Protein synthesis in skeletal muscle of neonatal pigs is enhanced by administration of β-hydroxy-β-methylbutyrate

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DURING THE NEONATAL PERIOD, an infant’s growth and protein turnover are higher than during any other period of life (5). The high growth rate of neonatal skeletal muscle is mainly due to the high rate of protein synthesis (5, 6). Many low-birth-weight (LBW) infants experience failure to thrive. The amino acid leucine stimulates protein synthesis in skeletal muscle of the neonate, but less is known about the effects of the leucine metabolite β-hydroxy-β-methylbutyrate (HMB). To determine the effects of HMB on protein synthesis and the regulation of translation initiation and degradation pathways, overnight-fasted neonatal pigs were infused with HMB at 0, 20, 100, or 400 μmol·kg body wt−1·h−1 for 1 h (HMB 0, HMB 20, HMB 100, or HMB 400). Plasma HMB concentrations increased with infusion and were 10, 98, 316, and 1,400 nmol/ml in the HMB 0, HMB 20, HMB 100, and HMB 400 pigs. Protein synthesis rates in the longissimus dorsi (LD), gastrocnemius, soleus, and diaphragm muscles, lung, and spleen were greater in HMB 20 than in HMB 0, and in the LD were greater in HMB 100 than in HMB 0. HMB 400 had no effect on protein synthesis. Eukaryotic initiation factor (eIF)4E·eIF4G complex formation and ribosomal protein S6 kinase-1 and 4E-binding protein-1 phosphorylation increased in LD, gastrocnemius, and soleus muscles with HMB 20 and HMB 100 and in diaphragm with HMB 20. Phosphorylation of eIF2α and elongation factor 2 and expression of system A transporter (SNAT2), system L transporter (LAT1), muscle RING finger 1 protein (MuRF1), muscle atrophy F-box (atrogin-1), and microtubule-associated protein light chain 3 (LC3-II) were unchanged. Results suggest that supplemental HMB enhances protein synthesis in skeletal muscle of neonates by stimulating translation initiation.

amino acid; protein metabolism; protein degradation; mTOR; infant

Protein homeostasis is the byproduct of a complex interplay of catabolic and anabolic pathways. Skeletal muscle serves as a reservoir of amino acids during periods of fasting and when dietary intake is unable to meet metabolic demands (11). The ubiquitin-proteasome and autophagy-lysosome pathways have been identified as being particularly important proteolytic pathways in skeletal muscle (11, 26, 41, 49). Polyubiquitination by ubiquitin ligases is the rate-limiting step of the ubiquitin-proteasome system, the two most important muscle-specific ubiquitin ligases being muscle atrophy F-box (atrogin-1) and muscle RING finger 1 protein (MuRF1). Microtubule-associated protein light chain 3 (LC3-II), a ubiquitin-like molecule, has been identified as a marker of the autophagosome (41), although increasing amounts of LC3-II may not be a direct measure of protein degradation (11).

The leucine metabolite β-hydroxy-β-methylbutyrate (HMB) has been tested for its potential effectiveness as a supplement to promote lean gain and strength in adults (19, 30). Studies suggest that HMB can mitigate protein degradation in the adult model (23, 25); however, no studies have evaluated the effects of HMB supplementation on protein synthesis and degradation in the neonatal model. Therefore, in this study we hypothesized that supplementation of HMB would 1) stimulate protein synthesis through activation of the mTOR signaling pathway, and 2) reduce protein degradation through inhibition of the ubiquitin-proteasome and autophagy-lysosome pathways in skeletal muscle of neonates.

MATERIALS AND METHODS

Animals. Four multiparous crossbred sows (Yorkshire × Landrace × Hampshire × Duroc; Agriculture Headquarters, Texas Department of Criminal Justice, Huntsville, TX) were housed, fed, and managed as previously described (15, 16). Piglets remained with the sow following birth and were studied at 5–7 days of age. Indwelling catheters were placed in the carotid artery and internal jugular under general anesthesia at 2 days of age (4). Piglets were allowed to recover

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postanesthesia and then were returned to the sow, where they remained until studied. The Animal Care and Use Committee of Baylor College of Medicine approved all experimental procedures. This study was conducted in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals.

