

A Muscle Hypertrophy Condition in Lamb (Callipyge): Characterization of Effects on Muscle Growth and Meat Quality Traits¹

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ABSTRACT: The present experiment was conducted to determine the effect of the callipyge phenotype on traits affecting muscle growth and meat tenderness. Dorset wethers (n = 40) that were either carriers or non-carriers were fed grain and slaughtered at 169 d of age. Callipyge phenotype did not affect ($P > .05$) slaughter weight, hot carcass weight, or weights of the heart, spleen, viscera, kidney-pelvic fat, head, and pelt; however, callipyge lambs had a higher dressing percentage and lighter lungs, liver, and kidneys ($P < .01$). Callipyge lambs had reduced fat thickness and marbling score and higher leg scores and longissimus area (34%). Adductor (30%), biceps femoris (42%), gluteus group (31%), longissimus (32%), psoas group (20%), quadriceps femoris (18%), semimembranosus (38%), and semitendinosus (26%) weights were higher in the callipyge phenotype ($P < .01$); however, phenotype did not affect ($P > .05$) weights of infraspinatus or supraspinatus. Longissimus pH and temperature declines were not affected ($P > .05$) by phenotype. Longissimus myofibril fragmentation index was lower at 1 (27%), 7 (35%), and 21 (37%) d postmortem and

Warner-Bratzler shear force was higher at 1, 7, and 21 d postmortem in the callipyge phenotype ($P < .01$). Shear force values of callipyge lambs at 21 d postmortem tended to be greater ($P = .12$) than shear force values of non-carriers at 1 d postmortem. Activities of calpastatin (83%) and m-calpain (45%) were higher in the callipyge ($P < .01$); however, μ -calpain activity was not affected ($P > .05$). Longissimus and semitendinosus RNA concentration, DNA content, RNA content, protein content, and the RNA:DNA ratio were higher ($P < .05$), but DNA concentration, protein concentration, and protein:DNA were not affected in the callipyge phenotype. The higher calpastatin activity associated with callipyge suggests that protein degradation may be reduced in the live animal. Additionally, the increased muscle DNA content associated with the callipyge phenotype suggests an increase in satellite cell proliferation, and results in an increased capacity of skeletal muscle to accumulate and maintain myofibrillar protein. These results suggest that both reduced rate of protein degradation and higher capacity for protein synthesis are consequences of the callipyge condition.

Key Words: Callipyge, Calpastatin, Calpain, Muscle Hypertrophy, Tenderness

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Introduction

Recently, empirical evidence for the existence of a gene in sheep causing extreme muscling has been identified. Preliminary evidence suggests that an autosomal dominant gene may be responsible for this major effect on muscling and composition (Cockett et al., 1993).

Compared with normal lambs, callipyge lambs have superior feed efficiency and carcass composition (Jackson and Green, 1993; Jackson et al., 1993a,b). An interesting aspect of this muscle hypertrophy is that, unlike double muscling in cattle, the sheep condition does not manifest itself until a few weeks after birth. Thus, dystocia is not a problem in carrier sheep. This muscle hypertrophy condition provides the sheep

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industry with a number of advantageous traits. However, the mechanisms through which this condition manifests itself are not known.

The objective of the present experiment was to determine the effects of the callipyge phenotype on measures of muscle growth and meat quality traits of lambs and to obtain indices of its mode of action.

Materials and Methods

I. First Experiment: Characterization of Callipyge Gene Effect

Animals. The Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee approved the use of animals in this study. Dorset wethers ($n = 40$) were selected at random from all the male progeny that resulted from the mating of rams that were known to be carriers of the callipyge gene with ewes that were believed to be non-carriers of the callipyge gene. Thus, theoretically 50% of the lambs should have been normal and 50% of the lambs should have been callipyge. To avoid bias, lambs were not preselected for any phenotypic criterion. Lambs were grain-fed and slaughtered in two groups (2 wk apart); the oldest half of the lamb crop was slaughtered in the first group. Slaughter age ranged from 159 to 177 d (mean = 169 d). At slaughter, the head, pelt, heart, lungs, liver, spleen, kidneys, kidney-pelvic fat, and viscera were weighed. Hot carcass weight was determined, and carcasses were chilled (-1°C) for 22 h.

Carcass Measurements and Muscle Dissection. At 22 h postmortem, carcasses were ribbed between the 12th and 13th ribs and leg score, actual and adjusted fat thickness, body wall thickness (at a point 7.6 cm from the lateral end of the longissimus), longissimus area, longissimus depth, marbling score (100 = Traces⁰, 200 = Slight⁰, 300 = Small⁰, 400 = Modest⁰), and lean color score (1 = dark red, 8 = pale pink) were recorded.

The right side of each carcass was dissected and individual weights of the adductor, biceps femoris, infraspinatus, longissimus, psoas group (psoas major and psoas minor combined), quadriceps femoris (rectus femoris, vastus medialis, vastus intermedius, and vastus lateralis combined), semimembranosus, semitendinosus, supraspinatus, and gluteus group (gluteus medius, gluteus profundus, and gluteus accessorius combined) were recorded. Muscle nomenclature was based on Tucker et al. (1952).

Semitendinosus Analyses. After slaughter and dressing (not more than 30 min postmortem), the semitendinosus was removed from the left side of the carcass, trimmed of any external fat, weighed, diced, frozen in liquid nitrogen, and pulverized. Samples were stored (-70°C) for up to 2 mo before determination of DNA, RNA, and protein concentrations.

Longissimus Analyses. After slaughter and dressing (not more than 30 min postmortem), a portion

(approximately 70 g) of the longissimus was removed from the fourth rib region of the left side of the carcass. Ten grams of the fresh prerigor longissimus sample was used for determination of the activities of the components of the calpain system and the remainder was diced, frozen in liquid nitrogen, and stored (-70°C) for up to 2 mo before determination of DNA, RNA, and protein concentrations.

