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

Chuang Xu1, Shi Shu1, Cheng Xia1,2*, Bo Wang1, Hong-You Zhang1 and Bao Jun2
1Department of Clinical Veterinary Medicine, College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, China
2Synergetic Innovation Center of Food Safety and Nutrition, Northeast Agricultural University, China

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<0.01), ALB (P<0.01), and GLO (P<0.01) 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 antioxiditive 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 explosed 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.
(DMI), were chosen around 14d postpartum from an intensive 2000 dairy cattle farm in Heilongjiang, China. The cows were considered to have ketosis if they showed clinical symptoms or no remarked signs and had plasma 3-hydroxybutyrate (BHBA) concentrations > 1.20 mmol/L. If the cows had no clinical signs and normal plasma BHBA concentrations of BHBA concentrations > 1.00 mmol/L, they were considered healthy controls (C) [1,9]. All the cows were fed a total mixed ration (TMR) at 7:00 am, 13:00pm and 19:00 pm daily during transition period, which consisted of concentrated feed, silage corn, brewer’s grain, cooked soybeans, Chinese bay, melon pulp, surface melon shell, and fat. The nutritional analysis was 57.10% dry matter (DM), 18% crude protein, 7.4×10^6 J/kg (DM) net energy for lactation (NEL), 5.70% fat, 36.80% neutral detergent fiber (NDF), 19.60% acid detergent fiber (ADF), 0.73% Ca, and 0.37% P.

In the early morning, whole blood samples were collected from the vena canalis mediana using sodium heparin, and then immediately centrifuged at 1,400 × g for 10 min at room temperature. The supernatants were aliquoted to 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 Juqiang Biotechnology Limited Company and Shanghai Desay Diagnostic System Limited Company in China using full automatic analyzer (Hitachi 7600, 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 NEFAs0]) [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 vain 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 Table 1, 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.

| Parameters* | Ketosis | Control | P-value |
|-------------|---------|---------|---------|
| **Glc (mmol/L)** | 2.70 ± 0.89 | 3.31 ± 0.17 | 0.003 |
| **NEFAs (mmol/L)** | 0.99 ± 0.32 | 0.51 ± 0.15 | 0.000 |
| **BHBA (mmol/L)** | 2.71 ± 1.29 | 0.62 ± 0.11 | 0.000 |
| **AST (U/L)** | 149 ± 40 | 93 ± 13 | 0.000 |
| **ALT (U/L)** | 22.86 ± 4.52 | 20.77 ± 4.25 | 0.171 |
| **CHE (U/L)** | 136 ± 17 | 155 ± 20 | 0.005 |
| **TBIL (μmol/L)** | 3.46 ± 1.35 | 2.83 ± 0.47 | 0.049 |
| **DBIL (μmol/L)** | 2.53 ± 0.78 | 1.82 ± 0.43 | 0.001 |
| **TP (g/L)** | 73.61 ± 3.69 | 77.32 ± 5.03 | 0.023 |
| **ALB (g/L)** | 32.48 ± 2.29 | 33.84 ± 2.24 | 0.087 |
| **GLO (g/L)** | 41.03 ± 3.74 | 43.37 ± 6.46 | 0.228 |
| **MDA (μmol/mL)** | 2.89 ± 0.48 | 2.29 ± 0.37 | 0.047 |
| **Ins (mIU/L)** | 13.87 ± 1.75 | 14.01 ± 2.01 | 0.043 |
| **RQUICKI (mIU/L)** | 0.36 ± 0.02 | 0.38 ± 0.02 | 0.017 |

*ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BHBA, β-hydroxybutyric acid; CHE, cholinesterase; DBIL, direct bilirubin; Glc, glucose; GLO, globulin; IBIL, indirect bilirubin; Ins, insulin; MDA, malondialdehyde; NEFAs, non-esterified fatty acids; SOD, superoxide dismutase; TBIL, total bilirubin; TP, total protein; RQUICKI, revised quantitative insulin sensitivity check index.

**Table 1:** Levels of fifteen parameters in plasma of experimented cows.

In addition, compared to C group, plasma levels of AST, DBIL in T group raised markedly (P<0.01), and TBIL increased significantly (P<0.05). Plasma concentration of CHE and TP decreased markedly in T group (P<0.01). However, ALT, IBIL, ALB and GLO did not change in both groups. It suggested that there still were some abnormal changes of liver function in dairy cows with ketosis.

Furthermore, plasma concentrations of MDA and SOD were significantly higher in T group than that in C group (P<0.01), RQUICKI is also lower (P<0.05). It suggested that the affected cows were in condition of oxidative stress and insulin sensitivity reduced.

Finally, nine cows with ketosis were divided into two groups of T1 (5 cows) and T2 (4 cows) on the basis of plasma glucose concentration at last time. The curves of glucose tolerance test in three groups of cows shown that there were the same curve changes, and plasma Glc concentration were higher in T1 group than those in C and T2 groups except for -15 min, but similar in C and T groups except for -15 min and 0 min. In Figure 1, plasma concentration of glucose in T1 group is significantly lower than that in C or T2 groups at 15 minutes before administration of 50% glucose, but increased markedly in T1 group at five time points after administration of 50% glucose (P<0.05). Furthermore, plasma concentration of glucose in T1 group was still up to 4.24 mmol/L at 120 min after administration of 50% glucose (P=0.006). However, there were no difference in plasma concentration of glucose between T2 group and C group. It may indicate that insulin resistance exists in some cows with ketosis.

**Discussions**

In brief, NEFA and BHBA are products of fat catabolism that can supply energy to body. Their increased levels in blood are symbols of negative energy balance, which predict a great amount of fat mobilization [1,9]. β-oxidation of NEFA occurs mainly in liver. Its marked increase may prompt the formation of reactive oxygen species.
(ROS) and development of oxidative stress [2,6]. 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, TBIL, CHE and TP, changed significantly in cows with ketosis, suggested that liver disfunction 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 ketosis, suggested that liver disfunction exists in the affected cows and cows with ketosis in the future.

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 can protect cell from oxidative lesion by catalyzing superoxide anion radical. Research [6,15] suggested that there was a negative correlation between SOD and MDA, but this association between antioxidase and lipid peroxidation was not significant during early lactation. Someone observed [5,7] that the active concentration of oxidant is consistent with peak value of MDA, it is considered as an adaptation phenomenon due to an increased oxidative stress. Wang et al reported that total anti-oxidative ability changed nonsignificantly in dairy cows affected ketosis, and mRNA expression of insulin receptor decreased [16,17]. There has always been no standardization to evaluate in a broken balance between oxidation and anti-oxidation in dairy cows with ketosis, so it is difficult to offer some detailed evidences on relationship of oxidative stress and ketosis [6,8]. Thus, in this study MDA and SOD increased markedly in cows with ketosis, suggested that cows with ketosis may experience an adaptation response to oxidative stress and antioxidation as human and rodents.

Finally, it needs a longer time to develop insulin resistance in human and rats, but insulin sensitivity changes markedly, especially from 3 weeks prepartum to 3 weeks postpartum, which is basically consistent with change of NEFA in blood [13,18]. In this study dairy cows with ketosis had high level of MDA and SOD, and lower RQUICKI, suggested that the affected dairy cows may have both oxidative stress and insulin resistance. However, it is still unclear whether cattle, like human and rodents, insulin resistance may reduce glucose utilization, in turn make fat mobilization increased, and then cause a vicious circle.

Our glucose tolerance test showed that some ketotic cows had 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.

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