Regulation of Phosphoenolpyruvate Carboxykinase (GTP) in Adipose Tissue in vivo by Glucocorticoids and Insulin

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1. The regulation of the synthesis of phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) in epididymal adipose tissue, liver and kidney in vivo was studied immuno-chemically. 2. Phosphoenolpyruvate carboxykinase (GTP) synthesis in adipose tissue is increased by starvation, diabetes and noradrenaline, and decreased by re-feeding and insulin. These changes were also seen in adrenalectomized rats and are qualitatively similar to those observed for the liver enzyme. This indicates the involvement of cyclic AMP as an inducer and insulin as a de-inducer in the regulation of phosphoenolpyruvate carboxykinase (GTP) in both tissues. (Induction and de-induction are defined as selective increase and decrease respectively in the rate of enzyme synthesis, regardless of the mechanism involved.) 3. Adrenalectomy had little effect on phosphoenolpyruvate carboxykinase (GTP) synthesis in liver and kidney, but increased the synthesis rate of the adipose-tissue enzyme. Starvation and adrenalectomy had additive effects in increasing the synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase (GTP). In adrenalectomized diabetic rats glucocorticoids increased phosphoenolpyruvate carboxykinase (GTP) synthesis in liver and kidney while decreasing enzyme synthesis in adipose tissue. De-induction of adipose tissue phosphoenolpyruvate carboxykinase (GTP) is therefore regulated independently by glucocorticoids and insulin. 4. Although liver, kidney and adipose-tissue phosphoenolpyruvate carboxykinases (GTP) are seemingly identical, there is an apparent tissue-specific differentiation in regulatory systems for the enzyme.

Phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) is present in the liver, kidney cortex and epididymal adipose tissue of the rat. Its physiological function in all these three tissues was described previously (Scrutton & Utter, 1968; Iynedjian et al., 1975; Reshef et al., 1972; Hopgood et al., 1973). In each of these tissues the cytosolic forms of the enzyme share similar catalytic and immunological properties (Ballard & Hanson, 1969; Longshaw & Pogson, 1972; Hopgood et al., 1973; Iynedjian et al., 1975), yet the hormonal regulation of their activities in vivo varies. This phenomenon indicates an apparent differentiation not only in the physiological regulation of phosphoenolpyruvate carboxykinase activity, but also in the mechanism of hormone action.

The glucocorticoids are known to affect the activity of phosphoenolpyruvate carboxykinase in all three tissues. In the liver they increase the activity and synthesis rate of the enzyme slightly, compared with cyclic AMP, and their effect is most apparent in diabetic animals or in liver-derived cells (Gunn et al., 1975a,b). Under most conditions in vivo, the effect of glucocorticoids on hepatic phosphoenolpyruvate carboxykinase is masked by enhanced insulin release, which subsequently decreases the rate of enzyme synthesis (Gunn et al., 1975a). In contrast both the activity and synthesis rate of the enzyme in the renal cortex are markedly increased by glucocorticoids (Iynedjian et al., 1975; Gunn et al., 1975a) and do not appear to be directly affected by insulin (Kamm et al., 1974; Iynedjian et al., 1975).

Insulin and glucocorticoids are also important in the regulation of phosphoenolpyruvate carboxykinase in adipose tissue. Adrenalectomy, starvation and diabetes all cause an increase in enzyme activity in this tissue, whereas injection of either insulin or glucocorticoid into adrenalectomized-diabetic animals decreases the activity of phosphoenolpyruvate carboxykinase (Reshef et al., 1969a,b, 1970). Therefore, in contrast with both the liver and kidney enzyme, phosphoenolpyruvate carboxykinase activity in adipose tissue appears to be decreased by both

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insulin and glucocorticoid acting independently. Hopgood et al. (1973) have shown that, when starved animals are re-fed, there is a decrease in the synthesis rate of adipose tissue phosphoenolpyruvate carboxykinase. This is circumstantial evidence indicating that insulin regulates this enzyme, as it does the liver enzyme, through changes in synthesis rate. In the present paper the separate effects of insulin and glucocorticoids on the synthesis rate of phosphoenolpyruvate carboxykinase in adipose tissue, liver and kidney have been investigated more thoroughly in vivo in adrenalectomized and diabetic animals.

