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Methylglyoxal: A newly detected and potentially harmful metabolite in the blood of ketotic dairy cows

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ABSTRACT

Ketosis causes serious economic losses for the modern dairy industry because it is a highly prevalent metabolic disease among cows in high-producing herds during the transition period. Due to some striking similarities between diabetes in humans and ketosis in dairy cows, there is potential for the use of methylglyoxal (MGO)—commonly used in human diabetics—as a biomarker in dairy cattle. However, currently no data are available about the presence of MGO in the serum of dairy cattle or about the characteristics of its production or its potential contribution in the pathogenesis of ketosis. To determine the potential origin and pathway of formation of MGO, cows in different metabolic conditions [i.e., non-subclinically ketotic dairy cows in early lactation (n = 7), subclinically ketotic dairy cows in early lactation (n = 8), overconditioned dry cows (BCS >4.25, n = 6), and nonlactating heifers (n = 6)] were selected. Serum MGO concentrations were determined and correlated with indicators of the glucose and lipid metabolism and with haptoglobin (Hp) as an inflammatory marker. The serum MGO concentrations in subclinically ketotic cows (712.60 ± 278.77 nmol/L) were significantly greater than in nonlactating heifers (113.35 ± 38.90 nmol/L), overconditioned dry cows (259.71 ± 117.97 nmol/L), and non-subclinically ketotic cows (347.83 ± 63.56 nmol/L). In serum of lactating cows, concentrations of glucose and fructosamine were lower than in heifers and were negatively correlated with MGO concentrations. Even so, concentrations of metabolic and inflammatory markers such as dihydroxyacetone phosphate, nonesterified fatty acids, β-hydroxybutyrate, acetone, and Hp were remarkably higher in subclinically ketotic cows compared with nonlactating heifers; these metabolites were also positively correlated with MGO. In human diabetics elevated MGO concentrations are stated to originate from both hyperglycemia and the enhanced lipid metabolism, whereas higher MGO concentrations in subclinically ketotic cows were not associated with hyperglycemia. Therefore, our data suggest MGO in dairy cows to be a metabolite produced from the metabolization of acetone within the lipid metabolization pathway and from the metabolization of dihydroxyacetone phosphate. Furthermore, the highly positive correlation between MGO and Hp suggests that this reactive compound might be involved in the proinflammatory state of subclinical ketosis in dairy cows. However, more research is needed to determine the potential use of MGO as a biomarker for metabolic failure in dairy cows.

Key words: methylglyoxal, subclinical ketosis, dairy cow, glycolipid pathway

INTRODUCTION

Subclinical ketosis is an important metabolic disorder in high-producing dairy cows, affecting 30 to 40% of animals, especially during early lactation (Ametaj et al., 2016). According to some authors, there are 2 distinct types of ketosis: type I, characterized by hypoglycemia-hypoinsulinemia, and type II, characterized by hyperglycemia-hyperinsulinemia (Holtenius and Holtenius, 1996). The latter type shows striking similarities with type II diabetes in humans because, like type II diabetes occurs in obese humans, it mainly occurs in overconditioned cows (Holtenius and Holtenius, 1996).

Negative energy balance (**NEB**) in early-lactating cows is characterized by a failure of hepatic gluconeogenesis to supply adequate glucose (**GLU**) for maintenance and lactation. An inadequate or poor adaptive response to NEB can lead to ketosis (Herdt, 2000). High concentrations of nonesterified fatty acids (**NEFA**) and low concentrations of GLU are furthermore accepted

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as major indicators of this NEB (Abuelo et al., 2014). Massive fat mobilization from different adipose depots drives NEFA into the liver via the blood circulation and leads to NEFA oxidation through fatty acid β-oxidation in hepatic mitochondria to produce energy (Drackley, 1999). When the oxidative capacity of the liver is exceeded due to an overload of NEFA, the excessive NEFA will transform into acetone (**AC**), acetoacetate, and BHB (ketosis) or be stored as triglycerides (**TG**) in hepatocytes (fatty liver; Bezerra et al., 2014).

