ABSTRACT

During the transition period, dairy cows often experience negative energy balance, which can induce metabolic and immunological disturbances. Previous work has shown that there is a relationship between the dysfunction of immune cells and the increase in blood nonesterified fatty acid (NEFA) concentration. Nevertheless, it is difficult to determine the exact effect of NEFA on the immune system, as other metabolic and hormonal perturbations occur simultaneously during the transition period. In the present study, we have determined the effect of NEFA on immune functions using an experimental model designed to assess the effects independently of energy balance, as well as hormonal and metabolic changes due to parturition. Six dry and nonpregnant cows were infused with either sterile water (control treatment) or a lipid emulsion (Intralipid 20%, Frenesius Kabi, lipid treatment) at a rate of 1 mL/kg per hour for 6 h according to a crossover design. Blood concentrations of NEFA, β-hydroxybutyrate (BHB), and glucose were measured every hour throughout the infusion period, and 1 and 18 h after the end of infusion. Proliferation and interferon-γ secretion of lymphocytes, phagocytosis, and oxidative burst of neutrophils and blood insulin concentration were evaluated before, during, and at the end of the infusion. For NEFA, BHB, and glucose, treatment × time interactions were present. When compared with the control condition, NEFA and BHB levels were greater in the plasma of cows infused with lipids from 1 h after the start of infusion until 1 h after the end of infusion. Glucose level also increased in response to lipid infusion from 2 h of infusion until 1 h after the end of treatment. For sterile water and lipid infusions, respectively, maximal concentrations were 0.06 ± 0.10 mM and 1.39 ± 0.10 mM for NEFA, 0.70 ± 0.05 mM and 1.06 ± 0.05 mM for BHB, and 4.56 ± 0.27 mM and 6.90 ± 0.27 mM for glucose. For all blood metabolites, there were no differences between treatments 18 h postinfusion. Lipid infusion significantly increased blood insulin concentration at 3 and 6 h of infusion. However, it returned to its basal concentration 18 h after the end of the infusion. Lymphoproliferation declined as early as 3 h after the start of the lipid infusion. At 3 and 6 h of infusion, lipid treatment significantly reduced INF-γ concentration in the culture cell supernatant. The lipid infusion did not affect neutrophil phagocytosis. Nevertheless, the efficacy of the response was affected by a reduction of neutrophils’ oxidative burst. These results confirm that NEFA inhibits immune functions independently of energy balance and other changes that occur during the transition period. They also indicate that high blood lipid concentration causes insulin resistance.

Key words: energy balance, lymphocyte, neutrophil, glucose, immunosuppression

INTRODUCTION

It is known that during the periparturient period, cows are at high risk of developing health problems. Indeed, 75% of diseases occur in the first month following calving, and 36% of deaths in the 60 d following parturition (Leblanc, 2010). Cows undergo physiological and endocrine changes and experience a state of negative energy balance (NEB). The mobilization of body reserves to meet the energy needs required for milk production leads to an increase in blood nonesterified fatty acid (NEFA) concentration. Previous work has shown a relationship between the dysfunction of lymphocytes after calving and the increase in
the concentration of NEFA in the blood (Carbonneau et al., 2012; Vanacker et al., 2017). Other studies have also shown that the addition of NEFA to cell culture media inhibits the functions of both neutrophils and lymphocytes (Ster et al., 2012; Vanacker et al., 2022). These suggest a causative relationship between the inhibition of immune functions and the increase in blood NEFA concentrations during the peripartum period.

During the periparturient period, cows also experienced insulin resistance. Insulin is required for glucose uptake into most peripheral tissues, such as adipose tissue, liver, and muscle, but not the mammary gland and the gravid uterus (De Koster and Opsomer, 2013). Therefore, this targeted insulin resistance can be considered as a homeorhetic adaptation to ensure that a sufficient supply of glucose is provided to the growing offspring before and after birth (Bell and Bauman, 1997). However, a strong insulin resistance that lasts over time is detrimental to cow health as it can lead to a more widespread mobilization of NEFA as a primary source of energy for other tissues (Bell and Bauman, 1997). As a result, the concentration of NEFA in blood increases, bringing with it a higher risk of health issues such as ketosis, abomasal displacement, hepatolipidosis, and impaired ovulation cycle (Bossert et al., 2008). Although insulin resistance is linked to obesity and can be induced by lipid infusion in lean humans (Liang et al., 2018), the etiology of this phenomenon in cows remains unclear.

