Comparative Biochemistry and Physiology, Part A 164 (2013) 101–110

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Fuel feeds function: Energy balance and bovine peripheral blood mononuclear cell activation

A. Schwarm [a], T. Viergutz [b], B. Kuhla [a], H.M. Hammon [a], M. Schweigel-Röntgen [a,*]

[a] Research Unit Nutritional Physiology "Oskar Kellner", Leibniz Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
[b] Research Unit Reproductive Biology, FBN, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

ARTICLE INFO

Article history:
Received 26 July 2012
Received in revised form 12 October 2012
Accepted 14 October 2012
Available online 22 October 2012

Keywords:
Oxygen consumption
Respiration
Energy metabolism
Lymphocyte
Leukocyte
Immune response
Cell cycle

ABSTRACT

A general phenomenon in *peripartum* mammals is the breakdown of (acquired) immunity. The incidence of parasite load, disease and inflammation often rise during the specific energetically demanding time of pregnancy and lactation. In this period, blood leukocytes display decreased DNA synthesis in response to mitogens *in vitro*. Leukocyte activation, the phase of the cell cycle preceding the DNA synthetic phase has hardly been investigated, but the few studies suggest that leukocyte activation may also be impaired by the limited energy/nutrient availability. Leukocyte activation is characterized by manifold processes, thus, we used the cellular oxygen consumption rate (OCR) as a measure of ATP turnover to support all these processes. We hypothesized that the activation of peripheral blood mononuclear cells (PBMC) – in terms of oxygen consumed over basal levels after *in vitro* stimulation – is altered by energy balance around parturition. We studied *peripartum* high-yielding dairy cows because they undergo substantial fluctuations in energy intake, energy output and body fat mass. We established a fluorescence-based test strategy allowing for long-term (≥24 h) quantification of O_2-consumption and studied the *peripartum* period from 5 weeks *ante partum* to 5 weeks *postpartum*. In addition, we determined cellular lactate production, DNA/RNA synthesis and cell size and zoo-technical parameters such as animal energy intake and milk yield were assessed, as well as selected plasma parameters, *e.g.* glucose concentration. The basal OCR of PBMC from pregnant, non-lactating cows (n = 6, −5 weeks *ante partum*) was 1.19 ± 0.15 nmol min^{-1} (10^7 cells)$^{-1}$ and increased to maximum levels of 2.54 ± 0.49 nmol min^{-1} (10^7 cells)$^{-1}$ in phytohemagglutinin (PHA)-stimulated PBMC. The basal OCR did not change over the *peripartum* period. Whereas the activation indices, herein defined as the PHA-induced 24 h-increase of OCR above baseline, amounted to 1.1 ± 0.3, 4.2 ± 0.3, 4.1 ± 1.1, 2.1 ± 0.3, and 2.7 ± 0.5 at weeks −5, −1, +1, +2, and +5 relative to parturition, respectively. Because the activation index was positively correlated to plasma glucose levels and to energy balance during late pregnancy (week −5/week −1) and transition to lactation (week −1/week +2), we conclude that PBMC activation is modulated by energy/nutrient availability. In future studies, the activation index should aid the identification of causal mechanisms of disparity in PBMC activation, such as attenuated ion transport or macromolecule synthesis.

1. Introduction

As the old maxim "feed a cold, starve a fever" insinuates, there is an important biological link between energy balance and immune function and, thus, disease susceptibility and recovery (Demas, 2004). Because optimal immune function requires energy, the animal's nutritional or energetic state can have a strong effect on its response to pathogens and parasites (Houston et al., 2007). During specific energetically demanding times – such as during pregnancy and lactation – immune function is generally compromised (Nelson et al., 2002). For example, Weinberg (1984) and Rosell and de la Fuente (2009) reported an increased risk of illness or death in rabbits and humans as pregnancy progressed. A general phenomenon in *peripartum* mammals is the breakdown of (acquired) immunity which has been determined for example by an increase in parasite egg output in feces (*e.g.* human: Lloyd, 1983, horse: Xiao and Herd, 1994, cow: Kloosterman et al., 1985, sheep: Festa-Bianchet, 1989; Beasley et al., 2010, pig: Connan, 1967, dog: Lloyd et al., 1983, rabbit: Molina et al., 1999, mouse: Selby and Wakelin, 1975) and by diminished blood leukocyte DNA synthesis in response to mitogens (*e.g.* human: Riley et al., 1989; Redwine et al., 2001, pig: Magnusson and Fossum, 1988, buffalo: Amerasinghe et al., 1994, cow: Ropstad et al., 1989, reviewed by Mallard et al., 1998, sheep: Burrells et al., 1978; Lacetera et al., 2001). One important question with respect to *peripartum*

