Investigation on the Relationship of Insulin Resistance and Ketosis in Dairy Cows

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Abstract

Ketosis is an important metabolic disease of dairy cows during the transition period, but it is fully unclear about its endocrine etiology. Our study is to clarify the relationship between oxidative stress, liver function, insulin resistance and ketosis in dairy cows. Sixteen ketotic Holstein cows (T) and twenty-four non-ketotic Holstein cows (C) were used as the experimental animals from an intensive dairy farm in Heilongjiang province, China. Blood samples from all experimental cows were collected at 14 days postpartum during morning fasting. Fifteen parameters for energy balance, liver function, oxidative stress, insulin sensitivity and glucose tolerance test between T and C were measured using commercial kits. Results showed that the concentration of plasma glucose (P<0.01) was lower in T compared with C cows, whereas there were marked increases in the concentration of plasma non-esterified fatty acids and beta-hydroxybutyric acid (P<0.01). The level of plasma AST (P<0.01), TBIL (P<0.05), ALT (P<0.05), and ALB (P<0.05) increased significantly in T cows compared with C cows, but plasma CHE (P<0.01) and TP (P<0.05) decreased significantly, and no significant change in plasma ALT, IBIL, ALB, and GLO. Level of plasma malondialdehyde (MDA) and superoxide dismutase (SOD) was significantly higher in ketotic cows than that of non-ketotic cows (P<0.05), but value of plasma revised quantitative insulin sensitivity index (RQUICKI) was lower significantly in T cows than that of C cows (P<0.05). Concentration of plasma Glc increased significantly in T cows compared with C cows during glucose tolerance test (P<0.05). Therefore, the ketotic cows were in condition of negative energy balance, suffered to certain extent from liver function abnormality, and experienced oxidative stress and low insulin sensitivity. Therefore, a closed relationship between ketosis and insulin resistance should be related to liver function and oxidative stress that can cause insulin resistance.

Keywords: Dairy cows; Ketosis; Negative energy balance; Liver function; Oxidative stress; Insulin resistance

Background

Ketosis in dairy cows is an important metabolic disorder that easily occurs in transition period if cows cannot adapt to the high glucose demand relative to their limited intake [1]. A great amount of non-esterified fatty acids (NEFAs) from fat mobilization of dairy cows affected ketosis may produce a great deal of oxygen radical, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can initiate oxidative stress [2]. The oxidative stress pathway activated will prompt insulin resistance (IR), insulin secretion injury, diabetes, and vasculopathy. The common soil hypothesis was proposed by Stern [3], and just cited and “revisited” by Ceriello and Motz [4], namely the oxidative stress is a common basis of IR, diabetes and vasculopathy, which has been verified widely in clinical, but its mechanism still is unclear. Malondialdehyde (MDA) is a degradation product of lipid peroxidation and its level in blood may be considered as an assessing indicator of lipid peroxidation degree [5]. Superoxide dismutase (SOD) is a measurable indicator of antioxidation [6]. MDA and SOD are common indicators to evaluate oxidative stress and antioxidative system. An unbalance happens between oxidative system and antioxidative system, which can cause body damaged is usually named as “oxidative stress” [7].

At present, it has been accepted nearly that euglycemic hyperinsulinemic clamp is a “golden standard” method to measure insulin resistance according to concentration of glucose and insulin in blood in animals [3,4], but it is complex, time-consuming, and expensive. So it is not usually used to investigate a herd metabolic disorder. Then some methods related closely to euglycemic hyperinsulinemic clamp, such as accurate glucose tolerance test and revised quantitative insulin sensitivity check index (RQUICKI), were established to easily assess insulin resistance, in particular RQUICKI [3,4]. In the morning, a stable balance will reach among insulin sensitivity in tissues and blood glucose, insulin, and NEFAs. It is a ready method to assess insulin resistance because of only measurement of blood glucose, insulin, and NEFAs [4,8]. It is not fully explicit what insulin resistance plays roles in the etiology of dairy ketosis, therefore in our experiments relationship between insulin resistance and ketosis was exploded by monitoring oxidative stress, liver function and insulin resistance in order to provide a new strategy for preventing ketosis in dairy farms in China in the future.

