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Technical note

Expression analysis of key somatotropic axis and liporegulatory genes in ghrelin- and obestatin-infused dairy cows

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Abstract

Ghrelin, an orexigenic hormone, is the endogenous ligand for the growth hormone secretagogue receptor (GHSR). Obestatin is produced from the same precursor peptide as ghrelin, and although obestatin was initially thought to promote actions opposite to those of ghrelin, many studies have failed to confirm this hypothesis. In the current study, multiparous cows were continuously infused with ghrelin (n = 10) or obestatin (n = 10) for 8 wk and compared to an untreated group (n = 10) to examine the effects of these hormones on somatotropic and liporegulatory gene expression. The expression of key genes was measured by quantitative real-time polymerase chain reaction. Growth hormone secretagogue receptor mRNA expression was altered in ghrelin- and obestatin-infused cows in a similar manner, as expression was increased at 4 wk, however it had decreased by 8 wk. Obestatin-infused cows presented with a significant decrease in the expression of ATP-binding cassette A1 (ABCA1) in adipose tissue, suggesting changes in cholesterol transport. Liver insulin-like growth factor (IGF) binding protein-3 mRNA displayed a week-by-treatment interaction, as expression was increased in control and obestatin-infused cows; however, expression decreased in ghrelin-infused cows. Adipose expression of hormone sensitive lipase (LIPE) mRNA was not altered by treatment or time, suggesting hormone infusion is not initiating lipolysis. The expression of lipogenic genes in adipose tissue increased with time in all groups, consistent with the general lactational profile of lipogenesis in dairy cows. These data indicate that continuous infusion of ghrelin or obestatin does not alter the expression of key somatotropic or liporegulatory genes in the lactating dairy cow, although obestatin infusion may alter cholesterol transport.

Keywords: Neuroendocrine; Somatotropic axis; Ghrelin; Obestatin; Negative energy balance; Early lactation

1. Introduction

Ghrelin is a potent orexigenic hormone that increases preprandially in blood, signaling the requirement for food intake. Composed of 27 amino acids in cows, ghrelin is produced and released principally from the abomasum, from where it enters the bloodstream and activates the growth hormone secretagogue receptor (GHSR), which is found in many tissues [1,2]. Activation of GHSR results in the release of growth hormone (GH) and a variety of other effects [3]. Ghrelin is a product of the cleavage of pre-proghrelin, a peptide that, when split, results in ghrelin and obestatin [4]. Obestatin is classified as an anorexigenic protein and reportedly has effects opposite to those of ghrelin. Recent studies, however, do not report consistent effects of this hormone [4,5], and no data are available on the effects of obestatin infusion on gene expression in dairy cows.

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In rodents, ghrelin increases food intake when administered peripherally, and it has been reported to induce adipose synthesis, reduce adipolysis [6], and regulate insulin secretion and glucose metabolism [3]. The responses to ghrelin administration in ruminants, however, are less well understood. Recent studies investigating ghrelin concentrations in cows indicate increased plasma ghrelin levels when cows are on restricted feed intakes, confirming that ghrelin concentrations increase when food is required [7]. In addition to elevated ghrelin, GH and nonesterified fatty acid (NEFA) concentrations were also increased, consistent with the reported findings of Roche et al [1]. ThidarMyint et al [8] confirmed these findings, reporting that intravenous ghrelin administration led to increased NEFA concentrations, and that blood GH was increased in a dose-dependent manner. Reported effects of ghrelin infusion on dry matter intake in ruminants is varied, however, in the h immediately post-infusion, the time spent eating and the dry matter intake of steers were increased [9]. Ghrelin also induces the release of adrenocorticotropin hormone (ACTH), leading to the release of cortisol [3]. This effect of ghrelin has been observed in dairy cows, as indicated by an increase in serum cortisol [10].

Studies focusing on dairy cows have reported that preprandial ghrelin surges are observed only during early lactation, when cows are in negative energy balance (NEB), indicating that the NEB seen during early lactation is affected by ghrelin [11]. The study described here was conducted in 2006 as part of a larger experiment, in which it was demonstrated that 8 wk of continuous ghrelin or obestatin infusion resulted in decreased body condition score (BCS) in ghrelin-treated cows [1]. Serum concentrations of insulin-like growth factor (IGF)-1 and glucose were also reduced, and NEFA concentrations were increased. The primary objective of the current study was to investigate the expression of key somatotropic and liporegulatory genes in the liver and adipose tissue of these cows, and, in particular, to assess whether gene expression changes in response to infusion of these hormones was indicative of a lengthened NEB in ghrelin-infused, early lactation dairy cows.

2. Materials and methods

2.1. Experimental design and treatments

The study was conducted in 2006 as part of a larger experiment described by Roche et al [1], and all procedures were approved by the Ruakura Animal Ethics Committee, Hamilton, New Zealand . A subset of 30 lactating Holstein-Friesian dairy cows was randomly chosen from 3 existing experimental herds to create an untreated control (CON) and 2 treatment groups (n = 10 each). Groups were generated by randomized block design in week 4 of lactation, ensuring that calving date, parity (4.3 ± 1.81), pre-experimental body weight (498 ± 51.2 kg), body condition score (BCS) (4.6 ± 0.38; [12]), and milk production (27.7 ± 3.88 kg of milk/d; 1.3 ± 0.17 kg of fat/d; 1.0 ± 0.14 kg of protein/d; 4.7 ± 0.060% fat; 3.7 ± 0.20% protein) were balanced across treatment. The treatments consisted of an 8-wk infusion with 0.007 μmol/kg of body weight$^{0.75}$/d of either (Dap [2,3-diaminopropanoic acid]-octanoyl^3)-human ghrelin ([Dap^3]-ghrelin; [GHRL group]) or synthetic bovine obestatin (OBE group). Obestatin and [Dap^3]-ghrelin were obtained from Peptides International Inc. (KY, USA), following the procedures of Roche et al [1]. The continuous subcutaneous infusion was administered via miniature implanted osmotic pumps (Alzet Model 2ML4; Braintree Scientific, Inc.; MA, USA) that released 2.5 μL of solution/h at a constant rate for 28 d. Cows were rotationally grazed as a herd for the duration of the experiment and had access to a fresh allocation of pasture twice daily. Pasture allowance of greater than 40 kg of dry matter per cow was available each day to ensure feed was not restricted [1].

2.2. Tissue sampling, RNA extraction and mRNA analyses

2.2.1. Collection of liver and adipose samples

Tissue biopsy samples were obtained from each animal 1 wk prior to infusion and at 4 and 8 wk post-infusion start date. Liver sample collection was performed as described by Lucy et al [13], resulting in samples of approximately 200 mg. Subcutaneous adipose samples were collected posterior to the shoulder blade, and approximately 10 cm down the withers. The site was clipped and cleansed with iodine before the local anesthetic 2% lignocaine was administered. A 3-cm incision was made through the depth of the skin, where approximately 150 mg of adipose was removed using forceps and a scalpel. All samples were placed in microcentrifuge tubes, frozen in liquid nitrogen, and stored at −80°C until analysis.

2.2.2. RNA extraction

Tissue biopsies were placed in Qiagen RLT buffer and homogenized using Lysing Matrix D tubes in a FastPrep instrument (MP Biomedicals; CA, USA). Total RNA was extracted using a Qiagen RNeasy kit. All samples were