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Lipoprotein lipase and glycerophosphate acyltransferase in ovine tissues are influenced by growth and energy intake regimen

Mark K. Andersen, James W. Bailey,* Carolina Wilken, and Daniel C. Rule

*Department of Animal Science, University of Wyoming, Laramie, WY; and *Department of Nutrition, University of Tennessee, Knoxville, TN*

The purpose of this study was to determine the effect of energy intake regimen on lipoprotein lipase (LPL) activity in muscle and adipose tissue, and on glycerophosphate acyltransferase (GPAT) activity in adipose tissue and liver of sheep. Six ewe lambs (31.3 ± 1.0 kg) per treatment consumed either a low- (LOW, 12.1 Mjoules ME/day) or a high-energy diet (HI, 19.2 Mjoules ME/day). Treatments LOW1 and HI were fed diets for 120 days. Treatment LOW2 was fed the low-energy diet until ewes attained the 120-day weight of HI ewes. Treatment CG (compensatory gain) was fed low-energy for 120 days and then fed high-energy for 30 more days. Growth rates and back fat thicknesses were consistent with energy intake regimen. Adipose tissue LPL activity was greater ($P < 0.05$) in animals fed the diet containing the greatest energy density (HI) compared with all other treatment groups. Skeletal muscle LPL activity was greatest for LOW1 and lowest for CG ($P < 0.05$); HI and LOW2 were intermediate. Cardiac muscle LPL activity was similar for LOW1 and CG, which were both greater ($P < 0.05$) than HI and LOW2. Adipose tissue GPAT activity was lowest for LOW1 and highest for CG ($P < 0.05$); HI and LOW2 were intermediate. Liver GPAT activity was similar for LOW1, HI, and CG, which were greater ($P < 0.05$) than LOW2. Results indicate that ovine adipose tissue LPL may play a role in maintenance of adiposity, and that skeletal and cardiac muscle LPL appeared to be regulated differently. Adipose tissue GPAT activity was consistent with back fat thickness, indicating that this enzyme may play a significant role in regulating ovine adipose tissue lipid accretion. Coordinate regulation of adipose tissue LPL and GPAT was not apparent. © Elsevier Science Inc. 1996 (J. Nutr. Biochem. 7:610–616, 1996.)

Keywords: ovine; lipoprotein lipase; glycerophosphate acyltransferase; adipose tissue; muscle; liver

Introduction

Lipoprotein lipase (LPL, EC 3.1.1.34) catalyzes hydrolysis of triacylglycerols (TG) of chylomicrons and very-low density lipoproteins (VLDL),[1] and has the same metabolic function in adipose tissue and muscle of most species.[2] Dietary fat consumption and hepatic de novo lipogenesis[3] provide the majority of VLDL- and chylomicron-TG available for oxidation and storage in human adipose tissue. As such, LPL plays a pivotal role in regulating lipid accretion in human adipose tissue.[1] The role of LPL in ovine adipose tissue is less well defined because diets are typically low in fat (≤3% by weight) and de novo lipogenesis occurs in adipocytes.[4] However, during metabolic extremes such as fasting,[5] lactation,[6] and maintenance compared with ad libitum energy intake,[7] adipose tissue LPL activity decreases. In ovine muscle, LPL presumably functions similarly to

Address reprint requests to Daniel C. Rule, Ph.D. at Department of Animal Science, University of Wyoming, Laramie, WY 82071-3684, USA.
Received June 26, 1995; accepted July 7, 1996.
Manuscript No. JA-1761 was approved by the University of Wyoming Agricultural Experiment Station.
Part of the data were presented at the American Society of Animal Science conference in 1992 (Proc. West. Sec. Amer. Soc. Anim. Sci., 43, 13.) and at the Federation of American Societies for Experimental Biology conference in 1993 (FASEB J., 7, A605).