Experimental

Male Wistar rats (6–8 weeks old) were used throughout the experiments. Adrenalectomized animals were maintained on 1% (w/v) NaCl and used 4–6 days after the operation. Diabetes was induced by subcutaneous injection of 18.5 mg of alloxan/100 g body wt. Only animals exhibiting marked glucosuria were used in the study. Bilateral adrenalectomy was performed on diabetic rats 3 days after alloxan treatment and the adrenalectomized–diabetic animals were used 3 or 4 days later provided that a high glucosuria was still evident.

Cortisol 21-phosphate (disodium salt) and noradrenaline were from Sigma Chemical Co., St. Louis, MO, U.S.A., and triamcinolone acetonide (9α-fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregn-4a,5α-diene-3,20-dione) from E.R. Squibb and Sons, New York, NY, U.S.A. Glucagon-free insulin was the gift of Eli Lilly and Co., Indianapolis, IN, U.S.A. The L-[4-3H]leucine (2.1–2.8 Ci/mmol) was from the Nuclear Research Centre, Negev, Beer-Sheva, Israel, and NaNH4CO3 (2–10 mCi/mmol) was from The Radiochemical Centre, Amersham, Bucks, U.K.

Liver, kidney and adipose-tissue cytosolic phosphoenolpyruvate carboxykinases are immunochemically identical (Ballard & Hanson, 1969; Hopgood et al., 1973; Lynedjian et al., 1975) and the same procedure was used for the immunoprecipitation of the enzyme from all three tissues. Specific antibodies to phosphoenolpyruvate carboxykinase from the cytosol of rat liver were produced in goats as described by Hopgood et al. (1973). To measure the synthesis rate of the enzyme, rats were injected with 100 μCi of [3H]leucine intraperitoneally and killed 30 min later. The liver, kidneys and epididymal adipose tissues were removed and homogenized in 0.25 M sucrose, and a cytosol fraction was prepared by centrifugation at 10000 g for 45 min. The activity of phosphoenolpyruvate carboxykinase was determined for each cytosol fraction (Ballard & Hanson, 1969). One unit of enzyme activity catalyses the fixation of 1 μmol of NaNH4CO3/min at 37°C. Radioactivity incorporated into phosphoenolpyruvate carboxykinase, isolated as an antigen–antibody precipitate, and into total cytosol protein was determined as described (Hopgood et al., 1973). Synthesis rates for phosphoenolpyruvate carboxykinase are expressed both as the radioactivity incorporated into the enzyme/30 min per mg of tissue and as the percentage of cytosol protein synthesis.

Results

Phosphoenolpyruvate carboxykinase activity in the epididymal adipose tissue of the rat is increased by injection of adrenaline or noradrenaline into intact animals, increased by bilateral adrenalectomy, and decreased by injection of cortisol or triamcinolone (Reshef et al., 1970; Reshef & Hanson, 1972). These changes are due, at least in part, to changes in the synthesis rate of the enzyme (Table 1). Further, these effects of noradrenaline and glucocorticoids on phosphoenolpyruvate carboxykinase are not due to large alterations in the rate of cytosol protein synthesis, for although adrenalectomy increased the incorporation of leucine into adipose-tissue proteins, the effect on the enzyme was greater (Table 1).

This increase in the activity and synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase in adrenalectomized rats is not reflected by complementary changes in either the activity of the hepatic enzyme (Reshef et al., 1969a,b, 1970) or the activity and synthesis rate of the renal enzyme (Lynedjian et al., 1975). Starvation of intact rats, however, increases both the activity and the synthesis rate of the enzyme in all three tissues (Hopgood et al., 1973; Tilghman et al., 1974; Gunn et al., 1975a). Therefore a comparison of the effects of glucocorticoids on the activity and synthesis rate of phosphoenolpyruvate carboxykinase in all three tissues in both adrenalectomized and starved animals might more clearly define the tissue differences in the regulation of the enzyme by glucocorticoids (Table 2).

