Effect of sire breed and genetic merit for carcass weight on the transcriptional regulation of the somatotropic axis in longissimus dorsi of crossbred steers

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ABSTRACT: The somatotropic axis plays an important role in postnatal growth, development, and differentiation of skeletal muscle. The aim of this study was to examine the effect of sire breed and sire EPD for carcass weight (EPDcwt) on the expression of components of the somatotropic axis in LM of beef cattle at slaughter. Crossbred Aberdeen Angus (AA; n = 17) and Belgian Blue (BB; n = 16) steers born to Holstein-Friesian dams and sired by bulls with either high (H) or low (L) EPDcwt were used in the study. Thus, there were 4 genetic groups [i.e., BBH (n = 8), BBL (n = 8), AAH (n = 8), and AAL (n = 9)]. Blood samples were collected via jugular venipuncture at regular intervals for analysis of plasma concentrations of IGF-1 and insulin. Total RNA was isolated from LM collected at slaughter, and the mRNA expression of IGF-1, IGF-2, their receptors (IGF-1R; IGF-2R), 6 IGFBP, acid labile subunit (ALS), and GH receptor (GHR) was measured by real-time reverse-transcription quantitative PCR. There was no effect of either sire breed or EPDcwt on concentrations of circulating IGF or insulin (P > 0.05). Gene expression of IGF-1R and IGFBP3 was upregulated in AA (P < 0.001) compared with BB, whereas IGF-1 was upregulated in H compared with L animals (P < 0.01). Correlation analysis indicated moderate positive associations between gene expression of IGFBP3 and IGF-1 (r = 0.54; P < 0.001) and IGF-1R (r = 0.48; P < 0.01). In addition, correlation analysis revealed that mRNA expression of IGFBP3 was moderately negatively associated with LM area per kilogram of carcass weight (r = −0.40; P < 0.05). Greater gene expression of IGF-1 and reduced transcript abundance of IGFBP3 in muscle may have a role in increased muscle growth potential in steers during the finishing period. These data will contribute to a better understanding of the molecular control of muscle growth at a tissue level in cattle.

Key words: bovine, gene expression, skeletal muscle, somatotropic axis

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INTRODUCTION

Bovine skeletal muscle is a tissue of significant economic importance to the global economy. Worldwide, beef production is projected to increase at a rate of 0.9 to 1.4% annually over the next decade (European Commission, 2010). The Irish Cattle Breeding Federation undertakes genetic evaluations for a range of performance traits across all of the main cattle breeds. Breeding value for carcass weight, an important trait reflecting lifetime growth, is estimated using a multi-trait animal model and is expressed as the EPD for carcass weight (EPDcwt; Campion et al., 2009b). Similar genetic evaluations for carcass characteristics are routinely conducted worldwide (Crews et al., 2004; Van Groningen et al., 2006), and sires can be ranked based on their EPDcwt.

Bernard et al. (2009) examined the effects of genetic selection in favor of increased muscle growth on gene expression in muscle of young bulls. These authors reported that many genes of the somatotropic axis were differentially expressed between bulls selected for high...
compared with low growth potential. In vivo and in vitro studies have shown that both IGF-1 and IGF-2 stimulate proliferation and differentiation of muscle cells through their interaction with IGF receptors (Jones and Clemmons, 1995; Oksbjerg et al., 2004). Consequently, the somatotropic axis is likely to be a promising target for candidate genetic markers for improving meat yield in cattle. To the knowledge of the authors, there is little published information available on comparisons of different breeds and within-breed genetic merit for carcass growth on the expression of component genes of the somatotropic axis in bovine muscle. Therefore, the objective of this study was to determine the effect of i) sire breed and ii) sire EPD$_{cwt}$ on the mRNA expression of genes of the somatotropic axis in LM in Aberdeen Angus (AA) and Belgian Blue (BB) cattle. The LM was selected due to its high commercial value.

**MATERIALS AND METHODS**

All procedures were carried out under license in accordance with the European Community Directive, 86-609-EC. Animals were slaughtered in a European Union-licensed abattoir (Meadow Meats, Rathdowney, Co. Laois, Ireland).

