Branched-Chain Amino Acid Aminotransferase Activity Decreases during Development in Skeletal Muscles of Sheep

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ABSTRACT The catabolism of branched-chain amino acid (BCAA) differs between sheep and monogastric animals. The transamination of BCAA seems to be affected by development of the sheep. We studied the developmental changes in the activity and expression of the BCAA aminotransferase (BCAT) isoenzymes in skeletal muscle of sheep. Five muscles were taken from fetus, newborn, preruminant and ruminant lambs. BCAT specific activity and the contribution of each BCAT isoenzyme (mitochondrial and cytosolic (BCATm and BCATc, respectively)) were quantified using radioenzymatic and immunoprecipitation assays. BCATm and BCATc mRNAs were assessed by real-time reverse transcription–polymerase chain reaction. BCAT specific activities were 62% (diaphragma) to 83% (longissimus dorsi) lower in the ruminant lamb than in the fetal sheep. BCATm and BCATc were both expressed in sheep skeletal muscle at all developmental stages. BCATc was mainly responsible for the developmental decrease in BCAT specific activity. BCATc specific activities were 77% (diaphragma) to 92% (longissimus dorsi) lower in the ruminant lamb than in the fetal sheep, whereas BCATm specific activities were only 36% (semimembranosus) to 56% (longissimus dorsi) lower. BCATc and BCATm mRNAs in the longissimus dorsi were not affected by development of the sheep. The developmental decrease in BCATc activity, and to a lesser extent in BCATm activity, probably involves posttranscriptional mechanisms in sheep. The present results are consistent with lower in vivo metabolism of BCAA in ruminant than in the fetal sheep.


KEY WORDS: • branched-chain amino acid aminotransferase • development • sheep • skeletal muscle

The first step in the catabolic pathway of the branched-chain amino acids (BCAA), leucine, isoleucine and valine, is the reversible transamination catalyzed by BCAA aminotransferase (BCAT), EC 2.6.1.42. The products of transamination are branched-chain α-ketoacids (BCKA), α-ketoisocaproate (KIC), α-keto-β-methylvalerate (KVM) and α-ketoisovalerate (KIV), respectively. We recently cloned the sheep cDNA of mitochondrial BCAT (BCATm) (Faure et al. 1999) and cytosolic BCAT (BCATc) (Bonfils et al. 2000). The second step is irreversible oxidative decarboxylation of BCKA catalyzed by the mitochondrial BCKA dehydrogenase enzyme complex (EC 1.2.4.4; Harris et al. 1997) forming the branched-chain acyl-CoA derivatives. This step commits the BCAA carbon skeleton to the degradation pathway. A unique feature of BCAA metabolism in mammals is its tissue specificity; most indispensable amino acids are degraded in the liver, whereas BCAA are metabolized extensively in extrahepatic tissues (Harper et al. 1984). BCAA contribute to the synthesis of dispensable amino acids (alanine, glutamine) that participate in interorgan exchanges of nitrogen within the body (Harper et al. 1984).

For the past four decades, the regulation of BCAA catabolic enzymes has been studied extensively in monogastric animals and in humans. Investigations of BCAA catabolism in ruminants are scarce, but there is converging evidence suggesting that BCAA catabolism is unique in ruminants. Indeed, several indirect measures of evidence, including arteriovenous differences in blood amino acid concentrations, whole body amino acid fluxes, in vitro BCAA oxidation measurements and enzymatic assays, support the hypothesis that BCAA catabolism is low in ruminant muscle (Papet et al. 1992, Teleni 1993). The reported values for BCAT specific activity in ruminant tissues were lower than those in rats (Bergen et al. 1988, Goodwin et al. 1987, Papet et al. 1988, Wijayasinghe et al. 1983). We recently showed that the expression of BCAT (mRNA and activity) in skeletal muscle, which is the primary site of BCAA transamination (Papet et al. 1988), was much lower in sheep than in rats (Faure et al. 1999). Studies carried out at several developmental stages (fetus, growing lamb, adults and pregnant ewes) suggest that BCAT activity decreases during development (Bergen et al. 1988, Faure et al. 1999, Goodwin et al. 1987, Liechty et al. 1987, Papet et al. 1988). Such a phenomenon does not occur in rats (Cappuccino et al. 1978, Kadowaki and Knox 1982). The present study...
was undertaken to determine the biochemical basis responsible for the decrease in BCAT activity in skeletal muscle during the development of sheep.

