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The growth hormone/insulin-like growth factor (somatotropic) axis plays a central role in both regulating the metabolism of nutrients that support lactation, and controlling key processes of reproductive efficiency. The current study compared circulating concentrations of insulin-like growth factor-I, and hepatic mRNA abundance of key genes associated with the somatotropic axis in cows divergent in genetic merit for fertility traits, but with similar genetic merit for milk production traits. Superior genetic merit for fertility traits was associated with greater circulating concentrations of insulin-like growth factor-I, greater hepatic expression of insulin-like growth factor-I and reduced hepatic expression of lower molecular mass binding proteins.

FERTILITY GENETIC MERIT AND SOMATOTROPIC AXIS

Genetic merit for fertility traits in Holstein cows. III. Hepatic expression of somatotropic axis genes during pregnancy and lactation

S. B. Cummins*, S.M. Waters†, A.C.O. Evans,† P. Lonergan,† and S.T. Butler*‡

* Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland.
† School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland.
‡ Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland.
ABSTRACT

The objective of this study was to characterise the circulating concentrations of insulin-like growth factor-I (IGF-I), and the hepatic expression of key genes regulating the somatotropic axis in cows divergent in genetic merit for fertility traits, but with similar genetic merit for milk production traits. A total of 11 cows with good genetic merit for fertility (Fert+) and 12 cows with poor genetic merit for fertility (Fert-) underwent liver biopsy by percutaneous punch technique on d 20 (± 6.7 d) prepartum and on d 2 (± 1.5 d), d 58 (± 3.7 d), d 145 (± 13 d) and d 245 (± 17.1 d) postpartum. Total RNA was isolated and the mRNA expression of growth hormone receptor (GHR 1A and GHRtot), IGF-I, janus tyrosine kinase 2 (JAK2), signal transducer and activator of transcription 5B (STAT5B), suppressor of cytokine signalling 3 (SOCS-3), acid-labile subunit (ALS), and IGF binding proteins (IGFBP1 to IGFBP6) were measured by RT-qPCR. During lactation, the circulating concentrations of IGF-I were 34% greater in Fert+ cows (P < 0.01). Fert+ cows had increased mean expression of IGF-I mRNA during the study; however, the differences in IGF-I mRNA abundance between Fert+ and Fert- cows was most pronounced at d 145 and d 245. The expression of IGFBP3 and ALS transcript was similar in Fert+ and Fert- cows for the duration of the study. Fert- cows, however, had greater (P < 0.05) expression of IGFBP2, IGFBP4, IGFBP5 and IGFBP6. There was no effect of genotype on mRNA abundance of GHR 1A, STAT5B, JAK2, or SOCS-3 (P > 0.05). These results demonstrate that genetic merit for fertility traits affects
hepatic expression of key genes of the somatotropic axis regulating the synthesis, bioavailability and stability of circulating IGF-I.

INTRODUCTION

The modern dairy cow undergoes a co-ordinated series of physiological adaptations to support milk synthesis (Bauman, 2000). This is most noticeable during the early postpartum period, when the energy demand associated with rising milk output is greater than energy supplied by dry matter intake. This energy deficit places the cow in a state of negative energy balance (NEB), resulting in mobilisation of body reserves (Baumen et al., 1980). The growth hormone/insulin-like growth factor (somatotropic) axis plays a central role in the regulation of nutrient partitioning and key processes controlling reproductive efficiency. Therefore, it is seen as a potential biological mechanism linking nutritional status and reproduction (Lucy et al., 2001; Veerkamp et al., 2003). Perturbations in metabolic status, indicated by changes in circulating concentrations of insulin-like growth factor-I (IGF-I), have been demonstrated to be associated with reproductive efficiency (Patton et al., 2007; Taylor et al., 2004; Wathes et al., 2003).

Pituitary-derived growth hormone (GH) stimulates hepatic IGF-I synthesis via a specific cell surface GH receptor (GHR). The resulting systemic increase in IGF-I inhibits GH secretion, thereby completing the feedback loop and maintaining the system in equilibrium (Le Roith et al., 2001). Under conditions of NEB and body condition score (BCS) loss, the GH-IGF axis becomes uncoupled. The liver becomes refractory to GH during NEB, whereby elevated circulating concentrations of GH fail to stimulate an increase in hepatic IGF-I synthesis (Lucy, 2008; Vicini et al., 1991).
The GH-IGF axis is regulated at multiple levels within the liver. The effect of GH on hepatic IGF-I synthesis is dependent on the abundance of cell surface GHR, and also the availability and activity of intracellular signalling proteins responsible for activation (janus tyrosine kinase 2 (JAK2), and signal transducer and activator of transcription 5B (STAT5B)) and regulation (suppressor of cytokine signalling 3 (SOCS-3)) (Jones and Clemmons, 1995; Ram and Waxman, 1999). Following hepatic IGF-I synthesis and release into circulation, plasma IGF-I concentrations are regulated by binding with one of the 6 IGF binding proteins (IGFBP1 to IGFBP6) and the presence of IGFBP acid-labile subunit (ALS), altering the half-life and potential function of IGF-I (Le Roith et al., 2001).