As found previously (Reshef et al., 1969a,b), the activity of phosphoenolpyruvate carboxykinase in adipose tissue is increased by starvation and adrenalectomy and further increased by a combination of both treatments. These changes are accompanied by comparable increases in the synthesis rate of the enzyme (cf. Tables 1 and 2). Under all three conditions both the activity and synthesis rate of phosphoenolpyruvate carboxykinase may then be decreased by injection of triamcinolone (Table 2). Again there was little change in the synthesis rate of adipose-tissue cytosol proteins (results not shown). Although triamcinolone tended to decrease the rate of protein synthesis in adipose tissue (see Table 1), the specific effect on phosphoenolpyruvate carboxykinase was greater, as is evident from the decrease in the relative rate of enzyme synthesis. There was extreme variability in the total incorporation of [3H]leucine into adipose-tissue protein, a situation possibly caused by
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Table 1. Effect of noradrenaline and glucocorticoids on the synthesis rate of phosphoenolpyruvate carboxykinase in adipose tissue

Fed rats were injected subcutaneously with noradrenaline (0.2 mg/100 g body wt.) and killed 5 h later. Fed adrenalectomized rats were injected intraperitoneally with cortisol (5 mg/100 g body wt.) and killed 10 h later. At 0.5 h before death all animals were injected with 100 μCi of [3H]leucine. Phosphoenolpyruvate carboxykinase was assayed and the synthesis rate determined as described in the Experimental section. Values are means ± S.E.M. of the numbers of observations in parentheses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phosphoenolpyruvate carboxykinase (munits/g fresh wt.)</th>
<th>Cytosol Phosphoenolpyruvate carboxykinase</th>
<th>Incorporation into phosphoenolpyruvate carboxykinase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (3)</td>
<td>62 ± 6</td>
<td>293 ± 4</td>
<td>6.8 ± 0.28</td>
</tr>
<tr>
<td>Noradrenaline (6)</td>
<td>157 ± 35</td>
<td>293 ± 67</td>
<td>6.51 ± 0.89</td>
</tr>
<tr>
<td>Adrenalectomy (3)</td>
<td>327 ± 50</td>
<td>472 ± 50</td>
<td>10.32 ± 1.93</td>
</tr>
<tr>
<td>Adrenalectomy + cortisol (3)</td>
<td>209 ± 10</td>
<td>276 ± 75</td>
<td>2.70 ± 1.22</td>
</tr>
</tbody>
</table>

Table 2. Tissue differences in the response of phosphoenolpyruvate carboxykinase activity and synthesis rate to adrenalectomy and starvation

Fed and 24 h-starved rats were injected subcutaneously with triamcinolone (5 mg/100 g body wt.) at time zero, with 100 μCi of [3H]leucine at 9.5 h and were killed at 10 h. Phosphoenolpyruvate carboxykinase activity (units/g fresh wt.) was assayed and the relative synthesis rate of the enzyme (%) determined as described in the Experimental section. Values are the means ± S.E.M. of three or more observations. N.D., not determined (see Gunn et al., 1975a).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adipose</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity (units/g fresh wt.)</td>
<td>Relative synthesis (rate %)</td>
<td>Activity (units/g fresh wt.)</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>0.31 ± 0.02</td>
<td>1.99 ± 0.42</td>
<td>4.34 ± 0.43</td>
</tr>
<tr>
<td>+ triamcinolone</td>
<td>0.09 ± 0.03</td>
<td>1.28 ± 0.20</td>
<td>3.75 ± 0.27</td>
</tr>
<tr>
<td>Starvation</td>
<td>0.21 ± 0.06</td>
<td>1.90 ± 0.29</td>
<td>10.43 ± 0.23</td>
</tr>
<tr>
<td>+ triamcinolone</td>
<td>0.07 ± 0.01</td>
<td>0.46 ± 0.13</td>
<td>8.67 ± 0.52</td>
</tr>
<tr>
<td>Adrenalectomy + starvation</td>
<td>0.45 ± 0.11</td>
<td>4.89 ± 0.90</td>
<td>11.05 ± 0.51</td>
</tr>
<tr>
<td>+ triamcinolone</td>
<td>0.23 ± 0.05</td>
<td>2.44 ± 0.50</td>
<td>11.35 ± 1.11</td>
</tr>
</tbody>
</table>