MATERIALS AND METHODS

Sheep and collection of blood and tissues. Male Limousine × Romanov × Ile de France lambs from a herd at Institut National de la Recherche Agronomique Clermont-Ferrand-Theix Center were studied at different developmental stages: fetus, newborn, preruminant and ruminant (Table 1). Three pregnant ewes were slaughtered at 141 d of gestation (term is 147 d), and the fetuses were removed immediately from the uteruses. Newborn, preruminant and ruminant lambs were born through noninduced births and were allowed to stay with their dams for 1 d. Six newborn lambs were killed the day after birth. Lambs included in the preruminant group were transferred into individual pens in the animal care facility of the center. They were fed twice daily and allowed free access to food. The diet was an artificial diet containing 60% milk (Agnodor; Sanders, Athos Mons, France). The composition of powdered diet (96 g/100 g dry matter) was 240 g crude protein, 240 g fat, 5 g cellulose and 75 g ash per kg, and the gross energy was 22 MJ/kg. Lambs included in the ruminant group were initially reared in the barn, where they had free access to the commercial milk, a commercial concentrate and hay. When they were ~38 d old (12 kg), they were transferred to individual pens in the animal care facility of the center and weaned through the complete withdrawal of milk. They were fed only hay (7.5 MJ metabolized energy and 77 g crude protein per kg) and a commercial concentrate (11.1 MJ) containing 176 g protein and 175 g fat per kg, Ucabcè; Lapeyrouse, France) for ~3 wk. Experiments complied with the Guide for the Care and Use of Laboratory Animals (National Research Council 1985). All sheep were anesthetized with pentobarbital injection and then exsanguinated. Blood was collected from each sheep and the sample. Protein was determined using the Bio-Rad protein assay kit.

BCAT activity assay. BCAT activity assays were performed as previously described (Faure et al. 1999). Substrates used in the present enzymatic assay were isoleucine (12 mmol/L) and [14C]KIC (0.5 mmol/L). The α-ketoacid specific radioactivity was ~350 dpm/mmol. Linearity with respect to incubation time and amount of extract used in the assay was established for each type of muscle sample. Protein was determined using the Bio-Rad protein assay kit (Richmond, CA) with bovine serum albumin as a standard. A unit of BCAT activity was defined as 1 nmol of BCAA formed/min at 37°C. BCAT specific activity was expressed as nmol/(min · mg protein).

Immunoprecipitation experiment. An antiserum raised against the sheep BCATm isoenzyme was used (Faure et al. 1999). Sheep tissue homogenates were adjusted to 0.5 mol NaCl/L, and immunoprecipitation was conducted as described elsewhere (Hall et al. 1993). The volume of antiserum needed to quantitatively neutralize BCATm was determined to be 25 µL per 200 µL of homogenate (see Results). The controls used preimmune serum. The BCATm specific activity was calculated from the percentage of immunoprecipitated activity for each sample. The BCATc specific activity was obtained by the difference between BCAT and BCATm specific activities. The accuracy of this methodology was confirmed by comparing these results with quantification of BCATc and BCATm activity after their separation using ion exchange chromatography (see Results).

Ion exchange chromatography. A 100,000 × g supernatant obtained from 2.5 g of fetal longissimus dorsi was applied to Fractogel EMD DEAE 650 (S) ion exchange column (1 × 10 cm; Merck, Darmstadt, Germany) equilibrated with 10 mmol potassium phosphate/L buffer (pH 7.5) containing 1 mmol dithiothreitol and 1 mmol benzamide per L (Faure et al. 1999). After the column had been washed with the equilibrium buffer, BCAT activity was eluted by application of 0–1 mmol/L NaCl linear gradient in the latter buffer. BCAT activity was quantified in the chromatographic fractions as above. Chromatography was carried out at 4°C. The column was attached to an L-6200 Intelligent Pump HPLC system equipped with an L-4200 UV Detector and an L-5200 Fraction Collector (Merck).