**Experimental Design and Animal Measurements**

This study used muscle samples harvested at slaughter from a larger study by Campion et al. (2009b). Briefly, in that study, male progeny (n = 114) of Holstein-Friesian dairy cows and sired through AI, by bulls of 2 contrasting beef breeds [i.e., AA (n = 56) and BB (n = 58)] were used. Within breed, progeny were classified as from sires of either high (H) or low (L) EPD$_{cwt}$. For AA-sired animals, 32 were of H EPD$_{cwt}$ and 24 of L EPD$_{cwt}$, whereas for BB 31 were of H and 27 of L EPD$_{cwt}$. There was no difference between the dams of the various genetic groups in estimated genetic merit for beef production and carcass weight (Campion et al., 2009a). The finishing diet consisted of a total mixed diet, based on the original blocking criteria of Campion et al. (2009b). The subgroups were as follows: i) AAH (n = 8), ii) AAL (n = 9), iii) BBH (n = 8), and iv) BBL (n = 8). Animals represented the progeny of 16 different sires (AA; n = 7 and BB; n = 9) and had a mean BW and age at slaughter of 591 kg (SD 62 kg) and 764 d (SD 37 d), respectively. Mean values for the subgroups used in our study for the main outcome traits were similar to that of Campion et al. (2009b), and there was no overdominance of any particular sires within group.

Animals were blood sampled (10 mL) for the analysis of plasma concentrations of IGF-1 and insulin via jugular venipuncture at approximately 7, 14, and 18 mo of age and again at 2 d before slaughter (24 mo of age). Lithium heparin evacuated vials (Cruinn Diagnostics, Dublin, Ireland) were used. Blood was centrifuged at 1,500 x g for 15 min at 4°C, and plasma was decanted and frozen at −20°C pending analysis. Concentrations of IGF-1 in blood plasma were determined by RIA after an acid-ethanol extraction procedure, as described by Spicer et al. (1988). Intraassay CV for IGF-1 quantification was 9.8, 8.2, and 15.9% for low, medium, and high standards, respectively. Interassay CV for IGF-1 was 5.4, 5.2, and 3.3% for low, medium, and high standards, respectively. Insulin concentrations were quantified by fluoroimmunoassay (AutoDELFIA, PerkinElmer Life and Analytical Sciences, Turku, Finland) and validated for bovine plasma (Ting et al., 2004). Intraassay CV for insulin was 5.9, 3.5, and 3.1% for low, medium, and high standards, respectively. Interassay CV was 11.5, 8.1, and 7.0% for low, medium, and high standards, respectively.

**Tissue Collection, RNA Extraction, and cDNA Synthesis**

Samples of LM were harvested from animals within 30 min of slaughter, washed in sterile Dulbecco’s PBS (Sigma-Aldrich Ireland Ltd., Dublin, Ireland), and snap-frozen in liquid nitrogen. All instruments used in tissue harvesting were sterilized and treated with 75% ethanol and RNAzap (Applied Biosystems, Warrington, UK). Samples were stored in dry ice for 1 h before being transferred to a −80°C freezer for long-term storage. Total RNA was isolated from muscle tissue using TRI Reagent (Sigma-Aldrich Ireland Ltd.) and chloroform, and the RNA was subsequently precipitated using isopropanol. The RNA quantity was determined by measuring absorbance at 260 nm on a Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). The 28s:18s ratio and RNA integrity number, indicators of RNA quality, were assessed using the Agilent Bioanalyzer with the RNA 6000 Nano LabChip kit (Agilent Technologies Ireland Ltd., Dublin, Ireland). Samples were treated with RQ1 RNase-free DNase (Promega, UK Ltd., Southampton, UK), and total RNA (1 μg) was then reverse transcribed to cDNA, with random hexamers, using the High Capacity cDNA Reverse Transcription kit (Applied Biosys-
Muscle messenger RNA expression of somatotropic axis

