Aspects of adipose-tissue metabolism in foetal lambs

Richard G. VERNON, James P. ROBERTSON, Roger A. CLEGG and David J. FLINT
Hannah Research Institute, Ayr KA6 5HL, Scotland, U.K.

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1. The mean volume of adipocytes, the rates of fatty acid and acylglycerol glycerol synthesis from various precursors (in vitro), the rates of oxidation of acetate and glucose (in vitro) and the activities of lipoprotein lipase and various lipogenic enzymes were determined for perirenal adipose tissue from foetal lambs during the last month of gestation. 2. The fall in the rate of growth of perirenal adipose tissue during the last month of gestation is associated with a diminished capacity for fatty acid synthesis and lipoprotein lipase activity, but no change in the rate of acylglycerol glycerol synthesis was observed. There was no fall in the activities of cytosolic acetyl-CoA synthetase or the NADP-linked dehydrogenases, suggesting that the decrease in the rate of fatty acid synthesis was due to an impairment at the level of acetyl-CoA carboxylase or fatty acid synthetase. 3. The rate of fatty acid synthesis from acetate was greater than that from glucose. The rate of fatty acid synthesis from glucose per adipocyte of foetal lambs was similar to that of young sheep. The characteristic metabolism of adipose tissue of the adult sheep is thus present in the foetus, despite the relatively large amounts of glucose in the foetal ‘diet’.

Perirenal adipose tissue in the newborn lamb has the characteristics of brown adipose tissue; it has an important role in non-shivering thermogenesis and hence survival (see Noble, 1979). The quantitative development of perirenal adipose tissue in the foetal lamb has been described by Alexander (1978), who showed that between about 70 and 120 days of gestation the tissue grew rapidly, but then developed more slowly until birth (at about 148 days of gestation). Metabolic changes during the development of perirenal adipose tissue in the foetal lamb have not been described.

In the present paper we report the rates of various metabolic pathways and the activities of several enzymes of perirenal adipose tissue from foetal lambs during the last 30 days of pregnancy, and show that the diminished rate of growth of the fat-pad during this period is associated with a fall in the rate of fatty acid synthesis and the activity of lipoprotein lipase. In addition we show that the characteristic preference for acetate rather than glucose as a precursor for fatty acid synthesis of the adult sheep is also present in the foetus.

Methods

Animals

Sheep were either Cheviot or Finn x Dorset Horn cross-breeds. They were 5–7 years old and were fed on hay ad libitum plus a cereal mix (425 g·day⁻¹ until day 105 of pregnancy, then increasing gradually to 1400 g·day⁻¹ at 130 days of pregnancy and thereafter). Gestation was 145 to 148 days. Ewes were killed at 10:00 h with a captive-bolt humane killer. Foetal lambs were removed from the mother and killed in the same way. Samples of perirenal adipose tissue were removed and put into 0.9% NaCl at about 35°C.

Measurement of metabolic activities

Pieces of adipose tissue weighing about 5 mg were cut with scissors. Samples of these pieces (total wt. approx. 50 mg) were transferred to Erlenmeyer flasks containing 3 ml of Medium 199 (with Earle’s salts, l-glutamine and 25 mm-Hepes [4-(2-hydroxy-methyl)-1-piperazine-ethanesulphonic acid], pH 7.3; Gibco–Biocult Ltd., Paisley, Scotland, U.K.), penicillin (10 μg·ml⁻¹), streptomycin sulphate (100 μg·ml⁻¹) and insulin (10 μg·ml⁻¹). Then 0.25 μCi of [U-¹⁴C]glucose (3.8 Ci·mol⁻¹; final concn. 5.5 mM) or of 1-¹⁴C]acetate (56–59 Ci·mol⁻¹; final concn. 2.2 mM) or of 1-¹⁴C-lactate (50 Ci·mol⁻¹; final concn. 3.3 mM) were added to the flasks, which were then incubated for 2 h at 37°C. The incorporation of ¹⁴C into fatty acids, acylglycerol glycerol or CO₂ was determined as described previously (Vernon, 1976). Concentrations of glucose and acetate used were saturating
for fatty acid and acylglycerol glycerol synthesis, but the concentration of L-lactate was sub-optimal (see the Discussion section). The rates of substrate conversion into products were uniform over the 2 h incubation period, except for the rate of incorporation of glucose carbon into fatty acids, which showed a tendency to accelerate. The rate of fatty acid synthesis from glucose increases by several fold during a 24 h incubation (Vernon, 1979). Previous estimates of the rate of fatty acid synthesis from glucose in adult sheep are based on the amount of $^{14}$C incorporated over either a 2 h or a 3 h incubation period, hence a 2 h incubation period was used in the present study for comparative purposes. All $^{14}$C-labelled compounds were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Bovine insulin (23.6 units·mg$^{-1}$) was kindly given by The Boots Drug Co., Nottingham, U.K.