At 24 h postmortem, the entire longissimus was removed from the right side of each carcass. The longissimus was cut into chops (2.54 cm thick) for determination of sarcomere length at 1 d postmortem, and Warner-Bratzler shear force, myofibril fragmentation index, and SDS-PAGE at 1, 7, and 21 d postmortem.

Longissimus pH and Temperature. At 0, 3, 6, and 9 h postmortem, pH and temperature were determined on the longissimus at the seventh rib region of the left side of the carcass. Also, pH was determined on longissimus chops at 1, 7, and 21 d postmortem. pH and temperature were determined with a digital handheld pH meter (Model 05669-00, Cole-Palmer, Chicago IL).

Sarcomere Length. Sarcomere length was determined on the longissimus muscle at 1 d postmortem according to Cross et al. (1980).

Myofibril Fragmentation Index. At 1, 7, and 21 d postmortem, Myofibril Fragmentation Index was determined on fresh longissimus muscle samples according to the procedure of Culler et al. (1978).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Myofibrils were prepared according to Goll et al. (1974). For titin gels and all western blot analysis, myofibrils were suspended in 5 mM Tris-HCl, pH 8.0 (Huff-Lonergan et al., 1995), and prepared for SDS-PAGE according to Wang (1982).

Myofibrillar proteins were resolved on 7.5 to 15% gradient gels (Koochmaraie, 1990b). For electrophoresis of titin and nebulin, continuous 5% gels (100:1 ratio of acrylamide to *N,N*-methylenebis[bisacrylamide] in 50% glycerol) were used. The SDS-PAGE analysis was conducted on all lambs. The lambs whose shear force and MFI values were nearest to the mean of each treatment were reanalyzed, photographed, and reported as Figure 1. Myofibrils from the same lambs were used for all western blot analyses.

Immunoblotting. Vinculin, α -actinin, and desmin were analyzed on 10% gels (37.5:1), and troponin-T was analyzed on 12.5% gels (37.5:1) with 4% (37.5:1) stacking gels. Discontinuous gels were run at 200 V for approximately 45 min. Gels (10 and 12.5%) were transferred to Immobilon-P (Millipore) membranes overnight at 4°C and 170 mA in buffer containing 25 mM Tris, 193 mM glycine, and 15% methanol. Continuous 5% gels (titin, nebulin) were transferred in Tris-glycine buffer containing .005% SDS and no methanol. Lanes containing molecular weight markers were stained with amido black. To prevent non-

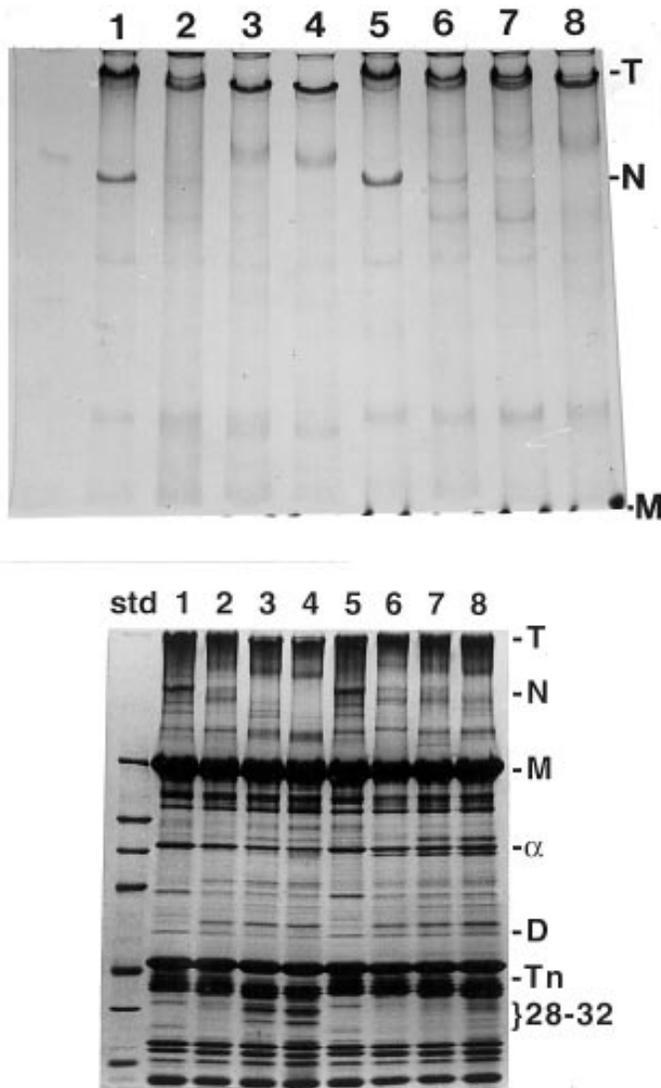


Figure 1. Effect of postmortem storage on longissimus myofibrillar proteins from callipyge and non-callipyge lambs. Top panel is a 5% continuous SDS-PAGE and bottom panel is a 7.5 to 15% gradient gel. First lane (Std) is molecular weight standards and contains myosin heavy chain, 200 kDa; *E. coli* β -galactosidase, 116.3 kDa; rabbit muscle phosphorase b, 97.4 kDa; bovine serum albumin, 66.2 kDa; hen egg-white ovalbumin, 45 kDa; bovine carbonic anhydrase, 31 kDa; soybean trypsin inhibitor, 21.5 kDa; and hen egg-white lysozyme, 14.4 kDa. In top and bottom panels, lanes 1, 2, 3, and 4 are control (non-callipyge lambs) at 0, 1, 7, and 21 d postmortem, respectively. In top and bottom panels, lanes 5, 6, 7, and 8 are callipyge lambs at 0, 1, 7, and 21 d postmortem, respectively. Each lane was loaded with 30 and 80 μ g of myofibrillar protein for the 5% and 7.5–15% gel, respectively. T = titin; N = nebulin; M = myosin heavy chain; α = α -actinin; D = desmin; Tn = troponin-T; 28–32 = degradation products with molecular mass of 28 to 32 kDa.