Methods

Animals and samples collection

All animals used in this experiment were treated according to the International Guiding Principles for Biomedical Research Involving Animals. In this study forty cows including sixteen ketotic cows (T) and twenty-four non-ketotic cows (C), which had average of 3.6 ages, 2-3 parities, 20-30 kg/d milk yields, and 11-16 kg/d dry matter intake...
In the early morning, whole blood samples were collected from the vena candelis mediana using sodium heparin, and then immediately centrifuged at 1,400 x g for 10 min at room temperature. The supernatants were aliquoted into Eppendorf tubes (1 mL plasma/tube) and stored at -80°C until analysis. Both clinical parameters and plasma metabolites were measured for all experimental animals. Clinical blood biochemical data included glucose (Glc), β-hydroxybutyric acid (BHBA), and non-esterified fatty acids (NEFAs) for assessing energy balance status, and aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), direct bilirubin (DBIL), cholinesterase (CHE), total protein (TP), and albumin (ALB) for measuring liver function, and then MDA and SOD for determine oxidative stress status. All blood parameters were detected by commercial kits from Beijing Jiuyang Biotechnology Limited Company and Shanghai Desay Diagnostic System Limited Company in China using full automatic analyzer (Hitachi 76000, Japan).

**Detection of insulin sensitivity detection**

According to concentration of Glc, NEFAs, and insulin (Ins) in plasma from T and C, difference of insulin sensitivity index (RQUICKI) can be measured by an formula (RQUICKI = 1/[logG0+logI0+log NEFAs 0]) [4,8]. Ins was measured using ELISA kit (RD Bovine) from Nanjing Jiancheng Biotechnology Limited Company by ELISA microplate reader (HuaDong Electron DG5033A).

Seven healthy cows and nine cows with ketosis, which from the above T and C cows, were restrained at different pens respectively for one day before glucose tolerance test [10]. Each cow was administrated rapidly 1000 ml of 50% glucose by jugular intravenous infusion for 10-15min. Blood samples were collected using a catheter by jugular vein at 15 minutes before administration of glucose, and at 0, 15, 30, 45, 60, 90, 120 minutes after completion of the injection.

**Statistical analyses**

All numerical data from two experiments were presented as means ± standard deviation (SD). Student's t-test and one-way analysis of variance (ANOVA) were used to evaluate differences between groups. In the entire research process, the significance level was set at p<0.05. Statistical analysis was performed using SPSS 17.0 software (SPSS Company, Chicago, IL, United States).

**Results**

In Table1, plasma concentrations of BHBA and NEFAs were significantly higher in T group than that in C group (P<0.01). The greater values for NEFA and BHBA indicate that T cows were in greater negative energy balance.
Excessive NEFAs can induce oxidative stress, which is able to break down a balance between antioxidative system and oxidative system, and may inhibit glucose intake through interfering with glucose transporter function, and interfere with insulin signal transduction pathway in liver and peripheral tissues [5,10]. When fat mobilized greatly, accompanied by lots of NEFA, TG may accumulate in liver, and then cause fatty liver, which can damage hepatocyte [11,12].

The current study showed that some parameters in plasma, such as AST, DBIL, TBLIL, CHE and TP, changed significantly in cows with ketosis, suggested that liver dysfunction exists in the affected cows because these abnormal parameters can reflect the abnormal status of liver function [13,14]. Therefore, once liver lesion for example fatty liver happens all kinds of metabolic regulation may be affected, which may induce insulin resistance, thus will affect glucose, protein and fat metabolism in dairy cows with ketosis.

In addition, in our studies the significant changes of MDA and SOD in plasma of dairy cows with ketosis indicated that the affected cows may suffer from the oxidative stress and enhance antioxidative ability. MDA has been usually used to assess oxidative stress. SOD cows may suffer from the oxidative stress and enhance antioxidative metabolism in dairy cows with ketosis.

Insulin resistance, some did not, which is in accordance with a report that insulin resistance exits in some dairy cows with high blood body ketone [12,14,17].

Our results demonstrated that dairy cows affected ketosis suffered from negative energy balance, liver dysfunction, oxidative stress and decreased insulin sensitivity, implied that there may be a close relationship of insulin resistance and dairy ketosis, but it need further exploring the correlations among measurements in the insulin resistant and cows with ketosis in the future.

Competing Interests

The authors declare that they have no competing interests.

Authors' contributions

BW, CX and HYZ designed the study. BW and ZLS did the laboratory work. CX, ZLS and SS coordinated the writing and editing of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors thank financial support from the Chinese National Nature Science Foundation (31001094) and Chinese National Nature Science Foundation (2013BAD21B01-2 and 2012BAD12B03-2).

References


