Conclusion

We have measured a- v differences across adipose and muscle tissues before and after a glucose load. The time course for the uptake of glucose by muscle is consistent with it being insulin sensitive; that for adipose tissue suggests it is not insulin sensitive. The glucose uptake by adipose tissue is quantitatively small, so adipose tissue lactate production can be only a small fraction of the total glucose ingested. Between 15 and 60 min after glucose ingestion, adipose tissue is able to store glucose in a form as yet unidentified.

Table 1. Stimulation by serum of lipase activity with Intralipid and triolein as substrates

<table>
<thead>
<tr>
<th></th>
<th>Lipase activity (units)</th>
<th>Without serum</th>
<th>With serum</th>
<th>With serum/without serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intralipid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 8.1</td>
<td>11 ± 5</td>
<td>100</td>
<td>10 ± 5</td>
<td></td>
</tr>
<tr>
<td>pH 7.5</td>
<td>5 ± 2</td>
<td>59 ± 12</td>
<td>13 ± 7</td>
<td></td>
</tr>
<tr>
<td>Triolein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 8.1</td>
<td>48 ± 20</td>
<td>66 ± 19</td>
<td>1.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>pH 7.5</td>
<td>50 ± 17</td>
<td>69 ± 16</td>
<td>1.4 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

Values shown are the means ± S.E.M. for eight normal subjects. An oral glucose load (75 g) was given at time zero. (a) Differences between arterialized and venous plasma glucose concentration across muscle (○) and adipose tissue (●) against time. (b) Arterialized plasma concentrations of glucose (●) and insulin (○) against time.

Fig. 1. Relationship between glucose a- v differences across forearm and adipose tissue and plasma concentrations of insulin and glucose

Lipases in heart and the effect of insulin

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Lipoprotein lipase (LPL) is synthesized within cardiac myocytes and then transported to the capillary endothelium. Other triacylglycerol lipases described in heart are an acid glycerol; Nilsson-Ehle (1985) and a serum-independent neutral triacylglycerol lipase described (Rogers et al., 1985) as substrates. Lipase activity is expressed in relation to the activity at pH 8.1 in the presence of serum, which is taken as 100.

Without Intralipid as substrate, serum resulted in a large stimulation of activity at both pH 7.5 and 8.1 (Table 1), although the activity was higher at pH 8.1. With triolein as substrate, serum produced only a small increase in activity against Intralipid, which is taken as 100.

With Intralipid as substrate, serum resulted in a large stimulation of activity at both pH 7.5 and 8.1 (Table 1),

Abbreviation used: LPL, lipoprotein lipase.

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by serum was similar for both triolein (from 13 to 58 units) and Intralipid (from 25 to 137 units). With the heart residue as enzyme source, LPL activity in the absence and presence of serum was 31 and 51 with triolein and 4 and 40 with Intralipid. With myocytes as the enzyme source, LPL activity in the absence of serum was 0.73 ± 0.12 units and 1.98 ± 0.45 units with triolein and 0.4 ± 0.15 units and 2.56 ± 0.18 units with Intralipid.

These results suggest that in the absence of serum both triolein and Intralipid are poor substrates for LPL, but that heart contains serum-independent enzymes for which triolein is a better substrate than Intralipid. Therefore, Intralipid was used in the further work on LPL.

In many studies, hormonal effects on LPL activity have only been observed when translocation of LPL out of the cell is brought about by either the hormone or heparin (Chohan & Cryer, 1980). The second part of this work investigated whether heparin altered total cellular LPL activity and the effect of insulin in this system.

Myocytes, prepared by the method of Piper et al. (1982), were incubated in medium M199 (1.4 × 10^5 viable cells/ml) with 1%/w/v defatted albumin and leupeptin (20 μg/ml) for 3 h at 37°C with additions as indicated. Samples were centrifuged for 20 s at 15,000 g in an Eppendorf microcentrifuge, the medium decanted and the cells resuspended in buffer containing heparin (5 i.u./ml). The cells were sonicated and all samples stored at −70°C. LPL activity was assayed at pH 8.1 against Intralipid and in the presence of heparin (10 i.u./ml). In each experiment incubations were carried out in duplicate. One unit of LPL activity released 1 μmol of free fatty acid/10^6 cells.