Preparation of adipocytes and measurement of $^{125}$I-labelled-insulin-binding activity

Adipocytes were prepared from foetal adipose tissue by collagenase digestion as previously (Flint et al., 1979), except that the tissue was not shaken during collagenase digestion; instead the incubation period was extended to 2 h, after which cells were released by gentle swirling of the flasks. The modification was found essential when preparing adipocytes from adult sheep. Adipocytes were washed twice with warm (about 37°C) Medium 199 before determination of their mean volume (Vernon, 1977). Adipocytes to be used for determining their capacity to bind $^{125}$I-labelled insulin were washed a further three times with Krebs-Ringer phosphate buffer (McKenzie & Dawson, 1969) with half of the quoted calcium concentration.

The binding of $^{125}$I-labelled insulin (specific radioactivity 120–150 Ci·g$^{-1}$, from The Radiochemical Centre) by adipocytes was measured as described previously (Flint et al., 1979), except that only one concentration of insulin (1 ng·ml$^{-1}$) was used. Results were corrected for non-specific binding (Flint et al., 1979); on average this amounted to 20 and 16% of the total $^{125}$I-labelled insulin bound for adipocytes from foetal and adult sheep respectively.

Enzyme assays

Samples of adipose tissue were homogenized by hand (approx. 10 strokes) in an all-glass homogenizer (clearance approx. 0.1 mm; Jencons Scientific Ltd., Hemel Hempstead, Herts., U.K.) at room temperature (approx. 20°C). Tissue was homogenized in 3 vol. of 300 mM-sucrose/30 mM-Tris/HCl/1 mM-EDTA/1 mM-reduced glutathione, pH 7.4. The homogenate was centrifuged at 70000 g for 60 min at 4°C. The resulting supernatant fraction was used for the assay of glucose 6-phosphate dehydrogenase, NADP-malate dehydrogenase, NADP-isocitrate dehydrogenase and acetyl-CoA synthetase as described previously (Vernon, 1976) and also for ATP citrate lyase by the method of Sere (1962), except that the pH was 7.4 and 1 mM-dithiothreitol was used instead of $\beta$-mercaptoethanol.

Further samples of adipose tissue were used for the assay of lipoprotein lipase as described previously (Flint et al., 1979).

The protein concentration of adipose tissue homogenates and supernatants was determined by the method of Wang & Smith (1975).

Statistical analysis

Results are expressed as means ± s.e.m. Statistical analysis was performed by Student's $t$ test.

Results

There was no significant change in the amount of lipid or 70000 g-supernatant protein per g wet wt. of perirenal adipose tissue during the last month of gestation (Table 1). There was a small (approx. 40%) increase in adipocyte mean volume, with a corresponding fall in the number of adipocytes per g of tissue (Table 1).

The rate of fatty acid synthesis per g of tissue from all precursors tested fell more than 50-fold over the period of study, primarily owing to a fall in the rate per cell (Table 2). The rate of fatty acid

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Table 1. Adipocyte mean volume, lipid concentration and number of adipocytes·g of tissue$^{-1}$ of perirenal adipose tissue from foetal lambs

Results are means ± s.e.m. for the numbers of observations in parentheses. * Significantly different from 30 days pre partum ($P < 0.05$).

