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Effects of the Callipyge Phenotype on Serum Creatinine, Total Cholesterol, Low-Density Lipoproteins, Very-Low-Density Lipoproteins, High-Density Lipoproteins, and Triacylglycerol in Growing Lambs

H. H. Meyer, A. Abdulkhaliq[1], S. L. Davis, J. Thompson, R. Nabioullin, Pai-yen Wu, and Neil E. Forsberg[2]

Department of Animal Sciences, Oregon State University, Corvallis 97331-6702

ABSTRACT: The goals of this study were to investigate the effects of the callipyge (CLPG) phenotype on serum creatinine and lipid profiles of growing lambs. Preliminary studies in our laboratories indicated that creatinine may have utility in distinguishing the CLPG phenotype and that expression of the CLPG gene altered concentrations of serum total cholesterol (TC). As a result, in this study, we examined the influence of the CLPG gene on concentrations of creatinine, TC, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triacylglycerol (TG) at varying stages of maturity in lambs. Ten homozygous (c/c) Polypay ewes were crossed with Dorset rams heterozygous for the CLPG gene (C/c). From this cross, 20 lambs (13 females and 7 males) were born, of which 11 were homozygotic (c/c) and 9 were heterozygotic (C/c; CLPG) based on muscle weights and longissimus dorsi (LD) area at slaughter. Blood samples were taken at monthly intervals and serum lipid constituents were assayed. At 1 mo of age, no differences ($P > .05$) in plasma lipids were detectable between phenotypes. However, at 2 mo of age, CLPG lambs had higher ($P < .01$) concentrations of TG, TC, HDL, and VLDL compared to homozygotic (c/c) lambs. Triglycerides and VLDL were elevated ($P < .05$) in CLPG lambs at 3 mo of age. By slaughter, no differences ($P > .05$) in serum lipid constituents were detectable between genotypes. Hence, the increase in serum TC is due to elevated levels of HDL and VLDL. These observations indicate that creatinine may be used to distinguish CLPG lambs and that the CLPG gene alters serum lipid profiles during the postnatal period.

Key Words: Callipyge, Lipid Metabolism, Very-Low-Density Lipoprotein, High-Density Lipoprotein, Low-Density Lipoprotein, Creatinine

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Introduction

A gene causing rapid postnatal muscle hypertrophy and a reduction in adipose tissue deposition in sheep was recently identified and is now referred to as "Callipyge" (**CLPG**; Cockett et al., 1993). The CLPG gene has been mapped to ovine chromosome 18 (Muggli-Cockett et al., 1993; Cockett et al., 1994); however, the function of the gene product and the nature of the mutation that leads to muscle hypertrophy have not yet been characterized.

Phenotypic characteristics of CLPG animals offer few hints regarding the fundamental nature of the CLPG gene. Several authors (Jackson et al., 1993a,b; Snowder et al., 1994), including ourselves (Meyer et al., 1995), have reported that the CLPG gene causes muscle hypertrophy and reduces adipose tissue deposition. In a recent study we documented that the CLPG gene not only affects postweaning muscle growth but also reduces adipose tissue deposition (Meyer et al., 1995). Kline and Whisnant (1994) reported that CLPG animals had enhanced IGF-I binding activity in certain muscles, and Koohmaraie et al. (1994) have suggested that the gene acts via a mechanism in common to that regulated by treatment of animals with β-adrenergic agonists. The disparate effects of the gene indicate that expression of the CLPG gene manifests itself in several tissues and affects several seemingly distinct physiological processes.

In the course of investigating effects of the CLPG gene on various blood parameters we discovered in preliminary studies that heterozygotic lambs (i.e., those expressing the CLPG phenotype) had serum creatinine and total cholesterol (**TC**) concentrations different from those of control (homozygous; c/c) lambs. The latter observation was particularly excit-

[1]Biol. Dept. Birzeit Univ., West Bank.
[2]To whom correspondence should be addressed.
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ing because it indicated that expression of the CLPG gene locus was also affecting lipid metabolism. Hence, the objectives of this study were to elucidate the effects of the CLPG gene on serum creatinine and lipid concentrations. Because our earlier work had indicated that the effects of the CLPG gene were transient, in this study we evaluated the effects of age on serum creatinine and lipid concentrations.

Our results document that the CLPG gene causes an age-related elevation in several serum creatinine and lipid constituents and we propose that the CLPG gene offers scientists a new model to understand the regulation of serum cholesterol concentrations.