RNA isolation and real-time quantitative reverse transcription polymerase chain reaction (PCR). Total RNA was isolated by extraction with guanidinium isothiocyanate (Chomczynski and Sacchi 1987). Single-stranded cDNAs were obtained through reverse transcription of µg total RNA using the Superscript preamplification system (Life Technologies, Gaithersburg, MD). The levels of BCATm and BCATc mRNAs were determined by real-time quantitative PCR using the fluorescence-based TaqMan methodology and a 7700 Sequence Detector System (PE Biosystems, Courtabœuf, France) (Heid et al. 1996, Holland et al. 1991). The amplification and product-reporting system used is based on the 5’ to 3’ exornucleic activity of the Tag DNA polymerase. In addition to the two amplification primers, as in conventional PCR, a dual-labeled fluorogenic hybridization probe is also included. One fluorescent dye serves as a reporter, and its emission is quenched by a second fluorescent dye. During the extension phase of PCR, the reporter is cleaved from the probe thus released from the quencher, resulting in an increase in fluorescent emission. The intensity of the fluorescent emission is plotted versus PCR cycle number to generate an amplification curve for each sample. The fractional cycle number at which the fluorescence is higher than a fixed threshold is defined as the threshold cycle (Ct). The standard procedure was performed with 5 µL cDNA diluted 6.7 times. Amplification primers and TaqMan probes were designed based on the 3’ end regions of sheep BCATm and BCATc cDNA sequences (Fig. 1; GenBank accession numbers AF050173 (Faure et al. 1999) and AF184916 (Bonfils et al. 2000), respectively) using Primer Express Oligo Design (version 1.0; PE Biosystems). The sense and antisense primers for amplification of BCATm were 5’-TGGCAATATGGAAGTAA-GAACGGG-3’ and 5’-GGCCAAGAAGCCTGGGT-3’ respectively (Oligo Express, Paris, France), and the TaqMan BCATm probe was FAM-5’-ACCCGGGAGCCTGGTAGCTTC-3’-TAMRA (Eurogentec, Liège, Belgium). The sense and antisense primers for amplification of BCATc were 5’-GGACAACTACGAGCTGTCG-3’ and 5’-CTAACATGAGCAGGACACCATGTT-3’ respectively (Oligo Express), and the TaqMan BCATc probe was VIC-5’-TGTTGGCCGTTGGGACACAGTGTG-3’-TAMRA (PE Biosystems). The cycling conditions included 2 min at 50°C, 10 min at 95°C for cycling and then 1 cycle at 95°C for 15 s and 60°C for 1 min.

### TABLE 1

<table>
<thead>
<tr>
<th>Characteristics of the sheep1</th>
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<tr>
<td>n</td>
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<tr>
<td>Age, d</td>
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<tr>
<td>Weight, kg</td>
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<tr>
<td>Growth rate, g/d</td>
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<tr>
<td>1 Values are means ± se; n = 5 or 6.</td>
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<tr>
<td>2 The age of the fetus is the difference between the term and the day of the collection of fetuses.</td>
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<tr>
<td>3 NM, not measured.</td>
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Skeletal muscle chromatography. All BCAT activity found in the 100,000 × g supernatant obtained from sheep longissimus dorsi was retained by the ion exchange Fractogel EMD DEAE column. Two peaks of BCAT activity eluted from the column at 150 and 400 mmol NaCl/L, indicating that the two BCAT isoenzymes are expressed in skeletal muscle from sheep (Fig. 2). The peak eluting at the lowest salt concentration corresponded to BCATm, because the sheep placental BCATm activity eluted at 150 mmol/L when the same protocol was performed (Faure et al. 1999). In agreement with the chromatographic data obtained for rat BCAT isoenzymes (Hall et al. 1993, Wallin et al. 1990), sheep BCATc activity eluted at the highest salt concentration.

Immunoprecipitation validation. The antiserum used to immunoprecipitate BCATm was raised against purified sheep BCATm (Faure et al. 1999). As shown in Fig. 3, the addition of increasing volumes of the antiserum to 200 µL sheep placenta homogenate, which exhibits only BCATm, resulted in a gradual neutralization of the enzyme. Complete neutralization was obtained with 20 µL of antiserum. Then, 25 µL of antiserum were used in all further assays to ensure quantitative immunoprecipitation of BCATm. Using homogenates of sheep longissimus dorsi in which both isoenzymes are expressed, we verified that the antiserum immunoprecipitated BCATm specifically and accurately. Indeed, the percentage of BCAT activity neutralized by the antiserum (25.8 ± 3.4, n = 3) was the same as the percentage of BCAT activity recovered in the BCATm peak from DEAE ion exchange chromatography (24.7 ± 3.4, n = 3, Fig. 2). Thus, whether BCAT isoenzymes are in the native conformation, the antiserum is specific for BCATm, and the immunoprecipitation method accurately determines the relative contribution of the two BCAT isoenzymes.