Real-Time Reverse-Transcription Quantitative PCR

Primers were designed to measure expression of the candidate and reference genes using the Primer3 software program (Rozen and Skaletsky, 2000). Primer specificity was established using the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). Primers for real-time reverse-transcription quantitative PCR (RT-qPCR; Table 1) were commercially synthesized (Sigma-Aldrich Ireland Ltd.). The PCR products generated by amplification were sequenced to verify their primer specific identity (Biochemistry DNA Sequencing Facility, University of Cambridge, Cambridge, UK). To determine relative gene expression, a suitable highly stable reference gene was required. Reference genes tested across all samples using RT-qPCR included glyceraldehyde 3-phosphate dehydrogenase (GAPDH), β-actin, hydroxymethylbilane synthase, and elongation factor 1α 2 (Byrne et al., 2005; Pérez et al., 2008). Data were analyzed using NormFinder, a model-based approach software (Andersen et al., 2004; accessible through MultiD Analyses AB, Gothenburg, Sweden) to measure the overall stability of the tested reference genes. The software calculates the intra- and intergroup CV and combines both coefficients to give a stability value, with a smaller value implying greater stability in gene expression. With a stability value of 0.54, GAPDH was chosen as the reference gene in this study, whereas elongation factor 1α 2, β-actin, and hydroxymethylbilane synthase were unsuitable, having stability measures of 0.87, 0.82, and 0.75, respectively. Other studies examining gene expression in LM tissue also used GAPDH as a reference gene (Kim et al., 2008; Natonek-Wiśniewska et al., 2009). The RT-qPCR reactions were carried out using SYBR FAST Green master mix (Applied Biosystems). Measurements were performed in triplicate using the Applied Biosystems Fast 7500 v2.0.1 instrument with the following cycling parameters: 95°C for 20 s; 40 cycles of 95°C for 3 s; 60°C for 30 s, followed by amplicon dissociation (95°C for 15 s; 60°C for 60 s; 95°C for 15 s, and 60°C for 15 s). Primer concentrations were optimized for each gene, and dissociation curves were examined for the presence of a single PCR product. The efficiency of the reaction was calculated using a 2-fold serial dilution of cDNA and generation of a standard curve. All PCR efficiency coefficients were between 0.9 and 1.0 and therefore deemed acceptable. The software package GenEx 5.2.1.3 (MultiD Analyses AB, Gothenburg, Sweden) was used for efficiency correction of the raw cycle threshold (Ct) values, interplate calibration based on a calibrator sample included on all plates, averaging of replicates, normalization to the reference gene, and the calculation of quantities relative to the greatest Ct.

Statistical Analysis

Data were checked for normality using the UNIVARIATE procedure of statistical analysis software (SAS Inst. Inc., Cary, NC). Relative gene expression data for IGF-2R, IGFBP5, and IGFBP6 were transformed as appropriate by raising to the power of λ (TransReg procedure; SAS). The remaining nonnormally distributed gene expression values were log-transformed using log2. Plasma analyte data were analyzed using repeated measures ANOVA (PROC MIXED, SAS) with sire breed, sire EPDcwt, and day of sampling included as fixed effects together with their interaction term as appropriate. Day of sampling was included as the repeated term, and an unstructured variance-covariance structure was selected. Gene expression data were analyzed using mixed models ANOVA (PROC MIXED). Sire breed and sire EPDcwt were included as fixed effects in the statistical model together with the interaction term, where appropriate. Sire was included as a random effect. The Tukey critical difference test was performed to determine the existence of statistical differences between treatment mean values. Spearman correlation coefficients among gene expression values and production traits were determined using the CORR procedure of SAS. The Spearman correlation procedure was the chosen method due to the nonparametric nature of the data.

RESULTS

Plasma Concentrations of IGF-1 and Insulin

No effect (P > 0.05) of sire breed or EPDcwt or their interaction was observed for plasma concentrations of IGF-1 or insulin (Table 2). Plasma concentrations of IGF-1 increased linearly between 7 and 24 mo of age. A similar trend was observed for circulating concentrations of insulin with levels increasing linearly over time throughout the lifetime of the animal.