655 Avenue of the Americas, New York, NY 10010

0955-2863/96/$15.00
PII S0955-2863(96)00119-2

other species in that restriction of dietary energy increases LPL activity in this tissue.[8]

Glycerophosphate acyltransferase (GPAT, EC 2.3.1.15) catalyzes the committed step in glycerolipid biosynthesis;[9] in adipose tissue, TG is the major product; and in liver, TG and phospholipids are produced. Few studies have investigated regulation of GPAT in ovine tissues. Adipose tissue GPAT activity has been studied during growth in sheep,[10] but effects of growth rate and energy consumption have not been determined. Regulation of ovine hepatic GPAT activity and TG production has not been reported. The purpose of the present study was to determine activities of LPL in adipose tissue and muscle, and of GPAT in adipose tissue and liver of lambs grown under a dietary energy intake regimen that caused differences in growth rate, but without metabolic extremes. This study also allowed for evaluation of reciprocal regulation of adipose tissue and muscle LPL activities as well as of coordinate regulation of LPL and GPAT in ovine adipose tissue.

Methods and materials

Animals

Twenty-four Columbia ewe lambs, average body weight 31.3 ± 1.0 (SEM) kg, were housed indoors in pairs in 2.4 m^2 pens; a 12-hr light/dark cycle was maintained. Feces and urine were expelled through openings in the floor of each pen and into a ventilated pit, which was cleared as needed. Animals were fed their daily rations each morning, and their well being checked again each afternoon or early evening. All lambs had free access to fresh water.

The daily ration of each lamb consisted of 30% corn, 30% barley, 22% dehydrated alfalfa pellets, 10% chopped alfalfa hay, 7% soybean meal, and 1% salt (NaCl). Lambs were assigned to one of two dietary energy intake groups. The high intake group (HI) consumed 1.8 kg of diet per day, and the low intake group (LOW) consumed 1.1 kg of diet per day. Daily energy intakes were calculated[11] as 19.2 and 12.1 Mjoules of metabolizable energy per day for the HI and LOW groups, respectively. The HI treatment group was fed the diet until ewes weighed 60 kg. Two LOW treatments were used. The LOW1 group was fed the diet for the same number of days as the HI animals, and the LOW2 group was fed the diet until body weights were 60 kg; thus, LOW1 was a low-energy, time-constant group, and LOW2 was a low-energy, body weight-constant group. A compensatory gain (CG) group consisted of animals that consumed 1.1 kg per day for the same number of days as the HI animals (essentially LOW1) after which time they consumed 1.8 kg per day until animals weighed 60 kg.

At 60 kg of body weight or after the designated number of days, lambs were slaughtered at the University of Wyoming Meat laboratory; the abattoir was located in the same building as the laboratory where assays were conducted. Lambs were killed by electrical stunning followed by exsanguination according the federal standards for humane lamb slaughter.[12] Abattoir procedures were conducted under the supervision of a Wyoming Department of Agriculture inspector. Some of each carcass was used for other research; remaining meat was available to consumers.

Tissue dissection and assays

Immediately after slaughter, skeletal muscle samples were dissected from the sternomandibularis. Subcutaneous adipose tissue was sampled perianal and adjacent the caudal vertebrae (tail head). Upon evisceration (approximately 15 min post mortem), cardiac muscle was sampled at the apex. Liver was sampled 3 cm distal to the gall bladder. Skeletal and cardiac muscle and liver samples were placed in 4°C Krebs-ringer phosphate (KRP) buffer (pH 7.4). For LPL assay, adipose tissue samples were placed in 37°C KRP, and for GPAT assay, adipose tissue samples were placed in 4°C KRP.

Total LPL was released from tissues by homogenizing (Tekmar Tissuemizer) 100 mg of each muscle and 200 mg of adipose tissue in 2.4 mL of KRP that contained 80 μL of heparin (4.0 mg/mL),[13] and then incubating the homogenate for 30 min at 37°C. Samples were centrifuged at 750 g for 15 min, and the clear supernatant fraction was used for LPL assay.[13] Triolein was used as substrate, and (9,10-^{3}H-oleate)-triolein was used as radiotracer. Ovine serum, used as a source of apolipoprotein-CII,[14] was heated to 60°C for 10 min to inactivate endogenous lipases, and aliquots stored at −80°C. Assays were conducted in triplicate at 37°C for 0, 5, and 10 min to quantify zero time values and to ensure that assays were linear with time. Reactions were terminated by addition of 3.3 mL of chloroform:methanol:heptane, 1.25:1.41:1 (vol:vol:vol) along with 1.0 mL of 0.05 mole/L bicarbonate (pH 10.5) to extract fatty acids hydrolyzed by LPL.[15] Samples were vortex-mixed, centrifuged at 750 g, and radioactivity in 1.0 mL of the upper phase quantified by liquid scintillation counting. LPL activity was calculated after correction for sample volume, upper phase volume, and specific activity.[13]

Tissue homogenization buffer (pH 7.4) consisted of 0.15 mol/L KCl, 10.0 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES), 1 mmol/L ethylenediaminetetraacetic acid (EDTA), and 1 mmol/L dithiothreitol. Per gram of adipose tissue and liver, 2 mL and 7 mL of buffer, respectively, were used for homogenate preparation. Homogenates were centrifuged at 10,000 g for 15 min at 4°C. The adipose infranatant and liver supernatant fractions were used for GPAT assays.