In humans, methylglyoxal (**MGO**)—a relatively new biomarker for type II diabetes—is currently more and more in use (Ogawa et al., 2010; Eberhardt et al., 2012; Matafome et al., 2013). Methylglyoxal is formed enzymatically from the triose phosphate intermediates [glyceraldehyde 3-phosphate (**GAP**) and dihydroxyacetone phosphate (**DHAP**)] produced during glycolysis or from the metabolization of ketone bodies or threonine in the lipid metabolism (Phillips and Thornalley, 1993; Richard, 1993; Thornalley, 1996). Prolonged hyperglycemia (125 mg/dL) in diabetics will result in an increase of MGO because hyperglycemia provokes an increased glycolysis (Sheader et al., 2001; Shamsaldeen et al., 2016). Production via the lipid metabolism occurs during metabolization of ketobodies, finally leading to the production of AC that metabolizes into acetol and subsequently MGO, a reactive carbonyl and dicarbonyl substance (Casazza et al., 1984; Bondoc et al., 1999). Moreover, MGO has been associated with inflammation because MGO-induced oxidative and carbonyl stress can lead to an increase in advanced glycation end products and inflammatory events (Vulesevic et al., 2016).

So far, to the best of our knowledge, no data are available concerning the MGO serum concentration in cattle. Due to the abovementioned striking similarities between diabetes type II and ketosis type II and to the seriously stressed lipid metabolism in periparturient dairy cows, it would be interesting to know whether this potentially harmful metabolite is also present in the blood of dairy cows. Furthermore, DHAP and GAP can be produced by glycerol through the gluconeogenic pathway in cattle (Goff and Horst, 2001), resulting in another potential pathway leading to the production of MGO.

The goal of the present research was to explore whether and how MGO is produced within the glycolipid metabolism in dairy cows. To obtain the first insights about the possible origin of this molecule in cattle, we included healthy, nonlactating heifers and lactating cows as well as subclinically ketotic cows. Furthermore, to evaluate the potential association of this reactive substance with subacute inflammation, we tested the association of this molecule with haptoglobin (**Hp**), a well-known subacute inflammation marker in transition cows.

MATERIALS AND METHODS

The study was carried out in strict accordance with the recommendations mentioned in *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011). All animal experimental procedures were approved by the Animal Care Commission of the College of Veterinary Medicine, Northwest A&F University (Yangling, China). Every effort was made to minimize animal pain, suffering, and distress and to reduce the number of animals used.

Animals

The present study took place from June 2015 to May 2016 in Shaanxi Huayin Dairy farm located at N 34°35′, E 109°55′. Cows (n = 27) were randomly selected from a 2,000-cow Holstein dairy herd with an average annual milk yield of 9,800 kg. Included cows were divided into 4 groups: 6 nonlactating heifers of 15 mo of age, 6 overconditioned dry cows (BCS >4.25; Edmonson et al., 1989), 7 non-subclinically ketotic cows (2 wk in milk, BHB <0.6 mmol/L, NEFA <0.4 mmol/L), and 8 recently calved cows that were subclinically ketotic (2 wk in milk, 1.2 mmol/L ≤ BHB ≤ 3.0 mmol/L; Duffield, 2000; Oetzel, 2004; Rutherford et al., 2016). Cows were fed TMR and had free access to water. The nutrient composition of the diets is presented in Table 1. The diets were formulated using the China Professional Manager Consultancy–Dairy software (version 3.1.05) from Cornell University (Ithaca, NY), University of Pennsylvania (Philadelphia), Miner Institute (Chazy, NY), and Lanzhou Precision Animal Husbandry Technology Co. Ltd. (Lanzhou, Gansu, China).

Blood Collection and Determination of Metabolites

Blood samples were taken during multiple herd visits when individual animals were at the requested period in lactation (DIM) and were collected from the jugular vein into plain vacuum tubes (Beckton Dickinson, Franklin Lakes, NJ) in the morning before the cows had access to fresh feed. In total, 10 mL of blood was gathered, kept on ice, and transported to the laboratory. Blood samples were centrifuged at 1,500 × *g* at 4°C for 10 min. The serum was transferred into sterile microtubes (Corning Inc., Corning, NY) and preserved at −80°C until analysis. Serum concentrations of BHB (mmol/L), NEFA (mmol/L), aspartate amino transferase (U/L), total bilirubin (**TBIL**; μmol/L), TG (mmol/L), and GLU (mmol/L) were colorimetrically