Previous studies have developed high fertility vs. low fertility animal models through diet manipulation (Grala et al., 2011), comparing cows at different stages of lactation (Gross et al., 2011), comparing cows that became pregnant to cows that failed to become pregnant (Rhoads et al., 2008) or cows of different genetic origin (New Zealand Holstein-Friesian vs. North American Holstein-Friesian) (Lucy et al., 2009; McCarthy et al., 2009) to determine whether hepatic regulation of the GH-IGF axis is related to fertility. While providing a valuable insight into the effects of energy status, lactation and genetic origin on characteristics of the GH-IGF axis, these studies are confounded by effects of age, nutrition, stage of lactation, BCS, among other factors.

Recently, we have reported that genetic merit for fertility traits had a pronounced effect on reproductive efficiency, BCS profile and circulating concentrations of IGF-I (Cummins et al., 2012). Cows with good (Fert+) genetic merit for fertility traits had greater circulating concentrations of IGF-I throughout lactation compared with cows with poor (Fert-) genetic merit for fertility traits. However, the underlying
physiological differences between Fert+ and Fert- cows which regulate the observed
differences in the circulating IGF-I concentration remain unknown. Therefore, the aim
of this study was to determine the effect of genetic merit for fertility traits on
transcriptional regulation of key genes controlling the hepatic GH-IGF axis during the
gestation/lactation cycle.

MATERIALS AND METHODS

Herd Establishment

Animals with high and low genetic merit for fertility traits were assembled during
autumn 2007 using official dairy evaluations published by the Irish Cattle Breeding
Federation, and has been outlined in detail by Cummins et al. (2012). In summary, the
national dairy cattle database was screened for nulliparous spring calving heifers that
were of > 75% Holstein ancestry, and had an EBV for milk production of between
+200 kg and +900 kg. Within this population, heifers with extreme negative (good
fertility) and positive (poor fertility) EBVs for calving interval were identified. A
further restriction was placed on heifer sire and maternal grandsire EBV for calving
interval. Good fertility (Fert+) heifers were restricted to animals where the sire and
maternal grand sire had negative EBVs for calving interval. Conversely, poor fertility
(Fert-) heifers were restricted to animals where sire and maternal grand-sire had
positive EBVs for calving interval. A total of 26 nulliparous Fert- and 26 nulliparous
Fert+ cows were screened free of infectious diseases, and were purchased and moved
to the Moorepark Animal and Grassland Research and Innovation Centre in Fermoy,
Co. Cork, Ireland (55°10’N 8°16’W). The total number of animals enrolled in the
current study was 23; of these 11 were Fert+ (3 first lactation and 8 second lactation)
and 12 were Fert- (4 first lactation and 8 second lactation). The subsample of animals selected for the current study calved between February 1 and May 5, and were representative of the larger groups of Fert+ and Fert- cows (range of sires, parity, etc.). The EBVs of two genotypes are summarized in Table 1.

Management System

For the duration of the study, animals were managed identically as one herd in a typical grass-based spring-calving production system. Before parturition, cows were housed in a free stall barn and given full time access to a total mixed ration (TMR) of grass silage (90%) and concentrate (10%). Following parturition, cows were turned out to grass in early February until mid-November and grazed under a rotational grazing system in a predominantly perennial ryegrass (Lolium perenne L.) sward. The mean daily herbage allowance was 14.3 ± 1.3 kg/d of DM/cow, and was supplemented with 4.0 ± 1.7 kg/cow/d of concentrate. The chemical composition of the diet is summarized in Table 2. Cows were milked twice daily at 0730 and 1630 h. Milk yield was recorded at each milking using electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). Milk composition (fat, protein and lactose) was determined weekly from successive evening and morning samples by mid-infrared reflectance spectroscopy using a FT6000 Milkoscan instrument (Foss Electric, Hillerød, Denmark). Cow liveweight was measured weekly and BCS (Edmonson et al., 1989) was assessed every 2-3 wk throughout the study.

Liver tissue sampling

Liver tissue was collected from all cows on d 20 (± 7 d) prepartum and d 2 (± 2 d), d 58 (± 4 d), d 145 (± 13 d) and d 245 (± 17 d) postpartum. A percutaneous punch
The technique was used to collect liver tissue as described by Smith et al. (2003). In summary, a biopsy site on the right flank of the cow was shaved and sanitized with 7.5% iodinated povidone and methylated spirits. A subcutaneous injection of lidocaine hydrochloride (2%) was used to anaesthetize the area. A 1 cm incision was made through the skin, and the biopsy instrument was used to pierce through the intercostal muscle and peritoneum. Once the liver was located, a 1 to 1.5 g sample of liver tissue was removed. The sample was washed in sterile PBS, snap-frozen in liquid nitrogen, and stored at –80 °C until further analysis. The incision site was sutured, and treated topically with Duphacycline Aerosol (3.6% Oxytetracycline hydrochloride: Norbrook Laboratories Ltd, Newry, Co Down, Northern Ireland).