specific antibody binding, membranes were blocked with 3% BSA in Tris-buffered saline, pH 7.4, containing .05% Tween-20 (TTBS) for 90 min. Antibody incubations were carried out in 1% BSA-TTBS at room temperature and membranes were washed three times with blocking buffer after each incubation. Membranes were incubated for 90 min with primary antibody as follows: anti-titin 1:200 (clone T12; Boehringer Mannheim), anti-nebulin 1:1,000 (clone NB2; Sigma), anti-vinculin 1:400 (clone V284; Boehringer Mannheim), anti- α -actinin 1:400 (clone BM-75.2; Sigma), anti-troponin-T 1:10,000 (clone JLT-12, Sigma), anti-desmin (clone D76; developed by D. A. Fischman and hybridoma cultures obtained from the Developmental Studies Hybridoma Bank). Secondary antibodies were alkaline phosphatase conjugates of anti-mouse IgG 1:1,000 (Sigma, A-5153) or IgM 1:2,000 (Sigma, A-9688). Antibody binding was visualized by exposure to BCIP/NBT (BIO-RAD).

Warner-Bratzler Shear Force. For determination of Warner-Bratzler shear force, longissimus chops were vacuum-packaged, aged (4°C) until 1, 7, and 21 d postmortem, frozen, and stored (–30°C) for up to 2 mo. Chops were thawed (4°C), broiled to an internal temperature of 40°C, turned, and broiled to an internal temperature of 75°C. Chops were cooled (4°C) for 24 h before removal of six cores (1.27 cm diameter) parallel to the longitudinal orientation of the muscle fibers. Each core was sheared once with a Warner-Bratzler shear attachment using an Instron Universal Testing Machine (Instron, Canton, MA) with a 50-kg load cell and crosshead speed of 5 cm/min.

Calpains and Calpastatin. Calpains and calpastatin were extracted from 10 g of longissimus immediately after slaughter and quantified according to Koohmaraie (1990a). Data were expressed as units per gram of muscle or units per milligram of extractable protein (specific activity). Protein concentration was determined after homogenization and centrifugation and before dialysis. Protein concentration was determined using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond CA).

Heated (Crude) Calpastatin. Calpastatin activity of fresh longissimus was determined at 7 and 21 d postmortem as described by Shackelford et al. (1994) with the following modifications: 100 mg/L of ovomucoid, 2 mM PMSF, and 6 mg/L of leupeptin were added to the extraction solution; samples were dialyzed after the first centrifugation.

DNA, RNA, and Protein. Concentrations of RNA were determined by the method of Munro and Fleck (1969). The DNA concentrations were determined according to Labarca and Paigen (1980) using Hoechst 33258 reagent. Protein concentrations were determined by the biuret method (Gornall et al., 1949).

Muscle Histochemistry. Longissimus and semitendinosus were obtained from the right side of each

carcass at 24 h postmortem. Several .7-cm³ samples were frozen on cork in liquid nitrogen-cooled isopentane and stored at -70°C. Transverse cryostat sections, 10µm thick, were cut and allowed to air-dry. Sections were stained according to the procedures for simultaneous staining of bovine muscle fiber types described by Solomon and Dunn (1988). A minimum of 200 fibers per lamb were classified as β -red, α -red, or α -white according to the classification of Ashmore and Doerr (1971). Fiber areas were measured by Microcomp PM (Southern Micro Instruments, Atlanta, GA) interactive image analysis for planar morphometry.

Apparent fiber number was determined (Swatland, 1984) on semitendinosus at two locations (3 cm from the origin or insertion). At each sample location, the area of the muscle was measured and two samples were obtained and frozen as described above. Fiber density was determined on four contiguous fields from each location. Fiber density and muscle area data were used to calculate apparent fiber number.

Statistical Analysis. Because these lambs could not be genotyped with respect to the callipyge gene, lambs were classified as callipyge or non-callipyge based on leg scores and longissimus area/kilogram of carcass. Data were analyzed using χ -square analysis to determine whether the segregation of phenotypes was different from the expected ratio of 1:1. The frequency of lambs classified as callipyge was 38%, which did not differ from the expected percentage of 50% ($P > .10$; $df = 1$). One-way ANOVA were conducted for a completely randomized design using the GLM procedure of SAS (1988).

II. Second Experiment. Quantitative Analysis of Calpastatin in Different Callipyge Lamb Tissues

Animals. The Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee approved the use of animals in this study. Dorset \times Composite III (1/2 Columbia, 1/4 Hampshire, 1/4 Suffolk) ewe lambs ($n = 20$) were selected to be clearly callipyge carriers or non-carriers based on visual and physical evaluation of muscling in the live lamb at 4, 8, and 12 wk of age. The lambs were fed grain and slaughtered at a live wt of 55 to 70 kg. One lamb of each genotype was slaughtered each week for 10 wk to allow extraction and quantification of calpastatin using fresh (unfrozen) tissues (Koochmaraie, 1990a).

Calpastatin. Within 30 min after slaughter, 10 g of tissue was homogenized in 3 volumes of 50 mM Tris, 10 mM EDTA, pH 8.3. The homogenization buffer contained 100 mg/L of ovomucoid, 2 mM PMSF, and 6 mg/L of leupeptin. The homogenate was centrifuged at $37,500 \times g$ for 90 min. The supernatant was dialyzed overnight against 40 mM Tris, 5 mM EDTA, pH 7.4. After dialysis, the extract was heated at 95°C (the entire dialysate was brought up to 95°C and then held

at 95°C for 10 min), cooled on ice, and centrifuged at $37,500 \times g$ for 60 min. The supernatant was loaded onto a 15×120 -cm column packed with DEAE-Sephacel. After loading, columns were washed with 40 mM Tris, 25 mM NaCl, 10 mM MCE, pH 7.4, until absorbance at 278 nm was less than .1. Calpastatin was then eluted with 40 mM Tris, 200 mM NaCl, 10 mM MCE, pH 7.4, and up to 12 5-mL fractions were collected. These fractions were screened for calpastatin activity, and active fractions were pooled and re-assayed (Koochmaraie, 1990a). DEAE-Sephacel-purified lung m-calpain was used as the source of m-calpain. Data are reported as units of calpastatin activity/gram of fresh tissue, and a unit is defined as the amount of calpastatin needed to inhibit one unit of m-calpain activity. One unit of m-calpain is defined as the amount of enzyme that catalyzes an increase of one absorbance at 278 nm in 60 min at 25°C.