Materials and Methods

Ten homozygous (c/c) Polypay ewes were mated to heterozygous (C/c) Dorset rams and 20 offspring were obtained, of which 13 were females and 7 were males. Lambs were born over a 16-d period (February 2 through February 20, 1994) and were raised at the Oregon State University Sheep Facility with their dams until weaning at approximately 3 mo of age. Male lambs were castrated by elastrator bands applied within 24 h of birth. Following weaning, lambs were allowed to graze in a common pasture and provided access to a commercial lamb creep feed. Lambs were maintained on full feed until slaughter at a target weight of 57 kg at approximately 8 mo of age. Lambs were humanely slaughtered following USDA guidelines in three groups at approximately equal size at the Oregon State University Meat Science Laboratory. After a 72-h chill, carcasses were sectioned between the 12th and 13th rib for measurement of longissimus muscle (**LD**) area.

At monthly intervals (1, 2, 3, 4, and 5 mo and just before slaughter), blood samples (10 mL) were taken by jugular puncture using Vacutainer tubes (Becton-Dickenson, Rutherford, NJ). Blood samples were stored overnight at 4°C, after which serum samples were separated by centrifugation, divided into two sample vials, labeled, and frozen at –20°C until analysis of sera constituents was possible.

Serum creatinine concentrations at all time points were analyzed using a commercial kit (555A, Sigma Chemical, St. Louis, MO). At slaughter, measurements of the weights of various hindlimb muscles and of the cross-sectional area of the LD were evaluated as means of distinguishing between the CLPG and non-CLPG phenotypes. Finally, concentrations of TC, high-density lipoproteins (**HDL**), and serum triacylglycerol (**TG**; triglycerides) were assessed at the local Good Samaritan Hospital, which used a Hitachi/Boehringer Mannheim Model 717 Chemistry Autoanalyzer (Hitachi/Boehringer Mannheim, Indianapolis, IN). From these data, very-low-density lipoproteins (**VLDL**) were calculated as serum TG/5. Total LDL were calculated as TC-HDL-VLDL. The basis for calculating LDL and VLDL in this manner is a standard laboratory approach in general use in hospitals and clinical laboratories for this purpose (Friedewald et al., 1972). Very-low-density lipoprotein content is a constant proportion of TG up to TG concentrations of 400 mg/dL (Friedewald et al., 1972).

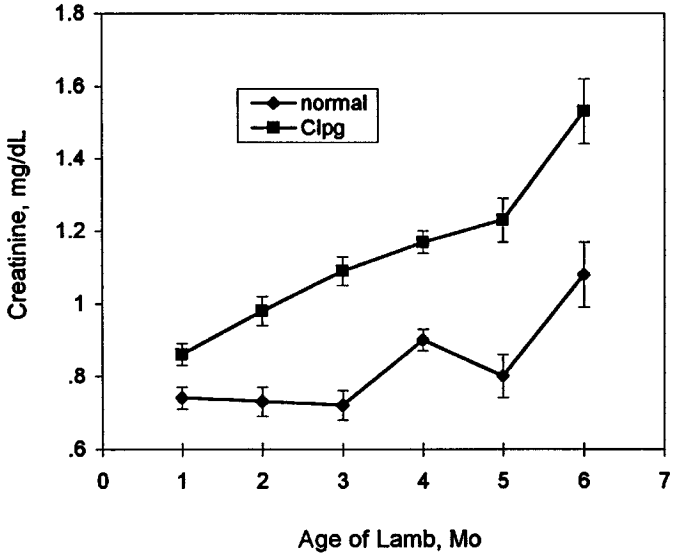

Figure 1. Effects of age and the Callipyge (Clpg) gene on serum creatinine concentrations (mg/dL). Data are least squares means ± SE.

Data were analyzed by least squares procedures (Harvey, 1987) regarding each monthly measure of each blood parameter as a separate trait (i.e., 36 data sets). All data sets were analyzed fitting lamb genotype, sex, and genotype × sex interaction as fixed effects and fitting lamb birth date as a covariate. Lamb genotype was assigned to each lamb on the basis of visual appraisal at 2, 3, and 4 mo of age; genotype was confirmed based on visual carcass appraisal, longissimus muscle area, and weight of individual dissected muscles following slaughter of lambs at uniform live weight.

