Lipogenesis in the Ruminant: Effect of Fasting and Refeeding on Fatty Acid Synthesis and Enzymatic Activity of Sheep Adipose Tissue$^{1,2}$


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ABSTRACT The metabolic control of lipogenesis was investigated in growing and fattening lambs by fasting and refeeding studies. Fasting state was monitored by indirect calorimetry measurements of respiratory quotient and methane production. At various time periods, rump adipose tissue was obtained by biopsy and used for determination of lipogenic capacity and the activities of relevant enzymes. The activity of acetyl CoA carboxylase very closely paralleled the progressive decrease in fatty acid synthesis by adipose tissue slices during starvation and the increase upon refeeding. Acetyl CoA synthetase and fatty acid synthetase activities also decreased substantially during fasting, but in fattening lambs, this decrease did not occur until after 48 hours of fasting. Additionally, the activities of acetyl CoA synthetase and fatty acid synthetase did not increase significantly with refeeding. In both groups of lambs the activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and NADP-isocitrate dehydrogenase decreased only moderately during starvation and had not increased significantly at the time of biopsy after refeeding. The results of these investigations indicate that acetyl CoA carboxylase is the rate limiting enzyme in fatty acid synthesis by ruminant adipose tissue. The correlation between in vitro lipogenic capacity and acetyl CoA carboxylase activity during fasting and refeeding was 0.95 and 0.93 with growing and fattening lambs, respectively. J. Nutr. 103: 1479-1488, 1973.

INDEXING KEY WORDS lipogenesis · sheep · adipose tissue · acetyl CoA carboxylase

The control of fatty acid synthesis has been extensively investigated in nonruminant species (see reviews by Tepperman and Tepperman [1], Hanson et al. [2], Flatt [3], and Vagelos [4]). However, relatively few studies have been conducted with ruminant animals. Investigations involving shifts in the dietary carbohydrate: lipid ratio indicated marked adaptations occurred in lipogenic enzymes of rat liver while those in calf liver remained unchanged (5). The lack of lipogenesis adaptation in calf liver is presumably related to the fact that adipose tissue rather than liver is the major site of fatty acid synthesis in ruminants (6, 7). Results from long-term starvation studies have established that NADPH enzymes in ruminant adipose tissue undergo only moderate decreases in activities during a fast of 21 to 27 days (8, 9). Acetate has been shown to be the predominant carbon source for fatty acid synthesis in ruminant adipose tissue (7, 10) but apparently no investigations into the effect of physiological state on enzymes directly concerned with acetate incorporation have been conducted.

The objective of this study was to investigate the control of fatty acid synthesis...
in ruminant adipose tissue. The effect of fasting and refeeding on the lipogenic capacity of sheep adipose tissue was measured by in vitro tissue incubations and results coupled with changes in the activity of enzymes associated with acetate incorporation into fatty acids and NADPH generation.

MATERIALS AND METHODS

Animals. Sheep were individually housed in a controlled environment unit and were fed ad libitum the diets shown in table 1. Two groups of sheep were involved in these investigations. Rambouillet wether lambs (growing lambs) averaging 39 kg body weight (range 37 to 42 kg) were used in experiment 1. The diet of growing lambs consisted of equal parts alfalfa pellets and concentrates. Experiment 2 involved white-face wether lambs (fattening lambs) averaging 52 kg body weight (range 46 to 55 kg). Fattening lambs were fed a complete pelleted diet comprised of 90% concentrates and 10% alfalfa.

Individual intakes were determined and expressed as an increment of maintenance digestible energy requirement. The maintenance requirement was calculated according to Garrett et al. (11) whereas the digestible energy of the dietary components was calculated using National Research Council values (12).

Physiological measurements. The fed and fasted state of each animal was monitored with a chamber open circuit respiration calorimetry system which has been previously described (13). Respiratory quotient (RQ) and methane production were determined in triplicate 20-minute measurements for each sheep within a 3-hour period immediately prior to each tissue biopsy. Pilot experiments established that results of these short-term fasting gaseous exchanges were very similar to those obtained from 12-hour measurements. Care was taken to assure steady-state concentration of exhaust gases before measurements were started, and all sheep were acclimated to the chamber prior to the experiment.

Blood was sampled from a jugular catheter at the time of each tissue biopsy. The plasma was analyzed for nonesterified fatty acids (NEFA) using the titrimetric method of Ko and Royer (14) with palmitic acid as the standard.