direct absorption of radioactive leucine into the tissue. For this reason, the changes in leucine incorporation into total tissue phosphoenolpyruvate carboxykinase do not readily show the trends that become apparent when the results are expressed as percentage of cytosol protein synthesis.

In marked contrast with its effect on adipose-tissue enzyme, triamcinolone increases both the activity and synthesis rate of renal phosphoenolpyruvate carboxykinase in intact (Lynedjian et al., 1975; Gunn et al., 1975a) as well as adrenalectomized rats (Table 2). Hepatic enzyme activity and synthesis rate, on the other hand, although increased by starvation, were little affected by adrenalectomy and decreased after injection of triamcinolone. We have previously reported (Gunn et al., 1975a) that administration of glucocorticoids results in insulin release and that this is the probable cause of the decrease in the rate of hepatic enzyme synthesis seen in both fed and starved animals. Insulin given directly to diabetic rats, or the release of insulin mediated either by re-feeding starved animals or by triamcinolone injection, produces a rapid fall in the rate of hepatic phosphoenolpyruvate carboxykinase synthesis (Hopgood et al., 1973; Tilghman et al., 1974; Gunn et al., 1975a). However, this effect did not appear to be as marked for the liver enzyme in starved adrenalectomized rats (Table 2). Since the activity and synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase is also decreased by insulin, re-feeding and triamcinolone (Reshef et al., 1970; Hopgood et al., 1973; Tables 1 and 2), we used starved, adrenalectomized rats to
Table 3. Effect of re-feeding on phosphoenolpyruvate carboxykinase synthesis in the adipose tissue and liver of adrenalectomized rats

Adrenalectomized rats were re-fed after 21 h starvation. At 2.5 h later the animals were injected with 100 μCi of [3H]leucine and were killed at 3 h. Re-fed animals (+) are compared with rats starved for 24 h (--). Phosphoenolpyruvate carboxykinase was assayed and the synthesis rate determined as described in the Experimental section. Values are the means ± S.E.M. of the number of observations in parentheses.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Re-feeding</th>
<th>Phosphoenolpyruvate carboxykinase (units/g fresh wt.)</th>
<th>10^3 × Radioactivity incorporated (d.p.m./g of tissue)</th>
<th>Incorporation into phosphoenolpyruvate carboxykinase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cytosol protein</td>
<td>Phosphoenolpyruvate carboxykinase</td>
</tr>
<tr>
<td>Adipose (2)</td>
<td>–</td>
<td>0.32</td>
<td>778</td>
<td>24.7</td>
</tr>
<tr>
<td>Adipose (2)</td>
<td>+</td>
<td>0.39</td>
<td>1212</td>
<td>22.0</td>
</tr>
<tr>
<td>Liver (5)</td>
<td>–</td>
<td>12.3 ± 0.32</td>
<td>1132 ± 95</td>
<td>37.8 ± 19.3</td>
</tr>
<tr>
<td>Liver (5)</td>
<td>+</td>
<td>10.32 ± 0.65</td>
<td>896 ± 29</td>
<td>11.7 ± 4.7</td>
</tr>
</tbody>
</table>

Table 4. Effect of insulin on phosphoenolpyruvate carboxykinase synthesis in the adipose tissue and liver of diabetic rats