The centrifugal preparation eliminated mitochondrial GPAT from adipose and liver assays. The 10,000 g supernatant fraction contains the cytosolic fraction as well as endoplasmic reticulum (microsomes) which, in adipocytes, is the primary location of GPAT activity.[16–18] In liver, GPAT activity is equally distributed between microsomal and mitochondrial fractions.[16,17] Mitochondrial and microsomal GPAT differ in substrate affinity, acyl group specificity, and sensitivity to thiol-group reagents.[17] In the present study, using the 10,000 g fraction for both tissues allowed for comparisons of GPAT activity and TG biosynthesis between these two tissues.

GPAT assays were conducted as previously described[19] except that only initial rates of activity (proportional with time) were reported in the present study. For adipose tissue, 0.2 mL of homogenate were combined with 0.2 mL of reaction mixture (pH 7.4) at a final concentration of 50 mmol/L HEPES, 20 mmol/L K_2HPO_4, 0.16 mmol/L CoA, 12 mmol/L ATP, 12 mmol/L $MgCl_2$, 1 mmol/L dithiothreitol, 0.5 mg/mL bovine serum albumin, 17.5 mmol/L glycerol-3-phosphate, 0.3 μCi of ^{14}C-glycerol-3-phosphate, and 0.6 mmol/L palmitate (potassium salt). For liver, 0.1 mL of homogenate was combined with 0.3 mL of the reaction mixture. Substrates and cofactors were at saturating concentrations.

Incorporation of glycerol-3-phosphate into total glycerolipids (GPAT activity) was proportional with time for 20 min, and did not plateau (steady state) until 60 min. Reactions were terminated after 10 min by addition of 3.0 mL of 1:2 (vol/vol) chloroform: methanol. Extraction of total lipids and separation of glycerolipids by thin-layer chromatography are described elsewhere.[18] GPAT assays described in the present study have been used previously,[18,19] and were adapted from earlier reports.[20–22]

Data expression and statistical analysis

Muscle LPL activities were expressed on a tissue weight basis. Adipose tissue LPL and GPAT activities were expressed per cell

number; cellular adipocyte concentrations were determined as described by Bailey et al.[23] Liver GPAT data were expressed per mg of total protein as determined by the biuret procedure.[24]

Data were analyzed by analysis of variance for the complete randomized design,[25] and differences between means were determined by Duncan's new multiple range test[25] when significant F values were encountered. Differences were considered significant at $P < 0.05$.

Results

Animal growth

Growth and feed efficiency data are presented in *Table 1*. HI ewes required 120 days to attain 60 kg of body weight; LOW1 ewes were lighter after 120 days ($P < 0.05$) indicating that 1.1 compared with 1.8 kg per day of feed consumption accomplished the desired effect. Animals fed 1.1 kg per day required an additional 60 days to attain a final weight of 60 kg (LOW2). Rates of body weight gain were similar for LOW1, LOW2, and CG (for 120 days) treatment groups. Rate of body weight gain by HI ewes was significantly greater than either of the LOW groups, as well as the CG ewes for the first 120 days. The greatest feed efficiency and rate of gain was achieved by the CG-150 treatment group between 120 and 150 days.

Depth of subcutaneous fat at the 12th rib in the HI and LOW2 treatment groups was more than double (0.56 and 0.47 cm, respectively) the LOW1 (0.21 cm) treatment group. CG ewes, however, had the greatest fat thickness (0.67 cm). For the first 120 days, CG ewes were grown on the same feed consumption regimen as LOW1 ewes. Thus, the rate of fat deposition from 120 to 150 days in CG ewes would have been far greater than either the LOW or HI ewe groups.

