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Relation of inflammation and liver function with the plasma cortisol response to ACTH in early lactating dairy cows

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ABSTRACT

In this study we examined the relationship between cortisol and inflammatory status in early lactating dairy cows after a stimulation test of the adrenal cortex. Twenty-four cows were grouped into quartiles (6 cows per each quartile) in accordance with the liver activity index (based on plasma concentration of negative acute phase proteins in early lactation); the quartiles were lower (LO; cows with the lowest liver functionality), intermediate lower, intermediate upper, and upper (UP; cows with the highest liver functionality). Each cow was injected i.v. with 20 μg of a synthetic analog of ACTH at 35 d in milk (DIM). Blood samples were taken to assess inflammatory status, and at 0, 30, and 60 min after ACTH challenge to measure total cortisol. The free cortisol fraction was analyzed in the LO and UP quartiles and the bound cortisol fraction was estimated as the difference between total and free cortisol. The LO, in comparison with the other quartiles, suffered a more severe inflammatory status, with the highest values of haptoglobin, reactive oxygen metabolites, and total nitric oxide metabolites and the lowest concentration of direct or indirect markers of negative acute phase proteins. The cows in the LO quartile had the highest values of plasma nonesterified fatty acids and β-hydroxybutyrate at 7 DIM, suggesting a more severe body lipid mobilization. The LO quartile cows showed the highest frequency of health problems and the lowest milk yield in the first 35 DIM. Thirty minutes after the ACTH treatment, the concentration of total cortisol was lower in LO in comparison to other groups. Similarly, the bound cortisol fraction was lower in LO versus UP. The adrenal response appeared inversely related with health status after calving (e.g., lower in LO cows, experiencing the most severe inflammatory status). The lower increase in cortisol after the ACTH challenge in cows with greater inflammation (LO quartile) seems a consequence of the lower availability of cortisol-binding globulin synthetized by the liver, but other mechanisms can be involved (e.g., rate of cortisol production, secretion, and metabolic clearance). Our data provide evidence that inflammation and metabolic changes reduce the concentration of circulating plasma cortisol during an acute stress. Hence, the acute phase response in dairy cows should be taken into account to interpret the results obtained from stimulation tests of the adrenal cortex.

Key words: acute phase response, cortisol-binding globulin, adrenocorticotropin (ACTH) challenge, dairy cow

INTRODUCTION

Dairy cows suffer inflammation-like conditions during the periparturient period, often without any clinical symptom of disease (Bertoni et al., 2008). The main physiological effects of these conditions concern a change of the liver function due to the stimulation of the acute phase response and a reduction of some usually produced plasma proteins (Fleck, 1989; Bruss, 1997; Gruys et al., 2005; Bionaz et al., 2007). This circumstance is characterized by the quick induction of the synthesis of positive acute phase proteins (**pos-APP**; e.g., haptoglobin, serum amyloid A) and the reduction of the negative acute phase protein (**neg-APP**) synthesis (e.g., albumin, apolipoproteins, retinol binding protein, transferrin, transthyretin; Ceciliani et al., 2012).

It is noteworthy that the cortisol concentration can be affected by the availability of the circulating cortisol-binding globulin (**CBG**) produced in the liver, which is the main carrier of the hormone (Tyrrell and Forsham, 1986). Interestingly, the CBG concentration is known to be reduced in cases of inflammatory conditions (Pugeat et al., 1989; Christ-Crain et al., 2007) to such an extent that Gruys et al. (2005) considered CBG as a neg-APP. Therefore, inflammation might reduce the maximum concentration of bound plasma cortisol after ACTH challenge by reducing the level of CBG. As free cortisol has a very short half-life (about 2.2 min in healthy humans; Dorin et al., 2012), the reduction of the CBG affects the maximum concentration of total plasma cortisol. This consideration agrees

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with the conclusion of Christ-Crain et al. (2007), who suggested caution in the interpretation of the ACTH challenge results, in humans affected by diseases of different severity, if only based on total serum cortisol concentration.