<table>
<thead>
<tr>
<th>Foetal age (days before birth)</th>
<th>25–35</th>
<th>1–4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipocyte mean volume (pl)</td>
<td>6.9 ± 0.9 (8)</td>
<td>9.6 ± 0.7 (6)*</td>
</tr>
<tr>
<td>Lipid concentration (mg·g of tissue$^{-1}$)</td>
<td>331 ± 22 (9)</td>
<td>334 ± 16 (6)</td>
</tr>
<tr>
<td>$10^6$× No. of adipocytes·g of tissue$^{-1}$</td>
<td>67.6 ± 12.4 (6)</td>
<td>38.7 ± 1.9 (6)*</td>
</tr>
<tr>
<td>70000 g-supernatant protein (mg·g of tissue$^{-1}$)</td>
<td>37.0 ± 1.9 (3)</td>
<td>37.8 ± 2.3 (6)</td>
</tr>
</tbody>
</table>
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The rate of oxidation of [1-14C]acetate to CO₂ per g of tissue or per fat-cell increased over the experimental period (Table 2). In contrast, the fall in the rate of oxidation of [U-14C]glucose to CO₂ per g of tissue was at least partly due to a fall in the number of fat-cells per g of tissue (Table 2).

Inclusion of insulin in the incubation medium increased the rate of fatty acid synthesis from [1-14C]acetate in adipose-tissue pieces from foetal lambs (18 to 7 days before term) by about 12% on average, but this increase was not statistically significant. Adipocytes from foetal lambs of a similar age bound 125I-labelled insulin, although the amount bound per cell was much less than that by adipocytes from maternal perirenal adipose tissue (Table 3). The amount of 125I-labelled insulin bound per unit area, however, was the same for both foetal and maternal adipocytes at the concentration of insulin used (1 ng·ml⁻¹).

Activities of the various enzymes assayed are summarized in Table 4. Of the three NADP-linked dehydrogenases examined, there was an increase in the activity of NADP–isocitrate dehydrogenase (per mg of protein or per cell) over the experimental period, but no significant change in the others. The activity of acetyl-CoA synthetase did not change significantly during the last month of pregnancy, whereas there was a significant fall in the activities of both ATP citrate lyase and lipoprotein lipase.

Discussion

Alexander (1978) showed that the mass of perirenal adipose tissue in foetal Merino sheep increased by about 34% over the last 3 weeks of pregnancy. A similar percentage increase in adipocyte volume was found in the present study over the last 4 weeks of pregnancy. The mean adipocyte volume of lambs at about 120 days of gestation was similar to that found by Broad et al. (1980) with Romney sheep, whereas the volume of the fat-cells just before term was the same as previously reported for newborn lambs (Vernon, 1977).

The capacity for acylglycerol glycerol synthesis per cell was maintained over the last 30 days of pregnancy and, as in the adult, glucose rather than lactate was the preferred precursor (see Vernon, 1980a). In contrast, the capacity to produce fatty acids for esterification, either by fatty acid synthesis or via

Table 2. Metabolic activities of adipose tissue from foetal lambs

<table>
<thead>
<tr>
<th>Age (days before birth)</th>
<th>Activity (μmol converted: 2h⁻¹·10⁶ cells⁻¹)</th>
<th>Activity (μmol converted: 2h⁻¹·g of tissue⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25–35</td>
<td>2.5–35</td>
<td>0.9 ± 0.4 (3)***</td>
</tr>
<tr>
<td>7–18</td>
<td>2.8 ± 1.9 (3)***</td>
<td>0.5 ± 0.2 (6)***</td>
</tr>
<tr>
<td>12</td>
<td>4.0 ± 0.6 (8)***</td>
<td>0.5 ± 0.1 (6)***</td>
</tr>
<tr>
<td>30</td>
<td>4.4 ± 0.1 (6)***</td>
<td>0.14 ± 0.02 (3)</td>
</tr>
<tr>
<td>40</td>
<td>4.9 ± 1.6 (4)***</td>
<td>0.4 ± 1.1 (5)***</td>
</tr>
</tbody>
</table>

Synthesis from acetate was greater than that from glucose or L-lactate (at the concentration used).