Diabetic rats were injected subcutaneously with 5 units of insulin/100 g body wt. (+) at time zero, with 100 μCi of [3H]leucine at 1.5 h and were killed at 2 h. Phosphoenolpyruvate carboxykinase was assayed and the synthesis rate determined as described in the Experimental section. Values are the means ± S.E.M. of the number of observations in parentheses.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Insulin</th>
<th>Phosphoenolpyruvate carboxykinase (units/g fresh wt.)</th>
<th>10^3 × Radioactivity incorporated (d.p.m./g of tissue)</th>
<th>Incorporation into phosphoenolpyruvate carboxykinase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cytosol protein</td>
<td>Phosphoenolpyruvate carboxykinase</td>
</tr>
<tr>
<td>Adipose (3)</td>
<td>–</td>
<td>0.29 ± 0.08</td>
<td>76 ± 19</td>
<td>1.14 ± 0.27</td>
</tr>
<tr>
<td>Adipose (3)</td>
<td>+</td>
<td>0.24 ± 0.05</td>
<td>243 ± 40</td>
<td>3.14 ± 0.21</td>
</tr>
<tr>
<td>Liver (6)</td>
<td>–</td>
<td>13.40 ± 0.60</td>
<td>585 ± 77</td>
<td>23.2 ± 2.1</td>
</tr>
<tr>
<td>Liver (6)</td>
<td>+</td>
<td>10.54 ± 0.59</td>
<td>281 ± 120</td>
<td>1.41 ± 0.41</td>
</tr>
</tbody>
</table>

Investigate the possibility of a glucocorticoid–insulin interaction being involved in the response of the hepatic and adipose tissue enzymes to re-feeding (Table 3).

Re-feeding starved adrenalectomized rats for 3 h (Table 3) resulted in a decreased rate of hepatic phosphoenolpyruvate carboxykinase synthesis, although this was not as marked as that seen previously (Hopgood et al., 1973; Tilghman et al., 1974) in intact animals. Similarly, the relative synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase was also decreased in re-fed adrenalectomized rats (Table 3), but as in intact animals the effect was slow in comparison with the liver (Hopgood et al., 1973). The difference in the response of the liver enzyme to re-feeding and glucocorticoids in starved intact or adrenalectomized rats might reflect the amount of insulin secreted during the different treatments. Compared with the liver enzyme, the slower response of adipose-tissue phosphoenolpyruvate carboxykinase to re-feeding might reflect differences in insulin concentration between the portal vein and peripheral vessels. Insulin injection into diabetic rats (Table 4) causes a rapid decrease in the synthesis rate of hepatic phosphoenolpyruvate carboxykinase, but has little effect, over the first 2 h of treatment, on the relative synthesis rate of the adipose-tissue enzyme.

The data in Tables 3 and 4 therefore indicate that phosphoenolpyruvate carboxykinase in both adipose tissue and liver is de-induced by insulin, that de-induction is slow in adipose tissue compared with liver, and that this effect of insulin in vivo is separate and independent of any direct effect of the glucocorticoids. On the other hand, the possibility exists that the action of glucocorticoids to decrease the synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase is mediated via insulin release (Gunn et al., 1975a). However, the difference in the response of the enzyme in adipose tissue, liver and kidney to adrenalectomy and triamcinolone injection (Table 2) indicates that, in contrast with the enzyme in the other two tissues, glucocorticoids act in the absence of insulin to decrease the synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase.

These alternatives are resolved in Table 5. Triamcinolone injection into adrenalectomized diabetic rats increased the activity and synthesis rate of phosphoenolpyruvate carboxykinase in both the liver and
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Table 5. Tissue differences in the response to triamcinolone of phosphoenolpyruvate carboxykinase synthesis in adrenalectomized diabetic rats

