Protein kinetics in callipyge lambs[1,2]

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ABSTRACT: The objectives for this experiment were to determine the effect of the callipyge phenotype on protein kinetics. We studied callipyge and normal lambs (n = 37) at 5, 8, and 11 wk of age (n = 4 to 7/group) to determine how protein kinetics are altered by this trait. Total protein, DNA, and RNA and calpastatin activity were measured in five skeletal muscles and in the heart, kidneys, and liver, and protein accretion rates were calculated. At 8 wk, the fractional synthesis rates of proteins in these tissues were measured in vivo using a primed, continuous 8-h infusion of [$^2H_5$]-phenylalanine. Fractional rates of protein degradation were estimated by differences. At 5 wk of age, muscle weights, protein mass, protein:DNA, RNA:DNA, and calpastatin activity were higher ($P < .05$) for callipyge, and protein mass differences continued to increase through 11 wk. At 8 wk, fractional rates of protein synthesis and degradation were lower ($P < .05$) in callipyge than in normal lambs. The organs of callipyge lambs exhibited reduced growth at 11 wk. Thus, enhanced muscle growth seems to be maintained in callipyge lambs by reduced protein degradation rather than increased protein synthesis. However, we cannot exclude the possibility that the initial onset of the callipyge condition may be caused by an increase in the fractional rate of protein synthesis.

Key Words: Calpastatin, Fiber, Sheep, Protein Synthesis, Protein Degradation

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Introduction

A gene mutation, callipyge, that results in enhanced skeletal muscle growth has been identified in sheep (Koohmaraie et al., 1995; Carpenter et al., 1996; Jackson et al., 1997a). Lambs that eventually express this phenotype are born with apparently normal muscle development, and the callipyge-associated muscle hypertrophy becomes noticeable at 4 to 6 wk of age (Koohmaraie et al., 1995; Jackson et al., 1997a). The increase in muscle mass is not common to all muscles, and growth of some muscles has been shown not to differ at all (Koohmaraie et al., 1995; Jackson et al., 1997b).

The callipyge phenotype increases muscle growth via hypertrophy and muscles from market-age lambs have higher DNA content suggestive of increased protein synthetic capacity and higher calpastatin activity suggestive of reduced protein degradation (Koohmaraie et al., 1995). However, the effect of the callipyge phenotype on protein turnover has not yet been studied. Therefore, the objective of the present study was to determine the relative roles of muscle protein synthesis and protein degradation in callipyge-induced muscle growth. To control for potential differences in general systemic factors between phenotypes, comparisons were made both for muscles whose weight is increased by the callipyge phenotype (longissimus and biceps femoris) and for muscles whose weight is not increased by the callipyge phenotype (infraspinatus and supraspinatus).

[1]Mention of a trade name, proprietary product or specific equipment is necessary to report factually on the available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Table 1. Pelleted diet[a]

Ingredient	g/kg diet (as fed)
17% Dehydrated alfalfa	250
Casein	180
Ground corn	412
Soybean meal (solvent extruded)	80
Methionine	4
Beet molasses	20
Soybean oil	50
Sulfur	2
Sodium chloride	1
Vitamin mix[b]	1
Dry matter	905
Crude protein	286
Energy, kcal/kg	3,090

[a]Beginning at 3 wk, lambs were allowed ad libitum access to the pellet diet.
[b]Contained: retinal acetate, 8.8×10^6 IU/kg mix; cholecalciferol, 8.8×10^5 IU/kg mix; α-tocopherol acetate, 8.8×10^2 IU/kg mix.

Materials and Methods

Animals and Experimental Design

The protocol was approved by the Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee. A breeding strategy that takes into account the paternal polar overdominance inheritance pattern of the callipyge gene was designed to yield 50% normal and 50% callipyge lambs (Cockett et al., 1996; Freking et al., 1998). All lambs were sired by Dorset rams heterozygous for the callipyge gene and expressing the muscle hypertrophy phenotype and from noncallipyge Romanov ewes. Lambs were allowed to suckle for the first day of life and then were transferred to a nursery. The nursery consisted of pens with raised floors, each equipped with an automatic milk dispenser. The lambs were provided with supplemental heat for the first few weeks and then were maintained at 20°C. Lambs were allowed ad libitum access to a commercial lamb milk replacer (Ultrafresh Lamb Milk Replacer, Land O'Lakes, Fort Dodge, IA), which was discontinued after approximately 4 wk of age. Beginning at 3 wk of age, the lambs also were given ad libitum access to a high protein/high energy pelleted diet designed to support maximal rates of growth (Table 1). At 4 wk of age, lambs were evaluated for muscle expression indicative of the callipyge phenotype. Body weights, without feed deprivation, were determined weekly beginning at 4 wk. The lambs (n = 37) were assigned to one of three groups to be studied at 5 (callipyge n = 4; normal n = 7), 8 (callipyge n = 7; normal n = 7), and 11 (callipyge n = 7; normal n = 5) wk of age. Final verification of each lamb's phenotype was determined by experienced evaluators on the basis of muscle definition of the skinned carcass.