BCAT specific activity. Muscle type and developmental stage affected the specific activity of BCAT with a significant interaction between the two variables (Fig. 4). The ranking

- **RESULTS**

- **Statistical analysis.** Statistical analyses were performed using StatView (version 5.0; SAS Institute, Cary, NC). The level of significance was set at P < 0.05. Log-transformed BCAT activity data, which had homogeneous variances, were analyzed by one- or two-way (muscle type × developmental stage) ANOVA. When a significant effect was detected, differences among groups were compared by the protected least significant difference Fisher test. The effect of the developmental stage on plasma levels and C2 data were evaluated with the nonparametric Kruskal-Wallis test, and multiple comparison of means was performed with the nonparametric Mann-Whitney U test.

- **FIGURE 1** Partial nucleotide sequence of sheep mitochondrial and cytosolic branched-chain amino acid aminotransferase (BCATm and BCATc, respectively) from sheep skeletal muscle. The fetal longissimus dorsi supernatant was loaded onto the Fractogel EMD DEAE 650 (S) column. BCATm and BCATc activities were eluted at 150 and 400 mmol NaCl/L, respectively.

- **FIGURE 2** Chromatographic separation of mitochondrial and cytosolic branched-chain amino acid aminotransferase (BCATm and BCATc, respectively) from sheep skeletal muscle. The fetal longissimus dorsi supernatant was loaded onto the Fractogel EMD DEAE 650 (S) column. BCATm and BCATc activities were eluted at 150 and 400 mmol NaCl/L, respectively.
order of the muscles, starting with the highest BCAT specific activity, was roughly as follows: DIA, LD, SM, TFL and ST. DIA exhibited a significantly higher activity than SM, TFL and ST at all developmental stages, whereas differences between DIA and LD were significant only in preruminant and ruminant sheep. BCAT specific activity was higher in LD than in ST at all stages of development; whereas SM and TFL activity levels did not differ, except in ruminants. In all muscles, BCAT specific activity decreased during development. Differences among the four developmental stages were significant in all muscles studied, except that the DIA and ST data from the newborns did not differ from their respective values in the fetal and ruminant sheep. Further ST data from the newborn and fetal sheep did not differ. From fetal to ruminant stages, BCAT specific activity decreased significantly by 92, 88, 83 and 77% in LD, TFL, SM, ST and DIA, respectively.

Muscle BCAT capacity. The BCAT capacity of each muscle [nmol/(min · 100 g body)] was evaluated by taking into account the contribution of the weight of each muscle to the body weight and the BCAT specific activity (Fig. 5). Developmental stage affected the BCAT capacity for all muscles studied, except ST. DIA, LD, SM and TFL capacities were significantly lower in ruminant sheep than in sheep at other developmental stages. TFL capacity was significantly lower in the preruminant than in the fetal and newborn sheep. Surprisingly, LD capacity was lower in the newborn than in the fetal and ruminant sheep. The sum of the capacities of the five muscles was also affected by the developmental stage; it was ~60% lower in the ruminant sheep than in the younger developmental stages.

BCATc and BCATm mRNAs. The quantification of BCATc and BCATm mRNAs was carried out for LD because it exhibited the greatest decrease in specific activity of both BCAT isoenzymes during development. The levels of BCATc and BCATm mRNA did not change in LD during the development of sheep (Table 2). In addition, there was no correlation between the activity of each BCAT isoenzyme and its respective mRNA level. The mRNA measurements were conducted using the same conditions for BCATc and BCATm, and the PCR efficiencies were the same. The fact that the CT
values were similar for both isoenzymes indicated that the levels of the two mRNAs were in the same range.

**Plasma BCAA and BCKA.** With the exception of leucine, developmental stage affected plasma BCAA and BCKA concentrations (Fig. 6). Plasma concentrations of valine, leucine and isoleucine were 140, 100 (not significant due to high variability, \( P = 0.06 \)) and 50% (\( P = 0.26 \)) higher in the newborn than in the fetal sheep, respectively, and returned to their respective fetal values in growing sheep. Similarly, plasma concentrations of KIV, KIC and KMV were 240, 246 and 126% (\( P < 0.05 \)) higher in the newborn than in the fetal sheep, respectively, and returned to their respective fetal levels in both preruminant and ruminant sheep. However, plasma valine and KMV concentrations were significantly higher in the ruminant than in the preruminant sheep.