Tissue biopsies and incubations. Tissue biopsies of subcutaneous rump adipose tissue (fat pad on top of the rump, supragluteal) were obtained as previously described (7, 15). In experiment 1 biopsies from each sheep were obtained immediately prior to fasting (zero time), at 60 and 112 hours of fasting, and at 48 hours after refeeding. The times of biopsy in experiment 2 were 0, 24, 48, 96, and 192 hours after the initiation of fasting and 192 hours after refeeding.

Adipose tissue, obtained by biopsies, was placed in 0.9% NaCl (maintained at 37°) and immediately prepared for in vitro incubations. Tissue slices (120 to 160 mg) were prepared using a Stadie-Riggs hand
microtome. Tissue slice incubations (triplicates) were conducted in 3 ml of Krebs-Ringer bicarbonate buffer (Ca²⁺-free; pH 7.4), under 95% O₂:5% CO₂ in a metabolic shaker for 3 hours at 37°C. Substrates were dissolved in the buffer at a concentration of 10 mM acetate and 5 mM glucose with each incubation containing 1 μCi acetate-1-¹⁴C and 0.3 unit insulin. After the incubations, the reactions were terminated, and the synthesized fatty acids were extracted and quantitated as previously described (7, 16).

**Enzyme determinations.** Adipose tissue was homogenized immediately after biopsy in 4 volumes of isotonic Tris-sucrose buffer (pH 7.4) comprised of 0.3 mM sucrose, 30 mM Tris-buffer, 1.0 mM EDTA and 1.0 mM glutathione. The homogenate was centrifuged at 105,000 × g for 60 minutes and the supernatant fraction (cytosol) used for subsequent enzyme assays. Enzyme activity measurements were conducted immediately at 37°C in the linear range of activity.

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), NADP-isocitrate dehydrogenase (EC 1.1.1.42), and NADP-malate dehydrogenase (EC 1.1.1.40) were assayed as previously described (16). Activities for these enzymes are expressed as nmoles nucleotide reduced per milligram cytosol protein per minute.

Fatty acid synthetase was assayed spectrophotometrically by following NADPH conversion to NADP⁺ (17). The concentrations of reaction components were 40 mM potassium phosphate buffer (pH 6.8), 0.1 mM malonyl CoA, 0.1 mM acetyl CoA, 0.3 mM NADPH, 0.4 mM EDTA, and 0.4 mM dithiothreitol. Fatty acid synthetase activity was expressed as nmoles nucleotide oxidized per milligram cytosol protein per minute.

Acetyl CoA synthetase (EC 6.2.1.1) was assayed by the method of Sladek et al. (18) where the acetyl CoA produced was trapped as acetoxyhydratase. Concentrations of reaction components were 200 mM potassium phosphate buffer (pH 7.5), 10 mM magnesium chloride, 10 mM potassium acetate, 10 mM potassium-ATP, 10 mM glutathione, 0.5 mM CoA, 200 to 400 μM hydroxylamine, and 1 μCi/ml acetate-1-¹⁴C. Reactions were allowed to proceed at 37°C for 10 minutes with CoA being omitted from the blank reaction. The reaction was terminated by the addition of 1 ml of 3 N acetic acid followed by heating for 5 minutes at 100°C. An aliquot of the supernatant was added to a column (140 mm × 10 mm) packed with Dowex 2X-8 (200 to 400 mesh) to allow separation of the unreacted acetate-1-¹⁴C and acetoxyhydratase-¹⁴C. Acetoxyhydratase was eluted from the column with five successive washes with 2 ml of acetic acid (0.8 N). The radioactivity was determined by counting each fraction in a toluene-triton cocktail and results expressed as nmoles acetate converted to acetoxyhydratase per milligram cytosol protein per minute.

Acetyl CoA carboxylase (EC 6.4.1.2) was assayed similar to the method of Chang et al. (19). For the activation of acetyl CoA carboxylase, cytosol plus water (0.24 ml), 0.01 ml of bovine serum albumin (240 mg/ml), and 0.25 ml of a stock solution (40 mM Tris buffer, 0.4 mM EDTA, 0.4 mM glutathione, 40 mM MgCl₂ and 40 mM sodium citrate; pH 7.3) were preincubated for 30 minutes at 37°C in stoppered scintillation vials. After preincubation, 0.5 ml of an incubation medium comprised of stock solution, sodium ATP, potassium bicarbonate, and acetyl CoA was added to the reaction vials. The final reaction volume was 1.0 ml (pH 7.3), and component concentrations were 20 mM Tris buffer, 0.2 mM EDTA, 0.2 mM glutathione, 20 mM MgCl₂, 20 mM sodium citrate, 2.0 mM sodium ATP, 0.2 mM acetyl CoA, 20 mM potassium bicarbonate (3 to 4 μM/ml) and bovine serum albumin (2.4 mg/ml).