Lipoprotein lipase activity

Effects of energy intake regimen on LPL activity are shown in *Figure 1*. In adipose tissue, LPL activity was over twice as great ($P < 0.05$) in tissue from HI ewes than LOW1 or LOW2 ewes indicating that ovine adipose tissue LPL activity was responsive to differences in energy intake and (or) differences in growth rate. LPL activity in adipose tissue of CG ewes was similar to activity in LOW1 and LOW2, which was unexpected because increases in fat thickness and body weight after the 30 days of high-energy feeding of this group were greater than in either of the LOW groups and the HI group.

Skeletal muscle LPL activity was greatest ($P < 0.05$) in LOW1 ewes (*Figure 1*). Prolonged feeding of the low-energy diet (LOW2) resulted in decreased LPL activity ($P < 0.05$) in skeletal muscle compared with LOW1. Activity in LOW2 ewes, however, was significantly greater than in skeletal muscle of HI and CG groups; LPL activity in skeletal muscle of the CG group was the lowest ($P < 0.05$). In cardiac muscle, LPL activity differences between LOW1 and HI were consistent with results in skeletal muscle in that enzyme activity was significantly lower in HI ewes. Cardiac muscle LPL activities were similar between LOW2 and HI treatments, and LOW1 and CG treatments. Therefore, prolonged feeding of the low-energy diet (LOW2) resulted in depressed LPL activity in both cardiac and skeletal muscles (LOW1 vs. LOW2). For CG ewes, either cardiac muscle LPL activity was refractory to the increase in energy consumption after 120 days on the low-energy diet, or enzyme activity rebounded during the 30-day consumption of the high-energy diet.

Glycerophosphate acyltransferase activity

Adipose tissue GPAT activity and TG synthesis rates are shown in *Figure 2*. GPAT activity in LOW1 was significantly lower than the HI and CG treatment groups. Enzyme activity in LOW2 was intermediate to LOW1 and HI, which suggests that as LOW2 ewes approached 60 kg of body weight, fat accretion occurred at a greater rate, even though these ewes were consuming lower daily energy. Adipose tissue is a relatively late-maturing tissue in growing meat animals;[26] therefore, an elevated rate of fat accretion would be expected at later stages of growth by animals consuming less energy. Subcutaneous fat thickness (*Table 1*) was intermediate in LOW1 and HI ewes, which was consistent with the differences in GPAT activity. GPAT activity in adipose tissue of CG ewes was not different from that in HI ewes; however, because CG ewes consumed the same amount of daily energy as LOW1 until 120 days, the in-

Table 1 Growth, feed efficiency, and back fat depth of lambs grown on different energy intake regimens

Item	Intake regimen[a]					
	LOW1	HI	LOW2	CG-120	CG-150	SEM[b]
Final body weight, kg	49.8[d]	60.5[c]	60.0[c]	50.3[d]	60.2[c]	2.1
Days fed	120	120	180	120	30	—
Rate of gain, kg/day	0.153[e]	0.244[d]	0.159[e]	0.161[e]	0.330[c]	0.018
Gain:feed	0.138[d]	0.136[d]	0.145[d]	0.146[d]	0.183[c]	0.006
Back fat depth, cm	0.21[e]	0.56[cd]	0.47[d]	—	0.67[c]	0.04

[a]LOW1 and LOW2 consumed 1.1 kg of diet per day; HI consumed 1.8 kg per day. CG-120 consumed 1.1 kg per day, then was switched to 1.8 kg per day (CG-150).
[b]Back fat depth was measured at the 12th rib after carcasses were chilled for 48 hr.
[c,d,e]Different superscripts indicate significant differences between data within a row ($P < 0.05$).
[f]Standard error of the mean, $n = 6$.