In a recent review, Horst et al. (2021) questioned the hypothesis that fatty acids are responsible for the immune deficiencies and insulin resistance observed during the periparturient period. Indeed, they believe that most of the studies conducted on this topic point too quickly to a cause-and-effect relationship between NEFA concentrations at parturition and immune dysfunction. Horst et al. (2021) propose an alternative explanation in which systemic inflammation is responsible for the immune dysfunction and poor health status of periparturient dairy cows. According to this explanation, the high NEFA and BHB concentrations are a consequence, and not the cause, of the problem.

Our hypothesis is that the increase in blood NEFA concentration observed during the transition period is at least partially responsible for the immune dysfunction and insulin resistance associated with that period. The aim of this study was to determine the effect of increasing blood NEFA concentration on metabolism, immunity, and insulin resistance independently of hormonal, environmental, and physiological changes as well as of NEB occurring during the peripartum period.

MATERIALS AND METHODS

Animals and Experimental Procedures

The study was conducted in accordance with the guidelines of the Canadian Council on Animal Care (1993), and the experimental protocol was approved by Sherbrooke Research and Development Centre animal care committee (#542). Six healthy, nonpregnant Holstein cows housed at Agriculture and Agri-Food Canada’s Sherbrooke Research and Development Centre (Sherbrooke, QC, Canada) were used (descriptive statistics of the cows are available in Supplemental Table S1; https://data.mendeley.com/datasets/fgbx-5czks; Lacasse and Vanacker, 2022). The cows were dried off at least 2 wk before the experiment. The cows were fed a TMR containing (on a DM basis) 75.5% dry hay, 9.1% corn silage, 10.2% soybean meal, 3.5% beet pulp, 0.4% urea, and 1.3% mineral supplement. The TMR contained (on a DM basis) 12.4% CP, 35.7% ADF, 55.9% NDF, 0.6% of Ca, 0.3% of P, and provided 1.20 Mcal/kg of NE\textsubscript{L}. Cows were infused with either sterile water (control treatment) or a lipid emulsion (Intralipid 20%, Fresenius Kabi, lipid treatment) at a rate of 1 mL/kg per hour for 6 h according to a crossover design. The dose was determined during preliminary assays to induce an increase in blood NEFA concentration of similar magnitude to that found in early postpartum period. There was a 68-h interval between the 2 infusion periods.

The cows were infused through 2 catheters placed in each jugular vein 4 d before the first infusion. Before insertion of the catheter, the skin was shaved and disinfected, first with 70% ethanol and then with 10% iodine. All catheter and infusion parts were previously gas sterilized (Andersen Sterilizers Inc.). The part of the catheters that was inserted into the jugular vein via a sterile 12-gauge trocar was a 22-cm silastic tube (size 1.016 mm i.d. \times 2.16 mm o.d., VWR). This tube was connected to a removable connector for silicone catheter (1.2 \times 2.0 mm, Vygon), which was attached to the cow’s skin with 2 stitches (Supramid #2, CDMV Inc.). As the infusion was scheduled for 4 d after, 2 mL of heparin at a concentration of 500 IU was pushed through the connector before closing to clean blood from the tube and avoid clotting. The device was then covered with sterile gauze and held in place with Elastoplast (CDMV Inc.) wrapped around the neck of the cow until the day of infusion.

The day of the infusion, the catheters were checked to ensure that they were still in place and functional. An extension made of flexible plastic tubing (Tygon...
1.016 mm i.d. × 1.78 mm o.d., VWR) was then connected to the catheter via a male-to-male Luer-lock system (V-893.00-R0, SURGI-PHARM) using a truncated 19-gauge needle as a connector. A larger piece of tubing (Masterflex Tygon E-Lab, E-3603, Pump Tubing, L/S 16, Cole-Parmer) was placed in a sterile glass bottle containing either sterile water or Intralipid through a typical 25-mL plastic serological pipette to keep it straight and in place. This tube was then coupled to a peristaltic pump (Masterflex L/S TM, model 75-19-15 cartridge pump from Cole-Parmer) and connected to the smaller tubing, which was connected to the catheter via another male-to-male Luer-lock system using a truncated 19-gauge needle as a connector. The flow rate was adjusted before beginning of the infusion by calculating the volume that was pumped into a 15-mL Falcon tube over a period of 30 s.