Results and Discussion

We have reported the effects of the CLPG genotype on weights of various muscles and cross-sectional area of the LD in a previous manuscript (Meyer et al., 1995). These data were used to distinguish animals that were homozygotic (c/c) and heterozygotic (C/c). Cross-sectional area of the LD muscle clearly distinguished two distinct populations with mean LD areas of 13.6 ± .6 and 23.0 ± .3 cm^2 in normal and CLPG animals, respectively. In addition, however, analysis of serum creatinine concentration (Figure 1) identified the identical groups of animals as heterozygotic

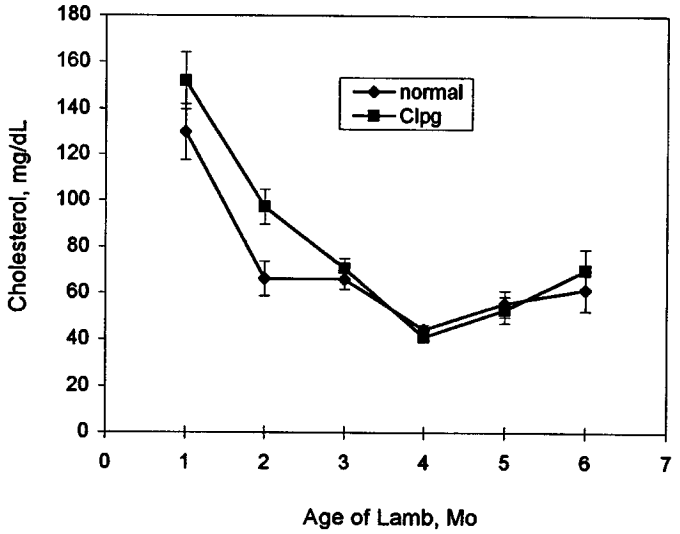

Figure 2. Effects of age and the Callipyge (Clpg) gene on serum total cholesterol concentrations (mg/dL). Data are least squares means ± SE.

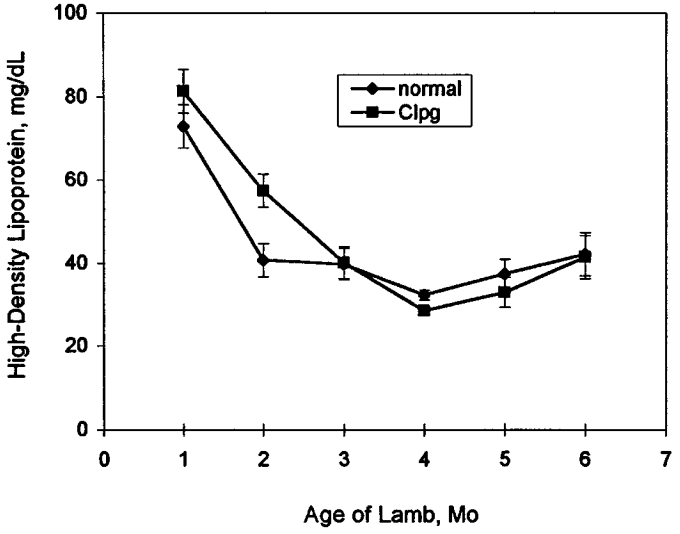

Figure 3. Effects of age and the Callipyge (Clpg) gene on serum high-density lipoprotein (HDL) concentrations (mg/dL). Data are least squares means ± SE.

and homozygotic. Specifically, creatinine concentrations were significantly ($P < .01$) elevated 15 to 50% in heterozygotic (CLPG) lambs from 1 mo of age through to slaughter.

Based on these results we now have two criteria that may be used for identification of the CLPG phenotype: LD area and serum creatinine concentration. Serum creatinine is typically used as an index of renal function. However, in this study, serum creatinine levels in CLPG animals were not sufficiently high to indicate that renal function was impaired. Instead, we propose that the elevation in serum creatinine was due to increased muscle mass in CLPG animals. Creatinine is a product of muscle creatine metabolism (Murray et al., 1993), and urinary creatinine excretion, therefore, has traditionally been used as an index of total muscle mass (Heymsfield et al., 1983; Xue et al., 1988). It follows, therefore, that serum creatinine concentration may also be influenced by muscle mass. If serum creatinine provides an index of muscle mass, these data indicate that expression of the CLPG gene and muscle hypertrophy begin in the early postnatal period and continue through to slaughter in CLPG lambs.

Effects of the CLPG gene on serum TC are shown in Figure 2. Total cholesterol concentration was elevated by 47% in CLPG lambs at 2 mo of age ($P < .01$). Following this time, however, TC concentration was unaffected by the CLPG gene.

Effects of the CLPG gene on HDL are shown in Figure 3. High-density lipoproteins were elevated by 39% at 2 mo of age in CLPG lambs ($P < .01$); however, at 4 mo of age, the CLPG gene caused a 15% reduction ($P < .04$) in serum HDL.