Expression of Genes of the Somatotropic Axis

Despite a sire breed × EPDcwt interaction being detected for IGFBP3, this interaction was found to be not significant (P = 0.07). Consequently, the effects of breed and EPDcwt are reported below (Table 3). There was a difference (P < 0.001) in transcript abundance for IGFBP3 between the breeds with expression greater in AA compared with BB; however, no effect (P = 0.24) of EPDcwt was observed in expression of IGFBP3. Similarly, transcript abundance of IGF-1R was greater (P < 0.001) in AA animals compared with BB. No difference (P > 0.05) was observed in gene expression of IGF-1R.
across EPDcwt groups. A difference in mRNA expression of IGF-1 was observed between EPD cwt groups with transcript abundance upregulated (P < 0.01) in H compared with L animals. There was no difference (P > 0.05) in gene expression across breed for IGF-1. Neither an effect of sire breed nor EPD cwt was detected (P >

Table 1. Bovine oligonucleotide primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>Forward: 5′: AGTTGGTGGATGCTCTCCAGT</td>
<td>NM_001077828</td>
</tr>
<tr>
<td></td>
<td>Reverse: 3′: CACTCATCACAGATTGCTGTC</td>
<td>NM_174087</td>
</tr>
<tr>
<td>IGF-2</td>
<td>Forward: 5′: ACCAAGACAGGACAGTCAACAC</td>
<td>NM_174554</td>
</tr>
<tr>
<td></td>
<td>Reverse: 3′: AGTGGTAGATGGAAGAAACAC</td>
<td>NM_174557</td>
</tr>
<tr>
<td>IGF-1R</td>
<td>Forward: 5′: AGGCCTCTCTCTCCAATCAATCAAT</td>
<td>NM_001146006</td>
</tr>
<tr>
<td></td>
<td>Reverse: 3′: GGGAGAGAATCCCAAGGAGA</td>
<td>NM_001040495</td>
</tr>
<tr>
<td>IGF-2R</td>
<td>Forward: 5′: ATGGCGGTATTGTGGATCAT</td>
<td>NM_176608</td>
</tr>
<tr>
<td></td>
<td>Reverse: 3′: GGATGTCGGCATGAATCTCT</td>
<td>NM_001034034</td>
</tr>
<tr>
<td>ACTB</td>
<td>Forward: 5′: ACTTGCGCAGAAAACGAGAT</td>
<td>NM_173979</td>
</tr>
<tr>
<td></td>
<td>Reverse: 3′: CACCTTCACCGTTCCAGTTT</td>
<td>NM_179797</td>
</tr>
</tbody>
</table>

1IGF-1R = IGF-1 receptor.
2IGF-2R = IGF-2 receptor.
3McCarthy et al. (2009).
4ALS = acid-labile subunit.
5GHR = GH receptor.
6GAPDH = glyceraldehyde 3-phosphate dehydrogenase.
7EEF1A2 = elongation factor 1 α 2.
8HMBS = hydroxymethylbilane synthase.
9ACTB = β-actin.

Table 2. Effect of sire breed and EPD for carcass weight (EPDcwt) on the plasma concentrations of IGF-1 and insulin

<table>
<thead>
<tr>
<th>Trait</th>
<th>Breed (B)2</th>
<th>EPDcwt3</th>
<th>Time (T), mo</th>
<th>P-value4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>BB</td>
<td>SED</td>
<td>H</td>
</tr>
<tr>
<td>IGF-1, ng/mL</td>
<td>249.5</td>
<td>275.3</td>
<td>49.5</td>
<td>255.7</td>
</tr>
<tr>
<td>Insulin, μIU/mL</td>
<td>12.3</td>
<td>14.5</td>
<td>1.54</td>
<td>13.5</td>
</tr>
</tbody>
</table>

aLeast squares means within a row without a common superscript differ (P < 0.05).
1Animals were blood sampled by jugular venipuncture at approximately 7, 14, and 18 mo of age, and again 2 d before slaughter at 24 mo of age.
2AA = Aberdeen Angus; BB = Belgian Blue.
3H = high for EPDcwt; L = low for EPDcwt.
4No statistically significant interactions (B × EPDcwt; B × T; EPDcwt × T) were observed.
Table 3. Effect of sire breed and EPD for carcass weight (EPD_{cwt}) on the relative expression of genes of the somatotropic axis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Breed 3</th>
<th>EPD_{cwt} 4</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>BB</td>
<td>SED</td>
</tr>
<tr>
<td>IGF-1</td>
<td>6.83</td>
<td>5.12</td>
<td>0.948</td>
</tr>
<tr>
<td>IGF-1R</td>
<td>17.2a</td>
<td>2.81b</td>
<td>2.833</td>
</tr>
<tr>
<td>IGF-2</td>
<td>16.1</td>
<td>20.9</td>
<td>3.407</td>
</tr>
<tr>
<td>IGF-3R</td>
<td>1.72</td>
<td>5.42</td>
<td>1.799</td>
</tr>
<tr>
<td>IGFBP4</td>
<td>57.9a</td>
<td>4.61b</td>
<td>10.231</td>
</tr>
<tr>
<td>IGFBP5</td>
<td>6.26</td>
<td>9.61</td>
<td>4.175</td>
</tr>
<tr>
<td>IGFBP6</td>
<td>22.1</td>
<td>24.1</td>
<td>8.496</td>
</tr>
<tr>
<td>IGF-2R</td>
<td>3.59</td>
<td>3.68</td>
<td>1.146</td>
</tr>
<tr>
<td>GHR</td>
<td>13.7</td>
<td>7.81</td>
<td>3.128</td>
</tr>
</tbody>
</table>

