AMP-Activated Protein Kinase is Negatively Associated with Intramuscular Fat in Beef Cattle

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Introduction
• Marbling is a major factor affecting beef quality grade in the present grading system (USDA, 1997).
• Increased marbling in young beef will garnish enormous economic returns (USDA Market Data, Dec 22, 2006).
• AMP-Activated Protein Kinase plays a central role in energy metabolism (Hardie, 2005).
• AMPK interacts with insulin/insulin like growth factor-1 (IGF-1) signaling which affects muscle growth mainly through the protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling pathway (Jakobsen et al., 2001; Latres et al., 2005).

Materials and Methods
• Cross-bred steers with high intramuscular fat (IMF) content (5.71 ± 0.19 %, n = 5) and low fat content (2.09 ± 0.12 %, n = 5) were selected from a study of 40 steers slaughtered at the University of Wyoming Meat Laboratory for analysis.
• Longissimus muscle samples were removed 10 min postmortem through the body cavity and immediately snap frozen in liquid nitrogen. Samples were then stored at -80 °C and used for immunoblotting and enzyme assay analysis.
• AMPK activity was measured using a SAMS peptide assay.
• Immunoblotting was performed using a Bio-Rad minigel system and antibodies were obtained from Cell Signaling (Danvers, MA) and Abcam Inc. (Cambridge, MA). Nitrocellulose membranes were visualized using ECL Western blotting reagents (Amersham Bioscience) and exposure to film (MR, Kodak, NY).
• Carcass parameters were measured 48 h postmortem, 9-10-11 rib sections were dissected according to Hankins and Howe (1944). Proximate analysis of longissimus muscle at the 13th rib was performed using modified AOAC (1990) procedures. Longissimus and semitendinosus muscles were dissected and weighed 7 d postmortem.

Objective
Our objective was to evaluate the relationship between AMPK and its associated signaling mediators, with marbling and growth of the longissimus muscle in beef cattle.

Results

Table 1. Carcass measurements of high and low intramuscular fat steers.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>High intramuscular</th>
<th>Low intramuscular</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCWT, kg</td>
<td>307.2 ± 12.7</td>
<td>334.0 ± 5.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Hot carcass weight, kg</td>
<td>1.63 ± 0.03</td>
<td>1.80 ± 0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>70.78 ± 0.36</td>
<td>5.09 ± 0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein, %</td>
<td>22.41 ± 0.22</td>
<td>36.9 ± 0.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat, %</td>
<td>5.71 ± 0.13</td>
<td>5.71 ± 0.13</td>
<td>0.98</td>
</tr>
<tr>
<td>Kidney, pelvic, and heart fat, %</td>
<td>0.87 ± 0.11</td>
<td>1.20 ± 0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>Ash, %</td>
<td>1.05 ± 0.02</td>
<td>1.10 ± 0.08</td>
<td>0.98</td>
</tr>
<tr>
<td>Warner-Bratzler shear force, kg</td>
<td>4.03 ± 0.44</td>
<td>81.4 ± 0.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Longissimus muscle area, cm²</td>
<td>70.5 ± 1.5</td>
<td>81.4 ± 1.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Longissimus muscle, %HCWT</td>
<td>1.63 ± 0.03</td>
<td>1.80 ± 0.05</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Figure 2. A. AMP-activated protein kinase (AMPK) activity in longissimus muscle of low Intramuscular (Low IMF) and high intramuscular fat (High IMF) cattle, B. AMPK and its phosphorylation at Thr 172 immunoblots in longissimus muscle of low Intramuscular (Low IMF) and high intramuscular fat (High IMF) cattle.

Figure 3. A. The ratio of phosphorylated Acetyl-CoA carboxylase (ACC) and total ACC in longissimus muscle B. ACC and its phosphorylation at Ser 79 immunoblots in longissimus muscle of low intramuscular (IMF) and high intramuscular fat (IMF) cattle.

Figure 4. Protein kinase B (Akt) and its phosphorylation at Ser 473 in longissimus muscle of low intramuscular (Low IMF) and high intramuscular fat (High IMF) cattle.

Figure 5. Mammalian target of rapamycin (mTOR) and its phosphorylation at Ser 2448 in longissimus muscle of low intramuscular (Low IMF) and high intramuscular fat (High IMF) cattle.

Discussion
• Our data demonstrated high intramuscular fat accumulation was associated with reduced lean growth in this set of beef cattle.
• The reduced lean growth in High IMF cattle seems not to be associated with mTOR signaling and MAPK signaling.
• The AMPK activity was lower in longissimus muscle with High IMF compared to Low IMF, demonstrating a negative relationship between intramuscular fat and AMPK activity. Therefore, our study indicates AMPK may be a molecular target to promote marbling in beef cattle.
• The mechanisms responsible for the negative association between intramuscular fat accumulation and lean growth, and the involvement of AMPK in this process warrant further investigation.

Acknowledgement
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References