Treatments and infusions. Prior to infusion study, piglets were removed from the sows and fed deprived for 12–14 h in a heated room (84°F). They were then randomly assigned to one of four treatment groups: HMB 0, HMB 20, HMB 100, or HMB 400 (n = 5–6/treatment group). Infusions were administered parenterally over 1 h. Piglets in the HMB 0, HMB 20, HMB 100, and HMB 400 groups received a priming infusion of either 0, 12, 60, or 240 μmol/kg body wt (BW) for 10 min prior to initiation of a 50-min infusion cycle during which they received either 0, 20, 100, or 400 μmol·kg BW⁻¹·h⁻¹ of their respective treatment. Blood samples were collected at the end of the 60-min infusion to measure blood glucose (YSI 2300 STAT Plus, Yellow Springs Instruments), insulin, HMB, amino acid, and keto acid concentrations.

Plasma and tissue HMB, α-keto acid, amino acid, and insulin concentrations. Plasma α-keto acids were analyzed by high-performance liquid chromatography (HPLC) as described elsewhere (34). Plasma HMB was analyzed by gas chromatography-mass spectrometry (GC-MS) as previously described (32). Muscle biopsies were analyzed for HMB content by a modification to the plasma HMB assay. Briefly, muscle tissue acidified homogenates were extracted with ethyl ether. The HMB was then back-extracted into 0.1 M phosphate buffer, dried, and analyzed by GC-MS. An HPLC method (PICO-TAG reverse-phase column; Waters, Milford, MA) was used for analysis of the individual plasma amino acid concentrations (2). Plasma radioimmunoreactive insulin concentrations were analyzed using a porcine insulin radioimmunoassay kit (EMD Millipore, Billerica, MA).

Tissue protein synthesis in vivo. A flooding dose of L-[4-3H]phenylalanine (1.5 mmol/kg BW, 0.5 μCi/kg; American Radiolabeled Chemicals, St. Louis, MO) was injected 30 min before organ collection to measure fractional protein synthesis rate (Ks) (21). Upon completion of the 1-h infusion, pigs were euthanized, and samples were obtained from the longissimus dorsi (LD), gastrocnemius, soleus, and diaphragm muscles, brain, duodenum, spleen, pancreas, kidney, skin, liver, left heart, lung, stomach, colon, and jejunum. Tissue samples were immediately frozen in liquid nitrogen and then stored at -70°C until analyzed as previously described (5).

Protein immunoblot analysis. Proteins were separated on polyacrylamide gel electrophoresis (PAGE). For each assay, analysis of the samples from all treatments was performed at one time on triple-wide gels (C.B.S. Scientific, San Diego, CA). Proteins were electrophoretically transferred to polyvinylidene difluoride transfer (PVDF) membranes (Pall, Jersey Village, TX), which were incubated with appropriate primary antibodies, washed, and exposed to an appropriate secondary antibody as previously described (8). For determination of the protein phosphorylations as well as the protein abundances, the data were corrected with β-actin. Primary antibodies that were used in the immunoblotting were: β-actin (Cell Signalling Technology, Danvers, MA), S6K1 Thr389 (Millipore, Bedford, MA), 4E-BP1 Thr37/46 (Invitrogen, Carlsbad, CA), eIF4A Ser75 (Cell Signalling Technology), eukaryotic elongation factor 2 (eEF2 Thr56, Cell Signalling Technology), system L transporter (LAT1; Cell Signalling Technology), MuRF1 (R&D Systems, Minneapolis, MN), (atrogin-1; ECM Biosciences, Versailles, KY), SNAT2 (Viva System Biology, San Diego, CA), and microtubule-associated protein-1 light chain 3 (LC3, Cell Signalling Technology).

Quantification of eIF4E·eIF4G complex. Anti-eIF4E monoclonal antibody (courtesy of Dr. Leonard Jefferson, Penn State University College of Medicine, Hershey, PA) was used to immunoprecipitate eIF4E·eIF4G complex from samples of fresh tissue. Amounts of eIF4G detected by antibody (Cell Signalling Technology) were corrected by the eIF4E (Cell Signalling Technology) recovered from the immunoprecipitate as previously described (14, 16, 27).