Effects of the CLPG gene on VLDL concentrations are shown in Figure 4. The VLDL were elevated by 53% in heterozygotic lambs at 2 mo ($P < .01$) and by 49% at 3 mo ($P < .04$) of age; however, no differences were noted at 1 mo of age or at ages approaching slaughter.

The CLPG gene exerted major effects on serum TG concentrations (Figure 5). Total serum TG were elevated by 55% at 2 mo of age ($P < .001$) and by 47% at 3 mo of age ($P < .05$). Total serum LDL were unaffected ($P > .05$) by the CLPG gene locus at any of the times blood samples were taken (Figure 6).

Collectively, these data indicate that the CLPG

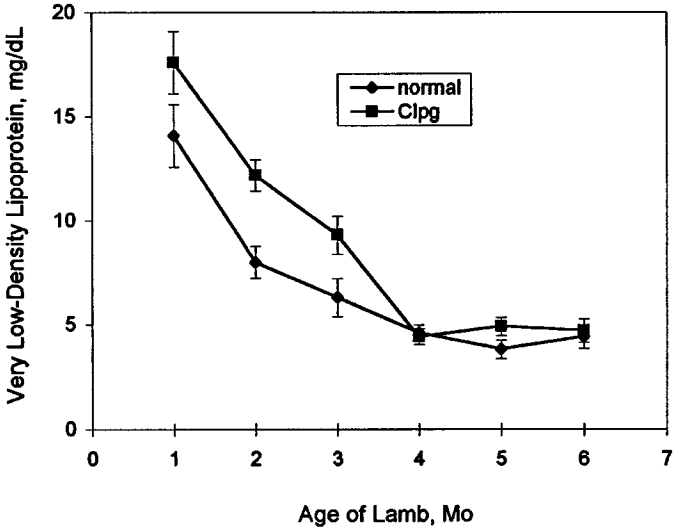

Figure 4. Effects of age and the Callipyge (Clpg) gene on serum very-low-density lipoprotein (VLDL) concentrations (mg/dL). Data are least squares means ± SE.

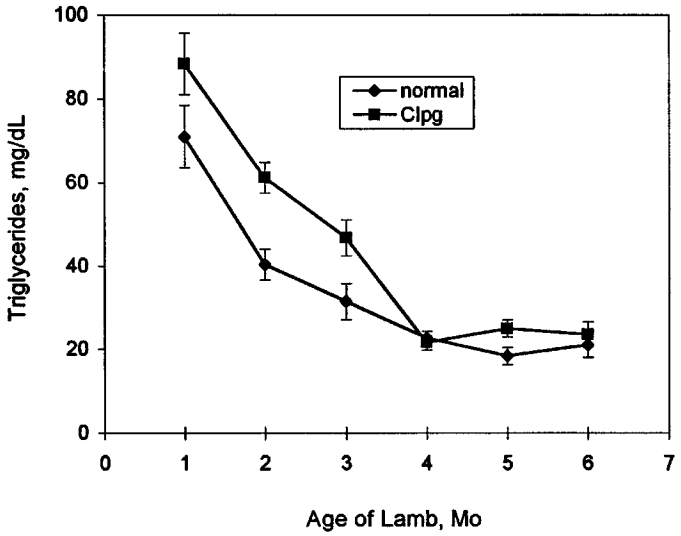

Figure 5. Effects of age and the Callipyge (Clpg) gene on serum triacylglycerol (TG) concentrations (mg/dL). Data are least squares means ± SE.

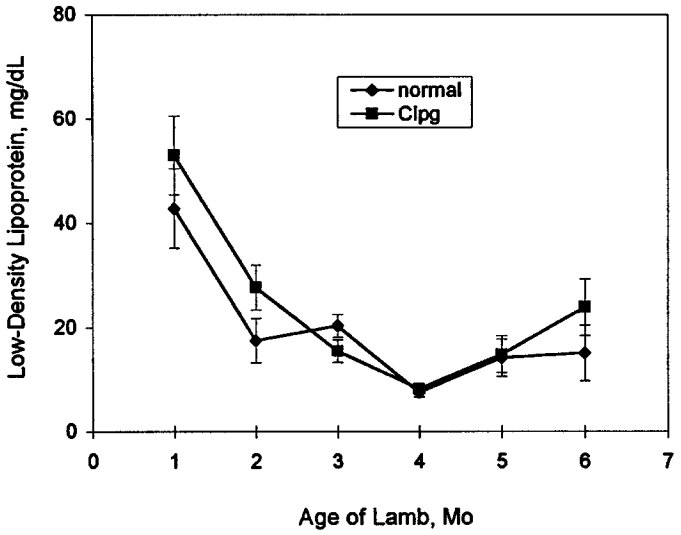

Figure 6. Effects of age and the Callipyge (Clpg) gene on serum low-density lipoprotein (LDL) concentrations (mg/dL). Data are least squares means ± SE.

gene causes major changes (39% to 55%) in serum lipid concentrations in growing lambs under 4 mo of age. Surprisingly, these effects were transient. In all cases, elevations in serum lipid constituents were detected at 2 and 3 mo of age, after which differences were not apparent. The exception to this was in our determination of LDL. Serum LDL were unaffected at any age by the CLPG gene locus.