**Blood Sampling and Laboratory Analysis**

Blood samples were collected to coincide with liver tissue collection timepoints. Blood samples were collected via coccygeal venipuncture into vacutainers containing lithium heparin as an anticoagulant (Becton Dickinson, Plymouth, UK). Samples were placed in a centrifuge for 15 min at 2,000 × g, plasma was decanted, and stored at -20 °C until further analysis. Plasma insulin concentration was determined using a solid-phase fluoro-immunoassay (AutoDELFIA, PerkinElmer Life and Analytical Sciences, Turku, Finland), with appropriate kits (Unitech BD Ltd., Dublin, Ireland). Inter- and intra-assay coefficients of variation were 17.5% and 8.5%, respectively. Blood plasma was analysed for circulating IGF-I concentrations and were quantified using a validated double-antibody radioimmunoassay, following ethanol:acetone:acetic acid extraction step as described by Enright et al. (1989). Inter- and intra-assay coefficients of variation were 15.6% and 15.8%, respectively.
**RNA extraction, cDNA synthesis and RT-qPCR**

Total RNA was isolated from 100 mg of frozen liver tissue using TRI Reagent (Sigma-Aldrich Ireland Ltd., Dublin, Ireland) and chloroform, and RNA was subsequently precipitated using isopropanol. The quantity and purity of RNA were determined by measuring absorbance at 260 nm on a spectrophotometer (NanoDrop Technologies, Wilminton, DE, USA). All RNA samples had a 260/280 nm absorbance ratio between 1.94 and 2.14. The 28s:18s ratio and RNA integrity number (RIN), an indication of RNA quality, were assessed by automated capillary gel electrophoresis using an Agilent Bioanalyser with the RNA 6000 Nano LabChip kit (Agilent Technologies Ireland Ltd., Dublin, Ireland). Ribonucleic acid samples had 28s:18s ratios ranging from 1.5 to 2.3 and RNA integrity numbers of between 7.5 and 9.3 and were deemed high quality and suitable for gene expression analysis. RNA samples were treated with RQ1 RNase-free DNase (Promega, UK Ltd., Southampton, UK) and purified using an RNeasy Mini kit as per the manufacturer’s instruction (Qiagen Inc. Valencia, CA, USA).

One µg total RNA was reverse transcribed to cDNA, with random hexamers, using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Warrington, UK) according to instructions supplied and stored at -20 °C.

Real-time quantitative PCR was used to measure expression of genes involved in the somatotropic axis, their transcriptional regulators, and reference genes. Primers were designed to specifically amplify templates of between 64 and 194 nucleotides overlapping exon-exon junctions where possible, using Primer-Blast software (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi). The sequences of primers used for each gene were commercially synthesized (Sigma-Aldrich Ireland Ltd., Dublin, Ireland) and are summarized in Table 3. The PCR products generated by
amplification were sequenced to verify their identity and confirm primer specificity (Eurofins MWG, Operon, Germany).

The stability of expression of candidate reference genes was investigated across all samples in the study. Reference genes included ß-Actin (*ACTB*), ribosomal protein S9 (*RPS9*), adenylate cyclase-associated protein 1 (*CAP1*), serine/arginine repetitive matrix 2 (*SRRM2*), 26S proteasome non-ATPase regulatory subunit 2 (*PSMD2*). The resulting expression data were analysed using geNorm software (version 3.5, Excel add-in Microsoft, Redmond, WA) as described by Vandesompele et al. (2002) to test the overall stability of the tested reference genes. The program estimates an expression stability value (*M*) for each reference gene (the lowest *M* value being the most stable). By performing a stepwise exclusion of reference genes with the highest *M* values, geNorm determines the minimal number of reference genes required for calculating an accurate normalization factor. In the current study, the highest stability was achieved by including three reference genes; *PSMD2*, *CAP1* and *ACTB* achieving a combined *M* value of 0.29.

All RT-qPCR reactions were performed using SYBR Fast Green mastermix (Applied Biosystems, Warrington, UK). Assays were carried out under identical conditions, and all samples were measured in triplicate using the Applied Biosystems Fast 7500 v2.0.1 instrument with the following cycle parameters (95 ºC for 15 s, 60 ºC for 60 s, 95 ºC for 15 s, and 60 ºC for 15 s). Primer and cDNA concentrations were optimized for each gene. The efficiency of the reaction was calculated using a 5-fold dilution series of cDNA to generate a standard curve. Dissociation curves were examined for the presence of a single PCR product. All PCR efficiency coefficients were between 0.9 and 1.08, 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 highest Ct.

Statistical Analysis

All statistical analyses and data handling were carried out using SAS (SAS Institute, 2006). Data were checked for normality and homogeneity of variance. Plasma hormone concentrations (IGF-I and insulin) and all gene expression data underwent transformation by raising the variable to the power of lambda. A Box-Cox transformation analysis was used to obtain the appropriate lambda value and normalise the distribution.