RESULTS

Plasma and tissue substrates and hormone concentrations. Plasma concentrations of HMB were significantly greater (P < 0.05) in the HMB 100 and HMB 400 group than in the HMB 0 group 1 h after the initiation of infusion (Table 1). HMB infusion increased HMB levels in the LD muscle and were greater in the HMB 400 than in the HMB 0 and 20 groups and highest in the HMB 400 group (P < 0.05). The circulating concentrations of the α-keto acids α-ketoisocaproic acid (KIC), α-ketoisovalerate (KIV), and α-ketomethylvalerate (KMV) were not altered by HMB infusion (Table 1). Infusion of HMB had no effect on the concentrations of leucine, BCAA, essential amino acids (EAA), or nonessential amino acids (NEAA) (Table 1). Plasma glucose concentrations were slightly increased (P < 0.05) in the HMB 20 and HMB 400 compared with the HMB 0 group.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>HMB 0</th>
<th>HMB 20</th>
<th>HMB 100</th>
<th>HMB 400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma HMB</td>
<td>8.8 ± 8.5</td>
<td>89.2 ± 8.5</td>
<td>316.3 ± 78.2</td>
<td>1,401.3 ± 85.6</td>
</tr>
<tr>
<td>Muscle HMB</td>
<td>30.2 ± 13.0</td>
<td>38.4 ± 15.3</td>
<td>123.3 ± 13.2</td>
<td>439.2 ± 14.2</td>
</tr>
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<td>Plasma KIV</td>
<td>21.8 ± 3.4</td>
<td>28.7 ± 3.3</td>
<td>24.3 ± 3.6</td>
<td>23.3 ± 3.1</td>
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<td>Plasma KMA</td>
<td>28.3 ± 4.3</td>
<td>37.4 ± 4.8</td>
<td>31.1 ± 4.5</td>
<td>30.0 ± 4.5</td>
</tr>
<tr>
<td>Plasma KIC</td>
<td>39.5 ± 7.3</td>
<td>44.0 ± 7.3</td>
<td>44.7 ± 6.7</td>
<td>41.8 ± 6.7</td>
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<tr>
<td>Plasma leucine</td>
<td>145 ± 15.6</td>
<td>170 ± 15.6</td>
<td>176 ± 14.2</td>
<td>160 ± 14.2</td>
</tr>
<tr>
<td>Plasma BCAA</td>
<td>558 ± 55.0</td>
<td>647 ± 58.0</td>
<td>628 ± 35.0</td>
<td>616 ± 35.0</td>
</tr>
<tr>
<td>Plasma NEAA</td>
<td>1,062 ± 97.0</td>
<td>1,188 ± 97.0</td>
<td>1,118 ± 88.0</td>
<td>1,146 ± 88.0</td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>81.5 ± 3.4</td>
<td>102 ± 3.4</td>
<td>90.0 ± 3.1</td>
<td>94.9 ± 3.1</td>
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<tr>
<td>Plasma insulin</td>
<td>1.4 ± 1.1</td>
<td>2.7 ± 1.2</td>
<td>2.2 ± 1.2</td>
<td>1.8 ± 1.1</td>
</tr>
</tbody>
</table>

Data are given as means ± SE; n = 5–6. 1Plasma and longissimus dorsi muscle concentrations in piglets infused with 0, 20, 100, or 400 μmol·kg⁻¹·h⁻¹ β-hydroxy-β-methylbutyrate (HMB) for 1 h. KIV, α-ketoisovalerate; KMA, α-ketomethylvalerate; KIC, α-ketoisocaproic acid; BCAA, branched-chain amino acids; EAA, essential amino acids; NEAA, nonessential amino acids. 2Plasma concentrations in mmol/ml. 3Skeletal muscle concentrations in mmol/g tissue. 4Plasma concentrations in mg/dl. 5Plasma concentrations in μU/ml. Labeled means in a row without a common letter differ, P < 0.05.
Plasma insulin concentrations were not significantly different among treatment (Table 1).

Protein synthesis. The fractional rates of protein synthesis in the LD, gastrocnemius, soleus, and diaphragm muscles were greater ($P < 0.05$) in the HMB 20 than in the HMB 0 baseline group (Fig. 1, A–D). Protein synthesis rate in the LD muscle was greater in the HMB 100 than in the HMB 0 group ($P < 0.05$), but in the gastrocnemius, soleus, and diaphragm muscles no differences between groups were found. Protein synthesis rates in skeletal muscles did not differ between HMB 400 and HMB 0.

