HIGH-ENERGY DIET AT ANTEPARTUM DECREASES INSULIN RECEPTOR GENE EXPRESSION IN ADIPOSE TISSUE OF POSTPARTUM DAIRY COWS

ZHIGANG ZHANG, JIANGUO WANG¹, RUIFENG GAO¹, WEIQIAN ZHANG, XINWEI LI¹,
 GUOWEN LIU¹, XIAOBING LI¹, ZHE WANG¹, AND XINGLIN ZHU¹

College of Veterinary Medicine, Northeast Agricultural University, Harbin, 150030, China

¹College of Veterinary Medicine, Jilin University, Changchun, 130062, China
 wangzhe500518@sohu.com

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Abstract

The objective of the study was to determine expression of gene of insulin receptor (INSR) in adipose tissue of postpartum dairy cows fed diets containing different amounts of energy at the antepartum period. Healthy pregnant dairy cows (n=45) on 21st d of the antepartum were divided into three groups differing in diet composition, namely: control group fed a normal diet, high energy group fed a high energy diet, and low energy group fed a low energy diet. Twenty-one days after parturition, INSR gene expression in adipose tissue was determined by internally controlled reverse transcriptase PCR. The level of INSR mRNA in adipose tissues of cows fed the high energy diet was substantially lower than that in cows fed normal or low energy diets. A relatively higher level of INSR mRNA in the adipose tissue of cows fed low energy diet may be beneficial for gluconeogenesis and lipogenesis, which can relieve an energy negative balance. Reduced level of INSR mRNA in adipose tissue of cows fed high energy diet indicates that the response to insulin has significantly decreased.

Key words: dairy cows, adipose tissue, insulin receptor, high energy diet, gene expression.

A major physiological characteristics of peripartum cows (the last 3 weeks before parturition and the first 3 weeks of lactation) is negative energy balance (NEB) (1). Dairy cows need to mobilise adipose tissue to compensate the relative deficit of glucose during the peripartum period, which is due to the NEB concomitant with the onset of lactation (3, 17). The rapid adaptation of key metabolic pathways is crucial to successfully undergo the transition from pregnancy to lactation (6). A failure for the adequate adaptation, may result in energy imbalances, leading to an increased risk of digestive, metabolic, or infectious disorders (*e.g.* displaced abomasum, retained placenta, ketosis, fatty liver, or mastitis) (2, 12), or even to a combination of disorders.

Insulin is a peptide hormone that initiates its biological effects by binding to specific insulin receptor (INSR) and activating it. This results in tyrosine phosphorylation of several substrates, including INSR substrate proteins (16). In addition to its functions in the liver, insulin also stimulates the synthesis and storage of fat, inhibits fat breakdown, and promotes glucose utilisation in adipose tissue (14). A reduction of INSR expression in early lactation participates in the development of NEB in dairy cows.

It is very important to understand the mechanisms underlying metabolic adaptations of early

lactation, as well as development of new strategies to optimise health and well-being of early lactating dairy cows (22). Regulation of energy intake may be an effective method for relieving NEB in dairy cows during early lactation (7). In the study, the effect of a high energy diet during the antepartum period on INSR gene expression in adipose tissue of postpartum dairy cows was investigated.

Material and Methods

Healthy Chinese Holstein pregnant dairy cows (n=45) were selected, and divided into three equal groups. The cows were 3-5-years of age, with 1-3 parities and mean body weight of 643 kg, and their initial body condition score were similar amongst the groups. As shown in Table 1, cows in control group were fed according to the Dairy Cows Breeding Standard of China (2000), the high energy group was fed 20% above the nutritional requirement (120%), and the low energy group was fed 80% of the nutritional requirement. The cows were housed in tie stalls individually. The experimental period was from day 21 antepartum to day 21 postpartum. After parturition, all cows were fed according to the Dairy Cows Breeding

Standard of China (2000), with *ad libitum* intake.

On day 21 postpartum, a subcutaneous adipose tissue (50–80 mg) was obtained from cows by biopsy at the caudation. All samples were washed with sterile saline, snap-frozen in liquid nitrogen, and stored at -80°C . The experimental procedure was approved by the Academic Committee of Jilin University.

