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The Presence of Growth Hormone Secretagogue Receptor (ghrelin receptor) in Metabolic Tissues of Beef Cattle with Differences in Composition of Gain

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The presence of growth hormone secretagogue receptor (ghrelin receptor) in metabolic tissues of beef cattle with differences in composition of gain[1]

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SUMMARY

Beef steers (n = 72) of similar age, weight (651 ± 3.1 lb), and genetic (Angus crossbred) background were used to determine the effects of growing diet composition (high-forage vs. high-concentrate) on the abundance of growth hormone secretagogue receptor (GHS-R or ghrelin receptor) in metabolically important tissues of beef cattle. At trial initiation (d 0), 8 steers were harvested for initial carcass composition. The remaining 64 steers were allotted, by weight, to pen and treatment was assigned randomly. Treatments were 1) a high-forage diet fed during the growing period (116 d) followed by a high-concentrate diet during the finishing period (117-209 d; GRW-FNSH) or 2) a high-concentrate diet fed for the duration of the trial (0-209 d; FNSH-FNSH). Steers were allowed ad libitum consumption regardless of dietary treatment. Eight steers per treatment were harvested on d 88, 116, 165, and 209. Immediately following harvest, liver, muscle (*sternomandibularis*), and subcutaneous adipose tissue samples were collected from each steer and immersed in liquid nitrogen. *Longissimus dorsi* samples were collected following a 48 h chill to establish a preliminary analysis of GHS-R abundance within an economically important muscle tissue. Protein separation and quantification was determined using SDS-PAGE and Western blotting techniques. Protein abundance was detected using the LI-COR® system and standardized to β-Actin. Protein abundance data were analyzed statistically using the GLM procedure of SAS comparing diet, harvest date, and their interaction. Protein abundance of GHS-R in *longissimus dorsi* tissue fluctuated relative to serial harvest date ($P < 0.001$), and was highest on d 88 in both treatment groups. The FNSH-FNSH steers had increased abundance of GHS-R in *longissimus dorsi* on d 88 and 116 compared with the GRW-FNSH steers. A dietary treatment by serial harvest day interaction ($P < 0.05$) occurred for protein abundance of GHS-R in subcutaneous adipose tissue. Abundance of GHS-R in subcutaneous adipose tissue of the GRW-FNSH was greatest on d 88, whereas abundance for the FNSH-FNSH treatment was greatest at the end of the finishing period (d 209). An interaction of dietary treatment and serial harvest day resulted ($P < 0.05$) for GHS-R abundance in liver tissue. The GRW-FNSH steers had increased liver GHS-R abundance following realimentation compared with the FNSH-FNSH steers which were on a continuous plane of nutrition. Protein abundance for liver GHS-R in both dietary treatments increased quadraticly ($P < 0.001$) throughout the feeding period. The GHS-R was not detected in *sternomandibularis* tissue. Overall liver GHS-R abundance increased in both dietary treatments following realimentation which is inconsistent with our hypothesis. Increased GHS-R abundance in various tissues of beef cattle while ghrelin concentrations are high and excess fat deposition is occurring warrants further investigation.

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INTRODUCTION

Ghrelin is a peptide hormone synthesized by abomasal and ruminal tissues of cattle and binds to the growth hormone secretagogue receptor (GHS-R; Hayashida et al., 2001; Gentry et al., 2003). The receptor to which ghrelin binds has been identified in a variety of animal tissues including hypothalamus, pituitary, adipose, liver, and skeletal muscle tissues (Wang et al., 2002). The GHS-R has two different isoforms (1A and 1B), however GHS-R 1A has been reported to be the biologically active receptor (Kojima et al., 1999). Previous research in our lab has demonstrated elevated plasma ghrelin concentrations for cattle deprived of feed and those treated to prolonged moderate nutrient restriction (Wertz-Lutz et al., 2006 and 2008). However, Jennings et al. (2008) reported increased ghrelin concentrations in the current experiment where cattle that were receiving adequate nutrients and depositing substantial amounts of fat. In an effort to explain how plasma ghrelin concentrations were elevated both with nutrient restriction and abundance, it was hypothesized that abundance of the GHS-R would be decreased in liver, muscle, and adipose tissues of cattle that were accumulating substantial fat. In support of the hypothesis that GHS-R abundance would decrease as animals become fatter, Kurose et al. (2005) reported decreased GHS-R expression in the hypothalamus of fatter sheep. French et al. (2006) also reported decreased GHS-R expression in the pituitary of fatter sheep. Despite the fact that the GHS-R has been identified in a variety of tissues that are important to livestock production, and it is responsive to nutritional manipulation, knowledge of the GHS-R presence in metabolically important tissues of beef cattle remains unknown. Therefore, the objectives of this trial were to 1) establish the presence of GHS-R protein in metabolically important tissues of beef cattle and 2) establish a relationship between energy intake during the growing period and abundance of GHS-R protein.