The effect of genotype on variables with repeated measures such as milk yield, BCS, body weight, plasma hormone concentrations of insulin and IGF-I, and gene expression data were determined using mixed models with cow nested within genotype as a random effect. A first-order autoregressive covariance structure with homogeneous variance provided the best fit for the data. Transformed data were used to calculate P-values, and the estimated group means and 95% confidence intervals reported are back-transformed values. The effect of genotype, time, parity, and their interactions were tested. Calving date was included as an adjustment variable and significant effects (P < 0.1) were maintained in the final model. Preplanned contrasts using the ESTIMATE statement were carried out to compare gene expression data between Fert+ and Fert- cows during three distinct time periods; the dry period (wk -3), early lactation (wk 1 and wk 8), and mid/late lactation (wk 20 and wk 35).

RESULTS
Production Variables and Plasma Hormone Concentrations

The effect of genotype on milk production, BCS, and circulating concentrations of insulin and IGF-I are illustrated in Figure 1. There was no effect of genotype \((P = 0.9)\) on mean daily milk yield, but the genotype by time interaction was significant. Parity had a significant effect on milk yield \((17.6 \pm 1.5 \text{ kg/d vs. } 21.6 \pm 1.0 \text{ kg/d for parity 1 and parity 2 respectively})\). Trends in BCS were similar \((P = 0.4)\) for both genotypes during the 5 sample periods. The lowest mean BCS was recorded during the third sample period \((\text{wk 8})\), with only modest increases observed thereafter until the end of lactation. There was no effect of genotype \((P = 0.8)\) on the mean body weight of Fert+ and Fert- cows over the duration of the study \((521 \pm 16 \text{ kg and } 527 \pm 15, \text{ respectively})\). No difference was observed in the pre-partum circulating IGF-I concentrations. During lactation, however, the circulating concentrations of IGF-I were 34\% greater in Fert+ cows. There was no effect of genotype on plasma insulin concentrations during wk -3, 8, 20 or 35 (relative to parturition); however, Fert+ cows had 89\% greater plasma insulin concentration during the first week of lactation compared to Fert- cows \((P < 0.05)\).

Gene Expression Profiles for Duration of the Study

The effect of genotype on gene expression data is presented in Figures 2, 3 and 4. There was an effect of genotype on \textit{IGF-I} transcript abundance during the study; Fert+ cows had greater \((P < 0.05)\) gene expression of \textit{IGF-I} \((\text{Figure 2A})\). No differences \((P = 0.9)\) in \textit{GHR 1A} transcript abundance was observed between genotypes \((\text{Figure 2B})\). Fert- cows had, however, greater \textit{GHRtot} transcript abundance \((P < 0.05)\). No differences \((P > 0.05)\) were observed in mean transcript abundance of
IGFBP1 and IGFBP3 between Fert+ and Fert- cows (Figure 3A and 3C). Fert- cows had greater ($P < 0.05$) mRNA abundance of IGFBP2, IGFBP4, IGFBP5 and IGFBP6 over the duration of the study (Figure 3B, 3D, 3E, 3F). The majority of the low molecular mass IGFBP expression differences between Fert+ and Fert- cows can be accounted for during wks -3, 1 and 8 relative to parturition. No differences ($P > 0.05$) between genotypes were observed for transcript abundance of JAK2, STAT5B, SOCS-3 and ALS during any of the 5 timepoints examined in this study (Figure 4).

**Genotype Contrasts of Gene Expression during the Dry Period, Early Lactation and Mid/Late Lactation**

There was no effect of genotype on IGF-I mRNA abundance during the dry period or early lactation ($P > 0.05$); however, Fert+ cows exhibited greater ($P < 0.01$) abundance of IGF-I mRNA during mid/late lactation compared to Fert- cows. There was no effect of genotype on transcript abundance of GHR 1A during any of the three time periods ($P > 0.05$). Fert- cows had greater expression of GHRtot mRNA during the dry period ($P < 0.01$) and early lactation ($P < 0.05$) compared with Fert+ cows, but no differences between genotypes were observed in mid/late lactation ($P > 0.05$). Expression of IGFBP1 and IGFBP3 mRNA were similar for both genotypes during the dry period, early lactation, and mid/late lactation ($P > 0.05$). Fert- cows had a greater ($P < 0.05$) abundance of IGFBP2 and IGFBP6 mRNA during the dry period and early lactation, but no difference was observed between genotypes during mid/late lactation. Fert- cows had greater ($P < 0.05$) mRNA abundance of IGFBP4 during early lactation, but no differences between genotypes were observed during the dry period or mid/late lactation ($P > 0.05$). Expression of IGFBP5 mRNA was
significantly greater \((P < 0.01)\) in Fert- cows at all three time points compared to Fert+ cows.

**DISCUSSION**

This study compared the mRNA abundance of key genes in the somatotropic axis in cows with extremes in genetic merit for fertility traits during the gestation/lactation cycle. Factors known to alter the somatotropic axis such as plane of nutrition, body weight, age, stage of lactation, genetic merit for milk production and proportion of Holstein genetics were similar for both genotypes. The current study attempted to elucidate the orchestrated physiological changes occurring in liver tissue that could explain the previously reported differences in circulating IGF-I and reproductive performance between Fert+ and Fert- cows (Cummins et al. 2012). Fert+ cows had greater circulating concentrations of IGF-I throughout lactation, greater IGF-I gene expression during mid/late lactation, and lower abundance of mRNA encoding lower molecular mass binding proteins during early lactation compared with Fert- cows. The present study indicated that genetic merit for fertility traits alters components of the somatotropic axis regulating synthesis, bioavailability and stability of circulating IGF-I.