* Corresponding author. Tel.: +49 38208 68682; fax: +49 38208 68652.
E-mail addresses: schwarm@fbn-dummerstorf.de (A. Schwarm), viergutz@fbn-dummerstorf.de (T. Viergutz), b.kuhla@fbn-dummerstorf.de (B. Kuhla), hammon@fbn-dummerstorf.de (H.M. Hammon), roentgen@fbn-dummerstorf.de (M. Schweigel-Röntgen).

1095-6433/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cbpa.2012.10.009

breakdown of immune cell function is whether or not a reduction in the DNA synthetic (S) phase of the cell cycle can be explained by impaired cell activation (G1, cell cycle phase preceding the S phase, Fig. 1A). Only few data on *peripartum* immune cell activation are available, however, these data support this idea. For example, interleukin-2 (IL-2) production has been reported to be attenuated in lymphocytes from gravid female primates (Coe, 2012). And IL-2 production by splenic (Shanks et al., 1997) and blood (Jaedicke et al., 2009) lymphocytes was found to be suppressed during lactation in rats. This production of cytokines (*e.g.*, IL-2) and the expression of cytokine receptors (*e.g.*, IL-2Rα) start 12 to 24 h post stimulation with a peak at 24 to 48 h (Gaulton and Williamson, 1994). Other energy consuming processes during cell activation constitute alterations in cytoskeleton and gene transcription, increased synthesis of specific proteins, and the stimulation of active transport processes (ATPases) (Crabtree and Clipstone, 1994). The ATP turnover, *i.e.* the conversion of nutrient to cellular energy (ATP) to support all these processes, is correlated to the cellular oxygen consumption rate (OCR). OCR of mitogen-activated immune cells has been reported *e.g.* for human PBMC and rat thymocytes (in other context than reproduction) and was shown to be altered by health status, mitogen concentration and glucocorticoid treatment (Krauss et al., 1999; Schmid et al., 2000). Because we were particularly interested in the role of energy balance on/in the regulation of/PBMC activation, we studied *peripartum* dairy cows, which undergo substantial fluctuations in energy intake, energy output and body fat mass. We hypothesized that alterations in PBMC activation indices occur with changes in energy balance around parturition. As a prerequisite, a test strategy was established for studying PBMC activation by the fluorescence-based measurement of oxygen consumed over several hours and days of cell culture, by using PBMC from pregnant, non-lactating cows. Because we expected an effect of nutrient/energy status on the critical ATP-dependent process of PBMC activation, oxygen consumption (as a measure of ATP production) was determined in mitogen-stimulated PBMC from cows at weeks −5, −1, +1, +2, and +5 relative to parturition. In addition, we determined cellular lactate production, DNA/RNA synthesis and cell size. Around parturition, mammals alternate between contrasting states of energy balance. The animals' energy balance was estimated *ante partum* from metabolizable energy intake and *postpartum* from netto energy intake for lactation and energy-corrected milk yield. Furthermore, descriptive parameters of the animal nutrient/energy status were assessed such as body weight, body condition score, backfat thickness, and selected plasma parameters, *e.g.* glucose concentration. The aim of this study was to uncover whether PBMC activation – in terms of oxygen consumed over basal levels after *in vitro* stimulation – is altered by energy balance.