In the heart, liver, stomach, duodenum, jejunum, colon, pancreas, kidney, brain, and skin, protein synthesis rates did not differ between groups (Table 2). However, protein synthesis rates in the lung and spleen were higher ($P < 0.05$) in the HMB 20 than in the HMB 0 group (Table 2).

Intracellular signaling components. Phosphorylation of S6K1 and 4EBP1 (Figs. 2 and 3, A–C) as well as formation of the active eIF4E·eIF4G complex (Fig. 4, A–C) in the LD, gastrocnemius, and soleus muscles were greater ($P < 0.05$) in the HMB 20 and HMB 100 groups than in the HMB 0 group. Infusion of HMB 20, but not HMB 100, compared with HMB 0, increased ($P < 0.05$) formation of the active eIF4E·eIF4G complex as well as the phosphorylation of S6K1 and 4EBP1 in the diaphragm (Figs. 2–4, D). Phosphorylation of eIF2a and eEF2 in the LD, gastrocnemius, soleus, and diaphragm muscles were unaffected by HMB treatment (Table 3).

The expressions of atrogin-1 and MuRF1 in the LD, gastrocnemius, soleus, and diaphragm muscles were unaffected by HMB treatment (Table 4). The LC3-II-to-total LC3 ratio in skeletal muscles was also unaffected by HMB treatment (Table 4). There was no effect of HMB on the abundance of the amino acid transporters LAT1 and SNAT2 in the LD, gastrocnemius, soleus, and diaphragm muscles (Table 5).

**DISCUSSION**

Neonates are highly responsive to anabolic stimulators such that even small variations in the systemic availability of these agents have significant effects on their muscle protein anabolism.
Amino acids, and particularly leucine, as well as insulin and glucose, have been identified as biochemical anabolic stimulants that result in increased protein synthesis in neonatal muscle (4, 7, 15, 35, 52). HMB, an active metabolite of the amino acid leucine, has been promoted as a nutritional supplement to enhance muscle mass and strength in adults (19, 30) and is commercially available enriched in nutritional supplements. However, there is a lack of evidence regarding HMB supplementation in the neonate. Since our previous work has shown that administration of either leucine (15, 16) or its α-keto acid KIC (17) can elicit a rapid increase in muscle protein synthesis in neonatal pigs, we hypothesized that HMB administration would induce a similar positive effect. The results in the current study reveal that HMB alone augments skeletal muscle protein synthesis in neonates in association with the activation of mTOR signaling.

In the present study, parenteral infusion of HMB stimulated protein synthesis in skeletal muscle of fasted animals compared with fasted controls. HMB is produced endogenously via leucine degradation in both animals and humans (31). Leucine supplementation to achieve circulating concentrations within the physiological postprandial range results in an increase in skeletal muscle protein synthesis (15). Leucine is unique in its ability to act as a nutrient signal that activates translation initiation (15), as supplementation with the other BCAA valine and isoleucine do not stimulate skeletal muscle protein synthesis (16). In the current study, 1-h infusion of HMB resulted in increased protein synthesis, similar to that in neonatal pigs infused with leucine (15, 17). Physiological levels of HMB typically range from 1 to 4 μmol/l but can increase 5- to 10-fold after leucine is fed (31, 33). In the current study, we achieved correspondingly higher circulating HMB levels that at our HMB 20 and HMB 100 doses were similar to those achieved in HMB supplementation studies in humans (54), but with leucine concentrations remaining in the fasting range. The maximal increase in protein synthesis in muscle was achieved at circulating HMB levels close to 100 nmol/ml (HMB 20), with higher circulating concentrations of ~300 nmol/ml (HMB 100) not having an additional effect. Interestingly, protein synthesis approached that seen in controls when circulating concentrations exceeded 1,000 nmol/ml at the HMB 400 dose. These results are supportive that HMB can be considered an alternative to leucine to augment muscle protein synthesis in the neonate, but HMB may be ineffective at very high doses.