a,bLeast squares means within a row without a common superscript differ (P < 0.05).

1Gene expression values were normalized to the reference gene after adjustment for efficiencies and interplate variation and converted to values relative to the greatest cycle threshold (Ct) within each data set.

2AA = Aberdeen Angus; BB = Belgian Blue.

3H = high for EPD_{cwt}; L = low for EPD_{cwt}.

4IGF-1R = IGF-1 receptor.

5IGF-2R = IGF-2 receptor.

6GHR = GH receptor.

0.05) for gene expression of IGF-2, IGF-2R, IGFBP4, IGFBP5, IGFBP6, or GH receptor (GHR). Even after 40 amplification cycles, gene expression of IGFBP1, IGFBP2, and ALS in LM remained undetected.

Correlation Between Expression of Genes in the Somatotropic Axis

Correlation analysis was carried out to examine potential associations between genes of the somatotropic axis (Table 4). In summary, IGF-1 gene expression values were positively correlated with expression of IGFBP3, IGFBP4, IGFBP5, and IGFBP6, whereas IGF-1R gene expression was negatively associated with IGFBP5 and IGFBP6 but positively associated with IGFBP3 and GHR. Gene expression of IGF-2 was positively associated with gene expression of IGFBP4 and GHR but negatively correlated with IGFBP6. Gene expression of IGF-2R was negatively correlated with expression of IGFBP3 and IGFBP5 but positively correlated with expression of IGFBP6. Finally, gene expression of IGFBP5 was positively correlated with expression of IGFBP3.

Correlation Between Expression of Genes and Animal Production Variables

Correlation analysis was carried out to examine the potential associations between expression of genes of the somatotropic axis and relevant accompanying animal production traits, recorded as part of the studies of Camion et al. (2009a,b). The Spearman correlation coefficients for these associations are presented in Table 5. In brief, BW at slaughter and carcass weight were negatively correlated with expression of IGF-2 and GHR, whereas both measures of animal weight

Table 4. Associations between expression of genes of the somatotropic axis in LM

<table>
<thead>
<tr>
<th>Gene</th>
<th>IGF-1</th>
<th>IGF-2</th>
<th>IGF-1R</th>
<th>IGF-2R</th>
<th>IGFBP3</th>
<th>IGFBP4</th>
<th>IGFBP5</th>
<th>IGFBP6</th>
<th>GHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-2</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-1R</td>
<td></td>
<td>0.09</td>
<td>0.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-2R</td>
<td>−0.33</td>
<td>0.17</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGFBP3</td>
<td>0.54***</td>
<td>−0.24</td>
<td>0.48**</td>
<td>−0.47**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGFBP4</td>
<td>0.45**</td>
<td>0.60***</td>
<td>0.09</td>
<td>−0.01</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGFBP5</td>
<td>0.44**</td>
<td>−0.21</td>
<td>−0.35</td>
<td>−0.69***</td>
<td>0.47**</td>
<td>0.05</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IGFBP6</td>
<td>0.47**</td>
<td>−0.52***</td>
<td>−0.48**</td>
<td>0.39*</td>
<td>0.32</td>
<td>0.03</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GHR</td>
<td>0.31</td>
<td>0.57***</td>
<td>0.39*</td>
<td>−0.26</td>
<td>0.31</td>
<td>0.24</td>
<td>0.18</td>
<td>−0.23</td>
<td></td>
</tr>
</tbody>
</table>

1Values presented are Spearman correlation coefficients (r) from unadjusted data (n = 33).