**Plasma metabolites and hormones.** Developmental stage affected plasma concentrations of glucose, lactate, nonesterified fatty acids, insulin, cortisol and T3 (Table 3). Plasma concentration of glucose was low in fetal sheep, was 261% greater in the newborn and was maintained at this level in the preruminant and ruminant sheep. In contrast, plasma lactate level was high in the fetal sheep and was 62% lower in the newborn and older sheep. Plasma free nonesterified fatty acid concentration increased by 13-fold at birth and then declined progressively to reach the fetal level in the ruminant sheep. Plasma concentrations of insulin, cortisol and T3 were 310 (not significant due to high variability, \( P = 0.08 \)), 231 and 487% higher in newborn compared with fetal sheep, respectively. Insulin levels were maintained at a high level in the preruminant and ruminant sheep, whereas cortisol and T3 decreased. Variations in plasma metabolites and hormones were consistent with the nutritional and physiological modifications occurring in ruminant species, but there were no correlations between plasma concentrations of any metabolite or hormone, and BCAT activities.

**DISCUSSION**

Here we present the first in-depth investigation of the developmental changes in BCAT activity in five sheep skeletal muscles of different metabolic types. The activity of BCAT was high in the most oxidative muscle (DIA), intermediate in LD and low in SM, ST and TFL (the most glycolytic muscle). This is consistent with data obtained in rats (Yang and Birkhahn 1997), but the reason for such a difference is unknown. In all muscle types, the specific activity of BCAT decreased during the development of sheep, as previously reported (Bergen et al. 1988, Goodwin et al. 1987).

Our results obtained using complementary techniques established that sheep differ from rats and humans. Indeed, sheep skeletal muscle expresses both BCAT isoenzymes, whereas BCATm is the sole isoenzyme expressed in skeletal muscle of rats and humans (Hutson et al. 1992, Suryawan et al. 1998). The DEAE chromatography of a homogenate of sheep muscle clearly showed two peaks corresponding to the two BCAT isoenzymes. The simultaneous expression of these BCAT isoenzymes was confirmed by the presence of BCATc and BCATm mRNA in sheep LD. The reason for such a species difference is unknown, but differences between monogastric and ruminant promoters and/or tissue specific transcription factors may be involved. The pattern of expression of BCAT isoenzymes in sheep muscle raises questions concerning their physiological roles. BCKA formed in the cytosol could either be released from the muscle or be transferred to and then decarboxylated in the mitochondria. BCKA formed in the mitochondria could be directly decarboxylated or transported out of the mitochondria.

It is important to note that the two BCAT isoenzymes do not change exactly the same during the development of sheep. That means that the decrease in their activities is not due to a passive dilution due to increases in the size and protein content of muscle fibers but rather due to specific regulatory mechanisms. Variation in BCAT activity reflects variation in BCAT protein (no regulation of activity at the protein level has been ever described), so activity is dependent on the rates of synthesis and degradation of the protein.

The specific activity of BCATc decreases at each developmental step and is almost quantitatively responsible for the decrease of BCAT activity. The mechanism involved appears to be independent of its mRNA level (the results obtained for LD are likely valid for the other muscles). The translation of BCATc may be decreased and/or its degradation may be increased in skeletal muscles during the development of sheep.

### TABLE 2

Real-time reverse transcription—polymerase chain reaction for BCATm and BCATc mRNA in longissimus dorsi at different developmental stages in sheep

<table>
<thead>
<tr>
<th></th>
<th>Fetus</th>
<th>Newborn</th>
<th>Preruminant</th>
<th>Ruminant</th>
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</thead>
<tbody>
<tr>
<td>BCATm(^1)</td>
<td>27.84 ± 0.15</td>
<td>27.86 ± 0.22</td>
<td>27.97 ± 0.11</td>
<td>27.86 ± 0.14</td>
</tr>
<tr>
<td>BCATc</td>
<td>29.54 ± 0.46</td>
<td>28.71 ± 0.81</td>
<td>28.46 ± 0.21</td>
<td>29.32 ± 0.20</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SE, \( n = 4–6 \), of CT (threshold cycle, defined as the fractional cycle number at which the fluorescence exceeded a threshold limit).

\(^2\) BCATm and BCATc, mitochondrial and cytosolic branched-chain amino acid aminotransferase, respectively.
Mechanisms involved in these putative regulatory points are unknown. The metabolic importance of the observed reduction of BCATc activity during the development of sheep is unclear. In rats and humans, BCATc is mainly expressed in brain. BCATc is involved in BCAA catabolism, but it may be involved also in other cellular processes related to cell cycle and apoptosis (Eden and Benvenisty 1999, Khodorov et al. 2000). The existence of such a function for sheep BCATc in muscle fibers is questionable.