The lambs were killed at 5 and 11 wk by captive bolt gun followed by exsanguination and at 8 wk with an i.v. overdose of sodium pentobarbital followed by exsanguination. Skeletal muscles and organ samples were rapidly removed, frozen in liquid nitrogen, and stored at −70°C until analysis. Samples for calpastatin activity were processed immediately without freezing. After all the samples for analysis had been removed, remaining muscles or organs were quantitatively dissected, and the total weight was determined. The muscles and organs that were collected included longissimus (LD), biceps femoris (BF), infraspinatus (IS), supraspinatus (SS), diaphragm (DIA), heart (HT), liver (LIV), and kidney (KID). The tissues and organs were analyzed for total protein, DNA, and RNA concentrations and calpastatin activity.

In the 8-wk-old group, the rate of protein synthesis in these same muscles and organs was determined in vivo. Two indwelling jugular catheters were placed percutaneously, using aseptic procedures, 48 h before the lambs were to be infused. The infusion catheter was advanced through the left jugular by a distance that would place the catheter tip in the superior vena cava; the sampling catheter was inserted a short distance into the right jugular so that it was above the infusion catheter. The catheters were flushed with heparinized saline, capped, and then secured to the skin with a suture. Prior to the start of the infusion, each lamb was transferred to an individual metabolic crate. To minimize separation anxiety, two to three crates of lambs were placed next to each other during an infusion; these were housed within the nursery in close proximity to the nursery pens. The lambs were allowed to eat throughout the period of infusion. A blood sample was collected, and then a primed, continuous infusion of [$^2H_5$]phenylalanine was administered for 8 h. The lambs then were killed, and tissue samples were taken as described above. The amount of [$^2H_5$]phenylalanine incorporated into total tissue proteins and in the cellular and plasma free amino acid pools was determined by mass spectrometry. On the basis of these measurements, total body phenylalanine flux and in vivo rates of protein synthesis, accretion, and degradation were estimated.

Protein, DNA, and RNA

Tissue samples were homogenized in chilled, deionized water. Aliquots of the homogenate were solubilized in .1 *M* NaOH at 37°C for 1 h and then analyzed for protein concentration (Lowry et al., 1951) using BSA for a standard. The DNA concentration was measured using the combined procedures of Cesarone et al. (1979) and Labarca and Paigen (1980); calf thymus DNA (Fraction V) was used as a standard. The remainder of the homogenate was acidified to .2 *M* perchloric acid to precipitate total RNA and protein. Total RNA was determined as described by Munro and Fleck (1969). The protein pellet and acid supernatant were used for the estimation of [$^2H_5$]phenylalanine enrichment as described below.

Calpastatin Activity

The procedure was carried out as previously described (Koohmaraie et al., 1995). Briefly, a fresh 5-g sample of tissue was homogenized in five volumes of homogenization buffer (50 m*M* Tris·HCl, 10 m*M* EDTA, .1 g/L ovomucoid, 6 mg/L leupeptin, pH 8.3) and centrifuged, and the supernatant was dialyzed. The dialysate was heated to inactivate calpain activity and centrifuged, and the calpastatin in the supernatant was purified using a DEAE-Sephacel column. Calpastatin activity was determined from the inhibition of purified m-calpain activity by the calpastatin extracted from the tissues. Data are reported as units of calpastatin activity per gram of tissue protein; a unit is defined as the amount of calpastatin needed to inhibit one unit of m-calpain activity.

Fiber Typing

Samples of the LD were frozen in liquid-nitrogen-cooled isopentane; 10 μm-thick transverse sections were cut with a cryostat, air-dried, and then stained for myofibrillar adenosine triphosphatase with acid (pH 4.35) and NADH-TR activities, a simultaneous combination staining procedure, as described by Solomon and Dunn (1988). A minimum of 200 fibers per lamb were classified as slow-oxidative (β-red), fast-oxidative/glycolytic (α-red), or fast-glycolytic (α-white). The areas of the same fibers were measured with Micromp PM (Southern Micro Instruments, Atlanta, GA) interactive image analysis of planar morphometry.

Protein Synthesis In Vivo

A sterile solution of [$^2H_5$]phenylalanine (98% enriched, Cambridge Isotope Laboratories, Woburn, MA) was prepared in 9 g/L NaCl. Lambs received an initial priming bolus dose equivalent to a 1-h infusion, followed by an 8-h constant infusion at the rate of 40 μmol·kg body weight^{-1}·h^{-1}. In a pilot study conducted beforehand, we had determined that the priming dose and infusion rate yielded a steady-state plasma tracer:tracee ratio within 40 to 60 min of the bolus injection, and this was maintained throughout the 8-h infusion. Heparinized blood samples were drawn at 0, 4, 5, 6, 7, and 8 h after the start of the infusion; they were centrifuged, and the plasma samples were snap-frozen. At the end of the 8-h infusion, lambs were killed and tissues were obtained as described above. Due to equipment failure during the infusion, protein synthesis measurements could not be obtained for one callipyge lamb.