MATERIALS AND METHODS

Animals

Experimental procedures were approved by the South Dakota State University (SDSU) Laboratory Animal Resource Committee on Animal Care. Seventy-two beef steers of similar age (8 mo old), weight (651 ± 3.1 lb), and genetic background (Angus Crossbred) were used in this experiment. At trial initiation (d 0), 8 steers were harvested for initial tissue sample collection.

Dietary Treatments

Ingredient and nutrient composition of diets are presented in Table 1. Dietary treatments were 1) a low-energy diet (GRW1 and GRW2) for the growing period (d 0-116) followed by a high-energy diet during the finishing period (d 117-209; GRW-FNSH) or 2) the high-energy diet (FNSH) for the duration of the experiment (d 0-209; FNSH-FNSH). Steers were fed twice daily (8:00 am and 3:00 pm) and were allowed ad libitum access to feed and water throughout the trial. Steers assigned to the FNSH-FNSH treatment received a diet formulated to target 3.5 lb/d rate of gain, whereas GRW-FNSH cattle were fed to target 1.7 lb/d rate of gain (NRC, 2000). At the 83-d intermediate weight, steers were assigned to the GRW-FNSH treatment were gaining faster (3.0 lb/d) than predicted. As a result, the low-energy diet (GRW1) was adjusted by increasing the grass hay content to 25% and lowering the dry-rolled corn content to 13% (GRW2), thus lowering the energy content from 0.50 to 0.45 Mcal of NEg/lb (DM basis). To achieve the desired target, the GRW2 diet was then fed to the GRW-FNSH steers from d 89 to 116, followed by the FNSH diet from d 117 to 209.

Eight steers from each treatment group were harvested at 4 time points in the growth trial. Harvest points were: 1) during the growing period (d 88), 2) the end of the growing period (d 116), 3) the point at which the FNSH-FNSH steers reached 0.4 in ribfat (d 165), and 4) the point at which the GRW-FNSH reached 0.4 in ribfat (d 209). To monitor targeted ribfat, ultrasound measurements were recorded periodically before harvest points 3 and 4.

Table 1. Dietary ingredient and nutrient content of diets [a]

	Dietary Treatments		
Item	GRW1	GRW2	FNSH
High moisture corn	-	-	26.3
Whole shell corn	-	-	53.7
Dry rolled corn	28.0	13.0	-
Soybean meal	5.0	5.0	5.8
No grain corn silage	50.0	50.0	10.0
Grass hay	10.0	25.0	-
Liquid supplement [b]	-	-	4.3
Supplement [b]	7.0	7.0	-
Calculated nutrient composition			
DM	42.4	42.6	67.8
Ca	0.39	0.47	0.63
P	0.32	0.31	0.34
CP	12.5	12.5	12.5
NE_m, Mcal/lb	0.80	0.75	1.23
NE_g, Mcal/lb	0.50	0.45	0.63

[a] % Dry matter basis.

[b] Provided vitamins and minerals to meet or exceed nutrient requirements (NRC, 2000), FNSH diet contained 29 g/T of Rumensin and GRW diets contained 25 g/T of Rumensin.

Sample Collection

Subcutaneous adipose tissue, *sternomandibularis*, and liver tissue samples were collected immediately following harvest for analysis of GHS-R. Tissue samples were weighed, placed in aluminum foil, and immediately immersed in liquid nitrogen and stored at -80° C until further analyses. To establish a preliminary analysis of abundance of GHS-R in an economically important muscle tissue, *longissimus dorsi* samples were collected following a 48 h chill and stored at -20° C. Postmortem proteolysis was expected to be greater in the *longissimus dorsi* samples collected after a 48-h chill compared with those immediately immersed in liquid nitrogen, however, it was assumed that degradation would be consistent across treatments. Thus, the *longissimus dorsi* samples were analyzed to provide preliminary data for GHS-R abundance it that tissue.