To quantify calpastatin, heated tissue extracts had to be loaded onto an ion-exchange column so that the background absorbance could be reduced. For skeletal muscle and some of the other tissues used, calpastatin could have been quantified without the ion-exchange step (Shackelford et al., 1994; Table 5); however, for some tissues, the background (i.e., A₂₇₈ associated with extract after 5% TCA, and centrifugation steps in the calpastatin assay [Koochmaraie, 1990a]) was too high and had to be loaded onto the ion-exchange column. The proteins responsible for an increase in background absorbance either do not bind to DEAE-Sephacel and are removed during washing or will not elute under conditions used. For each tissue, data were analyzed by one-way ANOVA using the GLM procedure of SAS (1988).

Results

Carcass Measurements and Muscle Dissection. The effects of the callipyge condition on carcass traits and dissected muscle weights are reported in Tables 1 and 2. At similar age and identical carcass weight, callipyge lambs had significantly higher dressing percentage, and lower liver, lung, and kidney weights. The callipyge phenotypes had lower fat thickness and marbling score ($P < .01$). Dissected muscle weights were higher by an average of 27.6%, ranging from 2.9% (supraspinatus) to 42.1% (biceps femoris). Callipyge lambs had higher weights of 8 out of the 10 muscles dissected.

pH and Temperature Effects. Normal lambs and those with callipyge phenotype had the same rate or pattern of pH and temperature decline in the longissimus (Table 3).

Callipyge Effects on Postmortem Proteolysis, Myofibril Fragmentation Index, and Shear Force. Indices of meat tenderness (proteolysis of myofibrillar proteins as determined by SDS-PAGE, western blotting, MFI, and Warner-Bratzler shear force) were determined at

Table 1. Effect of phenotype on body weight, hot carcass weight, dressing percentage, and weights of dress-off items

Trait	Normal	Callipyge	SEM	Change, %	<i>P</i> < <i>F</i>
Slaughter age, d	169.4	169.7	.8	.2	.82
Slaughter wt, kg	54.0	54.2	1.5	.3	.94
Heart wt, g	226.5	210.9	7.0	-6.9	.13
Lungs wt, g	673.7	551.8	25.9	-18.1	.01
Liver wt, g	985.7	873.0	30.8	-11.4	.01
Spleen wt, g	85.9	83.3	3.3	-3.1	.58
Viscera wt, g	11,193.0	10,888.2	319.4	-2.7	.51
Kidney-pelvic fat wt, g	566.2	455.9	48.6	-19.5	.12
Kidney wt, g	131.7	111.6	4.1	-15.3	.01
Head wt, g	2,294.5	2,311.8	55.1	.8	.83
Pelt wt, g	6,652.4	6,233.7	203.8	-6.3	.16
Hot carcass wt, g	27.9	29.1	.9	4.4	.35
Dressing percentage, %	51.3	53.6	.6	4.5	.01

various times postmortem to determine the effect of the callipyge condition on meat quality. It is well established that proteolysis of myofibrillar proteins leads to increased fragmentation of myofibrils and decreased shear force during postmortem storage (for review see Koohmaraie, 1988, 1992a,b, 1994). In non-callipyge lambs, MFI increased and shear force decreased during postmortem storage (Table 4). However, these changes were minimal in callipyge lambs, resulting in high shear force values even after 21 d of postmortem storage. The differences in these traits between callipyge and non-callipyge lambs were highly significant at all postmortem times ($P < .01$). Longissimus muscle shear force value of callipyge

lambs at 21 d postmortem was higher than that of non-callipyge lambs at 1 d postmortem ($P < .12$, Table 4).

In non-callipyge lambs, changes typical of postmortem aging occurred (for review see Goll et al., 1983; Koohmaraie 1988, 1992a,b). These include 1) titin degradation, 2) nebulin degradation, 3) desmin degradation, 4) degradation of troponin-T, and 5) simultaneous appearance of a group of polypeptides with molecular mass of 28 to 32 kDa (Figure 1 top and bottom panels). In non-callipyge lambs, most of the above changes occurred by 7 d of postmortem storage. However, both rate and the extent of degradation of these proteins were greatly reduced in lambs

Table 2. Effect of phenotype on carcass measurements and dissected muscle weights

Trait	Normal	Callipyge	SEM	Change, %	<i>P</i> < <i>F</i>
Carcass measurements					
Leg score	12.2	14.4	.2	18.0	— ^c
Actual fat thickness, mm	6.3	4.5	.4	-29.4	.01
Adjusted fat thickness, mm	6.3	4.4	.4	-30.6	.01
Bodywall thickness, mm	23.8	22.3	.7	-6.1	.18
Longissimus area, cm ²	14.8	19.8	.5	33.8	.01
Longissimus depth, mm	28.9	35.2	.8	21.8	.01
Marbling score ^a	262.5	155.6	14.8	-40.7	.01
Lean color score ^b	4.8	4.6	.2	-2.6	.62
Dissected muscle weights					
Adductor wt, g	148.0	192.4	4.8	30.0	.01
Biceps femoris wt, g	360.0	511.5	12.2	42.1	.01
Gluteus group wt, g	289.9	379.7	8.6	31.0	.01
Infraspinatus wt, g	197.4	211.0	8.0	6.9	.24
Longissimus wt, g	715.8	945.4	25.2	32.1	.01
Psoas group wt, g	172.5	207.6	4.8	20.4	.01
Quadriceps femoris wt, g	492.2	584.8	14.9	18.8	.01
Semimembranosus wt, g	345.1	477.3	10.9	38.3	.01
Semitendinosus wt, g	134.0	169.5	4.8	26.4	.01
Supraspinatus wt, g	143.3	147.5	4.3	2.9	.50
Total muscle wt, g	2,998.3	3,826.6	90.0	27.6	.01

^a100 = Traces⁰, 200 = Slight⁰, 300 = Small⁰, 400 = Modest⁰.