Based on the results obtained for LD, developmental variations in the specific activity of BCATm probably are not due to BCATm mRNA variations. Therefore, the points of regulation of BCATm likely are posttranscriptional as for BCATc. However, the mechanisms are probably specific to each isoenzyme because the variations in BCATm and BCATc activity follow different patterns. We have previously shown that in sheep, the high BCAT activity observed in placenta is associated with a higher level of BCATm mRNA than in muscle (Faure et al. 1999). Rats adapted to a 50% casein diet exhibit higher BCAT activity in muscle, kidney and brain than rats fed 6, 18 or 35% casein diets, but only muscle had a significantly higher level of BCATm mRNA (Torres et al. 1999). Lactation induces an increase in the expression of the BCATm gene in the mammary gland of rats (DeSanti et al. 1996). Taken together, these results indicate that the regulation of both rat and sheep BCATm is tissue specific and may involve transcriptional and posttranscriptional mechanisms. The signaling is not known, and in the present study, none of the metabolites and hormones measured seemed to be involved.

Despite a gradual decrease in the specific activity during the development of sheep, the BCAT capacity of the muscles studied decreased only at the ruminant stage. Considering that all the skeletal muscles combined account for 40% of body weight, the whole body muscle BCAT capacity would be ~4.8 μmol/(min · 100 g body) in the newborns through preruminants and only 1.8 μmol/(min · 100 g body) in ruminants. In vivo, both deamination and reamination occur, but this futile cycle is less intense in the ruminant, except at the fetal stage, than in monogastric animals. This is consistent with the fact that in food-deprived sheep, the amount of alanine released from the hindlimb muscle is much lower than values reported for humans (see Teleni 1993). Only 34–42% of metabolized leucine is transaminated to KIC and 6–18.5% of metabolized KIC is reaminated to leucine in preruminant or ruminant sheep (Nissen and Ostaszewski 1985, Oddy and Lindsay 1986, Pell et al. 1986), whereas these values reach 50–80% and 60–90%, respectively in humans, pigs or dogs (Helland et al.

### TABLE 3

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Fetus</th>
<th>Newborn</th>
<th>Preruminant</th>
<th>Ruminant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>1.6 ± 0.5c</td>
<td>5.9 ± 1.4ab</td>
<td>6.7 ± 0.2a</td>
<td>5.6 ± 0.2b</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>11.7 ± 0.9a</td>
<td>4.5 ± 0.6b</td>
<td>3.6 ± 0.2b</td>
<td>2.8 ± 0.4b</td>
</tr>
<tr>
<td>NEFA, μmol/L</td>
<td>40 ± 4c</td>
<td>574 ± 634a</td>
<td>256 ± 64b</td>
<td>64 ± 9c</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>93 ± 11b</td>
<td>383 ± 136ab</td>
<td>417 ± 175a</td>
<td>243 ± 48a</td>
</tr>
<tr>
<td>Glucagon, pmol/L</td>
<td>91 ± 6</td>
<td>134 ± 18</td>
<td>130 ± 22</td>
<td>98 ± 12</td>
</tr>
<tr>
<td>Cortisol, nmol/L</td>
<td>49 ± 7b</td>
<td>163 ± 59a</td>
<td>45 ± 9bc</td>
<td>25 ± 7c</td>
</tr>
<tr>
<td>T3, pmol/L</td>
<td>4.5 ± 0.5c</td>
<td>26.3 ± 3.7a</td>
<td>19.5 ± 0.9a</td>
<td>7.8 ± 0.7b</td>
</tr>
<tr>
<td>T4, pmol/L</td>
<td>38.1 ± 4.8</td>
<td>40.7 ± 5.8</td>
<td>36.6 ± 2.8</td>
<td>30.3 ± 0.6</td>
</tr>
</tbody>
</table>

1 Values are means ± se, n = 6 (fetus and newborn) or 5 (preruminant and ruminant). Values in a row not sharing a superscript letter are different, P < 0.05.
2 NEFA, nonesterified fatty acids; T3, triiodothyronine; T4, thyroxin.

In conclusion, sheep skeletal muscle exhibits the unique expression of both BCATc and BCATm at all developmental stages. BCATc is mainly responsible for the developmental decrease in BCAT activity. The expression of both enzymes appears to be regulated at the posttranscriptional level during the development, but the mechanisms involved are unknown. The present results are consistent with a lower in vivo BCAA oxidation rate in ruminant than in the fetal sheep.