**Blood Collection**

Blood samples were collected from one of the jugular catheters. Blood samples were taken just before the beginning of the infusion (h 0) and every hour during the infusion (h 1 to h 6). Two other blood samples were taken 1 h (h 7) and 18 h (h 24) after the end of the infusion. Samples were collected in EDTA-coated vacutainer tubes (Becton Dickinson) for metabolites and insulin determinations, in heparin-coated Vacutainer tubes for the phagocytosis and oxidative burst assays, and in uncoated tubes for the serum preparation required for the peripheral blood mononuclear cell (PBMC) proliferation and cytokine secretion assays.

**Blood Metabolite Assays**

Plasma BHB and NEFA concentrations were evaluated with a BHB reagent kit (Pointe Scientific Inc.) and an enzymatic colorimetric method using a Randox kit (Randox Laboratories Ltd.), respectively. Both of these methods are described by Vanacker et al. (2020); however, the 2 incubation times were increased to 10 min for the NEFA assay. Absorptions were read using a SpectraMax 190 microplate reader (Molecular Devices). The intra- and interassay coefficients of variation (CV) were respectively 3.63 and 3.45% for BHB, 3.48 and 5.41% for NEFA, and 1.33 and 0.94% for glucose.

**Blood Insulin Assay**

Plasma insulin concentration was measured by ELISA with an insulin reagent kit (Mercodia), according to the manufacturer’s instructions. Samples above the upper detection limit were diluted at a rate of 1/2. Absorptions were read using a SpectraMax 190 microplate reader (Molecular Devices). The intraassay CV was 1.62% and the interassay CV was 2.63%.

**Polymorphonuclear Leukocyte Phagocytosis and Oxidative Burst Assays**

The phagocytosis assay was performed in whole blood by flow cytometry using BioParticles Conjugated for Phagocytosis (Life Technologies Inc.), as described by Vanacker et al. (2017). The oxidative burst assay was also performed in whole blood by flow cytometry, as described by Vanacker et al. (2017).

**Peripheral Blood Mononuclear Cell Isolation**

To assess the effect of the serum from the treated cows on PBMC proliferation, PBMC were isolated from the jugular blood of 4 healthy lactating donor cows not included in the experiment. The blood was collected in EDTA-coated vacutainer tubes. Isolation of PBMC was performed as described by Vanacker et al. (2017).

**Peripheral Blood Mononuclear Cell Proliferation Assay**

To assess the effect of the serum from the treated cows on PBMC proliferation, PBMC isolated from 2 healthy cows were incubated for 72 h with 7.5% sera sampled at h 0, 3, or 6 from each cow in a 96-well plate (1 × 10⁵ cells/well) at 38.5°C as described by Ollier et al. (2016), except that the sera were not decomplemented. For each serum tested, the PBMC of 3 wells were incubated with concanavalin A (Sigma-Aldrich) at 1 µg/mL, and the PBMC of another 3 wells were incubated without the mitogen as a negative control. The assay was repeated for each cell donor cow. For the immune parameters (lymphoproliferation, phagocytosis, and oxidative burst), we used the same controls as described by Vanacker et al. (2017).

**Interferon-γ Production**

To assess the effect of the lipid infusion on PBMC cytokine secretion, isolated PBMC from the 4 healthy cows were incubated with sera (7.5%) collected from experimental cows at h 0, 3, and 6. They were stimulated with concanavalin A at a concentration of 1 µg/mL and incubated in a 24-well plate (1.2 × 10⁶ cells/well) at 38.5°C with 5% CO₂ for 24 h. This was done in duplicate. The supernatant was collected by pooling the 2 wells with the same condition and then
stored at −80°C until measurement. Supernatant INF-γ cytokine content was determined using a commercial kit, Bovine Interferon-γ specific ELISA Assay Kit (Bio-Rad, Life Science Group), in accordance with the manufacturer’s instructions. Preliminary assays were first performed to determine the optimal dilution of the supernatant. The dilutions varied between 1/12 and 1/25 depending on the donor cow. For INF-γ determination the quality control pool had to be diluted at a rate of 1/2, the intraassay CV was 1.30% and interassay CV was 9.20%.