2IGF-1R = IGF-1 receptor.

3IGF-2R = IGF-2 receptor.

4GHR = GH receptor.

*P < 0.05; **P < 0.01; ***P < 0.001.
were positively correlated with expression of IGFBP4. Preslaughter ultrasonically scanned LM depth and LM area were negatively associated with expression of IGFBP3 and GHR, whereas LM area per kilogram of carcass weight correlated negatively with expression of IGFBP4 and IGFBP3 but was positively associated with expression of IGFBP4.

**DISCUSSION**

The main objective of this study was to examine the effects of both sire breed and sire EPDcwt on the expression of constituent genes of the somatotropic axis in muscle tissue. There is clear evidence that breed type influences carcass characteristics including both yield and quality of salable meat from cattle (Keane and Moloney, 2010). The AA and BB sire breeds were selected because of their well-documented differences in carcass conformation, muscle composition, and maturation rates (early vs. late; Bellinge et al., 2005; Keane and Drennan, 2008; Dinh et al., 2010). Campion et al. (2009b) reported that LM area and LM area adjusted for carcass weight were greater for BB compared with AA animals sired by bulls. At slaughter, BB had heavier carcasses and greater dressing percentage compared with AA cattle. Preslaughter ultrasonically scanned LM depth, carcass LM, and LM area per kilogram carcass weight were all greater for BB compared with AA animals. In addition, ultrasonically scanned LM depth was greater in AAH compared with AAL.

The somatotropic axis, also known as the GH-IGF system, consists of peptide hormones, cell surface receptors, and binding proteins (Denley et al., 2005). The axis is critical in regulating postnatal growth, development, and differentiation of skeletal muscle (Clemmons, 1997; Duan and Xu, 2005; Duan et al., 2010). Muscle growth is mediated by the activation, proliferation, and differentiation of muscle cells and appears to also be modulated by mitotic and myogenic activity of locally produced IGF-1 (Philippou et al., 2007). Furthermore, muscle cell cultures have been shown to produce IGF and IGFBP.

The action of GH in regulating growth and development is mediated by its plasma membrane-bound receptor, GHR (Isaksson et al., 1985). Dauncey et al. (1994) and Katsumata et al. (2000) reported that mRNA expression of GHR in LM was upregulated in pigs growing at a slower rate than pigs with faster growth rates. In the current study, gene expression of GHR was not different across either sire breed or EPDcwt. Spurlock et al. (2006) found that after administration of clenbuterol, a β2-adrenergic receptor agonist, male mice experienced increased BW. Consequently, gene expression of GHR in muscle was downregulated compared with control mice. In addition, Castigliego et al. (2010) reported that expression of GHR was downregulated in bovine muscle after administration of recombinant bovine GH. The results of our correlation analysis support these findings (Dauncey et al., 1994; Katsumata et al., 2000; Spurlock et al., 2006) in that moderate negative associations were observed between expression of GHR and BW at slaughter, carcass weight, and LM area per kilogram of carcass weight.

In addition to circulating in blood, IGF are also regulated in tissues. In muscle cell lines and cultures, IGF-1

**Table 5. Associations between expression of genes of the somatotropic axis in LM and production variables**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Slaughter weight, kg</th>
<th>Carcass weight, kg</th>
<th>UMD, mm</th>
<th>LM area, cm²</th>
<th>LM area, cm²/kg</th>
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</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>0.01</td>
<td>-0.06</td>
<td>-0.02</td>
<td>-0.09</td>
<td>0.15</td>
</tr>
<tr>
<td>IGF-2</td>
<td>-0.46**</td>
<td>-0.43**</td>
<td>-0.23</td>
<td>-0.17</td>
<td>0.29</td>
</tr>
<tr>
<td>IGF-1R</td>
<td>-0.12</td>
<td>-0.25</td>
<td>-0.25</td>
<td>-0.55***</td>
<td>-0.41**</td>
</tr>
<tr>
<td>IGF-2R</td>
<td>0.37*</td>
<td>0.40*</td>
<td>0.26</td>
<td>0.41*</td>
<td>0.01</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>-0.11</td>
<td>-0.25</td>
<td>-0.37*</td>
<td>-0.63***</td>
<td>-0.40*</td>
</tr>
<tr>
<td>IGFBP4</td>
<td>-0.19</td>
<td>-0.15</td>
<td>0.05</td>
<td>0.06</td>
<td>0.37*</td>
</tr>
<tr>
<td>IGFBP5</td>
<td>-0.18</td>
<td>-0.23</td>
<td>-0.27</td>
<td>-0.22</td>
<td>0.11</td>
</tr>
<tr>
<td>IGFBP6</td>
<td>0.33</td>
<td>0.36*</td>
<td>0.34</td>
<td>0.28</td>
<td>0.07</td>
</tr>
<tr>
<td>GHR</td>
<td>-0.67***</td>
<td>-0.71***</td>
<td>-0.41*</td>
<td>-0.52**</td>
<td>0.21</td>
</tr>
</tbody>
</table>

