5% from 90 to 120 min on the Intralipid and control study days, respectively, and 2.5 ± 0.4% and 2.4 ± 0.4%, respectively, from 180 to 210 min. After the start of the insulin infusion, serum insulin concentrations were comparable from 30 to 240 min (118 ± 11 and 112 ± 9 m-units/l, respectively).

Plasma NEFA, serum triacylglycerol, blood glycerol, 3-hydroxybutyrate and intermediary metabolite concentrations

- The plasma NEFA, blood glycerol and 3-hydroxybutyrate and serum triacylglycerol concentrations are shown in Fig. 2. Fasting plasma levels of NEFA (408 ± 45 versus 369 ± 48 µmol/l), blood levels of glycerol
control study day plasma NEFA concentrations were suppressed completely during the insulin infusion to 50 ± 1 μmol/l. Plasma NEFA concentrations from 30 to 240 min were significantly different on the Intralipid and control study days (P < 0.001). On the Intralipid study day blood glycerol concentrations increased to 149 ± 14 μmol/l at time 0 min and then reached a steady-state concentration of 152 ± 2 μmol/l, whereas on the control day levels declined to 11 ± 1 μmol/l. The difference between study days was again significant (P < 0.001). Blood 3-hydroxybutyrate levels were also significantly different from 30 to 240 min on the 2 study days (P < 0.001). Serum triacylglycerol concentrations rose to 2.04 ± 0.20 mmol/l at time 0 min and then reached a steady-state concentration of 2.24 ± 0.20 mmol/l from 30 to 240 min. On the control study day, serum triacylglycerol concentrations decreased from 0 to 60 min and then reached a steady-state concentration of 0.62 ± 0.01 mmol/l during the insulin infusion (P < 0.001 versus the Intralipid study day).

Plasma lactate, pyruvate and alanine concentrations were comparable in the fasting state and at 0 min in both studies (Table 1). During the insulin infusion plasma concentrations of lactate and alanine were lower on the Intralipid study day (both P < 0.001) (Table 1), but the difference in plasma pyruvate levels did not reach statistical significance (Table 1).

Whole-body glucose uptake and substrate metabolism

Data on insulin-mediated whole-body glucose disposal, oxidative glucose disposal, non-oxidative glucose disposal and lipid oxidation during the Intralipid and saline infusions are shown in Fig. 3. During the equilibration period, lipid oxidation was unchanged by the infusion of Intralipid (0.94 ± 0.08 versus 0.94 ± 0.07 mg min⁻¹ kg⁻¹). Similarly, basal glucose oxidation was unaltered (1.58 ± 0.23 versus 1.58 ± 0.14 mg min⁻¹ kg⁻¹). However, during the insulin infusion, lipid oxidation was significantly higher from 180 to 210 min on the Intralipid study day (1.07 ± 0.07 versus 0.27 ± 0.08 mg min⁻¹ kg⁻¹, P < 0.001). This was accompanied by a significant decrease in the rate of insulin-stimulated whole-body glucose uptake to 6.17 ± 0.71 compared with 8.53 ± 0.77 mg min⁻¹ kg⁻¹ (P < 0.001). Over this period glucose oxidation was also decreased to 1.95 ± 0.21 compared with 3.77 ± 0.32 mg min⁻¹ kg⁻¹ (P < 0.001), but there was no significant change in the rate of non-oxidative glucose disposal (4.22 ± 0.57 versus 4.76 ± 0.49 mg min⁻¹ kg⁻¹).

On the control study day there were significant increases in the rates of whole-body glucose uptake (P < 0.005) and oxidative (P < 0.02) and non-oxidative (P < 0.05) glucose metabolism and a significant decrease in lipid oxidation (P < 0.02) after increased time exposure to insulin (180 to 210 min versus 90 to 120 min). On the Intralipid study day there were also significant increases in the rates of whole-body glucose uptake (P < 0.001) and non-oxidative glucose metabolism (P < 0.01), but no changes in glucose or lipid oxidation rates with time.
Table 1. Blood lactate, pyruvate and alanine concentrations during the basal period (fasting), before the start of the insulin infusion (0 min) and during the insulin infusion (180–210 min). Values are means ± s.e. Statistical significance: *P < 0.001 compared with control study day.