Uncoupling of the somatotropic axis is a common phenomenon in high-yielding dairy cows during the early postpartum period (Lucy, 2008). In the current study, plasma IGF-I concentrations declined in both genotypes following parturition, but the magnitude of decline was greater in the Fert- cows. Fert+ cows maintained a 34% greater concentration of plasma IGF-I throughout lactation compared to Fert- cows, agreeing with the large body of evidence linking peripheral IGF-I to reproductive...
parameters (Lucy et al., 1998; Patton et al., 2007; Wathes et al., 2007). Given that nutrition, dry matter intake, energy balance, and milk production were similar in both genotypes (Cummins et al. 2012), the observed differences in circulating IGF-I appear to be dependent on genetic merit for fertility traits.

The actions of GH on hepatocytes to synthesise IGF-I are transduced by cell surface GH receptors (GHR) (Lucy et al., 2001). The three most prevalent transcript variants for bovine GHR protein are GHR 1A, 1B and 1C (Jiang and Lucy, 2001). The abundance of GHR 1A mRNA has been shown to be influenced by plane of nutrition and dry matter intake, and the decline in GHR 1A expression is hypothesized to be a primary factor causing hepatic refractoriness to GH-dependent IGF-I synthesis during the early postpartum period (Lucy et al., 2001). In the current study, the profile of GHR 1A expression was similar to previous reports (Kobayashi et al., 1999); with a nadir shortly after parturition followed by a rise and plateau during mid lactation. No difference was observed between genotypes in GHR 1A mRNA abundance. Moreover, Fert- cows exhibited lesser circulating concentration of IGF-I but had greater abundance of total GHR mRNA, particularly in early lactation. Previous reports have highlighted the strong association between circulating IGF-I concentrations and abundance of GHR in cows with different degrees of NEB (Fenwick et al., 2008). In the current study, however, energy balance was similar between genotypes (Cummins et al. 2012), indicating that the observed differences in peripheral IGF-I concentration in Fert+ was dependent on factors other than GHR mRNA abundance. This is consistent with results reported by McCarthy et al. (2009) who compared hepatic gene expression in Holstein Friesian cows of New Zealand and North American ancestry that exhibited similar postpartum energy balance.
The binding of GH to the GHR initiates a signalling cascade by activating the JAK-STAT intracellular pathway. JAK2 protein causes phosphorylation of a DNA binding protein (STAT5B), which stimulates the transcription of GH dependent genes such as IGF-I (Carter-Su et al., 2000; Horvath, 2000). A negative feedback mechanism to down-regulate GH signalling is provided by SOCS3 (Ram and Waxman, 1999). In the current study, there was no effect of genotype on JAK2, STAT5B or SOCS3 mRNA abundance in liver tissue. Levels of JAK2 and STAT5B appeared to be unaltered by physiological state and remained relatively stable throughout the gestation/lactation cycle, with mRNA abundance of SOCS3 increasing only during the early postpartum period. The absence of changes in the mRNA abundance of JAK-STAT pathway genes in different physiological states agrees with the results from short-term feed restriction; where cows were fed 30% of maintenance requirements for 2 wk (Rhoads et al., 2007). While gene knockout studies in mice identified STAT5B as a key agent regulating GH-dependent IGF-I gene expression in the liver (Teglund et al., 1998; Woelfle and Rotwein, 2004), the current study demonstrates that peripheral concentrations of IGF-I were not dependent on changes in STAT5B mRNA expression. Down stream regulation of the JAK-STAT pathway by SOCS3 protein could, however, partially explain the early postpartum decline in circulating IGF-I concentration. Based on these findings, greater circulating concentrations of IGF-I observed in Fert+ cows were not as a result of alterations in the intracellular signalling pathways that were measured.

Following the successful translocation of the dimerized STAT5B protein to the nucleus, transcription of IGF-I mRNA can occur (Horvath, 2000). As the liver is the primary source of circulating IGF-I, there is a strong association between hepatic IGF-I mRNA and circulating concentrations of IGF-I (Radcliff et al.; 2003; Sjögren et
In the current study, Fert+ cows had greater transcription of IGF-I mRNA in mid and late lactation, which coincided with greater circulating IGF-I. During the early postpartum period, however, greater circulating concentrations of IGF-I in Fert+ cows were not due to greater IGF-I mRNA abundance. A similar lack of association was reported in a study comparing hepatic gene expression in Holstein-Friesian cows of New Zealand and North American ancestry during the early postpartum period (Grala et al., 2011). The authors suggested that post-translation mechanisms altered IGF-I protein synthesis during the early postpartum period. A similar possibility cannot be ruled out in the present study, and furthered investigation is warranted.