1Values presented are Spearman correlation coefficients (r) from unadjusted data (n = 33).
2UMD = preslaughter ultrasonically scanned LM depth.
3Expressed per kilogram of carcass weight.
4IGF-1R = IGF-1 receptor.
5IGF-2R = IGF-2 receptor.
6GHR = GH receptor.

\*P < 0.05; **P < 0.01; ***P < 0.001.
and IGF-2 were produced and secreted into the culture medium (Jones and Clemmons, 1995; Oksbjerg et al., 2004). Local production of IGF-1 in skeletal muscle is thought to play a predominant role in supporting normal muscle growth through autocrine or paracrine mechanisms, or both (Sjögren et al., 1999; Dayton and White, 2008). In addition, research has found that locally produced IGF-1 in muscle has a key role in myo-fiber regeneration and hypertrophy (Isgaard, 1992; Philippou et al., 2007). For example, Chen et al. (2011) reported that gene expression of IGF-1 was significantly less in LM tissue of growth-restricted piglets compared with control animals. In the current study, a statistically significant difference in mRNA transcript abundance for IGF-1 between H and L groups for EPDcwt was consistent with the findings of Campion et al. (2009b) who reported that animals of H EPDcwt produced heavier carcasses with greater LM area compared with animals of L EPDcwt. This increased muscle growth can possibly be attributed to the greater local IGF-1 gene expression observed because no statistically significant difference in plasma concentrations of IGF-1 or insulin was observed because no statistically significant differences were not different between sire breeds. In addition, there was no association between IGF-1R expression and circulating IGF-1 concentrations at slaughter (data not shown). Tilley et al. (2007) reported that IGF-1R mRNA abundance was greater in small fetuses compared with fetuses of average size. In addition, Micke et al. (2011) found that expression of IGF-1R was upregulated at slaughter in muscle of cattle that were smaller at birth and suggested that this increase in gene expression acts as a compensatory effect in lighter animals to promote muscle growth. The positive association between IGFBP3 and IGF-1R expression reported in the current study supports that finding.

Myostatin, a member of the TGF-β superfamily, is a negative regulator of muscle mass (McPherron et al., 1997). However, in the current study there is no value in measuring gene expression of myostatin to investigate its regulatory effect on muscle growth because mutations in the myostatin coding sequence result in a truncated protein and consequently muscle hypertrophy or double muscling occurs (McPherron et al., 1997; Fahrenkrug et al., 1999). The Piedmontese and BB are 2 such breeds in which this phenomenon is observed (McPherron et al., 1997). The myostatin mutation phenomenon and its effect on muscle growth and the somatotropic axis must be considered when attempting to address potential effects of sire breed or EPDcwt for crossbreds BB animals. Crossbred BB animals that are heterozygous for the double muscling myostatin mutation have increased muscle mass compared with their conventional counterparts (Casas et al., 2004). At slaughter, BB had heavier carcasses, greater dressing percentage, and greater muscle size compared with AA. The IGF-1 and myostatin signaling pathways work simultaneously to achieve a controlled but flexible system for controlling muscle growth (Otto and Patel, 2010). Kamanga-Sollo et al. (2003) reported that myostatin caused increased production (doubling) of IGFBP3 mRNA in porcine embryonic cells compared with control cultures, which consequently resulted in reduced cell proliferation. It has been reported that free IGFBP3, which is not bound to IGF, affects cells via IGF-independent methods (Hwa et al., 1999; Baxter, 2000). Several mechanisms have been proposed that characterize the relationship between IGFBP3 and myostatin (Dayton and White, 2008); however, the full process has yet to be elucidated. A potential mechanism derived from the work of Dayton and White (2008) postulated that IGFBP3 mediates the proliferation-suppression actions of myostatin by downregulating the production of co-repressors, Ski and SnoN. In the current study, because BB animals had decreased mRNA
transcript abundance of IGFBP3 compared with AA animals, we suggest that myostatin produced in the AA animals is fully functioning, resulting in greater mRNA transcript abundance of IGFBP3 in AA compared with the BB animals. In contrast, all of the BB cattle employed in this study should have been heterozygous for the myostatin mutation, resulting in smaller amounts of active myostatin, and therefore IGFBP3 expression remained low. Consequently, the decreased abundance of IGFBP3 may have contributed to the greater muscle mass in BB animals.