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<th>Intralipid study day</th>
<th>Control study day</th>
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<tr>
<td>Lactate</td>
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<tr>
<td>Fasting</td>
<td>562 ± 86</td>
<td>561 ± 76</td>
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<tr>
<td>0 min</td>
<td>532 ± 64</td>
<td>532 ± 90</td>
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<tr>
<td>180–210 min</td>
<td>645 ± 38*</td>
<td>833 ± 71</td>
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<tr>
<td>Pyruvate</td>
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<tr>
<td>Fasting</td>
<td>54.1 ± 8.2</td>
<td>55.3 ± 7.3</td>
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<tr>
<td>0 min</td>
<td>41.6 ± 4.8</td>
<td>44.6 ± 7.8</td>
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<td>180–210 min</td>
<td>53.8 ± 3.5</td>
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<tr>
<td>Alanine</td>
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<tr>
<td>Fasting</td>
<td>252 ± 22</td>
<td>277 ± 20</td>
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<td>0 min</td>
<td>224 ± 19</td>
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<tr>
<td>180–210 min</td>
<td>171 ± 24*</td>
<td>234 ± 41</td>
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GS activity, hexokinase activity and glycogen content

The activities of GS and hexokinase and the glycogen content are shown in Table 2. Intralipid had no apparent effect on the basal (13.1 ± 1.9 versus 11.4 ± 2.3%) or stimulated (30.8 ± 2.3 versus 27.6 ± 4.5%) activities of GS. Thus the degree of stimulation of fractional GS activity (stimulated minus basal) was identical on both study days (235% and 242% for the Intralipid and control study days, respectively). GS, in the basal state (1.46 ± 0.21 versus 1.21 ± 0.17 units/g) was similar on the Intralipid and control study days and did not change during the insulin infusion (1.29 ± 0.22 versus 1.09 ± 0.34 units/g). There was also no significant difference in basal or stimulated skeletal muscle glycogen content on both study days (Table 3). Skeletal muscle hexokinase activity did not change in the presence or absence of raised plasma NEFA concentrations or during the insulin infusion.

DISCUSSION

In the present study we have demonstrated that maintenance of plasma NEFA levels at approximately fasting values resulted in a significant increase in lipid oxidation during euglycaemic hyperinsulinaemia. Plasma NEFA were mainly derived from the action of endothelial lipoprotein lipase on Intralipid triacylglycerols. However, NEFA released at the endothelial cell surface need not necessarily enter the plasma pool and thus the plasma NEFA concentrations might not reflect the entry rate of NEFA into peripheral tissues. A 28% decrease in insulin-stimulated whole-body glucose uptake ensued, and this was the consequence of a 48% decrease in glucose oxidation and a small but non-significant decrease in non-oxidative glucose disposal.

These findings are in agreement with previous reports. However, the majority of these studies [11, 14, 15] were performed at considerably higher plasma NEFA concentrations than the current study, higher even than the fasting levels observed in newly presenting, untreated type 2 patients [5], thus making extrapolation to the diabetic state difficult. Even though the present study was performed at mildly elevated plasma NEFA levels, the steady-state plasma NEFA concentrations were twice those reported in untreated type 2 patients after a comparable time exposure to hyperinsulinaemia. Although care must be taken when extrapolating from this acute study which was performed at 4.0 mmol/l to the diabetic state, our data strongly suggest that the glucose–fatty acid cycle could operate in type 2 diabetes. However, on the basis of the present results it is most unlikely that this would account for more than 30% of the insulin insensitivity seen in type 2 diabetes.

In this study, the majority of the decrease in insulin-stimulated whole-body glucose disposal is accounted for by inhibition of glucose oxidation. In a number of previous studies, however, elevated plasma NEFA levels have also been reported to inhibit non-oxidative glucose disposal [8, 9, 15]. As non-oxidative glucose disposal is generally thought to reflect storage of glucose, these studies suggest that glycogen synthesis may be decreased. Most recently, the addition of palmitate to human skeletal muscle preparations has been demonstrated to inhibit glycogen synthesis, both basally and during insulin stimulation [28]. The mechanism underlying these observations is uncertain, but a direct effect of fatty acids on GS has been proposed as one possible explanation.

GS, the key regulatory enzyme in glucose storage, is normally regulated covalently by phosphorylation/dephosphorylation of specific serine residues, and allosterically by G6P [29]. In studies in vitro fatty acids may affect liver GS activity by causing dissociation into subunits [16]. As this would be expected to bring about decreased activity of GS by insulin in vivo, this
A mechanism has been put forward to explain the decreased non-oxidative glucose disposal rates observed in these studies [30]. In the present study, however, we have demonstrated that acutely raising plasma NEFA levels to approximately 820 μmol/l had no effect on fasting GS fractional activity. Maintenance of plasma NEFA levels at fasting values also had no demonstrable effect on insulin-stimulated GS activity. Furthermore, the consequent increased lipid oxidation rates had no significant effect upon insulin-stimulated non-oxidative glucose disposal. It could be argued that the steady-state plasma NEFA levels achieved in this study were not sufficiently high to affect GS activity. However, similar findings to ours have recently been reported in the presence of markedly elevated plasma NEFA levels [31], and it thus seems unlikely that fatty acids inhibit non-oxidative glucose disposal via this mechanism. Nevertheless, the effect of fatty acids could possibly be masked if GS and GS₁ were to decrease in parallel. This can be discounted though, as GS₁ activity was unaltered by Intralipid both basally and during insulin infusion.