Acetyl CoA carboxylase reactions were incubated for 10 minutes at 37°C in stoppered vials. Acetyl CoA was omitted for blank reactions. Reactions were terminated with the addition of 0.2 ml of 6 N hydrochloric acid. Unreacted bicarbonate-¹⁴C was driven off by the addition of crushed dry ice. Radioactivity was counted in 10 ml of toluene-triton cocktail. Acetyl CoA carboxylase activities are expressed as nmoles bicarbonate incorporated into acid

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⁵Three, 30-second bursts in Omnit-Mixer (Ivan Sovaill, Inc, Midland, Mich.).
stable products per milligram cytosol protein per min.

Cytosol protein was determined by the method of Lowry et al. (20) using bovine serum albumin as the protein standard. Under the conditions utilized for homogenization and centrifugation, the milligram cytosol protein per gram wet tissue averaged (mean ± SEM) 4.3 ± 0.2 for experiment 1 (four periods, three animals) and 6.5 ± 0.2 for experiment 2 (six periods, five animals).

Statistical analysis. Statistical analysis was conducted by analysis of variance for a randomized complete block (21). Values used in the analysis were expressed as a percent of the initial (zero time) value for each sheep. Simple correlations between in vitro fatty acid synthesis and enzyme activities were determined across sheep and time period. A z-test was performed to analyze the significance of the correlations (21).

RESULTS

In ruminant animals, the large reservoir of foodstuffs in the rumen and the slow rate of nutrient passage through the gastrointestinal tract results in the tissues receiving a relatively constant supply of nutrients throughout the day. Therefore, ruminant species require several days to achieve a fasted state as contrasted to several hours in most nonruminant species. In ruminants, it is necessary to monitor some physiological parameters to determine the degree of starvation. Sheep are generally considered to be in "fasting metabolism" when the respiratory quotient is less than 0.76 and methane production is below 2 liters/day (22).

Experiment 1. The three lambs used in experiment 1 had achieved a fasted state by 60 hours after food removal as indicated by methane production and respiratory quotient (table 2). Consistent with the necessity for lipid mobilization during starvation, the levels of plasma NEFA increased dramatically. Compared with initial levels, plasma free fatty acids increased 9- to 12-fold at 60 and 112 hours of fasting and decreased to prefasting levels with refeeding.

Acetate incorporation into fatty acids by adipose tissue slices from growing lambs is shown in table 3. Fatty acid synthesis had decreased 91% and 95% by 60 hours and 112 hours of starvation, respectively. Relative to the fasting level, adipose tissue lipogenesis increased dramatically by 48 hours after refeeding and reached a rate not significantly different from the prefasted rate.

An examination of enzymatic activities indicated acetyl CoA synthetase and acetyl CoA carboxylase activities decreased substantially with fasting (table 3). However, only acetyl CoA carboxylase activity increased with refeeding in a manner similar to fatty acid synthesis. The correlation across periods between in vitro fatty acid synthesis and acetyl CoA carboxylase activity was 0.95 for experiment 1 (correlation significant at P < 0.01).

The activities of enzymes involved the generation of reducing equivalents necessary to support fatty acid synthesis decreased less dramatically with fasting in the adipose tissue from growing lambs (table 3). Values ranged from a 40 to 50% decrease in activity for glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (P < 0.05) to 15% to 30% decrease in NADP-isocitrate dehydrogenase activity (insignificant). NADP-malate dehydrogenase activity decreased similar to other NADPH-generating enzymes although the activity was substantially lower.

Two additional sheep were fed normally throughout experiment 1 with physiological and tissue measurements being conducted at identical time periods as the fasted sheep (data not presented). These animals had relatively constant values for all parameters measured in tables 2 and 3. It was concluded from these controls that the biopsy technique and experimental procedures involved were not influencing the metabolic parameters investigated. Therefore, changes noted during fasting were due entirely to the food deprivation of the animals.