Once released from the cell, the majority (95–99%) of IGF ligands in circulation are bound to one of six potential binding proteins (IGFBP1 – 6), and the specific IGFBP that binds with IGF plays a critical role in the somatotropic axis by regulating physiological activity of IGFs (Le Roith et al., 2001; Rajaram et al., 1997; Renaville et al., 2002). Produced almost entirely in the liver, IGFBPs act as carrier proteins transporting IGFs out of circulation to target tissues, and also extend the half-life of IGFs by preventing rapid metabolic clearance. Once in circulation, non-bound (free) IGF-I has a half-life of less than 10 min. Half-life is increased to 30-90 min if IGF-I is bound to lower molecular mass binding proteins (IGFBP1, 2, 4, 5, and 6), and further increased to 12-15 hr if bound to the larger molecular mass binding protein IGFBP3 in a ternary complex with ALS (Guler et al., 1989; Jones and Clemmons, 1995). In addition, IGF-I has been shown to have a greater affinity to its binding proteins than to its receptor, thus creating a further mechanism altering bioavailability of IGFs to target cells (Duan, 2002). In the current study, mRNA abundance of all IGFBPs was affected by stage of the gestation/lactation cycle. Transcription of the more stable IGFBP3 and ALS declined during early lactation whereas mRNA abundance of the
lower molecular mass binding proteins (*IGFBP1*, 2, and 4) increased. The greater abundance of *IGFBP2* mRNA during early lactation observed in Fert- cows is consistent with the inhibitory role of IGFBP2 on circulating IGF-I (Fenwick et al., 2008; Gross et al., 2011; McCarthy et al., 2009).

A notable feature of the current study was the difference between genotypes in the abundance of low molecular mass binding protein mRNA (*IGFBP2, 4, 5, and 6*). For the duration of the study, Fert+ cows had similar abundance of *IGFBP3* and *ALS* mRNA, but had lesser abundance of mRNA encoding IGFBP2, 4, 5 and 6 compared to Fert- cows. The marked increase in the mRNA abundance of low molecular mass binding proteins during early lactation in Fert- cows could have arisen as a result of reduced circulating concentrations of insulin. Elevated circulating insulin concentrations during early lactation cause a rapid reduction in plasma concentrations of IGFBP-2 and other low MW binding proteins (Butler et al., 2003; Butler et al., 2004). Elevated insulin concentrations (4 to 8 fold increase) during early lactation have been demonstrated to increase *GHR IA* and *IGF-I* gene expression (Butler et al., 2003; Rhoads et al., 2004). The more modest difference between genotypes in circulating insulin concentrations during early lactation in the current study was not associated with concomitant differences in hepatic *GHR IA* or *IGF-I* transcript abundance. Collectively, the results indicate that expression of low molecular mass IGFBP are more sensitive to changes in circulating insulin concentrations than *GHR IA* or *IGF-I*. We hypothesize that the Fert- cows had an increased proportion of IGF-I bound to low molecular mass binding proteins in early lactation compared to Fert+ cows. As a consequence of the shorter half-life of IGF-I bound to low molecular mass binding proteins, circulating IGF-I was reduced in Fert- cows in early lactation. In mid- to late-lactation, differences between genotypes in the mRNA abundance of the
low molecular mass binding proteins were not as pronounced, but IGF-I gene expression was greater in Fert+ cows during this time compared to Fert- cows.

CONCLUSION

The current study demonstrates the effects that genetic merit for fertility traits have on the regulation of the somatotropic axis in liver throughout the gestation/lactation cycle in Holstein cows. The elevated circulating concentrations of IGF-I observed in Fert+ cows could partially be explained by the reduction in expression of lower molecular mass binding protein mRNA during the early postpartum period, resulting in a greater proportion of circulating IGF-I bound to the more stable ternary complex. Moreover, greater peripheral IGF-I concentrations in Fert+ cows is attributed to greater abundance of IGF-I mRNA during mid and late lactation, and appeared to be independent of abundance of hepatic mRNA for GHR or alterations in the JAK-STAT intracellular pathway. The current study indicates that the mechanisms responsible for increased IGF-I in cows with superior genetic merit for fertility traits differs depending on the stage of lactation.

ACKNOWLEDGEMENTS

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REFERENCES


Table 1. The mean estimated breeding value\(^1\) (and SD) for Fert+ and Fert- cows

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fert+</th>
<th>Fert-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>NAHF (%)</td>
<td>92.6 (5.92)</td>
<td>94.1 (6.05)</td>
</tr>
<tr>
<td>Milk (kg)</td>
<td>+498.5 (145.9)</td>
<td>+469.2 (118.9)</td>
</tr>
<tr>
<td>Fat (kg)</td>
<td>+22.3 (5.67)</td>
<td>+18.7 (7.39)</td>
</tr>
<tr>
<td>Protein (kg)</td>
<td>+19.4 (6.33)</td>
<td>+18.8 (7.31)</td>
</tr>
<tr>
<td>Fat (g/kg)</td>
<td>+0.081 (0.12)</td>
<td>+0.023 (0.125)</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
<td>+0.065 (0.048)</td>
<td>+0.065 (0.062)</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>+3.40 (0.85)</td>
<td>-0.04 (1.09)</td>
</tr>
<tr>
<td>Calving interval (d)</td>
<td>-6.34 (1.80)</td>
<td>+5.05 (2.15)</td>
</tr>
<tr>
<td>Sire calving interval (d)</td>
<td>-7.83 (4.97)</td>
<td>+7.14 (2.57)</td>
</tr>
<tr>
<td>Maternal grandsire calving interval (d)</td>
<td>-6.12 (2.68)</td>
<td>+5.60 (2.97)</td>
</tr>
</tbody>
</table>

\(^1\)All PTA’s were obtained from the Autumn 2007 official dairy evaluations published by the Irish Cattle Breeding Federation and multiplied by two to convert to EBV’s.
### Table 2. The mean (and SD) chemical composition of grass, grass silage, and concentrate (Conc).