Adrenalectomized diabetic rats were injected subcutaneously with triamcinolone (5mg/100g body wt.) and were killed 10 or 24h later. At 0.5h before death all animals were injected with 100μCi [3H]leucine. Phosphoenolpyruvate carboxykinase was assayed and the synthesis rate determined as described in the Experimental section. Values are the means ± S.E.M. of the number of observations in parentheses.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time after triamcinolone (h)</th>
<th>Phosphoenolpyruvate carboxykinase (units/g fresh wt.)</th>
<th>10^-3 × Radioactivity incorporated (d.p.m./g of tissue)</th>
<th>Incorporation into phosphoenolpyruvate carboxykinase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cytosol protein</td>
<td>Phosphoenolpyruvate carboxykinase</td>
<td></td>
</tr>
<tr>
<td>Adipose</td>
<td>0 (8)</td>
<td>0.40 ± 0.03</td>
<td>422 ± 48</td>
<td>10.68 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>10 (3)</td>
<td>0.15 ± 0.03</td>
<td>303 ± 26</td>
<td>4.27 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>24 (9)</td>
<td>0.05 ± 0.01</td>
<td>299 ± 48</td>
<td>2.99 ± 0.54</td>
</tr>
<tr>
<td>Liver</td>
<td>0 (16)</td>
<td>6.90 ± 0.61</td>
<td>1026 ± 58</td>
<td>19.3 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>10 (8)</td>
<td>6.63 ± 0.65</td>
<td>975 ± 74</td>
<td>21.3 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>24 (13)</td>
<td>9.44 ± 0.55</td>
<td>1216 ± 152</td>
<td>29.8 ± 3.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>0 (16)</td>
<td>7.04 ± 0.69</td>
<td>599 ± 59</td>
<td>16.1 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>10 (8)</td>
<td>11.34 ± 1.03</td>
<td>499 ± 38</td>
<td>33.3 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>24 (13)</td>
<td>17.29 ± 1.66</td>
<td>634 ± 79</td>
<td>33.4 ± 2.5</td>
</tr>
</tbody>
</table>

Discussion

The cytosolic forms of phosphoenolpyruvate carboxykinase in adipose tissue, liver and kidney share similar physical, catalytic and immunochemical properties (Ballard & Hanson, 1969; Longshaw & Pogson, 1972; Hopgood et al., 1973; Iynedjian et al., 1975; Meyuhas et al., 1976). This indicates that the primary structure of the enzyme might be identical in all three tissues, and further, that the organ-specific response of the enzyme might reside in the systems that control the tissue content of the enzyme. Consequently a comparative study of the regulation of the enzyme in the three tissues may shed some light on the differentiation of regulatory systems in the different organs. Here we show that the activity and synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase is increased by starvation, diabetes, adrenalectomy and noradrenaline, and decreased by re-feeding starved animals, insulin and glucocorticoids. These data, in addition to those previously reported from this laboratory for the enzyme in liver and kidney as well as adipose tissue, enable certain statements to be made about the tissue-specific regulation of the enzyme.

The regulation of phosphoenolpyruvate carboxykinase activity in adipose tissue, liver and kidney shares certain features common to all three tissues. Thus enzyme activity and synthesis rate are increased in diabetic and starved animals (Hopgood et al., 1973; Tilghman et al., 1974, 1975; Gunn et al., 1975a,b; Tables 1, 2 and 4). Characteristically, however, the regulation of phosphoenolpyruvate carboxykinase under these conditions, at least in the liver and kidney, is mediated by different primary effectors. Hepatic enzyme activity and synthesis rate are increased by glucagon, acting through cyclic AMP, and decreased by insulin (Tilghman et al., 1974, 1975; Gunn et al., 1975a,b), whereas the activity and synthesis rate of renal phosphoenolpyruvate carboxykinase are not directly affected by these effectors, but by the acid–base status of the animal (Gunn et al., 1975a; Iynedjian et al., 1975).

Evidence for a direct involvement of insulin in the regulation of adipose-tissue and liver cytosol phosphoenolpyruvate carboxykinase comes from experiments in isolated cell systems (Meyuhas et al., 1976;
Tilghman et al., 1975). In both cases insulin de-
induced the synthesis of the enzyme. Also in _vivo_, the
activity and synthesis of liver and adipose-tissue
enzymes are decreased by injecting insulin to diabetic
rats or by re-feeding starved animals (Reshef et al.,
1970; Hopgood et al., 1973; Tilghman et al., 1974;
Tables 3 and 4). However, in the liver the rate of de-
induction of phosphoenolpyruvate carboxykinase
synthesis exceeds that of the adipose tissue enzyme by
about an order of magnitude (Hopgood et al., 1973;
Tables 3 and 4). Whether this observation has any
tissue-specific regulatory significance remains to be
resolved.

