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High concentrations of fatty acids and β-hydroxybutyrate impair the growth hormone-mediated hepatic JAK2-STAT5 pathway in clinically ketotic cows

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ABSTRACT

The hepatic growth hormone (GH)–insulin-like growth factor (IGF)-I axis is essential for regulating intrahepatic lipid metabolism. Ketotic cows are characterized by high blood concentrations of fatty acids and β-hydroxybutyrate (BHB), which display lipotoxicity. The aim of this study was to investigate changes in the hepatic GH–IGF-I axis in ketotic cows and to determine the effects of fatty acids and BHB on the GH–IGF-I axis in calf hepatocytes. Liver and blood samples were collected from healthy (n = 15) and clinically ketotic (n = 15) cows. Hepatocytes were isolated from calves and treated with various concentrations of GH, fatty acids, and BHB. The results showed that clinically ketotic cows displayed a high blood concentration of GH, a low blood concentration of IGF-I, and decreased hepatic GHR1A expression as well as impaired hepatic Janus kinase 2 (JAK2)-signal transducer and activator of transcription 5 (STAT5) signaling. In vitro, GH treatment induced activation of the JAK2-STAT5 pathway to increase the mRNA expression and secretion of IGF-I in calf hepatocytes. More importantly, treatment with fatty acids or BHB significantly inhibited *GHR1A* mRNA and JAK2 protein expression, as well as the STAT5 phosphorylation level and phospho-STAT5 nuclear translocation; these effects markedly reduced *IGF1* mRNA expression and secretion in calf hepatocytes. In summary, these results indicate that high blood concentrations of fatty acids or BHB can impair the intrahepatic GH-mediated JAK2-STAT5 pathway and downregulate IGF-I expression and secretion in ketotic cows.

Key words: ketotic cows, growth hormone, insulin-like growth factor-I

INTRODUCTION

During the early lactation period, most dairy cows experience a negative energy balance (**NEB**) caused by decreased DMI and increased demand for energy to support milk production (Xu et al., 2015a,b). Severe NEB initiates fat mobilization and a subsequent increase in the blood concentrations of fatty acids and BHB (Liu et al., 2014), which may result in the development of ketosis and fatty liver. Neuroendocrine responses involving the hypothalamus-pituitary-liver axis may result in significant changes in the secretion of several hormones, including prolactin and growth hormone (**GH**; Elsasser et al., 2000). These hormones are believed to have a profound effect on the adaptation to NEB (Herdt, 2000). Interestingly, postpartum dairy cows with NEB display GH resistance, high blood concentration of GH, and low blood concentration of IGF-I (Radcliff et al., 2006).

Growth hormone is a pituitary hormone that directly or indirectly affects numerous aspects of animal lactation, growth, and reproduction (Lucy et al., 2001; Douglas et al., 2016; Geiger et al., 2016). Hepatocytes are the target cells of GH and are the main source of IGF-I (Thissen et al., 1994; Sheehy et al., 2017). Growth hormone binds growth hormone receptor (**GHR**), thereby activating Janus kinase 2 (**JAK2**)-signal transducer and activator of transcription 5 (**STAT5**) signaling pathways and increasing the expression and synthesis of IGF-I in hepatocytes (Barclay et al., 2011). Furthermore, IGF-I acts as an endocrine factor that controls GH secretion through a negative feedback loop (Le Roith et al., 2001). Therefore, low hepatic expression of GHR could lead to decreased liver IGF-I production and less GH negative feedback (Silva et al., 2017).

The hepatic GH–IGF-I axis is essential in the regulation of intrahepatic lipid metabolism and mammary milk production (Piechotta et al., 2012; Mense et al., 2015; Silva et al., 2015). Studies have shown that dairy cows display downregulated hepatic GHR, increased blood GH concentration, and decreased blood IGF-I

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Table 1. Nutrient composition of the diets

Item	Measurement
Ingredient (%)	
Corn silage	40.00
Corn	35.00
Wheat bran	8.00
Soybean meal	5.00
Sunflower	8.00
NaCl	1.00
Premix[1]	1.80
$NaHCO_3$	1.20
Total	100.00
Nutrient composition (% of DM)	
NE_L (MJ/kg)	6.70
CP	15.20
NDF	33.45
ADF	17.20
NFC	40.40
Ca	0.70
P	0.50