Phenylalanine enrichment in the plasma, the tissue-free pool, and the protein-bound fraction were isolated and prepared for gas chromatography-mass spectrometry (GCMS) as follows. Plasma was acidified with four volumes of 1 *M* acetic acid. The protein-free supernatant from each acidified tissue homogenate described above was neutralized with 4 *M* KOH and used for the determination of the tissue-free amino acid pool. Protein pellets were hydrolyzed in 4 *M* HCl (Ultrex grade) at 110°C for 24 h. After drying and several washings with water, the amino acids were resuspended in 1 *M* acetic acid. All amino acid samples were purified using cation exchange chromatography (Dowex AG50, 8% crosslinked, H^+ form) before derivatization. The amino acids derived from the plasma, tissue free pool, and the protein hydrolysates were converted to the n-propyl ester, heptafluorobutyramide derivative (Hachey et al., 1991). Isotopic enrichment was measured by negative chemical ionization GCMS by monitoring the ions at a mass-to-charge ratio of 383 [m+0] to 388 [m+5].

Calculations

The total protein, DNA, and RNA masses of each muscle and organ were calculated from their respective concentrations in the individual tissues and total tissue weight. The ratios, RNA:DNA, protein:DNA, and RNA:protein (indicative of the tissue's protein synthetic capacity) also were calculated.

The fractional synthesis rate (**FSR**) of the constitutive proteins was calculated from the tracer:tracee ratios of the samples at the end of the 8-h infusion according to the equation

$$\text{FSR (\%/d)} = (E_{bound} \times 24 \times 100)/(E_{free} \times t)$$

where E_{bound} is the enrichment of protein-bound [$^2H_5$]phenylalanine at the end of the infusion, E_{free} is the intracellular free [$^2H_5$]phenylalanine for each tissue at the end of the infusion, and t is the total time of the infusion in hours. The tracer:tracee ratio of the tissue-free phenylalanine at the start of the infusion was assumed to be the same as for plasma phenylalanine, which was very low. The absolute rate of protein synthesis was calculated as the product of fractional protein synthesis rate and the total protein mass of each muscle or organ at 8 wk of age. The translational efficiency (KRNA, mg protein synthesized/g RNA) of the cellular ribosomes (which constitute 85 to 90% of total RNA), was calculated from the quotient of fractional protein synthesis rate and the RNA:protein ratio.

The total protein contents of each muscle and organ were highly correlated to the live weight of the lamb ($r^2 > .96$ for all muscles; $r^2 > .92$ for all organs) with no separate, identifiable contribution of age to the variance in total tissue protein content. Thus, prediction equations were generated for each genotype to estimate individual muscle or organ protein masses from live weight. For those lambs studied at 8 wk, these prediction equations together with the individual lamb's body weight from 5 wk of age were used to derive the protein contents of muscles or organs at earlier ages. Protein accretion rates for each muscle and organ were then derived from the slope of the regression of protein mass vs age for each individual lamb. Fractional accretion rates

(FAR, %/d) were determined by dividing the absolute protein accretion rates by the protein mass of the tissue at 8 wk.

Fractional protein degradation rates were estimated from the difference between fractional protein synthesis rate and fractional protein accretion rate. Absolute protein synthesis and degradation rates (g/d) were calculated by multiplying the fractional rates by the total protein content of individual tissues.

Total body phenylalanine flux was calculated using the following equation:

$$\text{Flux } (\mu\text{mol}\cdot\text{kg body weight}^{-1}\cdot\text{h}^{-1}) = ([E_i/E_p] - 1) \times i$$

where E_i is the tracer:tracee ratio of the infusate; E_p is the value for the steady-state enrichment of the plasma calculated as the average of the 4, 5, 6, 7, and 8 h values; and i is the rate of infusion of phenylalanine in μmol·kg body weight^{-1}·h^{-1}.

Statistical Analysis

Preliminary analyses indicated that, for both normal and callipyge lambs, dissected tissue weights were more strongly correlated with live weight of the animal before slaughter than slaughter age. Therefore, all mass traits (protein mass, DNA mass, and RNA mass) were adjusted to a common live weight within age group to eliminate the error-inflating effects of variation in growth rate among animals. Within each phenotype, adjustment factors were derived by regressing each mass trait against live weight across all age groups. Because most experiments (Shackelford et al., 1998) have indicated similar growth rates for normal and callipyge, the normal and callipyge lambs were adjusted to a common live weight within each age group.

Protein turnover data were analyzed with one-way ANOVA. All other traits were analyzed as 2 (phenotype) × 3 (age group) factorially arranged, completely randomized design. When the interaction was not significant ($P > .05$), it was pooled into the error term. The significance of differences between callipyge and normal lambs in measures of protein turnover conducted only at 8 wk was tested with Student's t-test (two-way). Differences in response between muscles were determined with ANOVA using muscle and phenotype as the main effects. Differences with P-values less than .05 were considered significantly different. Values were expressed as means either with the pooled standard deviation used in the ANOVA when testing for age and phenotype effects, or ± SEM for the protein turnover data when only the phenotype effects are evaluated.