Protein Abundance

SDS-PAGE. Denatured protein from subcutaneous adipose tissue, *sternomandibularis*, *logissimus dorsi*, and liver tissue samples were loaded (30 µg protein) in duplicate on SDS-polyacrylamide resolving gels (Tris HCl gels, Bio-Rad Laboratories, Hercules, CA). A pre-stained MW standard (100 kDa to 20 kDa; Bio-Rad Laboratories) was loaded (1 µg) onto each gel. A positive control mouse brain tissue (normal tissue, 0 days old) was loaded onto each gel and subsequently blotted for GHS-R according to manufacturer specifications (ab7188, Abcam, Cambridge, MA).

Western Blotting for GHS-R. Blots designated for GHS-R 1A were incubated at room temperature with a polyclonal rabbit anti-GHS-R 1A (GHSR11-A, Alpha Diagnostic, San Antonio, TX) diluted 1:5,000. Following incubation in primary antibody for protein of interest, all blots were incubated with monoclonal rabbit anti-β-Actin (A 5060, Sigma-Aldrich, St. Louis, MO) diluted 1:15,000 for subsequent standardization. After incubation (1 h) with primary antibody, blots were then incubated for 30 min with a goat anti-rabbit secondary antibody (926-32221 IRDye 680; LI-COR, Inc., Lincoln, NE) diluted to 1:35,000. Bands were detected using the LI-COR ® system (LI-COR) according to the manufacturer's instructions. To account for lane-to-lane variation in protein load, intact GHS-R band intensity were quantified relative to the β-Actin band intensity within the same lane. Arbitrary units for protein abundance were calculated by averaging the duplicate sample quantity and dividing by the quantity of β-Actin within the same lane.

Statistical Analyses

Seventy-two steers were used at trial initiation. However, during the feeding period one steer from each treatment was removed due to illness unrelated to dietary treatment. Therefore, statistical analyses were performed on data from the remaining 70 steers. Individual steer was used as experimental unit. Protein abundance data were evaluated as a randomized complete design using the GLM procedure of SAS comparing diet, harvest date, and their interaction. Differences in least squares means that resulted from treatment were evaluated by using the interaction of treatment by harvest date as the error term option. Differences in treatment means were separated using least squares means with treatment by harvest date as the error term. Mean differences were considered significant at $P < 0.05$. Orthogonal polynomials for unequal spacing of serial harvest points were established using the IML procedure of SAS. The polynomials were used to establish linear, quadratic, and cubic relationships between harvest dates.

RESULTS AND DISCUSSION

Growth Hormone Secretagogue Receptor

Ghrelin is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R). In rodents, ghrelin has been reported to increase feed intake and result in adiposity (Tschöp et al., 2000). Kurose et al. (2005) demonstrated decreased expression of GHS-R in hypothalamic tissue of sheep fed to achieve high body fat. Likewise, French et al. (2006) demonstrated decreased GHS-R expression in genetically fat sheep compared with lean lines of sheep. The GHS-R also has been identified in tissues other than the hypothalamus including adipose, liver, and skeletal muscle tissue in various species (Wang et al., 2002). However, presence of the GHS-R in metabolically important tissue of beef cattle remains unknown. Western blot data (Figure 1) illustrated detectable bands for the positive control which was mouse brain tissue (normal tissue, 0 days old) as well as for bovine subcutaneous adipose tissue and liver tissue. Protein abundance of GHS-R in *sternomandibularis* tissues, which had minimal adipose tissue, was undetectable, therefore no statistical analyses were performed with this tissue. Western blot data (Figure 2) illustrated detectable bands for the positive control which was bovine liver tissue as well as for bovine *longissimus dorsi* tissue. *Longissimus dorsi* samples were homogenized whole muscle which included lean tissue, intramuscular adipose tissue and connective tissue. The GHS-R protein was detectable in subcutaneous adipose tissue, liver, and longissimus dorsi. The GHS-R protein was most prominent in liver tissue and much less abundant in subcutaneous adipose tissue and *longissimus dorsi*.