The main IGFBP secreted by skeletal muscle is IGFBP5 (Duan et al., 2010). Depending on tissue type and circumstance, IGFBP5 has the ability to inhibit or potentiate IGF action (Clemmons, 1997; Ewton et al., 1998; Schneider et al., 2002). In skeletal muscle, Mukherjee et al. (2008) concluded that IGFBP5 inhibited IGF-1 action. They noted that the majority of other studies analyzing IGFBP5 action on IGF-1 in muscle reported similar findings; however, they emphasized that many of these case studies involved overexpression of IGFBP5. Surprisingly, in our study IGFBP5 was not differentially expressed across sire breed or EPD_{wet}.

Lehnert et al. (2007) highlighted the gene expression pattern of IGFBP5 in LM of developing bovine fetuses, as well as newborn calves. The authors reported that expression of IGFBP5 was significantly reduced in newborn calves compared with d 60, 135, and 195 of fetal development, suggesting that IGFBP5 may play a role in early muscle development in the bovine. The animals in the current study had a mean slaughter age of 764 d. To examine the potentiating or inhibitory effects of IGFBP5, muscle sampling by biopsy collection would be required at key growth periods throughout the life of the animals, starting at an earlier age. Alternatively, Ning et al. (2007) proposed that IGFBP3 can compensate for the loss of a functioning IGFBP5 protein in mammary tissue. In that study, IGFBP5 knockout mice exhibited normal growth and body composition. In the current study, although IGFBP5 was highly expressed in both breeds, increased IGFBP3 expression in AA was possibly compensating for the lack of a change in gene expression of IGFBP5 in these animals. However, this theory warrants further investigation in bovine skeletal muscle because correlation analyses detected a positive relationship between gene expression of IGFBP5 and expression of IGFBP3.

This is the first study to examine and report differences in the expression of key somatotropic genes in the muscle of cattle of H or L EPD_{wet} and across breeds of such contrasting morphology and maturity type. We have demonstrated that increased IGF-1 expression in muscle tissue may serve to promote growth in vivo supporting many other research findings (Powell-Braxton et al., 1993; Clemmons, 2009). Our study supports the findings of previous research, whereby IGFBP3 was proposed to mediate the equilibrium between IGF-1 and IGF-1R to enhance IGF-1 effects, thus promoting growth. Together an increase in gene expression of IGF-1 and a reduction in transcript levels of IGFBP3 in muscle may play a role in greater muscle growth potential in steers during the finishing period. Consequently, IGFBP3 and IGF-1 may serve as potential candidates for future investigation of molecular markers for muscle growth including exploration of small RNA regulation, transcription factors, and copy number and SNP variation. Indeed, recent data from our own group have shown that a SNP in the promoter region of IGF-1, predicted to introduce binding sites for transcription factors HSF1 and ZNF217, was associated with increased cow carcass weight (Mullen et al., 2011). Future studies should focus on sequencing the entire IGF-1 and IGFBP3 genes and regulatory regions in large numbers of animals divergent in growth performance for SNP discovery and subsequent association studies. After appropriate validation, such markers could be incorporated into future cattle breeding programs to improve the accuracy of selection for muscle growth. However, as this study clearly demonstrates, the expression of these key genes varies between breeds, thus emphasizing the necessity to validate all markers for growth across breed.

**LITERATURE CITED**


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