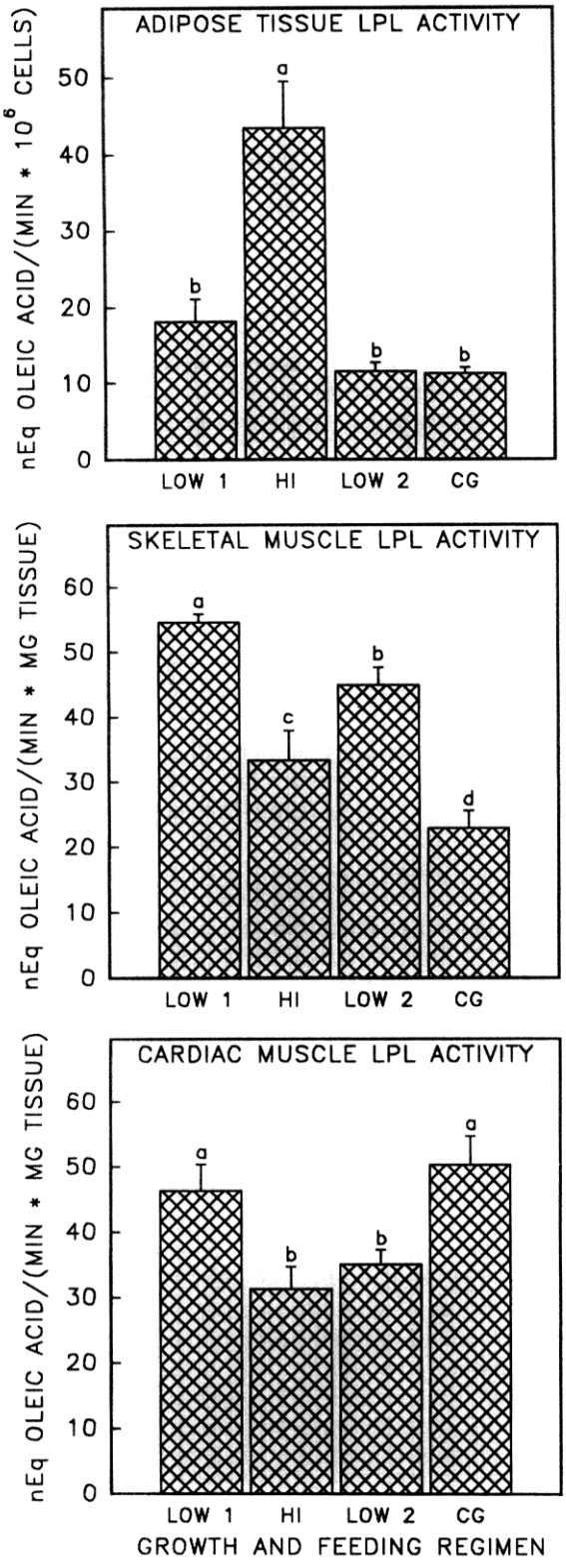

Figure 1 Effect of energy intake regimen on lipoprotein lipase activity in subcutaneous adipose tissue, skeletal (sternomandibularis) muscle, and cardiac (apex) muscle. Muscle data were expressed per mg of tissue. Treatment descriptions are given under "Methods and materials" section. Bars are mean ± SEM of six ewe lambs per treatment. Different superscripts indicate significant differences ($P < 0.05$).