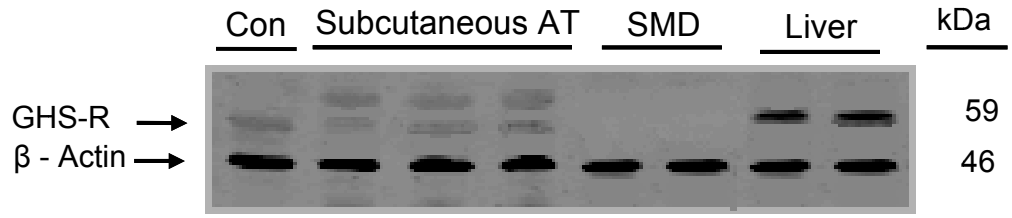

Figure 1. Validation of protein abundance of growth hormone secretagogue receptor (GHS-R) in subcutaneous adipose, liver, and *sternomandibularis* tissue in beef cattle. Con = control mouse brain tissue (normal tissue, 0 days old), AT = Bovine adipose tissue, SMD = *sternomandibularis* tissue, kDa = kilodalton.

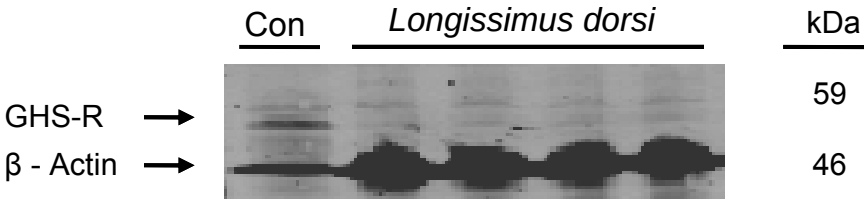

Figure 2. Validation of protein abundance of growth hormone secretagogue receptor (GHS-R) in *longissimus dorsi* tissue. Con = control bovine liver tissue, kDa = kilodalton.

Previous research in our lab has demonstrated elevated plasma ghrelin concentrations in cattle during acute feed deprivation as well as prolonged moderate nutrient restriction (Wertz-Lutz et al., 2006 and 2008). However, Jennings et al. (2008) established elevated plasma ghrelin concentrations in the current experiment in cattle consuming a high-grain diet ad libitum (FNSH-FNSH) which resulted in adequate nutrients. In an effort to explain how plasma ghrelin concentrations can be elevated both with nutrient restriction and nutrient abundance, it was hypothesized that the GHS-R abundance would be decreased in liver, muscle, and adipose tissue depots as cattle became fatter. Therefore, the hormone signal is increased but its receptor is not available to respond. Data from adipose tissue in the current experiment do not support this hypothesis.

There was an interaction of dietary treatment by serial harvest day ($P < 0.05$) for protein abundance of GHS-R in subcutaneous fat tissue (Figure 3A). Protein abundance of GHS-R was higher in subcutaneous fat tissue on d 88 and 209 compared with d 0, 116, and 165. Additionally, on d 88 GHS-R abundance was higher for GRW-FNSH compared with FNSH-FNSH steers. In contrast, GHS-R abundance was greater for the FNSH-FNSH steers compared with GRW-FNSH steers on d 209.