Statistical Analysis

Data were analyzed by ANOVA as a crossover design using the MIXED procedure of the SAS software package (SAS Institute Inc.). For most variables, the model included the fixed effect of treatments, sampling time, sampling day (period), block and treatment × time interaction. Sampling time was used as a repeated effect, and animal(treatment) was used as the subject. For PBMC function parameters, the model included the fixed effect of treatments, sampling time, sampling day (period), block and treatment × time, treatment × donor and treatment × donor × time interactions. Sampling time was used as a repeated effect and animal(treatment × donor) was used as the subject. The statistical model assumes (1) independence of observations, (2) normal distribution of the log 10 transformed data, and (3) homogeneity of variance. When multiple comparisons were made, the Tukey adjustment was used. When significant, the value before the start of the infusion was used as a covariable. Differences were considered statistically significant when \( P < 0.05 \) and were considered a trend when \( P < 0.10 \).

RESULTS

Blood Metabolite Assays

Lipid infusion caused a gradual increase in blood NEFA (treatment × time \( P < 0.001 \), Figure 1A), BHB (treatment × time \( P = 0.03 \), Figure 1B), and glucose (treatment × time \( P < 0.001 \), Figure 2A) concentrations. Concentrations of NEFA and BHB were already higher after 1 h (\( P < 0.001 \)) of lipid infusion, whereas that of glucose was higher after 2 h of lipid infusion. All metabolites reached a peak after 5 h, and their concentration decreased beginning at the end of the infusion. No significant difference was observed between the treatment and control 18 h after the end of the infusion (NEFA, \( P = 0.8 \); BHB, \( P = 0.5 \); glucose, \( P = 0.4 \)).

Blood Insulin

Lipid infusion also caused a gradual increase in blood insulin concentration (treatment × time \( P < 0.001 \), Figure 2B). Blood insulin concentration increased after 3 h of lipid infusion (\( P = 0.04 \)) and was 5 times greater after 6 h of infusion (\( P < 0.001 \)). At 18 h after the end of the infusion, blood insulin concentration was no longer elevated (\( P = 0.6 \)).

Polymorphonuclear Leukocyte Phagocytosis and Oxidative Burst Assays

The percentage of phagocytic neutrophils was not affected (\( P = 0.15 \), Figure 3A). The percentage of PMN producing an oxidative burst when stimulated with Phorbol 12-myristate 13-acetate was lower (\( P = 0.02 \), Figure 3B) during lipid infusion. However, the amount of reactive oxygen species produced was not affected by the treatments (\( P = 0.4 \), data not shown).

Peripheral Blood Mononuclear Cell Proliferation and Interferon-γ Production

Incubation of concanavalin A-stimulated PBMC with sera harvested during the infusion affected lymphoproliferation (treatment × time \( P = 0.005 \), Figure 4). Lymphoproliferation was decreased (\( P = 0.02 \)) after 3 h, but not after 6 h (\( P = 0.27 \)) of lipid infusion. Incubation of concanavalin A-stimulated PBMC with sera harvested during the lipid infusion decreased (\( P = 0.02 \)) secretion of INF-γ (Figure 5).

DISCUSSION

This study was aimed at evaluating the effect of a lipid infusion on blood metabolites and immune function of dairy cows. The experiment was designed to mimic the blood metabolites profile of periparturient cows independently of the NEB, hormonal changes, and other physiological changes occurring during the periparturient period. The experiment was carried-out in nonlactating cows for practical reasons and this could be considered a limitation of this study.

When infused with the lipid, blood NEFA concentration increased and reached a peak of 1.39 mM after 5 h of infusion. The week before parturition, NEFA concentrations are around 0.5 to 1.0 mM and reach concentrations of around 0.8 to 1.2 mM the day of the parturition. Indeed, NEFA concentrations at the end of the infusion period are comparable to what is observed in high-yielding cows after parturition. Infusion of lower doses of lipids to dry (Pires et al., 2007;
Caixeta et al., 2017) or lactating (Chelikani et al., 2003; Lamp et al., 2018) cows also increased, albeit to a lesser degree, the concentration of NEFA. The increase in NEFA concentration was sufficient, as in the transition period, to saturate the liver’s capacity to completely oxidize the acetyl CoA generated by the β-oxidation of fatty acids, as shown by the increase of BHB. Blood ketone bodies increases when the amount of fatty acids taken by the liver exceed its capacity to completely oxidize or esterified them. In our experiment, blood BHB concentrations increased from the beginning of the infusion, with a peak at 5 h of infusion and a concentration of 1.06 mM. Cows with a BHB concentration above 1.2 mM are considered to be