Experiment 2. Since the changes in fatty acid synthesis were essentially complete at the 60-hour biopsy in experiment 1, a second study was undertaken to more clearly establish the time sequence of changes in the lipogenic capacity and the activity of relevant enzymes during starvation. Additionally, larger lambs were used and fed at
a higher plane of nutrition in order to give
higher initial rates of fatty acid synthesis.
Previous investigations have shown that
the in vitro rate of fatty acid synthesis in
rump adipose tissue is approximately four-
fold greater in fattening lambs as compared
with growing lambs (7).

The effect of fasting on physiological
parameters in the fattening sheep is shown in
table 4. Plasma free fatty acid levels in-
creased dramatically by 24 hours after food
depprivation and continued to increase
throughout the fasting period. Both respira-
tory quotient and methane production de-
creased progressively during fasting and
increased upon refeeding. Methane produc-
tion was less than 2 liters/day by 96 hours
of starvation while a respiratory quotient of
less than 0.76 was achieved by 192 hours
of food deprivation.

The effects of fasting and refeeding on
acetate incorporation into fatty acids by
adipose tissue slices from fattening lambs
is shown in table 5. Acetate incorporation
into fatty acids after 48, 96, and 192 hours
of fasting represented 43%, 19%, and 3% of
the initial rate, respectively. Refeeding for
192 hours resulted in a fivefold in-
crease in lipogenesis relative to the rate at
192 hours of fast; however, this represented
only 15% of the initial (zero time) rate.
Upon refeeding, animals were very slow
to start eating. Intake on day 8 of refeeding
represented only 80% of the mainte-
nance requirement (table 4). It would
seem logical that refeeding after an 8-day
fast would require reestablishment of the
rumen microbial population prior to any
tissue adaptations.

The activities of enzymes involved in
acetate incorporation into fatty acids and
NADPH generation are shown in table 5.
Acetyl CoA carboxylase activity decreased
progressively and significantly with starva-
tion and was the only enzyme activity that
tended to increase with refeeding (P < 0.10)
in a manner similar to the lipogenic ca-
pacity of adipose tissue. The correlation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fasted (hr)</th>
<th>Refed (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Intake (increment of maintenance)</td>
<td>1.4 ± 0</td>
<td>0</td>
</tr>
<tr>
<td>Plasma NEFA (μmoles/liter)</td>
<td>86 ± 8a</td>
<td>767 ± 56b</td>
</tr>
<tr>
<td>Respiratory quotient</td>
<td>1.01 ± 0.03a</td>
<td>0.76 ± 0.01b</td>
</tr>
<tr>
<td>Methane production (liters/24 hr)</td>
<td>29.6 ± 4.5</td>
<td>1.6 ± 0.3</td>
</tr>
</tbody>
</table>

1 Values represent an average of means ± SEM for three lambs (90 kg weight); ND equals not determined. Means with unlike superscripts differ significantly (P < 0.05). 2 Calculated on digestible energy basis.
### Table 4

**Physiological changes in falling lambs during fasting and refeeding (experiment 2)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fasted (hr)</th>
<th>Refed (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake (increment of maintenance)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.6 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0</td>
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<tr>
<td>48</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>96</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>192</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma NEFA (µmoles/liter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>61 ± 9a</td>
<td>1250 ± 174d</td>
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<tr>
<td>24</td>
<td>441 ± 104b</td>
<td>ND</td>
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<td>48</td>
<td>641 ± 156c</td>
<td></td>
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<tr>
<td>96</td>
<td>801 ± 176d</td>
<td></td>
</tr>
<tr>
<td>192</td>
<td></td>
<td></td>
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<tr>
<td>Respiratory quotient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.01 ± 0.01a</td>
<td>1.06 ± 0.04a</td>
</tr>
<tr>
<td>24</td>
<td>0.85 ± 0.01b</td>
<td>0.72 ± 0.01c</td>
</tr>
<tr>
<td>48</td>
<td>0.80 ± 0.02c</td>
<td>0.78 ± 0.02a</td>
</tr>
<tr>
<td>96</td>
<td>0.80 ± 0.02c</td>
<td>0.78 ± 0.02a</td>
</tr>
<tr>
<td>192</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methane production (liters/24 hr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14.3 ± 4.7a</td>
<td>9.2 ± 1.2a</td>
</tr>
<tr>
<td>24</td>
<td>3.1 ± 0.3b</td>
<td>3.1 ± 0.3b</td>
</tr>
<tr>
<td>48</td>
<td>2.6 ± 0.2b</td>
<td>1.8 ± 0.3b</td>
</tr>
<tr>
<td>96</td>
<td>1.8 ± 0.3b</td>
<td>1.0 ± 0.1b</td>
</tr>
</tbody>
</table>

1 Values represent an average of means ± sem for five lambs (32 kg body weight); ND equals not determined. Means with unlike superscripts (a, b, c, d) differ significantly (P < 0.05). 2 Calculated on digestible energy basis.