The other striking feature is the tissue-specific
response of cytosol phosphoenolpyruvate carboxy-
kinase in all three tissues to glucocorticoids. Thus the
activity and synthesis of both kidney and liver
enzymes are increased by triamcinolone (Table 5;
Gunn et al., 1975a; Iynedjian et al., 1975), but the
effect on the liver enzyme is small and apparent only
in diabetic rats (cf. Tables 2 and 5; Gunn et al.,
1975a). Moreover removal of corticosteroids, by
adrenalectomy of intact rats, has little effect on the
response of the two enzymes to starvation and is only
apparent in diabetic rats.

In contrast, the activity and synthesis of adipose-
tissue phosphoenolpyruvate carboxykinase are mar-
kedly decreased by glucocorticoids and increased by
adrenalectomy. They are independent of insulin re-
lease as is indicated by the glucocorticoid effect on
diabetic rats (Table 5) and by a direct effect of these
hormones on adipose-tissue organ culture (Meyuhas et
al., 1976). This tissue-specific response of adipose tissue
to glucocorticoids may be associated with a decreased
protein synthesis caused by the hormone in this tissue.
Measurements of leucine concentration in the amino
acid pool of adipose tissue (Table 6) enable one to
determine its true specific radioactivity and hence the
amount incorporated into cytosol proteins. We note
that the tissue concentration of free leucine is not
significantly changed by triamcinolone treatment of
adrenalectomized diabetic rats. Since the radioactivity
in the trichloroacetic acid-soluble fraction is also not
affected (results not shown), and hence the specific
radioactivity of tissue leucine, it implies that cortico-
steroids decrease the synthesis of total cytosol
proteins in adipose tissue. This is not the only meta-
abolic activity so affected by corticosteroids in adipose
tissue. Decreased glucose transport (Munck, 1962)
and a decreased incorporation of $^3$H] Juridine (O.
Meyuhas & L. Reshef, unpublished work) have been
demonstrated in adipose tissue _in vitro_. The decreased
synthesis of adipose-tissue phosphoenolpyruvate
carboxykinase caused by glucocorticoids is therefore
probably a resultant expression of one or all of these
effects. Why the response of this enzyme to gluco-
corticoids is more sensitive than that of other cytosol
proteins (as is evident from the change in the relative
rate of its synthesis) remains to be resolved.

The data reported here therefore indicate that
adipose-tissue phosphoenolpyruvate carboxykinase,
in contrast with the enzyme in liver and kidney, is
de-induced by both glucocorticoids and insulin acting
independently of one another. Enhanced insulin
release after triamcinolone injection into non-diabetic
animals will therefore lead to the more rapid de-
induction of adipose-tissue phosphoenolpyruvate
carboxykinase, evident in comparing Tables 2 and 5,
whereas a combination of adrenalectomy and star-
vation leads to additive effects in terms of increased
enzyme activity and synthesis rate (Table 2).

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**Table 6. Effect of triamcinolone on leucine concentration in
amino acid pools of adipose tissue from adrenalectomized
diabetic rats**

<table>
<thead>
<tr>
<th>Triamcinolone</th>
<th>Free leucine (μmol/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−(3)</td>
<td>0.024</td>
</tr>
<tr>
<td>−(4)</td>
<td>0.028</td>
</tr>
<tr>
<td>+(4)</td>
<td>0.044</td>
</tr>
<tr>
<td>+(4)</td>
<td>0.024</td>
</tr>
</tbody>
</table>

References


1976
ADIPOSE-TISSUE PHOSPHOENOLPYRUVATE CARBOXYKINASE


Reshef, L., Meyuhas, O., Boshwitz, Ch., Hanson, R. W. & Ballard, F. J. (1972) Isr. J. Med. Sci. 8, 372–381