Serum lipids provide the primary mechanism for the transport of lipids between organs of synthesis and utilization in animals. Very-low-density lipoproteins are synthesized in the liver for the export of triacylglycerol to extra-hepatic tissues (Murray et al., 1993). Low-density lipoproteins represent a final stage in the extra-hepatic catabolism of VLDL. High-density lipoproteins are involved in VLDL and chylomicron metabolism and also in cholesterol transport. A major function of HDL is to act as a repository for specific apolipoproteins required for the metabolism of chylomicrons and VLDL (Murray et al., 1993). Also, HDL facilitates the transport of extra-hepatic cholesterol back to the liver (Murray et al., 1993). Total cholesterol represents the summation of all forms of cholesterol present in the serum. In human medicine, an elevation in HDL is a desirable health-related goal because HDL concentrations are negatively correlated with coronary atherosclerosis (Murray et al., 1993). Conversely, an elevation in serum LDL concentration is interpreted negatively because there exists a positive correlation between serum LDL concentration and coronary atherosclerosis (Murray et al., 1993). Triacylglycerol was estimated for the purposes of calculating VLDL content of sera samples. By itself, its diagnostic value is limited. Generally, it is elevated in Type II and IV hyperlipoproteinemias (Friedewald et al., 1972) and, therefore, can be used to assist in distinguishing between the various types of hyperlipoproteinemias.

The elevation in serum VLDL, although significant, could not account for the significant increase in TC. However, these data indicate that either hepatic output of triacylglycerol to extra-hepatic tissues is elevated in CLPG animals or that extra-hepatic utilization of VLDLs is reduced in CLPG animals. If the latter hypothesis is correct, it is possible that reduced adipose tissue deposition and increased VLDL in CLPG lambs could be due to impaired utilization of VLDL by adipose tissue.

High-density lipoprotein concentration was increased in CLPG animals to approximately the same extent as total cholesterol. Hence, we propose that the elevation in TC in CLPG animals was mediated primarily by an increase in HDL and, to a lesser extent, by an increase in VLDL.

Of interest is that the elevations in blood lipid constituents were transient. Differences were noted primarily at 2 and 3 mo of age after which differences were not apparent. These data suggest that the CLPG gene is regulated in a transient, developmental manner.

In conclusion, we found that CLPG lambs may be distinguished from normal lambs on the basis of serum creatinine concentrations. Using this and LD area to identify heterozygotic animals, we determined that CLPG lambs had higher concentrations of serum lipids (total cholesterol, HDL, VLDL, and TG) at 2 and 3 mo of age. Differences in these parameters were not detected in older lambs. The elevation in total cholesterol was due primarily to an increase in HDL and, to a lesser extent, to an increase in VLDL.

The limitation of this study is that we were unable to use existing genetic marker tests for identification of lambs harboring the Callipyge gene because we used multiple rams as sires in this study. However, the extreme hypertrophy of Callipyge animals, particularly in the loin, provided an unambiguous and unequivocal method of ascertaining lamb genotype.

Implications

One of the challenges of working with Callipyge (CLPG) animals is that it has not been possible to unequivocally identify animals expressing the CLPG phenotype until slaughter. Although visual appraisal and scoring of hypertrophy in live animals is useful, in our experience we did encounter some situations in which animals we scored as CLPG were, in fact, normal. Similarly, we also encountered situations in which we scored animals as normal and later determined them to possess the CLPG phenotype. On the basis of this research, CLPG animals may be distinguished more reliably from non-CLPG animals based on serum creatinine concentrations. This provides a rapid, non-invasive means for identifying CLPG lambs in the early postnatal period. This report demonstrates that expression of the CLPG phenotype affects not only parameters of animal growth but also affects serum lipid profiles. The CLPG lambs had elevated levels of total cholesterol, high-density lipoproteins, very-low-density lipoproteins, and triacylglycerol compared to normal lambs. These elevations were detected in lambs at 2 and 3 mo of age but not in older lambs. Hence, the CLPG gene exerts its effects on serum lipid profiles in an age-dependent manner.

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