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<thead>
<tr>
<th>Feed</th>
<th>Forage</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dry period</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP (g/kg DM)</td>
<td>145.0 (16.8)</td>
<td>142.1 (7.5)</td>
</tr>
<tr>
<td>NDF (g/kg DM)</td>
<td>504.0 (26.6)</td>
<td>234.5 (7.5)</td>
</tr>
<tr>
<td>ADF (g/kg DM)</td>
<td>361.4 (31.6)</td>
<td></td>
</tr>
<tr>
<td>Ash (g/kg DM)</td>
<td>79.9 (2.3)</td>
<td>57.9 (3.7)</td>
</tr>
<tr>
<td>Crude Fibre (g/kg DM)</td>
<td></td>
<td>86.3 (6.07)</td>
</tr>
<tr>
<td><strong>Lactation</strong>²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP (g/kg DM)</td>
<td>217.5 (37.8)</td>
<td>154.3 (13.2)</td>
</tr>
<tr>
<td>NDF (g/kg DM)</td>
<td>424.0 (40.6)</td>
<td>270.0 (32.0)</td>
</tr>
<tr>
<td>ADF (g/kg DM)</td>
<td>235.7 (21.1)</td>
<td></td>
</tr>
<tr>
<td>Ash (g/kg DM)</td>
<td>84.0 (8.6)</td>
<td>95.5 (11.3)</td>
</tr>
<tr>
<td>OM digestibility (g/kg OM)</td>
<td>805.9 (39.9)</td>
<td></td>
</tr>
<tr>
<td>Crude Fibre (g/kg DM)</td>
<td></td>
<td>106.5 (11.79)</td>
</tr>
</tbody>
</table>

¹Forage consisted of grass silage
²Forage consisted of grass
<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence 5'-3'</th>
<th>Amplicon size (bp)</th>
<th>Accession number</th>
</tr>
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| RPS9 | Forward: CCTCGACCAAGAGCTGAAG  
Revers: CCTCCAGACCTCAGTTCGTTTCT | 64  | NM_001101152.1 |
| SOCS3 | Forward: CAGCCTGCGCCTCAAGAGC  
Reverse: AAGTGCGCGCCTCGCAGG | 185 | NM_174466.2 |
| JAK2 | Forward: TGGCAATGAACAAACAAGGA  
Reverse: ATCTCACTGGCCACCCATTACATC | 100 | XM_865133.2 |
| STAT5B | Forward: TGCACTCGCCCATATTCTGTA  
Reverse: AGTCCGACGCTCCTCAAAATGT | 137 | NM_174617.3 |
| CAP1 | Forward: AGGCCTGTGCTTCAAGTGGTCCC  
Reverse: ACAAAAGAACCAGTAGTCCTCAGG | 121 | NM_001035010.1 |
| SRRM2 | Forward: CCAGCCTGCGCCTCAAGAAG  
Reverse: CTCGTAACCTCCCTGCTCTCCTCA | 194 | XM_587832.5 |
| PSMD2 | Forward: CTGTGGCTGGGCTGCTCACC  
Reverse: CCACATCCACTGCTGGCCC | 183 | NM_001101197.1 |
| ACTB | Forward: ACTTGCGCAGAAAACGAGAT  
Reverse: CACCTTCACCGTTCCAGTTT | 123 | BT030480 |
| IGF-I | Forward: ATGCCCAAGGCTCAGAAG  
Reverse: GGTGCGATCTATTCTTCACT | 115 | NM_001077828.1 |
| IGFBP1 | Forward: ACCAGCAGAGAATTGTC  
Reverse: CTGATGCGATCTCCAGAGAT | 119 | NM_174554.2 |
| IGFBP2 | Forward: ACATCCCCAATCTGAGC | 114 | NM_174555.1 |
| IGFBP3 | Forward: ACGACACACAAGAATCTCCCTCCTC | 101 | NM_174556.1 |
| IGFBP4 | Forward: ATGGTGCGTATGGAGGAGGT | 106 | NM_174557.3 |
| IGFBP5 | Forward: AAAGAGCTGACCACTGTCGA  
Reverse: CCCCCGGGTATATTCATCCTTC | 100 | NM_001105327.1 |
| IGFBP6 | Forward: GGAGGAGACGCGAGGAGGTA  
Reverse: GAATGGTGAAGGTCGAGCGAT | 100 | NM_001040495.1 |
| ALS | Forward: CTCTCTGGCTGGCAGCTC | 111 | NM_001075963.1 |
| GHRtot | Forward: ATGGCCTGACGAGGATCAT  
Reverse: AGGATGCTGCGATGAAATTC | 121 | NM_176608.1 |
| GHR 1A | Forward: CCAGCCTCTGTTCCAGAGGTGT  
Reverse: TGCCACTGCCAAGGCTCAAC | 87 | AY748827.1 |