There was a 50% fall in the rate of acylglycerol glycerol synthesis per g of tissue from either glucose or lactate over the last 30 days of pregnancy, but this was due to a fall in the number of adipocytes per g of tissue (Table 2). The rate of acylglycerol glycerol synthesis from glucose was greater than from L-lactate.
lipoprotein lipase activity, declined, suggesting that it is this, rather than the ability to synthesize triacylglycerol, which becomes limiting during the last month of gestation. A similar fall in the lipoprotein lipase activity and the rate of fatty acid synthesis was found in adipose tissue from foetal guinea pigs during late pregnancy, but in this species the rate of acylglycerol glycerol synthesis fell also (Jones, 1976).

The diminished ability to synthesize fatty acids is probably due to an impairment in the conversion of acetyl-CoA into fatty acids, for the capacity for acetyl-CoA synthesis from acetate by cytosolic acetyl-CoA synthetase, and the capacity to produce NADPH by oxidation of glucose 6-phosphate, malate or isocitrate, were all maintained or increased. A very low rate of fatty acid synthesis was found in all perirenal adipose tissue from newborn lambs (Vernon, 1975). The present study shows that this is at least partly due to a gradual decline in the rate of fatty acid synthesis over the last month of gestation, rather than to a very rapid decline in the rate just around parturition.

The mechanisms responsible for the fall in lipoprotein lipase activity and the rate of fatty acid synthesis are not known. Alexander (1978) suggested that the diminished rate of growth of the fat-pad was due to sub-optimal nutrition of the lamb, probably owing to a limitation at the level of placental transport. Endocrine factors may be involved. Serum growth-hormone (somatotropin) concentrations are high in foetal lambs during late pregnancy (Bassett et al., 1970; Gluckman et al., 1979), and hypophysectomy of foetal lambs promotes the growth of fat-depots (Liggins & Kennedy, 1968; Gemmell & Alexander, 1978; Alexander, 1978).

Acetate was a better precursor than glucose for fatty acid synthesis in adipose tissue from foetal lambs, as in that from adult ruminants (see Vernon, 1980a). The relative rates of fatty acid synthesis from acetate and glucose (allowing for glucose contributing two acetyl-CoA molecules, compared with one from acetate) are about 7:1 in adipose tissue from foetal lambs (35–7 days before term) compared with 10:1–100:1 found in adipose tissue from adult ruminants (see Vernon, 1980a). This difference appears to arise from a lower rate of fatty acid synthesis from acetate per fat-cell and in some cases a higher rate of glucose from foetal lambs. Comparisons are complicated, however, as rates of fatty acid synthesis in adipocytes from adult sheep change with age (see Vernon, 1980a; also Hood & Thornton, 1980). In addition, in most studies results were not expressed on a per-cell basis. Vézinhet & Nouguès (1977) reported rates of fatty acid synthesis from acetate and glucose equivalent to 340 and 8 μmol·2 h⁻¹·10⁶ cells⁻¹ respectively for perirenal adipocytes from 100-day-old Merino sheep (these would be true ruminants, but still growing); these rates fell to 50 and 3 μmol·2 h⁻¹·10⁶ cells⁻¹ for acetate and glucose respectively from cells from foetal lambs, as in that from adult ruminants (see Vernon, 1980a). The relative rates of fatty acid synthesis from acetate and glucose (allowing for glucose contributing two acetyl-CoA molecules, compared with one from acetate) are about 7:1 in adipose tissue from foetal lambs (35–7 days before term) compared with 10:1–100:1 found in adipose tissue from adult ruminants (see Vernon, 1980a). This difference appears to arise from a lower rate of fatty acid synthesis from acetate per fat-cell and in some cases a higher rate of glucose from foetal lambs. Comparisons are complicated, however, as rates of fatty acid synthesis in adipocytes from adult sheep change with age (see Vernon, 1980a; also Hood & Thornton, 1980). In addition, in most studies results were not expressed on a per-cell basis. Vézinhet & Nouguès (1977) reported rates of fatty acid synthesis from acetate and glucose equivalent to 340 and 8 μmol·2 h⁻¹·10⁶ cells⁻¹ respectively for perirenal adipocytes from 100-day-old Merino sheep (these would be true ruminants, but still growing); these rates fell to 50 and 3 μmol·2 h⁻¹·10⁶ cells⁻¹ for acetate and glucose respectively from cells from

Table 3. Binding of ¹²⁵I-labelled insulin to perirenal adipocytes from foetal and maternal sheep