There was no loss of cellular activity during the 3 h incubations in the absence of additions (4.3 ± 0.75 units at the start of the incubations compared with 4.5 ± 0.94 units after 3 h, n = 4). The addition of heparin (5 i.u./ml) to the medium resulted in a significant decrease in cellular activity to 3.3 ± 0.66 units. In the majority of experiments, increased LPL activity was not detected in the medium because of the small number of cells used. In the presence of insulin (25 munits/ml) and heparin the decline in cellular activity was almost abolished (4.0 ± 0.94 units). The maintenance of cellular activity in the presence of insulin could be due to decreased enzyme degradation, activation of preformed enzyme or enzyme synthesis (Eckel et al., 1984; Vanner et al., 1985). Insulin produced a 75% increase in protein synthesis (as measured by incorporation of ^3H]leucine) in myocytes incubated under similar conditions for 3 h. Whether insulin stimulates LPL synthesis is under investigation.


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Glucagon increases mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A synthase activity in vivo by desuccinylating the enzyme

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Lowe & Tubbs (1985) demonstrated that mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase (EC 4.1.3.5) purified from ox liver is potentilly inhibited by succinyl-CoA. In the presence of physiological concentrations (Barratt et al., 1976; Siess et al., 1980) of succinyl-CoA, the enzyme rapidly inactivates itself by catalysing self-succinylatation at the active site (Lowe & Tubbs, 1985). The succinylated enzyme can be reactivated and desuccinylated in the presence of acetyl-CoA. Lowe & Tubbs (1985) showed that the half-time for reactivation of the succinyl enzyme in the presence of 100 μM-acetyl-CoA was 17 min, essentially the same as the half-time for desuccinylatation (14 min) under the same conditions. On the basis of these experiments in vitro and those of others (Bremer, 1968; Dashti & Oimto, 1979; Siess et al., 1980), Lowe & Tubbs (1985) suggested that glucagon might increase the ketogenic flux through the HMG-CoA cycle by lowering the intramitochondrial succinyl-CoA concentration and therefore the succinylatation state of HMG-CoA synthase. This hypothesis is feasible (see Quant et al., 1987a, b), but remains unsubstantiated.

We have observed (Quant et al., 1987a) increased HMG-CoA synthase activity and decreased intramitochondrial succinyl-CoA levels in extracts prepared from rapidly frozen livers of short-term (10 min) glucagon-treated or mannheptulose-treated rats. These effects persisted in isolated mitochondria validating the use of mitochondria as a model system in which to examine the effects of hormones on HMG-CoA synthase. In subsequent investigations (Quant et al., 1988) we demonstrated that succinyl-CoA inhibits HMG-CoA synthase activity in isolated mitochondria and that the differences in the activity of the enzyme in mitochondria isolated from glucagon-treated or mannheptulose-treated rats and sham-injected control rats can be explained entirely by the differences in the prevailing succinyl-CoA levels. These findings confirmed that succinyl-CoA controls HMG-CoA synthase activity and therefore strongly supported the hypothesis of Lowe & Tubbs (1985). The aim of the experiments described in this paper was to establish whether the changes in activity of hepatic mitochondrial HMG-CoA synthase, that occur in vivo in response to prevailing succinyl-CoA levels, are caused by changes in the degree of succinylation of the enzyme.

Table 1 demonstrates that the enzyme present in extracts prepared from rapidly frozen livers of control sham-injected fed rats is substantially succinylated and can therefore be desuccinylated by incubation in medium known to desuccinylate HMG-CoA synthase. By contrast, the enzyme in extracts prepared from glucagon-treated or mannheptulose-treated fed rats is more active. It is less succinylated, and its activity is not significantly increased by incubation in desuccinylation medium. Incubating the extracts prepared from control rats in desuccinylation medium stimulated the...