Alternatively, it has been suggested that NEFA may exert their effect on non-oxidative glucose disposal via the glucose–fatty acid cycle. Elevation of plasma NEFA levels during the infusion of insulin leads to an increase in lipid oxidation. This in turn results in an increase in intracellular acetyl-CoA and citrate concentrations producing inhibition of pyruvate dehydrogenase and phosphofructokinase activities, respectively. Glucose oxidation and glycolysis are inhibited and G6P accumulates. Earlier steps of glucose metabolism are inhibited, eventually leading to decreased glucose transport into skeletal muscle, and thus decreased, glycogen synthesis may result. Randle et al. [7], however, originally hypothesized that the converse might occur, and proposed that decreased rates of glucose flux through the oxidative pathway would favour redirection of glucose metabolism towards glycogen synthesis. A number of studies have demonstrated that the glycogen content of rat heart, diaphragm and skeletal muscle preparations increased when plasma NEFA levels were elevated, thus supporting their hypothesis [32, 33]. Our data demonstrate for the first time that the glycogen content of human skeletal muscle was unaffected by increased plasma NEFA concentrations, both basally and during hyperinsulinaemia. In fact, there was a small non-significant increase in muscle glycogen content during the Intralipid infusion, whereas non-oxidative glucose disposal decreased slightly.

It might be considered that a type 2 statistical error is present when interpreting the skeletal muscle enzyme data given the small number of subjects who underwent paired biopsy. However, we estimated that the power of the study to show activation of GS during either clamp was approximately 90% for a two-sided 5% significance test, indicating that the likelihood of a type 2 error is small. Further calculations based upon the muscle biopsy data of five of the subjects indicated that we would need as many as 110 subjects to observe a true difference in GS.

Fig. 3. Basal and insulin-stimulated rates of lipid oxidation, glucose oxidation, glucose disposal and glucose storage during a 240 min euglycaemic clamp performed on the control (○) and on the Intralipid (△) study days. Values are means ±SEM.
Activation between the two studies at the 5% level in a two-sided test. In view of the invasive nature of the study it was felt unjustified to submit further subjects to biopsy.

Additional support for the operation of the glucose–fatty acid cycle comes from the intermediary metabolic data. On the Intralipid study day, decreased glucose flux through the glycolytic pathway is suggested by the lower clamp blood pyruvate and lactate concentrations. The significant decrease in blood alanine concentrations is also consistent with decreased flux through the glycogenic pathway, as pyruvate is a major precursor of alanine. Our data therefore confirm the findings, at much higher plasma NEFA concentrations (= 3 mmol/l), of Ferrannini et al. [14]. Their suggestion that decreased blood alanine levels are secondary to decreased alanine release from muscle rather than increased liver alanine uptake has been recently confirmed [34]. Overall, reduced glucose flux through the glycolytic pathway would be expected to favour redirection of any glucose taken up towards the storage pathways, and decreased glycogen synthesis would only arise when glucose uptake became rate-limiting.

In the present study we did not measure the activity of pyruvate dehydrogenase, the key regulatory enzyme of glucose oxidation. Previous work on human pyruvate dehydrogenase in vivo has produced discrepant findings, with some groups reporting activation by insulin [35], whereas others have found no change [36]. These discrepancies may well reflect the complexity of the enzyme system and assay methodology. We have measured skeletal muscle hexokinase activity, an important regulatory enzyme in the glycogenic pathway, and demonstrated that elevated plasma NEFA levels had no direct effect on its activity. However, preparation of the muscle extract and dilution in the assay will have decreased the concentration of its allosteric inhibitor, G6P. Unfortunately, it is extremely difficult to examine the effect of fatty acids upon the glycolytic and oxidative pathways in human skeletal muscle in vivo, as the use of freeze-clamp techniques to determine metabolic crossover points is not practicable. Furthermore, it is unlikely that needle-biopsy specimens will be snap-frozen in under 2 s [37]. Muscle metabolism will continue during this period, again making interpretation of data extremely difficult.

In summary, the present study has demonstrated that maintenance of plasma NEFA levels at fasting values decreased insulin-stimulated glucose metabolism by approximately 30%. This was due to a 48% decrease in glucose oxidation and a non-significant decrease in glucose storage. Elevated plasma NEFA concentrations had no demonstrable direct effect on the measured activity of GS either basally or during insulin stimulation. Similarly, skeletal muscle glycogen content was also unaffected by the elevated plasma NEFA levels.

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