<table>
<thead>
<tr>
<th>¹²⁵I-labelled insulin bound</th>
<th>Foetal</th>
<th>Maternal</th>
</tr>
</thead>
<tbody>
<tr>
<td>molecules/μm²</td>
<td>5.4 ± 0.8 (4)</td>
<td>395 ± 58 (5)</td>
</tr>
<tr>
<td>molecules/cell</td>
<td>0.16 ± 0.02 (4)</td>
<td>0.12 ± 0.02 (5)</td>
</tr>
<tr>
<td>Adipocyte mean volume (μl)</td>
<td>255 ± 54 (4)</td>
<td>3143 ± 614 (5)</td>
</tr>
</tbody>
</table>

Table 4. Enzyme activities of perirenal adipose tissue from foetal lambs

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity (nmol·min⁻¹·mg of protein⁻¹)</th>
<th>Activity (nmol·min⁻¹·10⁶ cells⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25–30</td>
<td>1–4</td>
</tr>
<tr>
<td>Glucose 6-phosphate dehydrogenase</td>
<td>46.0 ± 2.6 (3)</td>
<td>31.5 ± 5.0 (4)</td>
</tr>
<tr>
<td>NADP–malate dehydrogenase</td>
<td>18.4 ± 2.4 (3)</td>
<td>26.0 ± 2.5 (4)</td>
</tr>
<tr>
<td>NADP–isocitrate dehydrogenase (cytosol)</td>
<td>363.0 ± 68.4 (3)</td>
<td>734.2 ± 50.9 (4)*</td>
</tr>
<tr>
<td>Acetyl-CoA synthetase (cytosol)</td>
<td>15.0 ± 1.0 (3)</td>
<td>17.5 ± 1.3 (4)</td>
</tr>
<tr>
<td>ATP citrate lyase</td>
<td>8.9 ± 1.5 (3)</td>
<td>4.0 ± 0.3 (4)*</td>
</tr>
<tr>
<td>Lipoprotein lipase</td>
<td>9.6 ± 2.5 (3)</td>
<td>2.6 ± 0.2 (9)**</td>
</tr>
</tbody>
</table>

Adipocytes were prepared from foetal and maternal perirenal adipose tissue from sheep 7–18 days pre partum and the binding of ¹²⁵I-labelled insulin (1 ng·ml⁻¹) was measured as described in the text. Non-specific binding was subtracted as described in the text. Results are means ± S.E.M. for the numbers of observations in parentheses.
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250-day-old sheep. Preliminary estimations for 6–8-month-old Cheviot and Finn × Dorset Horn cross-breeds gave rates of fatty acid synthesis from acetate of 72–104 μmol·2·h⁻¹·10⁶ cells⁻¹ and from glucose of 1.6 μmol·2·h⁻¹·10⁶ cells⁻¹. Studies with older animals are confined to subcutaneous adipose tissue and show that the rate of fatty acid synthesis from acetate increased from 46 μmol·2·h⁻¹·10⁶ cells⁻¹ in 170-day-old Merino sheep to 320 μmol·2·h⁻¹·10⁶ cells⁻¹ by 17 months of age (Hood & Thornton, 1980), whereas for our own breeds a rate of fatty acid synthesis of 52 μmol·2·h⁻¹·10⁶ cells⁻¹ was found for 3–4-year-old ewes (Vernon et al., 1980).

The rate of fatty acid synthesis from acetate in adipocytes from foetal lambs during the last 30 days of gestation is thus in general lower than rates found in cells from adult animals. In addition, acetyl-CoA synthetase activity (per mg of protein) is also higher in perirenal adipose tissue from 6–8-month-old sheep than in foetal lambs (Vernon, 1976).

In contrast with fatty acid synthesis from acetate, the rate of acetate oxidation increased during late pregnancy, and by birth it was similar to the rate previously reported for newborn lambs (Vernon, 1975). Preliminary estimates indicate a rate of acetate oxidation of about 40 μmol·2·h⁻¹·10⁶ cells⁻¹ for perirenal adipose tissue from 6–8-month-old sheep, which is similar to the rate observed just before term.