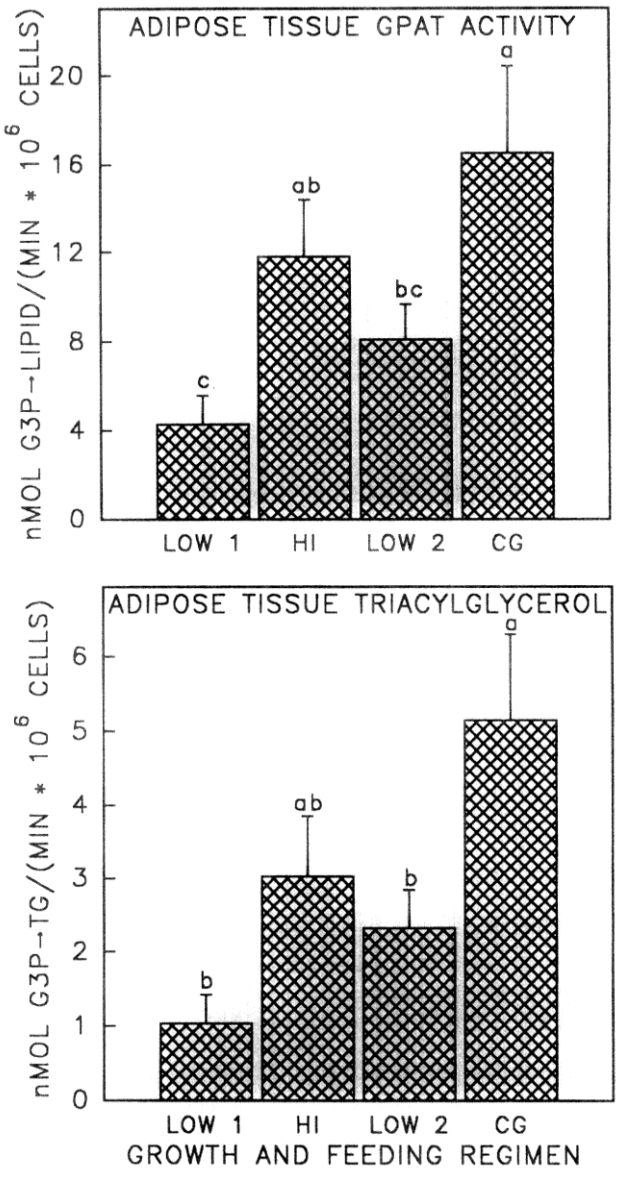

Figure 2 Effect of energy intake regimen on glycerophosphate acyltransferase activity and triacylglycerol production rates in adipose tissue homogenates in vitro. Treatment descriptions are given under "Methods and materials" section. Bars are means ± SEM of six ewe lambs per treatment. Different superscripts indicate significant differences ($P < 0.05$). For triacylglycerol production, for treatments CG vs. HI, $P = 0.07$; for HI versus LOW1, $P = 0.09$.

crease in GPAT activity in adipose tissue of CG ewes during their last 30 days was quite large compared with the LOW1 ewes. This difference in GPAT activity was consistent with the greater subcutaneous fat thickness observed in the CG group compared with the others. Triacylglycerol synthesis rates (*Figure 2*) during GPAT assay changed with treatment similarly to that of GPAT activity, suggesting that adipose tissue GPAT activity was consistent with TG production.

Liver GPAT activity (*Figure 3*) was similar ($P > 0.05$) for LOW1, HI, and CG treatment groups, and significantly lower for the LOW2 treatment. Because LOW2 ewes con-