Results

Growth

Body weights of callipyge and normal lambs were not significantly different at any age (3.14 ± .61 kg, 8.53 ± .68, 15.12 ± 1.27, and 22.51 ± 2.80 kg at birth, 5, 8, and 11 wk of age, respectively) and increased linearly from 5 to 11 wk of age (2.20 ± 1.11 kg/wk). Similarly, all muscle weights increased with age, as did their protein, RNA, and DNA masses (Table 2). The callipyge phenotype was associated with increased ($P < .001$) protein mass for longissimus and biceps femoris but not for infraspinatus and supraspinatus. These results are consistent with previous observations of the effect of the callipyge phenotype on weights of those muscles in market-weight lambs (Koohmaraie et al., 1995; Jackson et al., 1997c). The increase in protein mass in callipyge longissimus and biceps femoris was predominantly due to an absolute increase in tissue weight (17.1% increase, $P < .001$ for LD; 21.4% increase, $P < .001$ for BF). To a lesser extent, the increase in protein mass in callipyge longissimus and biceps femoris was caused by an increase in protein concentration (4.5% increase, $P < .01$ for LD; 3.4% increase, $P < .03$ for BF). There was a phenotype × age interaction on longissimus and biceps femoris protein mass such that the magnitude of the difference in protein mass between normal and callipyge increased with age. The interaction was particularly pronounced for biceps femoris, for which the difference in protein mass between phenotypes increased from a 2.0-g effect (17.9% change) at 5 wk to a 8.7-g effect (28.5% change) at 11 wk. In market-age (169 d) lambs, Koohmaraie et al. (1995) observed a 42% increase in biceps femoris weight associated with the callipyge phenotype.

Muscle DNA (Table 2) content increased with age ($P < .001$) for all muscles. Muscle DNA content was not affected ($P > .05$) by phenotype. The protein:DNA ratio (Table 3) increased with age for all muscles. In both longissimus and biceps femoris, the protein:DNA ratio was greater for callipyge relative to normal lambs.

Total RNA mass (Table 2) increased with age in all muscles. For both longissimus and biceps femoris, RNA content was greater ($P < .001$) in callipyge than normal lambs. The increase in total RNA content associated with the callipyge phenotype was proportionally greater for biceps femoris than for longissimus ($P < .05$). For both longissimus and biceps femoris, RNA:DNA ratio was higher for callipyge (Table 3). The callipyge phenotype did not affect RNA:protein ratio in any tissue.

Protein mass of liver and kidneys were lower for callipyge lambs (Table 4); however, phenotype did not affect heart protein mass. These results are consistent with previous observations (Koohmaraie et al., 1995) of the effect of the callipyge phenotype on weights of those organs in market-weight lambs. For each organ, the slope of the regression line between organ protein mass and body weight was significantly lower for callipyge lambs. The changes in organ protein masses tended to be accompanied by parallel changes in DNA and total RNA masses. Thus, values for protein:DNA and RNA:protein were similar among phenotypes (Table 5).

Table 2. Total protein, DNA, and RNA contents of longissimus (LD), biceps femoris (BF), infraspinatus (IS), supraspinatus (SS), and diaphragm (DIA) in normal (N) and callipyge (C) lambs at 5, 8, and 11 wk of age

Muscle	Age: 5 wk[x] N n = 7	5 wk[x] C n = 4	8 wk[x] N n = 7	8 wk[x] C n = 4	11 wk[x] N n = 7	11 wk[x] C n = 4	Pooled SD (df = 34)	Phenotype	Age	Phenotype × Age
	Total protein, g									
LD	20.5[e]	24.4[e]	34.6[d]	42.8[c]	55.4[b]	68.6[a]	3.5	***	***	*
BF	11.2[e]	13.2[e]	19.9[d]	24.6[c]	30.5[b]	39.2[a]	2.0	***	***	***
IS	5.7[d]	5.5[d]	9.8[c]	9.2[c]	16.3[a]	14.9[b]	1.1	†	***	NS
SS	4.4[c]	3.8[d]	6.8[b]	6.6[b]	10.5[a]	10.1[a]	.5	*	***	NS
DIA	3.8[c]	3.8[c]	6.8[b]	6.6[b]	9.7[a]	10.1[a]	.6	NS	***	NS
	Total DNA, g									
LD	178[e]	205[e]	306[c]	255[d]	424[b]	469[a]	33	NS	***	**
BF	119[c]	120[c]	178[b]	190[b]	303[a]	321[a]	32	NS	***	NS
IS	52[d]	55[d]	104[b]	80[c]	129[a]	125[a]	13	†	***	*
SS	44[c]	39[c]	62[b]	61[b]	87[a]	79[a]	7	†	***	NS
DIA	38[c]	40[c]	67[b]	63[b]	81[a]	80[a]	6	NS	***	NS
	Total RNA, g									
LD	120[e]	150[d]	244[c]	257[c]	305[b]	356[a]	25	***	***	NS
BF	63[d]	79[d]	128[c]	141[c]	163[b]	211[a]	15	***	***	*
IS	32[d]	34[d]	58[c]	54[c]	88[a]	79[b]	5	*	***	*
SS	32[d]	28[d]	47[c]	41[c]	72[a]	65[b]	6	**	***	NS
DIA	28[d]	29[d]	54[b]	46[c]	68[a]	71[a]	7	NS	***	†

[a,b,c,d,e]Within a row, means not bearing a common superscript letter differ ($P < .05$).
[x]Adjusted to a common live weight of 9.3, 15.3, and 22.9 kg at 5, 8, and 11 wk, respectively.
†$P < .10$.
*$P < .05$.
**$P < .01$.
***$P < .001$.