^b1 = dark red, 8 = pale pink.

^cNo statistical analysis conducted, because leg score and longissimus area kilogram of carcass was the basis for phenotypic classification.

Table 3. Effect of phenotype on longissimus muscle pH and temperature

Trait and time postmortem	Normal	Callipyge	SEM	Change, %	<i>P</i> < <i>F</i>
pH					
0 h	6.5	6.5	.04	-.9	.36
3 h	6.2	6.1	.05	-.6	.60
6 h	5.9	5.9	.04	.3	.81
9 h	5.8	5.8	.02	.7	.21
24 h	5.6	5.6	.02	.6	.23
168 h	5.6	5.6	.01	.1	.99
504 h	5.7	5.7	.02	.5	.37
Temperature, °C					
0 h	39.1	39.3	.2	.7	.47
3 h	17.3	17.9	.4	3.5	.32
6 h	10.2	10.4	.3	2.0	.68
9 h	6.4	7.1	.2	10.0	.07

expressing callipyge characteristics (Figure 1 top and bottom panel). To verify these findings, we conducted western blot analysis and probed the blots with antibodies against myofibrillar proteins known to be degraded with postmortem storage: 1) troponin-T, 2) desmin, 3) vinculin, 4) nebulin, and 5) titin (Figure 2). Western blot analysis confirmed that the rate and extent of degradation of these proteins were substantially reduced in meat from lambs with the callipyge phenotype. The level of degradation detected in callipyge longissimus at 21 d postmortem was similar to that of non-callipyge lambs at 1 d postmortem for most of these proteins. No apparent degradation of α -actinin occurred in either callipyge or non-callipyge muscles. A polypeptide with the molecular mass of 95 to 105 kDa (Figure 1 bottom panel, the band just below α -actinin) accumulates to a greater extent in callipyge than it does in the non-callipyge muscle. At present, we do not know the origin of this protein, but it could originate from any myofibrillar proteins with the molecular mass of 105 kDa and above.

Calpains and Calpastatin Activities. It is now well established that calpain-mediated degradation of myofibrillar proteins is responsible for postmortem tenderization of meat (for review see Koohmaraie, 1988,

1992a,b, 1994; Koohmaraie et al., 1995a). Furthermore, it has been proposed that the calpain proteolytic system plays an important role in degradation of muscle proteins in the live animal (for review see Goll et al., 1989) and perhaps muscle growth and development through calpain-mediated degradation of the transcription factors c-Fos and c-Jun (Hirai et al., 1991; Carillo et al., 1994). It was, therefore, of interest to determine the activities of the components of this proteolytic system in callipyge lambs (Table 5). At death, activity of μ -calpain was the same in callipyge and non-callipyge muscle, but activity of both m-calpain (45.4%) and calpastatin (82.8%) was higher in callipyge than in non-callipyge muscle. Callipyge had no effect on extractable protein concentration, so similar results were obtained when data was expressed as specific activity.

Previous work indicated that the rate of calpastatin inactivation is highly related to the extent of postmortem tenderization (for review see Koohmaraie et al., 1995a). Thus, we also measured calpastatin activity after 7 and 21 d of postmortem storage. Carrier lambs had 86 and 108% higher calpastatin activity after 7 and 21 d of postmortem storage, respectively (Table 5).

Table 4. Effect of phenotype on longissimus myofibril fragmentation index, Warner-Bratzler shear force, and sarcomere length

Trait and time postmortem	Normal	Callipyge	SEM	Change, %	<i>P</i> < <i>F</i>
Myofibril fragmentation index					
1 d	59.5	43.4	1.6	-27.0	.01
7 d	79.1	51.1	2.0	-35.3	.01
21 d	82.4	52.2	1.8	-36.6	.01
Warner-Bratzler shear force, kg					
1 d	7.5	10.9	.4	44.8	.01
7 d	4.7	10.1	.3	112.2	.01
21 d	3.3	8.2	.2	144.7	.01
Sarcomere length, μ m					
	1.62	1.61	.02	.5	.79

Nucleic Acid and Protein Concentration and Content. To gain some understanding of what muscle characteristics may be altered in lambs that display the callipyge phenotype, the DNA, RNA, and protein concentration and content were measured in the longissimus and semitendinosus (Table 6). The callipyge phenotype had similar effects in longissimus and semitendinosus, which included no effect on protein and DNA concentration, higher RNA concentration by approximately 12%, dramatically higher DNA, RNA, and protein content, higher RNA:DNA ratio, lower protein:RNA, and no effect on protein:DNA.

Effects of Fiber Type Distribution, Size, and Number. Longissimus from callipyge lambs had smaller β -red fiber areas (22.6%) and larger α -red (46.6%) and α -white fiber (45.1%) areas than non-callipyge lambs. The percentage of α -red fibers was lower (25.4%) and the percentage of α -white fibers was higher (28.4%) in callipyge lambs. These changes resulted in callipyge lambs having higher overall average fiber area (48.0%) than normal lambs (Table 7). This difference in fiber area probably explains the 32.1% higher longissimus weight in callipyge lambs. The fiber size differences in longissimus due to the callipyge phenotype are remarkably similar to the effects of a β -agonist on beef longissimus (Wheeler and Koohmaraie, 1992). However, contrary to our results with callipyge lambs, no effect on fiber type distribution due to a β -agonist was reported (Wheeler and Koohmaraie, 1992).