Primer design and DNA fragment amplification were performed as previously described (12, 13). Total RNA from adipose tissue was isolated using a Trizol RNA purification kit in accordance with the manufacturer's instructions. Isolated RNA was diluted 40 times and analysed spectrophotometrically at 260 and 280 nm. Only samples with $\text{OD}_{260\text{ nm}}/\text{OD}_{280\text{ nm}} > 1.9$ were retained for further analysis (24). Synthesis of cDNA and internally controlled RT-PCR were performed as previously described for detection of the INSR mRNA

(12, 13). Image processing and grey-scale analysis were performed with the TANLON gel image analysis system (Shanghai, China).

Concentrations of glucose and non-esterified fatty acids (NEFA) in serum were determined using commercially available kit (Nanjing Jian Cheng Institute of Bioengineering, China). Serum concentrations of β -hydroxybutyrate (BHBA) were measured by BHBA kit (Randox, United Kingdom). These assays were analysed using a Hitachi 7170 auto-analyser.

Data are presented as mean values and standard deviations (SD). Statistical significance was determined using one-factor analysis of variance followed by Duncan's least significant difference *post-hoc* test. The statistical package SPSS for Windows (version 18.0; SPSS, Chicago, USA) was used for statistical analysis. $P < 0.05$ was considered as statistically significant.

Table 1
Nutritional composition of the diet of different groups

Ingredient	Ratio of raw materials			
	Antepartum			Postpartum
	C	H	L	
Supplement feed as concentrate (%):	60.76	60.62	49.47	52.72
Corn	17.13	16.3	15.11	27.06
Bean meal	0.0	0.0	0.0	4.92
Cottonseed meal	1.0	2.0	0.0	2.0
Bean oil	0.0	1.98	0.0	1.96
Plumery powder	13.96	11.04	27.77	5.84
Wheat bran	4.15	4.06	4.15	0.0
Sunflower meal	1.0	1.0	1.5	1.0
Brushite	1.0	1.0	1.0	1.0
Compound additive premix	0.0	1.0	0.0	1.0
Rocks powder	1.0	0.8	1.0	1.0
Gravy salt	0.0	0.2	0.0	1.5
Baking soda	60.76	60.62	49.47	52.72
Roughage (%) :				
Corn silage	15.16	15.43	16.24	45.28
Beet dregs	12.11	24.67	0.0	20.9
Goat grass	72.73	59.9	83.76	33.82
Ratio of concentrate to roughage	37.53:62.47	42.83:57.17	34.04:65.96	43.78:56.22
Nutrient intake of 650 kg dairy cows				
Energy units of dairy cows (NND/d, per cow)	21.2	25.43	17.1	30.9
Dry matter (kg/d, per cow)	11.89	12.76	10.5	22.98
Crude protein (g/d, per cow)	1,117.0	1,353.0	908.7	2,895.0
Crude fibre (g/d, per cow)	2,818.0	2,736.0	2,675.0	4,551.0
Calcium (g/d, per cow)	48.05	56.8	40.28	142.4
Phosphours (g/d, per cow)	44.9	52.45	37.44	101.2

C - control group; L - low energy diet group; H - high energy diet group

Results

Based on the grey-scale analysis, the ratio of the grey-level between the PCR products of IR and the β -actin genes indicated the relative changes in expression of the IR gene in the tested samples (23). As shown in Table 2 and Fig. 1, there was a significant difference in gene expression among the three groups. INSR expression was reduced in the adipose tissue of cows fed a high energy diet compared with the control

group ($P<0.05$). Moreover, INSR mRNA levels in adipose tissues of cows fed the low energy diet were the highest among the three groups. Serum glucose and NEFA of cows fed high energy diet were higher than that of other groups. There was no difference in serum BHBA between cows from control group and low energy diet group, but there were significant differences when they were compared with a high energy diet group.