Table 3. Protein:DNA, RNA:DNA, and RNA:protein ratios of longissimus (LD), biceps femoris (BF), infraspinatus (IS), supraspinatus (SS), and diaphragm (DIA) in normal (N) and callipyge (C) lambs at 5, 8, and 11 wk of age

Muscle	Age: 5 wk N n = 7	5 wk C n = 4	8 wk N n = 7	8 wk C n = 7	11 wk N n = 5	11 wk C n = 7	pSD* (34*)	Phenotype†	Age
	Protein/DNA, g/mg								
LD	113	116	124	171	139	149	17	<.001	<.001
BF	100	110	113	129	113	122	10	<.020	<.001
IS	106	101	95	107	120	116	9	NS	<.001
SS	95	98	107	111	122	127	9	NS	<.001
DIA	97	99	99	106	121	124	8	NS	<.001
	RNA/DNA, g/g								
LD	.68	.74	.87	1.06	.72	.78	.16	<.05	NS
BF	.58	.68	.72	.75	.60	.66	.09	<.05	NS
IS	.64	.62	.58	.62	.65	.62	.05	NS	NS
SS	.69	.72	.74	.71	.83	.81	.08	NS	<.001
DIA	.71	.74	.81	.73	.86	.90	.07	NS	<.001
	RNA/protein, mg/g								
LD	6.05	6.15	7.12	6.22	5.22	5.20	.74	NS	<.001
BF	5.83	6.19	6.36	5.83	5.36	5.41	.45	NS	<.001
IS	6.08	6.17	6.09	5.77	5.42	5.39	.42	NS	<.001
SS	7.31	7.11	6.93	6.40	6.81	6.44	.80	NS	NS
DIA	7.37	7.45	8.12	6.88	7.07	7.26	.76	NS	NS

†Phenotype × age interaction was not significant ($P > .05$).
*Pooled standard deviation with error degrees of freedom.

Table 4. Total protein, DNA, and RNA contents of heart (HT), kidney (KID), and liver (LIV) in normal (N) and callipyge (C) lambs at 5, 8, and 11 wk of age

Organ	5 wk[x] N n = 7	5 wk[x] C n = 4	8 wk[x] N n = 7	8 wk[x] C n = 4	11 wk[x] N n = 7	11 wk[x] C n = 4	Pooled SD (df = 34)	Phenotype	Age	Phenotype × Age
	Total protein, g									
HT	7.2[c]	8.1[c]	11.0[b]	10.0[b]	14.1[a]	13.4[a]	1.1	NS	***	†
KID	6.8[d]	5.8[d]	10.3[c]	9.6[c]	15.6[a]	13.5[b]	1.0	***	***	NS
LV	38.5[e]	32.3[e]	84.0[c]	66.2[d]	118.2[a]	102.7[b]	6.4	***	***	†
	Total DNA, g									
HT	70[c]	83[bc]	135[a]	139[a]	90[b]	85[bc]	16	NS	***	NS
KID	285[cd]	265[d]	330[c]	307[cd]	527[a]	455[b]	43	*	***	NS
LV	1,040[d]	1,047[d]	1,309[c]	1,268[c]	2,382[a]	2,141[b]	163	†	***	NS
	Total RNA, g									
HT	108[b]	137[a]	109[b]	103[b]	161[a]	147[a]	19	NS	***	*
KID	221[de]	198[e]	272[c]	244[cd]	438[a]	379[b]	33	**	***	NS
LV	1,087[e]	984[e]	1,864[c]	1,641[d]	2,790[a]	2,514[b]	176	**	***	NS

[a,b,c,d,e]Within a row, means not bearing a common superscript letter differ ($P < .05$).
[x]Adjusted to a common live weight of 9.3, 15.3, and 22.9 kg at 5, 8, and 11 wk, respectively.
†$P < .10$.
*$P < .05$.
**$P < .01$.
***$P < .001$.

Muscle Histomorphometry

The cross-sectional area of all fibers increased with age and, for the fast-oxidative/glycolytic and fast-glycolytic fibers, the areas were significantly greater in callipyge lambs (Table 6). There was no age × phenotype interaction. There was no effect of callipyge on the slow-oxidative fibers. There was a shift in the fiber-type distribution in callipyge lambs from fast-oxidative/glycolytic to fast-glycolytic. This shift also was present by 5 wk of age. The effects of the callipyge phenotype observed herein for 5-, 8-, and 11-wk-old lambs are consistent with previous observations made in market-age lambs (Koohmaraie et al., 1995; Carpenter et al., 1996).