### Table 5

**In vitro lipogenesis and enzyme activities of rump adipose tissue from falling lambs during fasting and refeeding (experiment 2)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fasted (hr)</th>
<th>Refed (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro incubations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipogenesis</td>
<td>1361 ± 370a</td>
<td>200 ± 414b</td>
</tr>
<tr>
<td>Enzyme activities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetyl CoA synthetase</td>
<td>12.2 ± 1.3a</td>
<td>2.8 ± 0.4c</td>
</tr>
<tr>
<td>Acetyl CoA carboxylase</td>
<td>13.9 ± 2.6a</td>
<td>2.8 ± 0.3c</td>
</tr>
<tr>
<td>Fatty acid synthetase</td>
<td>64 ± 7a</td>
<td>3.6 ± 0.3c</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>133 ± 16b</td>
<td>29 ± 1b</td>
</tr>
<tr>
<td>6-Phosphoglucone dehydrogenase</td>
<td>55 ± 14b</td>
<td>65 ± 6c</td>
</tr>
<tr>
<td>NADP-isocitrate dehydrogenase</td>
<td>230 ± 30b</td>
<td>33 ± 3b</td>
</tr>
<tr>
<td>NADP-malate dehydrogenase</td>
<td>24 ± 2b</td>
<td>170 ± 10b</td>
</tr>
</tbody>
</table>

1 Values represent an average of means ± sem for five lambs. Means with unlike superscripts (a, b, c) differ significantly (P < 0.05). 2 Mean cytosol protein in mg per g wet tissue ± sem for the respective time period samples were: 0.4 ± 0.6; 6.5 ± 0.8; 6.6 ± 0.5; 6.9 ± 0.7; 6.8 ± 0.8; 6.2 ± 0.8. Lipogenesis results expressed as nmoles acetate incorporated into fatty acids per 100 mg tissue per 3 hr. 3 Enzyme activities expressed as nmoles/mg cytosol protein/min.
between acetyl CoA carboxylase activity and in vitro lipogenesis across time periods was 0.93 (correlation significant at $P < 0.01$). However, in the adipose tissue from fattening lambs, acetyl CoA carboxylase activity did not decrease to the very low levels during fasting that were found in experiment 1 with growing lambs.

Acetyl CoA synthetase and fatty acid synthetase activities also decreased during fasting. However, there was a 48-hour time lag before changes in activities of these two enzymes followed the decrease in lipogenic capacity of adipose tissue. The activities of acetyl CoA synthetase and fatty acid synthetase then decreased approximately 40% by 96 hours of fasting but remained unchanged after 8 days of refeeding relative to the levels at 192 hours of fast.

Similar to experiment 1, fasting of fattening lambs resulted in only slight decreases in adipose tissue enzymes associated with NADPH generation (table 5). The activity of NADP-malate dehydrogenase in sheep adipose tissue (experiments 1 and 2) was low relative to other NADPH enzymes although it decreased in a like manner during starvation. A low activity of NADP-malate dehydrogenase had previously been reported in ruminant adipose tissue and when coupled with the very low carbon flux through the citrate-cleavage cycle rules out this enzyme as an important source of NADPH for these species (7, 8, 10).

**DISCUSSION**

Results of the present investigation demonstrate that fatty acid synthesis in ruminant subcutaneous adipose tissue adapts to changes in physiological state. In vitro fatty acid synthesis by adipose tissue decreased progressively during starvation and increased with refeeding in growing and fattening lamb studies (tables 3 and 5). The length of fast required to achieve a postabsorptive state was quite different in experiments 1 and 2 (tables 2 and 4). However, in both experiments when "fasting metabolism" was achieved (that is RQ less than 0.76 and methane production less than 2 liters/day) the rate of acetate incorporation into fatty acids represented less than 10% of the initial values.