Table 2

Effect of different energy diet on INSR mRNA levels in adipose tissue of cows on day 21 postpartum (mean $\pm$ SD)

Index	Groups		
	C	L	H
INSR mRNA abundance relative to β -actin	0.65 $\pm$ 0.05 ^a	0.78 $\pm$ 0.1 ^b	0.54 $\pm$ 0.02 ^c

^{a,b,c} Mean values within a line with unlike superscript letters were significantly different ($n=15$, $P<0.05$). C - control group; L - low energy diet group; H - high energy diet group

Table 3

Effect of different energy intake on serum glucose, NEFA, and BHBA of dairy cows on day 21 postpartum (mean $\pm$ SD)

Index	Groups		
	C (n=15)	L (n=15)	H (n=15)
Glucose (mM)	3.6 $\pm$ 0.22 ^a	3.97 $\pm$ 0.35 ^b	3.31 $\pm$ 0.4 ^c
NEFA (mM)	4.63 $\pm$ 0.33 ^a	3.62 $\pm$ 0.29 ^b	5.68 $\pm$ 0.65 ^c
BHBA (mM)	0.35 $\pm$ 0.01 ^a	0.38 $\pm$ 0.23 ^a	0.73 $\pm$ 0.05 ^b

Different superscript letters indicate significant differences at $P<0.05$ in the same line.

C - control group; L - low energy diet group; H - high energy diet group

Fig. 1. PCR products of InsR in adipose tissues of different group dairy cows
M - DL-2000 marker; 1 - high energy group, 2 - control group, 3 - low energy group

Discussion

The transition from pregnancy to lactation in dairy cows involves extensive metabolic and endocrine changes to accommodate parturition and lactogenesis (6, 15). Energy demands during early lactation cannot be fulfilled entirely by dietary intake and, therefore, energy has to be mobilised from body reserves, mainly from adipose tissue (5). This process can result in increased circulating concentrations of NEFA and ketone bodies, and in a relatively low concentration of glucose (21). Glucose, NEFA, and BHBA are the key indexes of energy metabolic balance (25). On the basis of the results of this study, low energy diet during antepartum could alleviate the NEB of dairy cows postpartum. Contrarily, use of high energy diet at the antepartum period aggravated the NEB of dairy cows postpartum.

Insulin, which is secreted from pancreatic β -cells, plays a central role in the metabolism of mammals (4, 19). When INSR expression decreases, adipose tissues fail to respond effectively to circulating insulin (11). During NEB, decrease in both insulin and glucose levels, and elevated NEFA equally contribute to reduced insulin sensitivity (8, 18).

Insulin resistance in adipose tissue reduces the normal effects of insulin on lipid metabolism and results in a reduced uptake of circulating lipids and increased hydrolysis of stored triglycerides (20). Increased mobilisation of stored lipids in adipocytes elevates blood NEFA levels (11). Activation of the INSR in muscle and adipose tissue leads to an increase in the GLUT-4 on the surface of cells in these tissues, and therefore, it increases the rate of glucose entry into the cells (10). Imano *et al.* (9) have discovered that a decrease in the number of cell surface INSRs can cause insulin resistance. A study on the effects of NEFA and BHBA on the expression of INSR in bovine adipocytes cultured *in vitro* would help to define this problem in more detailed way.

Results of the study suggest that the high-energy diet at antepartum reduced the abundance of INSR mRNA in the adipose tissue of postpartum dairy cows, and increased adipose mobilisation, thereby further aggravating the NEB of cows. In contrast, feeding the low energy diet at antepartum increased the abundance of INSR mRNA in the adipose tissue of postpartum cows. This resulted in inhibition of fat mobilisation, promotion of glycogen synthesis, and maintenance of relatively constant blood glucose concentrations, thereby alleviating NEB. A decrease in INSR mRNA levels reduced the inhibitory effect of insulin on lipolysis in adipose tissue of dairy cows.

Summarising, in dairy cows fed a low energy diet at antepartum, INSR expression at postpartum increases, which may be beneficial for gluconeogenesis and lipogenesis and in compensating for the NEB that occurs in early lactation. Dairy cows fed a high energy diet at antepartum have decreased INSR expression in adipose tissue at postpartum, suggesting a possibility of reduction in responsiveness to insulin.

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