Protein Turnover at 8 Weeks of Age

For lambs studied at 8 wk, absolute daily rates of protein accretion (Table 7) were greater for longissimus and biceps femoris of callipyge lambs. The differ-

Table 5. Protein:DNA, RNA:DNA, and RNA:protein ratios of heart (HT), kidney (KID), and liver (LIV) in normal (N) and callipyge (C) lambs at 5, 8, and 11 wk of age

Organ	5 wk N n = 7	5 wk C n = 4	8 wk N n = 7	8 wk C n = 7	11 wk N n = 5	11 wk C n = 7	pSD* (37*)	Phenotype†	Age†
	Protein/DNA, g/mg								
HT	105	104	91	84	164	154	12	NS	<.001
KID	23	22	32	32	30	30	2	NS	<.001
LIV	35	34	66	57	50	48	5	NS	<.005
	RNA/DNA, g/g								
HT	1.70	1.70	.92	.85	1.80	1.67	.22	NS	NS
KID	.77	.77	.85	.83	.83	.84	.06	NS	<.05
LIV	1.02	.99	1.47	1.34	1.17	1.19	.11	NS	<.05
	RNA/protein, mg/g								
HT	16.2	16.3	10.1	10.1	11.0	10.8	1.3	NS	<.001
KID	33.2	34.8	26.6	25.5	28.0	28.0	2.2	NS	<.001
LIV	29.5	29.3	22.4	23.5	23.5	24.9	1.7	NS	<.001

†Phenotype × age interaction was not significant ($P > .05$).
*Pooled standard deviation with error degrees of freedom.

Table 6. Muscle fiber areas and fiber-type distribution in longissimus of normal (N) and callipyge (C) lambs at 5, 8, and 11 wk of age

	Age								
	5 wk[x]		8 wk[x]		11 wk[x]				
Fiber type	N n = 7	C n = 4	N n = 7	C n = 7	N n = 5	C n = 7	pSD* (34*)	Phenotype†	Age†
	Cross-sectional area, μm^2								
SO	510	545	672	548	785	755	152	NS	<.005
FOG	573	888	771	1,126	842	1,399	226	<.001	<.005
FG	977	1,275	1,132	1,443	1,375	1,868	313	<.005	<.005
Average	755	1,075	940	1,250	1,040	1,598	242	<.001	<.005
	Fiber-type distribution, %								
SO	12	10	8	7	11	8	3	NS	<.05
FOG	40	36	41	37	49	39	7	<.02	NS
FG	48	55	51	56	40	53	8	<.005	<.05

[x]Adjusted to a common live weight of 9.3, 15.3, and 22.9 kg at 5, 8, and 11 wk, respectively.
*Pooled standard deviation with error degrees of freedom.
†Phenotype × age interaction was not significant ($P > .05$).

ence between callipyge and normal lambs was greater, proportionally, for the biceps femoris muscles. The difference in absolute rates of protein accretion between phenotypes was approximately proportional to the difference in protein mass between phenotypes; consequently, fractional protein accretion rates were not different between phenotypes (Figure 1). In longissimus and biceps femoris, both the fractional protein synthesis rate and the fractional protein degradation rate were reduced ($P < .05$) for callipyge lambs. Thus, in longissimus and biceps femoris, protein accretion seems to be much more efficient for callipyge lambs.

In longissimus, the lower fractional protein synthesis rate was associated with a decrease in K_{RNA} (Table 7). For those muscles that did not exhibit a callipyge-associated increase in protein mass (infraspinatus, supraspinatus, and diaphragm), the fractional protein accretion, synthesis, and degradation rates were similar among callipyge and normal lambs.

Table 7. Absolute rates of protein accretion, synthesis, and degradation and translational efficiency (K_{RNA}) of longissimus (LD), biceps femoris (BF), infraspinatus (IS), supraspinatus (SS), diaphragm (DIA), heart (HT), kidneys (KID), and liver (LIV) in normal (N; n = 7) and callipyge (C; n = 6) lambs at 8 wk of age

	Accretion, g protein/d		Synthesis, g protein/d		Degradation, g protein/d		K_{RNA}, g/g	
Tissue	N	C	N	C	N	C	N	C
LD	.69 (.09)[a]	.93* (.05)	2.44 (.18)	2.34 (.27)	1.76 (.15)	1.41 (.27)	10.40 (.50)	8.41 (.90)
BF	.41 (.03)	.63* (.06)	1.24 (.05)	1.40 (.06)	.82 (.06)	.77 (.09)	10.27 (.49)	9.07 (.90)
IS	.21 (.02)	.21 (.01)	.55 (.04)	.51 (.04)	.34 (.04)	.30 (.04)	9.75 (.51)	8.93 (.65)
SS	.12 (.01)	.13 (.01)	.39 (.02)	.48 (.04)	.27 (.02)	.35 (.04)	8.74 (.32)	1.54 (.64)
DIA	.12 (.01)	.13 (.01)	.40 (.02)	.39 (.01)	.28 (.01)	.25 (.01)	7.59 (.18)	8.09 (.13)
HT	.17 (.01)	.14 (.02)	1.42 (.07)	1.19 (.13)	1.25 (.06)	1.04 (.11)	13.12 (.25)	10.80* (.96)
KID	.20 (.02)	.16 (.02)	5.16 (.31)	4.68 (.25)	4.96 (.30)	4.53 (.25)	19.72 (.43)	17.98* (.62)
LIV	2.12 (.16)	1.56* (.17)	34.00 (1.01)	38.60* (1.82)	30.88 (1.14)	37.04* (1.84)	18.96 (1.38)	21.99 (.92)

[a]Parenthetical values are the SEM.
*Phenotypes differ ($P < .05$). Phenotypes tend to differ ($P < .06$).