2. Materials and methods

2.1. Animals, feeding, and measurement of zootechnical data

Six multiparous (from third to fifth lactation) German Holstein high-yielding dairy cows (≥10,000 kg during the previous lactation) were randomly selected from trial animals as part of a larger joint research project. Dairy cows originated from a farm in Steinhagen, Germany. Cows were monitored from 7 weeks prior to the 5th week after calving in the period from December 2009 to July 2010. All cows were clinically healthy at the start of the experiment. Housing and feeding management were the same for all cows. Cows were fed *ad libitum* a dry-off (−7 to −4 week), close-up (−3 week until calving), and lactation diet (after calving), in meals of equal size at 0700 and 1500 h. This typical feeding strategy for dairy cows adapts the energy density of the diet to the energy demands of the cow and minimizes fat gain *prepartum*. Feed intake was measured on an individual basis. Feed ingredients and the chemical composition of the different diets (Supplementary data 1) followed the recommendations of the German Society of Nutrition Physiology (GfE, 2001). Cows

Hours after treatment	4	24	48	72
Cell diameter, µm				
Control	6.5 ± 0.1	6.4 ± 0.2	6.2 ± 0.1	6.3 ± 0.2
PHA-stimulated	6.9 ± 0.2	7.5 ± 0.3	6.9 ± 0.2	7.0 ± 0.3
P value	*0.012*	*0.007*	*0.006*	*0.018*
DNA+RNA content, pg cell^{-1}				
Control	-	0.41 ± 0.22	0.31 ± 0.09	0.45 ± 0.19
PHA-stimulated	-	0.54 ± 0.20	0.47 ± 0.10	0.82 ± 0.36
P value		*0.286*	*0.032*	*0.014*

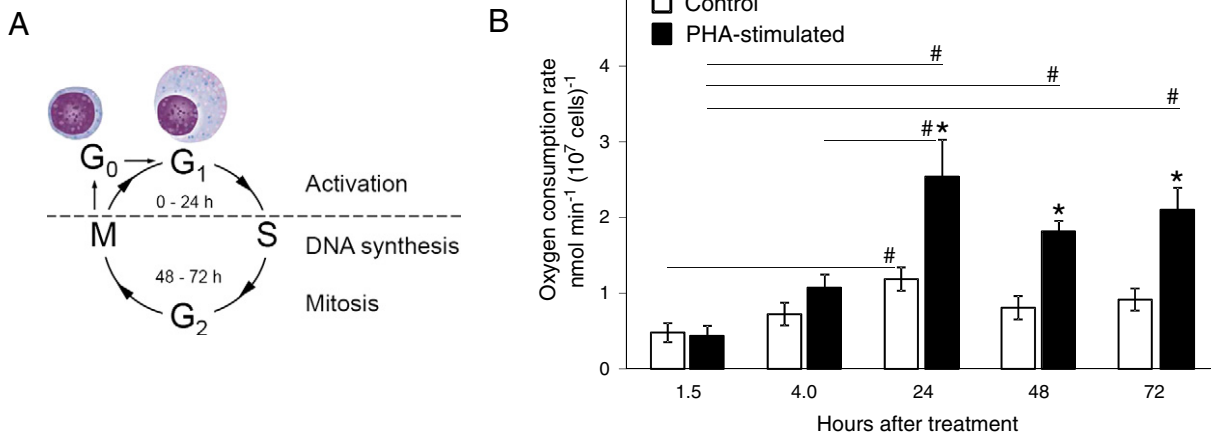

Fig. 1. A: Periods of the first cell cycle of peripheral blood mononuclear cells (PBMC) after a mitogen stimulus (Epifanova and Terskikh, 1969, p 86): G1, activation; S, DNA synthesis; G2, pre-mitotic; M mitosis; G0, quiescent cells. B: Oxygen consumption rate of PBMC from six cows measured 1.5 h, 4 h, 24 h, 48 h, and 72 h after treatment with or without phytohemagglutinin (PHA, 4 µg mL^{-1}). PBMC were obtained at week −5 before expected calving. 1.9×10^6 PBMC mL^{-1} in RPMI-1640 medium were stimulated at 1.5 h after seeding. Data are means ± SE. Asterisks indicate significant differences between control and PHA-stimulated cells (*$P<0.05$, Student's paired *t*-test). Crosses indicate significant differences between time points of each group (#$P<0.05$, one-way RM-ANOVA followed by Holm–Sidak post hoc test; Control: $F=3.96$, $P=0.016$; PHA-stimulated: $F=9.70$, $P<0.001$). In the top: cell diameter, DNA + RNA content and the *P* values for between treatment comparisons (Student's paired *t*-test) are depicted for selected time points. Note: The 24 h time point refers to the peak activation period of the cell cycle.