Semitendinosus from callipyge lambs had higher α -red (98.6%) and α -white fiber (51.8%) areas than that of non-callipyge lambs (Table 7). In the semitendinosus, the percentage of α -red fibers was lower (12.8%) and the percentage of α -white fibers was greater (10.0%) in the callipyge lambs than in control lambs. The effects of the callipyge condition on fiber area were larger and on fiber distribution smaller in semitendinosus than in longissimus. These differences resulted in a greater difference in overall average fiber area (61.8%) for semitendinosus than for the longissimus. However, the percentage difference in average fiber area was more than twice the difference in semitendinosus weight (26.4%) in callipyge lambs relative to non-callipyge lambs. This may be partially explained by the tendency ($P < .08$) for lower (11.4%) apparent fiber number in semitendinosus of callipyge lambs than in normal lambs. These data also indicate that the higher muscle weights in callipyge carrier lambs was almost certainly due to muscle fiber hypertrophy and not hyperplasia.

Quantitative Analysis of Calpastatin in Different Callipyge Lamb Tissues. To determine whether the difference in calpastatin activity was a general phenomenon or specific to affected tissues, calpastatin activity was determined in several tissues that represent a range of callipyge effect (Tables 1, 2).

Calpastatin activity was not uniformly higher in all these tissues (Table 8). Liver, lung, brain, and kidney calpastatin activities were not affected in callipyge lambs. Among the skeletal muscles, the effect of the callipyge phenotype seems to be proportional to its effect on muscle weight. Muscles whose calpastatin activity was affected greatly also had the greatest muscle weight increase (longissimus, biceps femoris, and semimembranosus). Conversely, muscles whose calpastatin activity was not affected by the callipyge phenotype also had no change in muscle weight (supraspinatus, infraspinatus). Calpastatin was higher ($P < .01$) in the triceps brachii by 68.6%. Although triceps brachii were not weighed in this experiment, we have data from the ewe siblings indicating that triceps brachii weights are 20.4% heavier in callipyge lambs (unpublished data). Psoas major was intermediate with respect to both effects.

Discussion

A muscle hypertrophy condition was first identified in sheep over a decade ago. It has since been shown that this condition is probably caused by a single gene. The gene is now called callipyge, and its locus has been mapped to chromosome 18 in sheep (Cockett et al., 1993). Jackson and Green (1993) and Jackson et al. (1993a,b) reported that birth weights, weaning weights, and rate of gain were not affected by this gene, but muscle mass was higher by 32.3%. The difference in muscle mass seems to be slightly more universal in leg, loin, and rack muscles than in shoulder muscles (Jackson and Green, 1993; Jackson et al., 1993a,b).

A heavy muscle condition also has been identified and selected for in cattle and is called double muscling. There are, however, distinct differences between the callipyge in sheep and double muscling in cattle. Double muscling in cattle 1) is due to muscle hyperplasia (increase in muscle fiber number during prenatal growth and development); 2) increases birth weights and can cause dystocia, particularly in purebred animals; 3) seems to be controlled either by a single dominant-recessive gene (e.g., Piedmontese and Belgian Blue) or by a multiple gene complex with additive effects (breeds other than Piedmontese and Belgian Blue, e.g., Limousin); and 4) causes a generalized increase of approximately 20% in muscle mass (Hendricks et al., 1973; Hanset and Michaux, 1985a,b, 1986; Hanset et al., 1987, 1989). With the exception of the muscle mass effect, the condition in sheep is entirely different from that in cattle. Although much is known about the callipyge effect on carcass composition, very little is known about its mode of action and its effects on muscle growth and metabolism.