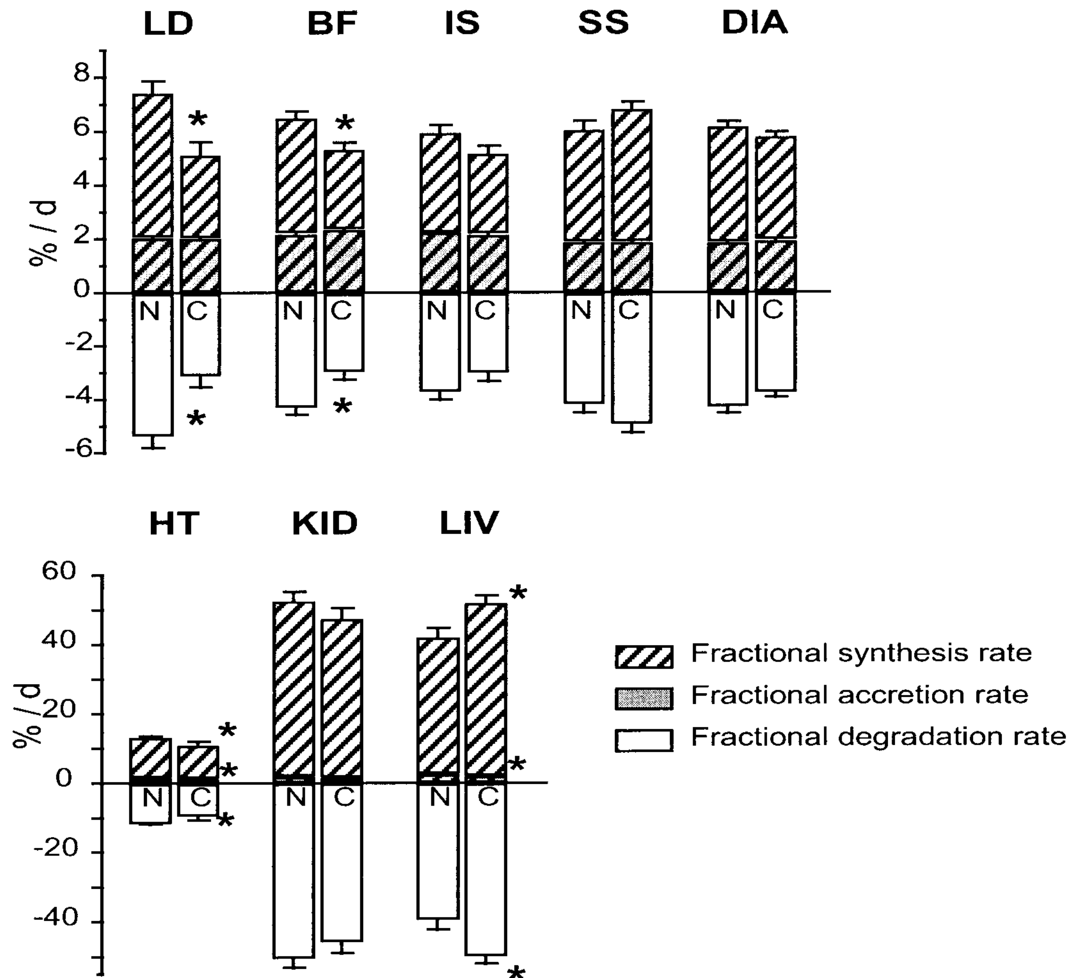

Figure 1. The fractional rates of protein synthesis, accretion, and degradation in (a) the longissimus (LD), biceps femoris (BF), infraspinatus (IS), supraspinatus (SS), and diaphragm (DIA); (b) the heart (HT), kidneys (KID), and liver (LIV), of normal (N, n = 6) and callipyge (C, n = 7) lambs at 8 wk of age. The error bars represent ± 1 SEM; *, $P < .05$ for normal vs callipyge.

The most marked change in protein turnover was in the liver. In callipyge lambs, there was a decrease in both the fractional and absolute rates of protein accretion. Both the absolute and fractional rates of protein synthesis and protein degradation were higher for callipyge liver. However, the increase in synthesis rate was smaller than the increase in degradation rate, leading to the net reduction in accretion.

A small, albeit significant, reduction in fractional protein synthesis, degradation, and accretion was evident for the heart. There was a tendency for protein accretion, synthesis, and degradation to be lower in the kidney of callipyge lambs. For both heart and kidney, K_{RNA} was lower in callipyge lambs.

Estimates of whole-body phenylalanine flux were not different between normal and callipyge lambs (211 ± 7 and 185 ± 16 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, respectively; $P > .1$).

Calpastatin Activity

In longissimus and biceps femoris, calpastatin activity was greater for callipyge lambs (Table 8). For longissimus, there was a magnitudinal phenotype × age interaction on calpastatin activity in which the effect of phenotype on calpastatin activity was particularly large at 5 wk of age. Calpastatin activity decreased with age in all the organs, but there was no effect of callipyge.