[1]The premix provided the following per kg of diet: vitamin A 200,000 IU, vitamin D 70,000 IU, vitamin E 1,000 IU, Fe 2,000 mg, Cu 600 mg, Zn 2,400 mg, Mn 1,300 mg, I 6 mg, Co 7 mg.

concentration during the first week of lactation compare with prepartum period (Lucy et al., 2001; Bernier-Dodier et al., 2011; Gross et al., 2011). Additionally, Fenwick et al. (2008) demonstrated that plasma protein and hepatic mRNA for IGF-I were reduced in cows with severe NEB induced by feed restriction on the second day of lactation compared with cows with mild NEB (Fenwick et al., 2008). Thus, these studies demonstrated that the GH-IGF-I axis is uncoupled in early-lactating cows.

Lipotoxicity is defined as lipid- and lipid metabolite-induced lean tissue dysfunction (Erikci and Hotamisligil, 2016). Dairy cows with clinical ketosis display high blood concentrations of fatty acids and BHB. Lucy et al. (2001) reported that GH resistance further promoted lipolysis in adipose tissue and was associated with an increase in fatty acid concentration in the blood during lactation (Lucy et al., 2001). Our previous studies indicated that fatty acids and BHB displayed lipotoxicity in liver tissues (Shi et al., 2014; Song et al., 2014; Deng et al., 2015; Song et al., 2016); however, the effects of fatty acids and BHB lipotoxicity on the GH–IGF-I axis of calf hepatocytes were not characterized. Therefore, the aim of the current study was to investigate (1) changes in the hepatic GH–IGF-I axis in ketotic cows and (2) the effects of fatty acids and BHB on the GH–IGF-I axis in calf hepatocytes, as well as the mechanisms underlying these effects.

MATERIALS AND METHODS

Animals

The Ethics Committee on the Use and Care of Animals of Jilin University approved the study protocol (Changchun, China; 2015 clinical trial [2015–121]). To ensure that the cows used in this study had no comorbidities, all cows received a routine physical examination. A TMR that met the animals' nutritional requirements was fed ad libitum once per day at 1100 h; the basal diet formulation is shown in Table 1. We chose lactating Holstein cows with similar numbers of lactations (median = 3, range = 2 to 4) and DIM (median = 6 d; range = 3 to 9 d) from a 1,000-cow dairy farm located in Changchun, Jilin Province, China. The cows were classified as suspected clinical ketosis by veterinarians if feed intake, milk yield, or both were reduced and a nitroprusside test for ketone bodies in milk was positive (Duffield et al., 2009; Piechotta et al., 2012); subsequently, the blood concentration of BHB in these cows was measured. According to the clinical symptoms and serum BHB concentration (Oetzel, 2004), 15 clinically ketotic cows whose serum BHB concentration were higher than 3 m*M* and 15 healthy cows whose serum BHB concentration were less than 0.6 m*M* were chosen for the experiments. Characteristics of the ketotic and healthy cows are presented in Table 2.

The milk yield was recorded on 3 consecutive days at 0530 and 1500 h. Blood samples were withdrawn

Table 2. The basic description of healthy and ketotic cow (data were analyzed using non-parametric tests)

	Ketosis (n = 15)		Control (n = 15)		
Item	Median	Interquartile range	Median	Interquartile range	*P*-value
BW (kg)	657	648, 665	625	589, 639	0.0002
Milk production (kg of milk/cow per day)	25.6	24.3, 26.4	30	29.3, 31.2	<0.0001
DMI (kg/d)	20.1	19, 20.5	21.8	20.2, 22.6	0.0020
BCS	3.16	3.05, 3.2	2.71	2.54, 2.74	<0.0001
Glucose (m*M*)	2.17	2.05, 2.34	4.13	4.01, 4.27	<0.0001
Fatty acids (m*M*)	1.29	1.11, 1.47	0.27	0.18, 0.3	<0.0001
BHB (m*M*)	3.87	3.6, 4.04	0.36	0.27, 0.38	<0.0001