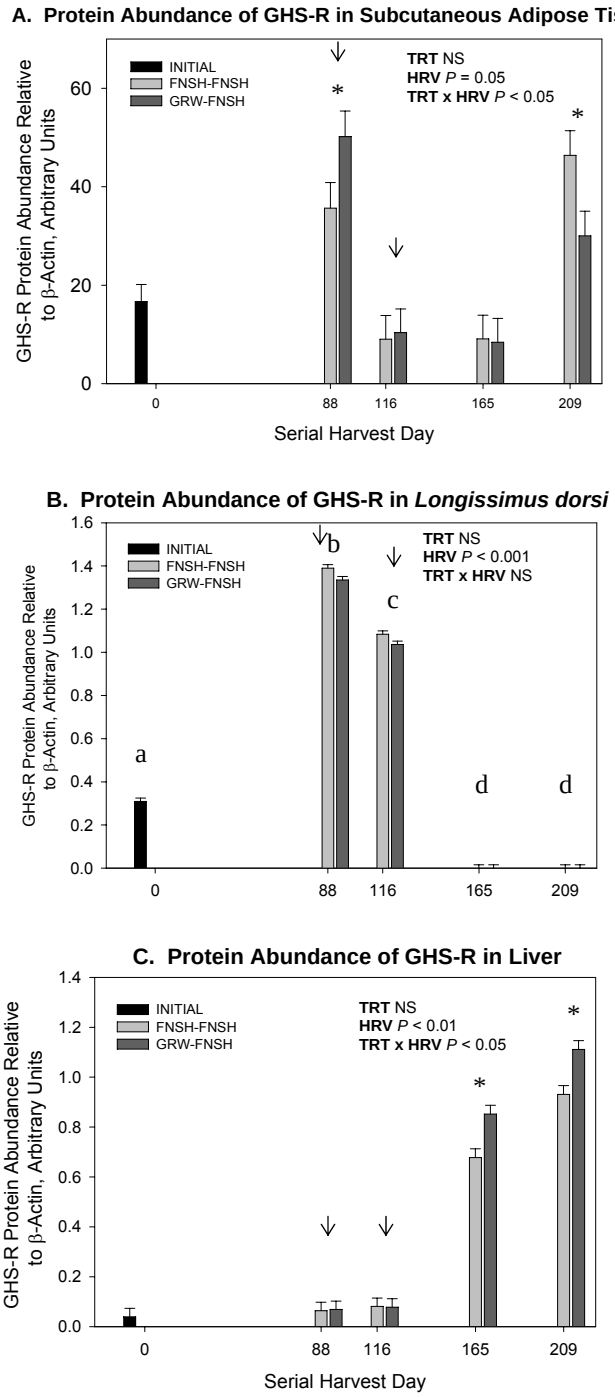

Figure 3. Protein abundance of growth hormone secretagogue receptor (GHS-R) in subcutaneous adipose tissue (A), *longissimus dorsi* (B), and liver tissue (C) in beef cattle. INITIAL = serial harvest at the beginning of feeding period, TRT = FNSH-FNSH = steers receiving a high-energy diet during the entire feeding period, GRW-FNSH = steers receiving a low-energy diet during the growing period, followed by a high-energy diet during the finishing period; NS = not significant. The first arrow indicates when energy content of the growing diet received by the GRW-FNSH steers was reduced further. The second arrow indicates when the GRW-FNSH steers were switched to the high- energy diet to begin the finishing period. ^{a,b,c,d} Means within serial harvest day that do not have common superscripts differ, $P < 0.05$ as a result of harvest date. * Within a harvest point, FNSH-FNSH vs. GRW-FNSH differ as a result of dietary treatment, $P < 0.05$.

Protein abundance of GHS-R in *longissimus dorsi* tissue was highest on d 88 in both treatment groups ($P < 0.001$), intermediate on d 0 and 116, and not detectable on d 165 and 209 (Figure 3B). *Longissimus dorsi* samples were collected after a 48 h carcass chill. It was assumed that postmortem proteolysis would be similar between treatment groups, however the delayed tissue collection may have influenced total abundance of the GHS-R protein. Protein abundance of GHS-R was detected in *longissimus dorsi* muscle after a 48-h chill, but not *sternomandibularis* muscle collected at harvest. These data suggest GHS-R identified in the *longissimus dorsi* during the growing period could be from intramuscular adipose tissue present within that muscle.

Liver GHS-R abundance did not support the hypothesis that elevated plasma ghrelin concentrations would be associated with decreased receptor abundance. An interaction of dietary treatment and serial harvest day resulted ($P < 0.05$) for GHS-R abundance in liver tissue (Figure 3C). Protein abundance for GHS-R in both dietary treatments increased quadradicly ($P < 0.001$). The GRW-FNSH steers had increased liver GHS-R abundance following realimentation compared with the FNSH-FNSH steers which were on a continuous plane of nutrition. Abundance of liver GHS-R was increased for both dietary treatments during the finishing period, however the increase was greater ($P < 0.05$) for GRW-FNSH steers compared with FNSH-FNSH steers. Davies et al. (2009) reported ghrelin stimulated the uptake of lipids in rat hepatocytes, allowing a two-fold increase of triglyceride content. In rodents, ghrelin infusion also has been reported to induce lipogenic gene expression favoring triglyceride deposition in liver tissue compared with skeletal muscle (Barazzoni et al., 2005). Gauna et al. 2005 also reported ghrelin stimulated the production of glucose from hepatocytes in vitro. These data suggest that ghrelin and GHS-R could play a key role in energy homeostasis by directly affecting liver metabolism.

Cattle experiencing differential composition of gain have differences in plasma ghrelin and leptin concentrations as well as differences in abundance of GHS-R in metabolically important tissues. Therefore, role of GHS-R in composition of gain and maintenance energy requirements in beef cattle warrant further investigation.

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