In agreement with previous findings (Jackson et

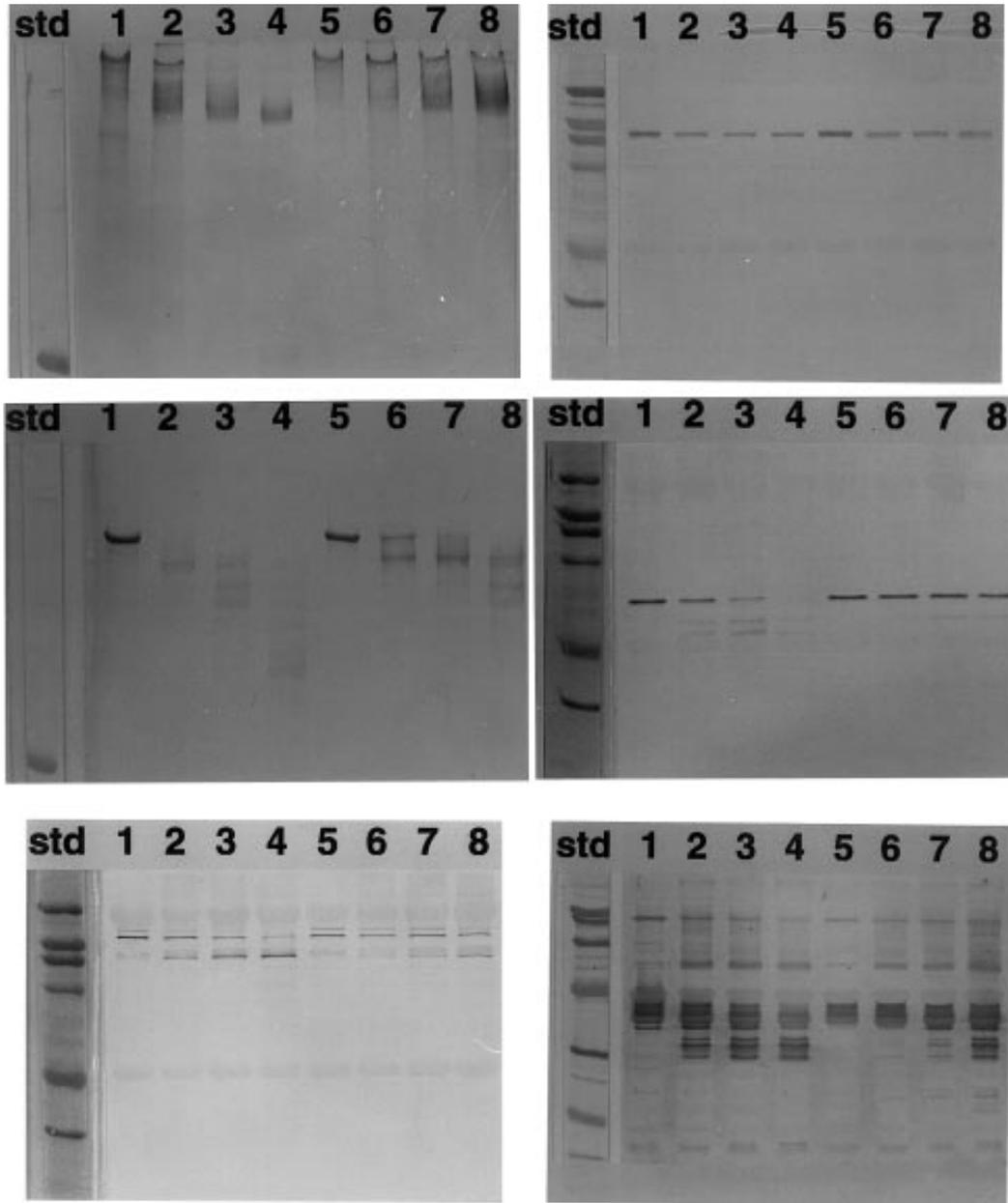


Figure 2. Western blot analysis of titin (top, left), nebulin (middle, left), vinculin (bottom, left), α -actinin (top, right), desmin (middle, right), and troponin-T (bottom, right) degradation during postmortem storage. Myofibrils from longissimus muscle of normal (lanes 1 to 4) or callipyge (lanes 5 to 8) lambs were prepared from samples taken at 0 h (lanes 1 and 5), 1 d (lanes 2 and 6), 7 d (lanes 3 and 7) and 21 d (lanes 4 and 8) of postmortem storage at 4°C. Each lane was loaded with 20 μ g of myofibrillar protein, electrophoresed and blotted as described in Materials and Methods. Standard (std) lanes consist of molecular weight markers including myosin (200 kDa), β -galactosidase (116.25 kDa), rabbit muscle phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), hen egg-white ovalbumin (45.0 kDa), bovine carbonic anhydrase (31 kDa), and soybean trypsin inhibitor (21.5 kDa). Only the 200-kDa marker is seen in the titin and nebulin blots. The 200-kDa marker did not transfer efficiently and is, therefore, not clearly visible in the troponin-T blot.

al., 1993a), our results indicate that all major leg and loin muscles, and at least one of the shoulder muscles, are heavier in callipyge lambs. Jackson et al. (1993a) reported that triceps brachii weights were heavier in callipyge lambs. Our unpublished data from the ewe

mates to the wethers used in this experiment indicated triceps brachii weights were 20.4% heavier in callipyge lambs. These data do not support the interpretation that the muscling effect is exclusive to leg muscles (Cockett et al., 1993).

Table 5. Effect of phenotype on longissimus activities of the components of the calpain proteolytic system

Trait	Normal	Callipyge	SEM	Change, %	<i>P</i> < <i>F</i>
At death					
μ-Calpain activity	.9	.9	.1	-3.9	.65
m-Calpain activity	1.2	1.7	.1	45.4	.01
Calpastatin activity	3.2	5.8	.2	82.8	.01
Protein concentration, mg/g	113.5	111.0	3.0	-2.2	.55
Heated calpastatin activity ^a					
7 d postmortem	1.2	2.2	.1	86.0	.01
21 d postmortem	.9	1.9	.1	108.0	.01

^aHeated calpastatin data was converted to column equivalents by using the following equation: column calpastatin activity = .2 + .53 × heated calpastatin activity.

Our results indicate that longissimus from lambs showing callipyge characteristics have extremely high shear force, even after 21 d of postmortem storage. Thus, longissimus from callipyge lamb must be tenderized before consumption. We are currently evaluating the effect of callipyge on tenderness of other muscles. These results demonstrate that the reduced rate of calpain-mediated postmortem proteolysis is responsible for the reduced rate and extent of postmortem tenderization. Western blot analysis of several myofibrillar proteins known to be involved with postmortem tenderization demonstrates that postmortem proteolysis is delayed by as much as 20 d in callipyge lambs. It is important to note that the same changes occurred in both callipyge and non-callipyge muscles; the only apparent difference is the rate at which these proteins are degraded.

Unlike β-adrenergic agonist L_{644,969} (**BAA**)-induced muscle hypertrophy (Koochmaraie et al., 1991), callipyge-induced muscle hypertrophy is associated

with a greater (34.6% in longissimus and 28.6% in semitendinosus) muscle DNA content. This indicates a greater increase in satellite cell activity. Indeed, our preliminary data suggest that significantly more satellite cells can be extracted from semitendinosus but not supraspinatus of callipyge lamb (Doumit and Koochmaraie, unpublished data). This difference in DNA content, together with higher RNA content (46.9% in longissimus and 43.2% in semitendinosus), suggests that an increased capacity to synthesize and maintain protein may be associated with greater muscle mass observed in callipyge lamb.

Similar to BAA-induced muscle hypertrophy (Koochmaraie et al., 1991), callipyge-induced muscle hypertrophy is associated with higher calpastatin activity, and a lower rate and extent of postmortem proteolysis and, thus, lower tenderization. We suggest, therefore, that a reduced rate of muscle protein degradation may also contribute to the greater muscle protein accretion in callipyge lamb.