In adult animals and humans, HMB has been extensively evaluated alone and in combination with other amino acids, with and without exercise, as a supplement to augment lean body mass and function (23, 25, 30, 31, 33, 37, 50, 54). Previous studies from our laboratory have shown that raising to fed levels either amino acids (7) or KIC (17) can independently stimulate skeletal muscle protein synthesis. In our study, muscle protein synthesis increased in response to HMB despite that circulating amino acids and KIC concentrations remained in the fasting range and were not altered during the 1 h HMB infusion. Since HMB is produced via transamination of leucine to KIC followed by irreversible KIC oxidation (31, 39, 48), an
increase in KIC would not be expected in the current study. Although a slight increase in circulating glucose was noted in the HMB 20 and HMB 400 treatment groups, it is unlikely that these small changes in glucose concentrations contributed to the increase in protein synthesis, as HMB 400 infusion did not affect protein synthesis. On the basis of these results, we conclude that the increase in protein synthesis in response to HMB administration was not elicited by changes in amino acids, KIC, or glucose. In agreement with these findings, recent studies have shown that enteral supplementation of the free-acid form of HMB resulted in an increase in protein synthesis in young men (50) and that HMB supplementation of previously untrained males during resistance exercise training demonstrated a tendency to increase strength and lean body mass compared with placebo (30). Furthermore, in vitro HMB supplementation has been shown to stimulate protein synthesis in murine myotubes (12). In our study, circulating HMB levels of 100 nmol/ml increased protein synthesis in both glycolytic and oxidative skeletal muscles and in the diaphragm (a muscle of mixed composition). However, HMB supplementation did not alter protein synthesis in heart muscle after 60 min of exposure. Previously, we reported a stimulatory effect of acute parenteral leucine on protein synthesis in young men (50) and that HMB supplementation of previously untrained males during resistance exercise training demonstrated a tendency to increase strength and lean body mass compared with placebo (30). Furthermore, in vitro HMB supplementation has been shown to stimulate protein synthesis in murine myotubes (12). In our study, circulating HMB levels of 100 nmol/ml increased protein synthesis in both glycolytic and oxidative skeletal muscles and in the diaphragm (a muscle of mixed composition). However, HMB supplementation did not alter protein synthesis in heart muscle after 60 min of exposure. Previously, we reported a stimulatory effect of acute parenteral leucine on protein synthesis in the heart (16); however, the response to leucine was less profound in the heart than in skeletal muscle and could reflect differences in the tissues’ sensitivity to anabolic agents.

While leucine and HMB have been demonstrated to stimulate skeletal muscle protein synthesis, less is known regarding their effects on visceral tissues. Although enteral leucine supplementation has been shown to stimulate protein synthesis in visceral organs of neonatal (29, 45) and weaned pigs (53), acute parenteral administration of leucine does not stimulate protein synthesis in liver (15, 44). In the present study, HMB did not affect protein synthesis in visceral tissues, except for the lungs and the spleen in the HMB 20 and HMB 100 groups. Longer-term studies are needed to identify whether there is an effect of HMB on protein synthesis in visceral organs of the neonate.

In this study, the increase in skeletal muscle protein synthesis in response to HMB was associated with activation of mTOR-dependent translation initiation factors, as has been previously described for leucine (14–17). Infusion of HMB for 1 h resulted in augmented phosphorylation of S6K1 and 4EBP1 and increased abundance of the active eIF4E·eIF4G complex, events consistent with leucine’s effects in skeletal muscle (14, 15, 43). The maximal increase in mTOR signaling was achieved at the HMB 20 dose, with the HMB 400 dose having no effect. Stimulation of mTOR-dependent translation by HMB has been shown recently in humans (50) and can be antagonized by the mTOR inhibitor rapamycin in myotubes (12).

Similarly to our previous study using short-term administration of leucine (44, 45), HMB supplementation had no effect on the phosphorylation of the tRNA-ribosome binding regulator eIF2α in skeletal muscle. Likewise, in the current study, HMB did not change the phosphorylation of eEF2, which regulates peptide elongation, consistent with our previous work.
with leucine in healthy neonatal pigs (44, 45, 51). In contrast, the phosphorylation of eEF2 has been shown to be attenuated by leucine in normal humans (9, 22), whereas HMB has been shown to antagonize the increase in the phosphorylation of eIF2α and eEF2 induced by cachectic stimuli in myotubes (12), suggesting a mechanism by which HMB attenuates depression of protein synthesis in catabolic settings.