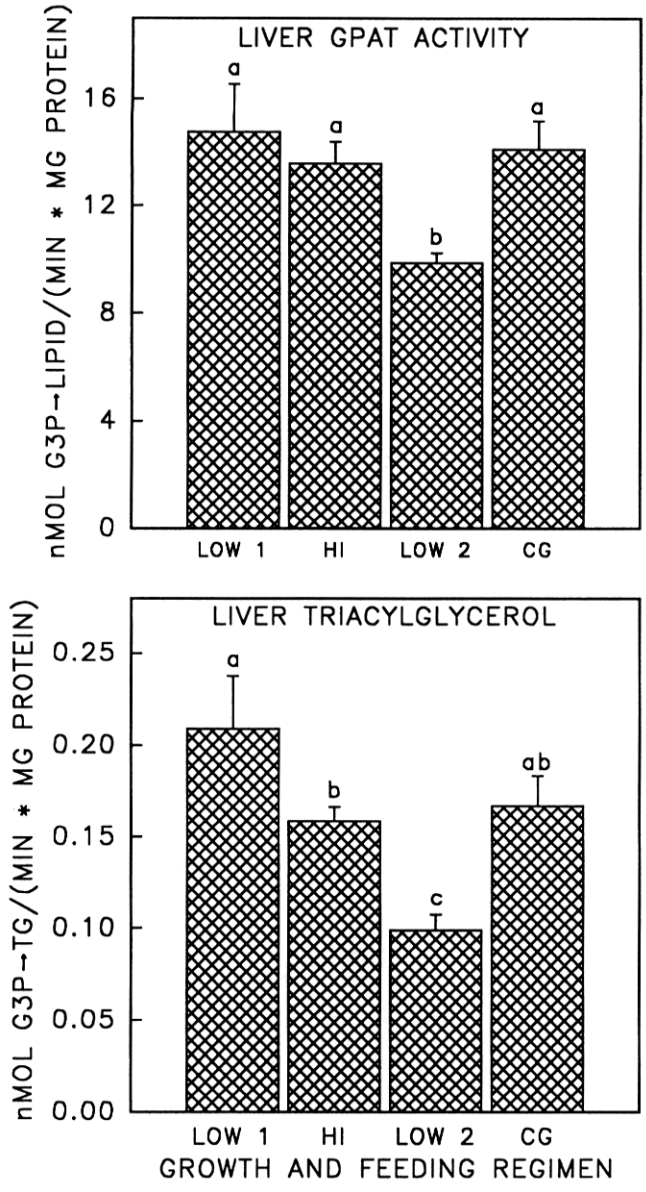

Figure 3 Effect of energy intake regimen on glycerophosphate acyltransferase activity and triacylglycerol production rates in liver homogenates in vitro. Treatment descriptions are given under "Methods and materials" section. Bars are means ± SEM of six ewe lambs per treatment. Different superscripts indicate significant differences ($P < 0.05$).

sumed the same daily energy as LOW1, but for a longer time, consumption of 1.1 kg of diet per day for the longer duration was responsible for reduced liver GPAT activity. However, changing dietary regimens from the lower to the higher daily energy consumption did not affect liver GPAT activity because LOW1 and CG activities were similar.

Production of TG by liver homogenates was similar for LOW1 and CG ewes, but HI values were lower than for LOW1; LOW2 ewes had the lowest liver TG production rate ($P < 0.05$). The treatment effects on TG production followed the same pattern as for GPAT activity, suggesting that treatment effects on liver (non-mitochondrial) GPAT activity were associated with changes in TG synthesis.

Discussion

The present study found that growth rate was related to daily energy consumption. An accelerated growth rate was observed in animals fed 1.8 kg of diet per day compared with 1.1 kg per day, and more so when daily energy intake was increased from 1.1 to 1.8 kg per day. Compensatory gain was first demonstrated by Osbourne and Mendel[27] in cattle and later in sheep and cattle by Meyer and coworkers.[28–30] Reports regarding effects of CG on body composition, however, are conflicting.[29,31,32] In the present study, fat thickness was greatest in CG ewes. Turgeon et al.[33] reported greater fat deposition in sheep that experienced CG during later stages of growth towards market body weight. This response was consistent with results of the present study because energy intake of the CG ewes was elevated during the later stage of growth.

Lipoprotein lipase

The growth differences and energy intake regimen employed in the present study has provided a novel model for study of LPL and GPAT regulation in ovine tissues. Both enzymes have been extensively studied in rats, and, for LPL, in humans. Far fewer studies on ruminant species have been reported for either enzyme. For LPL, several studies have reported effects of energy intake and lactation, but adipose tissue was the major focus. Greater energy intake caused increased LPL activity in bovine[5,34] and ovine[7] adipose tissue, changes that were likely associated with greater insulin secretion.[35] Up-regulation in adipose tissue and down-regulation in mammary tissue of LPL in dairy cows,[6,36,37] as well as increased LPL activity in adipose tissue of cows after the peak of lactation,[38] have been reported. These studies[6,36,37] indicate that LPL is an important enzyme in lipid metabolism of ruminants; however, it must be taken into consideration that these measurements were made under extreme metabolic conditions (fasting, heavy lactation). In the present study, changes in LPL activity were observed under less extreme conditions because the differences in daily energy intake were enough to cause different rates of growth and fat deposition without growth inhibition or extensive mobilization of body energy stores.

Reciprocal regulation of LPL was apparent in ewes when LOW1 and HI groups were compared (*Figure 1*) because greater and lower LPL activities occurred in adipose tissue and muscle tissue, respectively. The inverse relationship between adipose tissue muscle LPL activities due to energy consumption has been reported for other species.[1,8] However, reciprocal regulation of LPL between adipose tissue, skeletal muscle, and cardiac muscle may be less distinct because starvation and realimentation did not cause truly opposite effects in these tissues in rats.[39] In the present study, it was shown that LPL activity was sensitive to less dramatic changes in energy intake than previously reported for fasting.[5]

In adipose tissue, CG ewes did not exhibit greater LPL activity than either of the LOW groups. This observation was unexpected because of the greater fat thickness of the CG ewes. The difference in LPL activity between LOW1

and HI ewes would suggest that LPL would have been sensitive enough to respond to CG as well as to contribute to the increased fat deposition. However, whether or not acute changes occur in ovine LPL under similar nutritional conditions is not known. These results suggest that increased energy consumption during the later stages of body growth requires more than 30 days to induce an increase in adipose tissue LPL enzyme activity.