1 RPS9 = ribosomal protein S9, SOCS3 = suppressor of cytokine signalling 3, JAK2 = janus tyrosine kinase 2, STAT5B = signal transducer and activator of transcription 5B, CAP1 = adenylate cyclase-associated protein 1, SRRM2 = serine/arginine repetitive matrix 2, PSMD2 = 26S proteasome non-ATPase regulatory subunit 2, ACTB = ß-Actin, IGFBP1 = IGF binding protein-1, IGFBP2 = IGF binding protein-2, IGFBP3 = IGF binding protein-3, IGFBP4 = IGF binding protein-4, IGFBP5 = IGF binding protein-5, IGFBP6 = IGF binding protein-6, ALS = acid-labile subunit, GHRtot = growth hormone receptor, GHR 1A = growth hormone receptor 1A variant.
Figure 1. Cummins
Figure 2. Cummins
Figure 3. Cummins
Figure 4. Cummins
Figure 1. Circulating concentrations of IGF-I and insulin, and the mean daily milk yield and body condition score (BCS) during the gestation/lactation cycle of Fert+ and Fert- cows. Vertical bars indicate 95% confidence intervals.

(A) Circulating concentrations of IGF-I and insulin: Plasma IGF-I concentrations were significantly greater in Fert+ cows (P < 0.001) during lactation. The mean (95% confidence intervals in parentheses) circulating IGF-I concentration were 146.6 ng/mL (126.0, 168.8) and 110.0 ng/mL (93.7, 127.1) for Fert+ and Fert- cows, respectively. No effect of genotype was observed for plasma insulin concentrations (P = 0.1). The mean (95% confidence intervals in parentheses) circulating insulin concentration were 2.96 uIU/mL (2.41, 3.62) and 2.34 uIU/mL (1.90, 2.85) for Fert+ and Fert-, respectively. No genotype × wk or genotype × parity interactions were observed for IGF-I or insulin.

(B) Mean daily milk yield and BCS: No effect of genotype or genotype × parity interactions was observed for daily milk yield (P > 0.05). There tended to be a significant genotype × wk interaction (P < 0.05) for daily milk yield during the study period. No genotype (P = 0.4), genotype × wk (P = 0.3) or genotype × parity (P = 0.8) effects were detected for BCS (SED = 0.09 units).

Figure 2. Relative abundance of mRNA in liver for (A) IGF-I, (B) GHR 1A, and (C) GHRtot during wk -3, 1, 8, 20 and 35 relative to parturition in Fert+ and Fert- cows. Vertical bars indicate 95% confidence intervals.

(A) IGF-I: IGF-I mRNA abundance was significantly greater in Fert+ cows (P < 0.05) over the duration of the study. No genotype × wk or genotype × parity interaction existed (P > 0.05). (B) GHR 1A: No genotype, genotype × wk or genotype × parity interaction effects were detected for transcript abundance of GHR 1A (P > 0.05). (C) GHRtot: Transcription of GHRtot was significantly greater in Fert- cows (P < 0.05) over the duration of the study.

Figure 3. Relative abundance of mRNA in liver for (A) IGFBP1, (B) IGFBP2, (C) IGFBP3, (D) IGFBP4, (E) IGFBP5, and (F) IGFBP6 during wk -3, 1, 8, 20 and 35 relative to parturition in Fert+ and Fert- cows. Vertical bars indicate 95% confidence intervals.

(A) No effect of genotype was detected for transcript abundance of IGFBP1 (P = 0.3). (B) IGFBP2 mRNA abundance was significantly greater in Fert- cows (P = 0.003) over the duration of the study. (C) No effect of genotype was detected for mean transcript abundance of IGFBP3 during the 5 sample time-points (P = 0.17). (D) IGFBP4 mRNA abundance was significantly greater in Fert- cows (P = 0.027) over the duration of the study. (E) Fert- cows had significantly greater IGFBP5 mRNA than Fert+ cows throughout the study period (P < 0.001). (F) Abundance of IGFBP6 mRNA was significantly greater in Fert- cows (P = 0.013) over the duration of the study. No genotype × wk or genotype × parity interactions existed for any of the six IGFBPs (P > 0.05). a denotes differences at P < 0.1, and b denotes differences at P < 0.05.

Figure 4. Relative abundance of mRNA in liver for (A) JAK2, (B) STAT5B, (C) SOCS-3, and (D) ALS during wk -3, 1, 8, 20 and 35 relative to parturition in Fert+ and Fert- cows. Vertical bars indicate 95% confidence intervals.

There was no effect of genotype, genotype × wk or genotype × parity interaction on mRNA abundance of JAK2 (P = 0.3), STAT5B (P = 0.9), SOCS-3 (P = 0.5) and ALS (P = 0.7)