The amino acid transporters SNAT2 and LAT1 modulate amino acid entry inside the cell and have been implicated in mTOR activation (46). Silencing of SNAT2 has been associated with decreased cellular levels of glutamine and leucine and thus linked to decreased mTOR activity and protein synthesis (18). Likewise, LAT1 abundance has been demonstrated to be positively associated with mTOR activation (42). In the current study, HMB did not affect SNAT2 and LAT1 abundances. The lack of effect of HMB on SNAT2 and LAT1 expression may be due to the short study period. Further investigation into the role of amino acid transporters and their effect on protein synthesis in response to HMB is thus certainly warranted.

In the current study, we did not identify effects of HMB on indexes of the ubiquitin-proteasome degradation pathway, the ubiquitin ligases atrogin-1 and MuRF1, or a marker of the autophagy-lysosome system, the LC3-II/total LC3 ratio.

![Fig. 4. Abundance of eIF4E-eIF4G in LD, gastrocnemius, soleus, and diaphragm muscles of piglets infused with 0, 20, 100, or 400 μmol·kg⁻¹·h⁻¹ HMB for 1 h. Values are means ± SE; n = 5–6 per treatment. a,b,cValues not sharing superscripts differ (P < 0.05).](image)

Table 3. Phosphorylation of eIF2α and eEF2 in skeletal muscle of piglets infused with 0, 20, 100, or 400 μmol·kg⁻¹·h⁻¹ HMB for 1 h

<table>
<thead>
<tr>
<th></th>
<th>HMB 0</th>
<th>HMB 20</th>
<th>HMB 100</th>
<th>HMB 400</th>
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<td>eIF2α, AU</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>LD</td>
<td>0.83 ± 0.15</td>
<td>0.85 ± 0.15</td>
<td>0.89 ± 0.14</td>
<td>0.76 ± 0.14</td>
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<tr>
<td>Gastrocnemius</td>
<td>1.32 ± 0.21</td>
<td>1.42 ± 0.21</td>
<td>1.28 ± 0.19</td>
<td>1.25 ± 0.19</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.79 ± 0.15</td>
<td>0.80 ± 0.15</td>
<td>0.81 ± 0.14</td>
<td>0.77 ± 0.14</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>1.09 ± 0.18</td>
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<tr>
<td>eEF2, AU</td>
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<tr>
<td>LD</td>
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<td>Gastrocnemius</td>
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<td>1.23 ± 0.21</td>
<td>1.12 ± 0.19</td>
<td>1.09 ± 0.19</td>
</tr>
<tr>
<td>Soleus</td>
<td>1.32 ± 0.31</td>
<td>1.60 ± 0.31</td>
<td>1.55 ± 0.29</td>
<td>1.41 ± 0.29</td>
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<tr>
<td>Diaphragm</td>
<td>0.81 ± 0.16</td>
<td>0.84 ± 0.16</td>
<td>0.87 ± 0.14</td>
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</tbody>
</table>

Data are given as means ± SE; n = 5–6. AU, arbitrary units; LD, longissimus dorsi; eIF2α, eukaryotic initiation factor 2α; eEF2, eukaryotic elongation factor 2. Labeled means in a row without a common letter differ, P < 0.05.
Table 5. Amino acid transporters in muscle of neonatal pigs in response to 1-h HMB supplementation

<table>
<thead>
<tr>
<th></th>
<th>HMB 0</th>
<th>HMB 20</th>
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<tr>
<td>LAT1, AU</td>
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<td>LD</td>
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<td>Gastrocnemius</td>
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<td>Soleus</td>
<td>0.83 ± 0.14</td>
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<td>0.90 ± 0.14</td>
<td>0.82 ± 0.14</td>
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<tr>
<td>Diaphragm</td>
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<td>SNAT2, AU</td>
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<td>0.95 ± 0.12</td>
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<td>0.84 ± 0.11</td>
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<td>Soleus</td>
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<td>0.86 ± 0.14</td>
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<tr>
<td>Diaphragm</td>
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<td>0.64 ± 0.11</td>
<td>0.59 ± 0.10</td>
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Data are given as means ± SE; n = 5–6. LAT1, system L transporter 1; SNAT2, system A transporter 2. Labeled means in a row without a common letter differ, P < 0.05.
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HMB AND PROTEIN SYNTHESIS

AUTHOR CONTRIBUTIONS


REFERENCES