Food deprivation resulted in major changes in the activities of the enzymes involved in acetate incorporation into fatty acids by sheep adipose tissue (tables 3 and 5). Of these enzymes, only acetyl CoA carboxylase activity decreased with fasting and tended to increase with refeeding in a manner paralleling in vitro fatty acid synthesis. Despite the difference between growing lambs (experiment 1) and fattening lambs (experiment 2) in the prefasted lipogenesis rate and acetyl CoA carboxylase activity, the direct relationship holds.

Acetyl CoA synthetase and fatty acid synthetase activities also decreased in sheep adipose tissue with fasting, but only after a 48-hour time lag (table 5). Results from rat liver studies indicated that a 48- to 72-hour fast resulted in approximately a 50% reduction in acetyl CoA synthetase activity (23, 24). The authors are not aware of investigations with nonruminant tissues establishing the time sequence of changes in the activities of these two enzymes although a similar type of time lag has been reported with other enzymes. NADP-malate dehydrogenase and ATP-citrate lyase activities in rat and chicken liver decreased markedly with food deprivation but this change occurred several hours after the decrease in lipogenic capacity of the tissue (25, 26).

Although quantitative measurements are lacking, it has been postulated that the pentose phosphate cycle and the isocitrate pathway are the major sources of NADPH in ruminant adipose tissue (7) and mammary gland (16, 27, 28). Food deprivation results in only moderate decreases in the activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and NADP-isocitrate dehydrogenase in ruminant adipose tissue (8, 9, and present study). There is a tendency in ruminant adipose tissue for the dehydrogenases to have a higher activity when higher rates of fatty acid synthesis are obtained (7). Additionally, the activities of NADPH-producing enzymes are greater in adipose tissue from ruminants fed concentrate diets relative to those fed hay diets (8, 29, 30). Thus, the adaptative changes in glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and NADP-isocitrate dehydrogenase activities appear to occur over a period of weeks in ruminant adipose tissue and support the concept suggested by several investigators (31–34) that an increased...
or decreased use of an enzymatic pathway will result in a similar change in activities of enzymes associated with that pathway. In this regard it is interesting to note that in cow and sheep mammary tissue the initiation and cessation of lactation results in only moderate, gradual changes in the activities of NADPH-generating enzymes.

Acetyl CoA carboxylase has been postulated as the rate-limiting enzyme in fatty acid synthesis primarily because of numerous properties of this enzyme that are generally considered characteristic of regulatory enzymes (4, 35). Acetyl CoA carboxylase purified from ruminant adipose tissue (36) also exists in an inactive protomeric form and an active polymeric form with the equilibrium being affected by factors such as citrate and free fatty acids similar to acetyl CoA carboxylase from other species. The present study supports the concept that acetyl CoA carboxylase is indeed the regulatory enzyme in fatty acid synthesis in ovine adipose tissue. The close relationship between acetyl CoA carboxylase activity and in vitro lipogenic capacity found in the present study is shown in figure 1. In experiment 1 the correlation across time periods between in vitro fatty acid synthesis and acetyl CoA carboxylase activity was 0.95 whereas the correlation for experiment 2 was 0.93. A similar close relationship \( r = 0.97 \) between in vitro lipogenesis and acetyl CoA carboxylase activity also exists in cow mammary tissue during the initiation of lactation.

Investigations with rat liver indicate that the changes in acetyl CoA carboxylase activity that occur during fasting and refeeding are a result of dramatic alterations in the synthesis and degradation rates of this enzyme (35, 37). Presumably changes in the synthesis and degradation rates of acetyl CoA carboxylase are also occurring in ruminant adipose tissue during food deprivation and are reflected in alterations

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Fig. 1 Relationship between in vitro fatty acid synthesis and acetyl CoA carboxylase in rump adipose tissue from growing (X) and fattening lambs ( ) during fasting and refeeding.

\[ \text{Acetyl CoA Carboxylase Activity (nmol/mg cytosol protein per min)} \]

\[ \text{Acetate Incorporation into Fatty Acids (nmol/100 mg tissue per 3 hr)} \]

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in the activity of this enzyme (tables 3 and 5).

The results of the present study would suggest that in sheep adipose tissue, acetyl CoA carboxylase is the pacemaker enzyme in fatty acid synthesis. However, in metabolic control studies it must be remembered that the activity of an enzyme in the cell may be regulated by other factors such as concentration of substrates, activators, and inhibitors. Presumably, the effect of these factors may be masked in an in vitro assay of an enzyme conducted under conditions of $V_{\text{max}}$.

LITERATURE CITED