Figure 1. Blood concentration of (A) nonesterified fatty acid (NEFA) and (B) BHB in cows infused with a lipid solution (Intralipid 20%, Frenesius Kabi, 1 mL/kg per hour; orange line; n = 6) or sterile water (control; blue line; n = 6) for 6 h. Data presented as LSM ± SEM. *P ≤ 0.05; **P < 0.01; ***P ≤ 0.001.
in a state of hyperketonemia (Duffield, 2000; McArt et al., 2013; Shin et al., 2015). Although Chelikani et al. (2003) reported that BHB increased within 3 h of lipid infusion, Caixeta et al. (2017) reported only a trend, whereas Lamp et al. (2018) reported no increase in BHB concentration. The variability of results between the lactating cows appears to be related to energy balance, as the increase in BHB was greater in cows in early lactation compared with those in late lactation (Chelikani et al., 2003). However, in the present study as well as in the study by Caixeta et al. (2017), the different responses are most likely related to the dose of lipids infused, as the cows were neither lactating nor gestating.

**Figure 2.** Blood concentration of (A) glucose or (B) insulin in cows infused with a lipid solution (Intralipid 20%, Frenesius Kabi, 1 mL/kg per hour; orange line; n = 6) or sterile water (control; blue line; n = 6) for 6 h. Data presented as LSM ± SEM. *P ≤ 0.05; **P < 0.01; ***P ≤ 0.001.
Increased NEFA and BHB blood concentrations are believed to play a key role in periparturient immune dysfunction (Hammon et al., 2006; Duffield et al., 2009; Leblanc, 2010; Ster et al., 2012). Because the infusion of lipids was able to increase the levels of these 2 metabolites to concentrations comparable to those found in the blood of periparturient cows, we can conclude that the animal model we have developed is representative of the metabolic profile of periparturient cows. Thus, our model would be suitable for testing approaches aimed at reducing peripartum immune dysfunction and metabolic dysfunctions caused by the rise of blood NEFA and BHB concentrations independently of hormonal, environmental, and physiological changes, and NEB occurring during the peripartum period.

Lymphoproliferation and INF-γ production decreased in PBMC incubated with sera collected after 3 h of lipid infusion. Carbonneau et al. (2012), Ster et al. (2012), and Vanacker et al. (2017) observed that serum collected during the peripartum period had a negative

**Figure 3.** Percentage of (A) polymorphonuclear cells positive for phagocytosis and (B) oxidative burst assayed from the blood of cows infused with a lipid solution (Intralipid 20%, Fresenius Kabi, 1 mL/kg per hour; orange bars; n = 6) or sterile water (control; blue bars; n = 6) for 6 h. Data presented as LSM ± SEM. *P ≤ 0.05.
effect on PBMC proliferation and that this effect was correlated with the serum’s NEFA and BHB content. Supplementation of cell culture medium with NEFA or BHB has shown that low concentrations of NEFA led to a drastic decrease in proliferation, whereas only high concentrations of BHB affected proliferation (Ster et al., 2012). In the same study, adding NEFA to serum collected at 61 DIM to reach the level present in 5 DIM serum decreased proliferation to the level observed with 5 DIM serum, whereas a similar supplementation with BHB had no effects. Accordingly, other studies reported that high NEFA concentrations inhibited the proliferation of ovine (Lacetera et al., 2002) and bovine PBMC (Lacetera et al., 2004). The production of INF-γ was decreased in PBMC collected during the peripartum period (Loiselle et al., 2009) as well as in PBMC incubated with serum collected during this same period (Ster et al., 2012). As for proliferation, INF-γ secretion also decreased as the NEFA concentration in the medium increased (Lacetera et al., 2004; Ster et al., 2012). Therefore, impairment of PBMC functions during the transition period appears to be related to the increase in blood NEFA concentration.