In farm animals, the ACTH challenge is the main stimulation test used to demonstrate the sensitization of the adrenal cortex, and it is proposed to assess the welfare status (Broom, 1988; Mormède et al., 2007). The interpretation of this test remained uncertain and controversial, as reviewed by Mormède et al. (2007), who concluded "effects are not consistent across the studies that use similar treatments." In fact, several factors interfere with the adrenal responsiveness (e.g., age, environment, health status, social interaction), and a few of them can be clearly identified under the habitual conditions of life. Among that, we hypothesize that, in dairy cows, the CBG concentration is reduced during an acute-phase response (e.g., periparturient time when clinical and subclinical disorders are more frequent), and this decreases the total plasma cortisol response during a stimulation test of the adrenal cortex. To test this hypothesis we investigated the adrenal response (measured as the maximum plasma concentration of free and total cortisol) in early lactating cows grouped by different inflammatory conditions, evaluated with the liver activity index (**LAI**; which includes albumin, cholesterol, and vitamin A; Bertoni et al., 2008) and challenged with ACTH.

MATERIALS AND METHODS

Animals and Housing

This study complied with Italian laws on animal experimentation (DL n.116, 27/01/1992) and ethics. The experiment was carried out in a commercial dairy farm in northern Italy, during the autumn and winter seasons and involved 24 multiparous Italian Friesian lactating cows in their first 40 DIM. The cows had free access to water and feed. The diet was administered ad libitum once a day (0800 h) as a TMR. During the dry period, the diet included 33.6% corn silage, 21.4% alfalfa hay, 27.7% grass hay, 17.3% wheat straw (DM basis), and 100 g/cow per day of mineral-vitamin supplement. Ten days before calving, cows received a close-up ration composed of 42.3% corn-silage, 15.3% alfalfa hay, 11.2% grass hay, 6.3% wheat straw, and 24.9% concentrate (DM basis). After calving, the cows received a TMR composed of 32.1% corn-silage, 19.8% alfalfa hay, 4.4% grass hay, 10.5% high-moisture corn, and 33.1% concentrate, which included micro-mineral and vitamin supplements (DM basis). Cows were milked twice a day every 12 h (0400 and 1600 h).

Prechallenge Controls and Grouping

For 4 wk after calving, each subject was monitored daily for health status and milk yield. The incidence of clinical diseases was recorded (retained placenta, milk fever, lameness, mastitis, ketosis, diarrhea). At 7 ± 1, 14 ± 2, and 35 ± 2 DIM, individual blood samples (Vacuum Li-Heparin, Vacuette, Kremsmünster, Austria) were taken from the jugular vein before feed distribution. These samples were used to assess the concentration of albumin, cholesterol (index of lipoprotein), and vitamin A (index of retinol binding protein) and to calculate LAI for each cow, according the procedures previously described by Bertoni et al. (2008), with the exception of the third blood sample taken at 35 DIM instead of 28 DIM. Briefly, data of the 3 blood parameters were transformed into units of standard deviation obtained for each cow by the mean value of the herd population of each plasma parameter subtracted from each cow value at 7, 14, and 35 DIM and divided by the corresponding standard deviation. The final LAI of each cow is the result of the arithmetical mean of the 3 partial values obtained from the 3 selected blood variables at the 3 bleedings. Changes in neg-APP and LAI values in periparturient cows are closely related with specific inflammatory markers [e.g., haptoglobin, syalic acid, reactive oxygen metabolites (**ROM**); Trevisi et al., 2010b], as well as to impaired performance (Bertoni et al., 2008). Thus, we used LAI to differentiate the inflammatory condition among cows during their first month of lactation. Cows with lower LAI values had worse inflammatory status than cows with higher LAI values. Accordingly, the 24 cows were grouped into quartiles based on their LAI: upper quartile (**UP**; n = 6), cows with the highest values of LAI; intermediate upper quartile (**INUP**; n = 6); intermediate lower quartile (**INLO**; n = 6); and lower quartile (**LO**; n = 6), cows with the lowest values of LAI.

ACTH Challenge

At the end of the first month of lactation (35 ± 2 DIM), the 24 cows were challenged with ACTH. Cows were managed as usual on the day of the challenge. Approximately 3 h after the morning meal, cows were gently restrained in the headlock. The ACTH challenge protocol was reported previously (Trevisi et al., 2010a). Briefly, a dose of Synacthen (20 μg = 2 IU diluted in a total volume of 2 mL of physiological saline solution) was injected into the jugular vein. The Synacthen is a synthetic analog of ACTH (Novartis Pharma AG, Stein, Switzerland). The injected dose was defined in previous experiments (Bertoni et al., 2005) as the smallest amount of Synacthen able to reach the maxi-