The various observations described above indicate that the rate of fatty acid synthesis from glucose is higher in adipocytes from young growing sheep than in those from foetal lambs, and even at 6–8 months of age the rate per cell is at least 50% of that of cells from foetal lambs. Furthermore, the activity of NADP-malate dehydrogenase (per mg of protein) of the foetal lamb was only about twice that of 6–8-month-old sheep (Vernon, 1976) and preliminary results show an ATP citrate lyase activity of 3–6 nmol·min⁻¹·mg of protein⁻¹ for 6–8-month-old sheep. Thus the, albeit low, capacity for fatty acid synthesis from glucose in sheep adipose tissue appears to be maintained until at least 250 days of age. This is in marked contrast with fatty acid synthesis from glucose in ox liver, which was about 5-fold lower in the mother than in the foetus, along with a corresponding fall in the activities of NADP-malate dehydrogenase and ATP citrate lyase (Hanson & Ballard, 1968). However, the rate of fatty acid synthesis from acetate was also markedly lower in maternal than in foetal ox liver, indicating a general fall in the capacity for fatty acid synthesis and reflecting the commitment of the ruminant liver to gluconeogenesis (Ballard et al., 1969).

The low rate of fatty acid synthesis from glucose in ruminant tissues has been attributed to a low ATP citrate lyase activity (Ballard et al., 1969), but we have suggested that pyruvate dehydrogenase is at least as important in restricting the flux of glucose carbon to fatty acids in adult sheep (Robertson et al., 1980). The relative importance of ATP citrate lyase and pyruvate dehydrogenase in restricting the flux of glucose carbon to fatty acids in adipose tissue from foetal lambs is not known. The ratio of NADPH-isocitrate dehydrogenase to ATP citrate lyase activity (1 : >0.02) of foetal lamb adipose tissue, like that of adult sheep (Vernon, 1980b), is very low and would favour citrate metabolism via the isocitrate dehydrogenase cycle (Saggerson, 1974).

Fatty acid synthesis from L-lactate has been demonstrated in adipose tissue from adult ruminants (see Vernon, 1980a) and foetal lambs (Table 2). The physiological significance of this pathway in adult ruminants is uncertain, as high concentrations of L-lactate are required to saturate it in vitro (much higher than the concentration of L-lactate in the blood) (see Vernon, 1980a). An L-lactate concentration of 100 mM was required to saturate the pathway in adipose tissue from foetal lambs (results not shown). However, rates of fatty acid synthesis from L-lactate at a concentration of 3.3 mM, which is similar to the plasma L-lactate concentration in our lambs (2–3 mM), were comparable with the rate of fatty acid synthesis from glucose (Table 2). There is a net transfer of L-lactate as well as glucose from the mother to the foetal lamb (Meschia et al., 1980), so L-lactate may be a physiologically significant precursor for fatty acid synthesis in foetal lambs.

Insulin has little or no effect on the rate of fatty acid synthesis, glucose oxidation or glucose conversion into acylglycerol glycerol in ruminant adipose tissue in vitro (see Vernon, 1980a), but the reason for this is still unclear. Adipose tissue from foetal lambs thus resembles that of the adult in its apparent insensitivity to insulin in vitro.

In many respects the metabolism of adipose tissue from foetal lambs closely resembles that of adult sheep, both in its enzyme activities and in its preference for acetate rather than glucose as a substrate for fatty acid synthesis and also oxidation. This contrasts with the marked differences in the use of glucose and acetate for oxidation in the whole animal. The foetal lamb receives relatively large quantities of glucose from the mother, sufficient to sustain 50–70% of foetal oxidative metabolism (Battaglia & Meschia, 1978), whereas there is relatively little transfer of acetate to the foetal lamb; glucose and not acetate is thus the major fuel for oxidation in the foetal lamb (Battaglia & Meschia, 1978; Girard et al., 1979). On the other hand, the adult sheep receives little or no glucose from its diet (Lindsay, 1978) and acetate is the major fuel for oxidation (Lindsay, 1975).
The results suggest that, although the low capacity for fatty acid synthesis from glucose in adipose tissue of the adult ruminant appears to be readily explicable in terms of the relative availabilities of acetate and glucose from the diet, other factors must also be involved.

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References

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