Table 6. Effect of phenotype on longissimus nucleic acid and protein concentration and abundance

Trait	Normal	Callipyge	SEM	Change, %	<i>P</i> < <i>F</i>
Longissimus					
DNA concentration, μg/g	1,091.9	1,116.2	19.3	2.2	.38
RNA concentration, μg/g	496.5	554.5	10.5	11.7	.01
Protein concentration, mg/g	223.1	230.7	4.3	3.4	.22
DNA content, mg	780.7	1,050.8	27.6	34.6	.01
RNA content, mg	355.4	522.0	14.2	46.9	.01
Protein content, mg	160.1	218.8	7.3	36.6	.01
RNA:DNA, mg/mg	.46	.50	.01	9.5	.02
Protein:DNA, mg/mg	204.7	208.5	5.0	1.8	.60
Protein:RNA, mg/mg	450.4	420.1	10.1	-6.7	.04
Semitendinosus					
DNA concentration, μg/g	993.7	1,013.5	18.0	2.0	.44
RNA concentration, μg/g	449.5	507.4	11.3	12.9	.01
Protein concentration, mg/mg	230.7	238.5	4.2	3.4	.20
DNA content, mg	133.1	171.2	5.1	28.6	.01
RNA content, mg	60.1	86.1	2.8	43.2	.01
Protein content, mg	31.1	40.3	1.3	29.9	.01
RNA:DNA, mg/mg	.45	.51	.01	11.6	.02
Protein:DNA, mg/mg	232.7	236.4	4.5	1.6	.56
Protein:RNA, mg/mg	515.8	476.5	12.9	-7.6	.04

Table 7. Effect of phenotype on muscle fiber sizes and distributions and apparent fiber number

Muscle and fiber type	Normal	Callipyge	SEM	Change, %	<i>P</i> < <i>F</i>
	Fiber area, μm^2				
Longissimus					
β -Red	1,025	793	44	-22.6	.01
α -Red	1,072	1,571	70	46.6	.01
α -White	1,574	2,284	130	45.1	.01
Overall	1,288	1,905	93	48.0	.01
Semitendinosus					
β -Red	826	848	52	2.6	.77
α -Red	772	1,534	60	98.6	.01
α -White	1,133	1,720	77	51.8	.01
Overall	961	1,556	73	61.8	.01
	Fiber type distribution, %				
Longissimus					
β -Red	10	9	1	-9.4	.40
α -Red	46	34	1	-25.4	.01
α -White	44	57	1	28.4	.01
Semitendinosus					
β -Red	11	11	1	.0	.99
α -Red	39	34	1	-12.8	.01
α -White	50	55	1	10.0	.01
	Apparent fiber no.				
Semitendinosus	763,788	676,449	33,443	-11.4	.07

Callipyge lamb muscle is yet another model that supports degradation of key myofibrillar proteins as the principal cause of meat tenderization during postmortem aging (Koochmaraie, 1988, 1992a,b; Goll et al., 1991; Koochmaraie et al., 1995a; Taylor et al., 1995) and that calpain-mediated postmortem proteolysis is responsible for postmortem tenderization (Koochmaraie et al., 1986, 1988a,b; Koochmaraie, 1988, 1992a,b). It has been suggested that in addition to the calpain system, other proteolytic systems (i.e., cathepsins) are involved in meat tenderization (Yu and Lee,

1986; Calkins and Seideman, 1988; Zeece et al., 1992). These authors suggest that calpains are responsible for the majority of early (few days) postmortem proteolysis and that cathepsins are responsible for postmortem proteolysis beyond 5 to 7 d (Yu and Lee, 1986; Calkins and Seideman, 1988; Zeece et al., 1992). However, digestion of myofibrils with calpains or cathepsins results in hydrolysis of different proteins (Goll et al., 1983; Koochmaraie, 1992a,b). Thus, if both proteolytic systems were involved in meat tenderization, one would expect to

Table 8. Effect of callipyge phenotype on calpastatin activity of various tissues^a

Tissue	Normal	Callipyge	SEM	Change, %	<i>P</i> < <i>F</i>
Heart	21.6	17.7	2.7	-17.7	.34
Spleen	6.1	5.6	.6	-8.1	.54
Supraspinatus	5.2	5.0	.6	-3.6	.82
Liver	9.3	9.2	.6	-.4	.97
Masseter	3.3	3.4	.3	2.1	.86
Infraspinatus	3.1	3.2	.2	3.7	.73
Lung	12.4	13.9	1.2	11.8	.41
Brain	1.4	1.6	.1	16.5	.21
Kidney	4.7	5.6	.5	19.0	.18
Psoas major	2.4	2.9	.2	19.3	.13
Triceps brachii	2.6	4.5	.4	68.6	.01
Longissimus	2.0	4.2	.2	105.9	.01
Biceps femoris	2.0	4.4	.3	123.9	.01
Semimembranosus	2.8	6.2	.4	125.8	.01

^aUnits per gram of tissue.

see a calpain-mediated pattern of degradation initially, followed by a cathepsins-mediated pattern of degradation (presumably following loss of lysosomal integrity). In muscle from callipyge lambs that tenderize very little, troponin-T, desmin, vinculin, nebulin, and titin are all degraded, but the extent of degradation after 20 d is comparable to that seen after only 1 d in lambs that do not display the callipyge phenotype and undergo normal postmortem tenderization. Although the rate of postmortem myofibrillar protein degradation is delayed in muscle from callipyge lambs, the degradation products arising from individual proteins seem identical to those seen initially in non-callipyge lambs. Furthermore, α -actinin, which is degraded by cathepsin L but not calpains, is not degraded in either callipyge or normal lambs within 21 d postmortem. Collectively, these findings suggest that the calpain system is primarily responsible for postmortem proteolysis resulting in meat tenderization.

The results of the second experiment indicate that the higher calpastatin activity is not a general phenomenon and it occurs only in the tissues affected by the callipyge condition. Furthermore, the magnitude of the difference in the calpastatin activity seems to be proportionate to the muscle weight increase (Tables 5, 8). Therefore, callipyge lambs represent yet another model that shows a relationship between higher calpastatin activity and muscle growth and, thus, provides further support for the involvement of the calpain proteolytic system in the regulation of myofibrillar protein turnover in the living animal (for review see Goll et al., 1995).

Implications

These results demonstrate that the callipyge phenotype is manifested as a major effect on carcass composition (less fat and greater muscle mass). Muscle weight was on average 27.6% higher. The major problem with this condition is its negative effect on meat tenderness. Lower meat tenderness results from reduced rate and extent of postmortem proteolysis. Thus, it is likely that callipyge meat must be tenderized before consumption, otherwise meat toughness could preclude the use of these lambs in the sheep industry. We are examining various tenderization methods to enhance tenderness of callipyge lamb.

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