Discussion

Results reported herein provide insight into the effects of the callipyge phenotype on protein metabolism in a select group of skeletal muscles and organs. By and large, in those muscles whose mass was not affected by the callipyge phenotype, protein metabolism was similar among phenotypes. However, for those muscles whose mass was increased by the callipyge phenotype, the fractional rates of protein synthesis and degradation were reduced. This finding suggests that the muscling advantage of callipyge is maintained through a reduction in the rate of protein degradation rather than an increase in the rate of protein synthesis.

Because the present experiment was conducted at a time point at which the protein masses of normal and

Table 8. Calpastatin activity of longissimus (LD), biceps femoris (BF), infraspinatus (IS), supraspinatus (SS), diaphragm (DIA), heart (HT), kidneys (KID), and liver (LIV) of normal (N) and callipyge (C) lambs at 5, 8, and 11 wk of age

	Age								
	5 wk		8 wk		11 wk				
Tissue	N n = 7	C n = 4	N n = 7	C n = 7	N n = 5	C n = 7	pSD* (31*)	Phenotype†	Age†
	Calpastatin activity, U m-calpain/g protein								
LD	17[c]	45[a]	21[c]	32[b]	15[c]	26[b]	9	<.001	<.02
BF	21	26	18	24	12	20	8	<.03	<.02
IS	28	29	33	27	19	21	10	NS	NS
SS	29	33	26	26	21	32	10	NS	NS
DIA	33	49	42	40	31	38	14	NS	NS
HT	298	321	189	194	142	154	80	NS	<.001
KID	45	47	35	36	35	33	12	NS	<.05
LIV	228	177	85	108	57	71	54	NS	<.001

[a,b,c]Within LD values that do not share a common superscript letter differ ($P < .05$).
†Phenotype × age interaction was significant for LD ($P < 0.03$); age effect present only in callipyge lambs.
*Pooled standard deviation with error degrees of freedom.

callipyge lambs had already begun to diverge, we do not yet know whether the mechanism responsible for the initial difference in protein mass among phenotypes is the same as the mechanism responsible for maintaining that difference. Although we did not detect a difference among phenotypes in the fractional protein accretion rate of longissimus or biceps femoris, there must have been a difference among phenotypes at some earlier age for the protein mass of those muscles to be greater in callipyge at 8 wk. We cannot exclude the possibility that earlier differences in the fractional rate of protein accretion may have been caused by an increase in the fractional rate of protein synthesis. It would be of merit to investigate the effect of the callipyge phenotype on protein metabolism in the perinatal period. However, in preliminary attempts to study protein metabolism in newborn lambs, we observed numerous technical difficulties inherent to newborn lambs that precluded us from studying newborn lambs in this experiment.

Koohmaraie et al. (1995) showed that differences among muscles in the level of callipyge-induced hypertrophy were strongly correlated with the level of callipyge-induced increase in calpastatin activity. In the present experiment, differences among muscles in the level of callipyge-induced increase in muscle protein mass was correlated with calpastatin activity (r = .96), fractional degradation rate (r = –.75), and fractional synthesis rate (r = –.79). The negative relationship between the effect of the callipyge phenotype on fractional synthesis rate and muscle hypertrophy supports the hypothesis that the muscling advantage of callipyge is maintained through a reduction in the rate of protein degradation rather than an increase in the rate of protein synthesis.

The mechanism underlying the enhanced protein deposition in the longissimus and biceps femoris of callipyge lambs can only be inferred on the basis of the 8-wk protein turnover data, together with the growth and compositional measurements at 5 and 11 wk of age. By 8 wk of age, the longissimus and biceps femoris in callipyge lambs seemed to have attained a new steady state in which higher muscle masses were sustained by lower fractional protein degradation rate. This response is similar to that observed in the skeletal muscles of rats after chronic administration of the β-agonist clenbuterol (Reeds et al., 1986; Maltin et al., 1989; Hesketh et al., 1992); after a transient period of enhanced muscle growth, a larger muscle protein mass was associated with a proportionally higher RNA content, but no change in RNA:protein ratio, whereas fractional protein synthesis rate and fractional protein degradation rate were significantly lower than in controls. In the β-agonist model, the decrease in fractional protein degradation rate seemed to be more protracted than the increase in fractional protein synthesis rate and contributed to the more chronic anabolic response. Studies of β-agonist treatment in lambs have yielded similar findings (Nash et al., 1994; Mersmann, 1998). The reductions in protein degradation have been associated with reductions in protease activities and an elevation in calpastatin activity (Mersmann, 1998). Thus, the reductions in the fractional rates of protein turnover in callipyge longissimus and biceps femoris at 8 wk might represent an eventual adaptation to the hypertrophied condition, rather than the cause of the hypertrophy. The greater proportion of fast-twitch fiber mass in the callipyge lambs may also have contributed to the reduction in fractional protein synthesis rate and fractional protein degradation rate because these fibers have lower turnover rates than slow-twitch fibers (Garlick et al., 1989).

Implications

The muscling advantage of callipyge seems to be maintained through a reduction in the rate of protein

degradation rather than an increase in the rate of protein synthesis. This provides a basis for the development of strategies to select for or to alter body composition in meat animals. Although decreasing the rate of muscle protein turnover results in favorable effects on the rate and efficiency of muscle growth, this may not be the most desirable method to alter muscle growth in meat animals given the associated negative effects on meat tenderness.

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