Skeletal muscle LPL responded differently than adipose tissue to when animals were switched from 1.1 to 1.8 kg of daily intake (CG group). Thus, in skeletal muscle, the increase in available energy during CG caused reduced need for skeletal muscle LPL to an even greater extent than was observed in the HI group. In contrast, after the increase in food consumption by the CG treatment group, cardiac muscle LPL activity was unchanged. Compared with skeletal muscle LPL activity, this result would appear unusual. These results are similar to those of other investigators,[39] however, who reported increased LPL activity in cardiac muscle of 24-hr starved rats, where enzyme activity remained elevated 6 hr post-realimentation. The contrasting relationship between skeletal muscle and cardiac muscle LPL activity in the CG ewes indicates that regulatory factors affecting these ovine tissues may not be similar.

Glycerophosphate acyltransferase

Adipose tissue GPAT activity increased in ewes with higher daily energy intake and in ewes switched to higher energy intake at 120 days. These results suggest that adipose tissue GPAT activity is related to ovine fat deposition in vivo. This result was in contrast to previous work[10] in which growing ewes fed a high-energy diet did not respond with increasing GPAT activities in adipose tissue. In bovine adipose tissue, however, Bouyekhf et al.[40] reported greater GPAT activity in animals with greater carcass fat. The present study employed an end point closer to mature body size and greater age than previous growth studies; thus, age as well as physiological maturity may contribute to discrepancies observed in previous ovine studies.[10] Older rats and larger rat adipocytes had greater GPAT activities than adipocytes from young rats as well as from smaller adipocytes.[41,42] Adipocyte diameters of animals from the present study were not significantly affected by treatment (range 115 to 122 μm, data not shown); however, adipocytes of ewes of treatments HI and CG tended to have the largest diameters.

Ovine hepatic TG biosynthesis would occur from recycling of fatty acid through glycerolipid biosynthesis because de novo fatty acid synthesis does not occur in this tissue in sheep.[4] The direction in which treatments affected liver GPAT activity were similar to that of TG production in the liver homogenates, indicating that TG production may have been altered to compensate for changes in fatty acid cycling into VLDL-TG. Differences in TG production rates between LOW1 and LOW2 may represent differences in total body lipid metabolism because at the earlier growth phase of LOW1, fat accretion was low; however, fatty acid turnover was likely greater as indicated by greater muscle LPL activity in LOW1 ewes. When ewes were closer to mature body weight, and adipose tissue fat accretion was increasing (LOW2), liver TG synthesis rates may have decreased because adipose tissue fatty acid turnover was probably directed more toward storage rather than toward mobilization. For HI and CG ewes, the greater daily feed intake may have provided sufficient energy for both adipose tissue lipid storage and liver lipid esterification, resulting in similar liver GPAT activities and TG production rates.

Overall, the present study provides evidence that both LPL and GPAT activities respond to variation in growth rate and (or) dietary energy intake regimen in ovine tissues. Generally, adipose tissue and muscle LPL activities appeared to vary in a reciprocal fashion. Perhaps the major limitation of the study was that no acute changes that may have occurred shortly after dietary energy intake was increased were evaluated. On the other hand, because ruminants do not consume large amounts of lipid, and synthesize fatty acids de novo in adipocytes, the function of ovine adipose tissue LPL may be for maintenance of adiposity. Subsequently, much time may be required to stimulate ovine adipose tissue LPL enzyme induction. Treatment effects on LPL and GPAT activities were not completely consistent; thus, coordinate regulation of these enzymes does not appear to occur.

In conclusion, LPL activity in ovine adipose tissue and muscle, and GPAT activity and TG production in ovine adipose tissue and liver, changed in response to growth rate and energy intake regimen. Activity of adipose tissue GPAT varied in accordance with changes in fat thickness, which supports the enzyme's role in production of TG, the storage form adipocyte lipid. Changes in skeletal and cardiac muscle LPL, for the most part, were consistent with expectations regarding differences in energy intake; however, CG did not support lower cardiac muscle LPL activity, or greater adipose tissue activity. This effect suggests that LPL in adipose tissue may not support accretion of lipid, but may instead support maintenance of the adipose tissue mass. Although the catalytic function of LPL in sheep is the same as for other species, the role of adipose tissue LPL in the overall regulation of ovine lipid metabolism is yet to be determined.

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