Infusion did not produce any observable effect on PMN phagocytosis, but the number of PMN able to produce an oxidative burst was reduced by lipid infusion. Kehrli et al. (1989) reported that PMN functions are impaired during the peripartum period. Hammon et al. (2006) also reported that high NEFA blood concentrations after parturition were associated with decreased PMN functions. Nevertheless, the increased PMN demand during this period results in the rapid mobilization of bone marrow PMN and an increase in the proportion of immature PMN in circulation. However, this cannot explain the results of the present study, because cows were dry and not gestating. In vitro, the presence of high concentrations of NEFA was shown to adversely affect oxidative burst of PMN, but the concentrations of NEFA required were much greater (Ster et al., 2012). This finding suggests that PMN are less sensitive to NEFA than are PBMC, but as shown by our results, PMN may nevertheless be affected by the very high NEFA levels reached in the early postpartum period.

The lipid infusion caused an exponential increase in blood glucose concentration, despite an increase in insulin concentration. Although we have not performed a glucose tolerance test, this suggests the establishment of insulin resistance, because the increase in insulin secretion observed in response to lipid treatment does not translate to a reduction of glucose level. Insulin resistance is commonly observed during the peripartum period; nevertheless, glucose concentration remains low (De Koster and Opsomer, 2013). Although peripheral tissues become insulin resistant, this is not the case for the mammary tissue, which allow the utilization of glucose by the mammary gland for milk production (De Koster and Opsomer, 2013). In the present experiment,
the absence of demand for milk production allows an increase of glucose in the circulation. Accordingly, although intravenous infusion of lipids increased insulin without increasing glucose concentration in lactating cows (Chelikani et al., 2003; Caixeta et al., 2017), it increased both insulin and glucose in dry cows (Pires et al., 2007). In the latter study, insulin resistance was confirmed by an intravenous glucose tolerance test. In humans, obesity is a well-known cause of insulin resistance. Therefore, this suggest that the insulin resistance around parturition in dairy cows traces its origins to the increase in the concentration of NEFA. The exact mechanism by which NEFA induce insulin resistance in dairy cows is still unclear, but it surely involves decreased insulin sensitivity in peripheral tissue and has some characteristics in common with type 2 diabetes in humans (Youssef and El-Ashker, 2017). In lean nondiabetic human subjects, infusion of a low dose of lipids induces insulin resistance and a pro-inflammatory response (Liang et al., 2018). These authors also reported that the infusion of lipids upregulated toll-like receptor-4 (TLR4) levels and downstream signaling in monocytes. The TLR4 mediates LPS-induced inflammatory response (Chow et al., 1999); however, fatty acids are also potent activators of this receptor (Shi et al., 2006). In bovine adipose tissue explants, LPS induces insulin resistance (Chirivi et al., 2022); suggesting that the TLR4 pathway is part of the mechanism by which insulin resistance is induced in dairy cows.

In a recent review, Horst et al. (2021) argued against the view that an increase of NEFA is responsible for the immune dysfunction observed in dairy cows during the periparturient period. They hypothesized that immune dysfunction stems from traumas to the uterus, mammary gland, and gastrointestinal tract and which allow the transfer of pathogen-associated molecular patterns (such as LPS) and damage-associated molecular patterns, which would be at the origin of the systemic inflammation that naturally occurs during this period. Thus, increased NEFA, hyperketonemia, and hypocalcemia are homeorhetic adaptations to parturition. Horst et al. (2021) based their hypothesis on the fact that most studies concerning the effect of NEFA on immune function are either in vitro studies or examine the association between blood metabolites concentrations and transition cow problems instead of looking for a cause-and-effect relationship using a controlled environment. However, in the present experiment, inflammation or adaptation to parturition was ruled out, given that cows were in a stable physiological state and that the crossover design makes it possible to control for individual variations. Accordingly, intravenous infusion of lipids to steers decreases the cortisol and interleukin-6 response to an LPS challenge (Burdick Sanchez et al., 2015). We also observed

Figure 5. Concentration of INF-γ in the culture cell supernatant of peripheral blood mononuclear cells from 4 donor cows incubated with sera of cows infused with a lipid solution (Intralipid 20%, Frenesius Kabi, 1 mL/kg per hour; orange bars; n = 6) or sterile water (control; blue bars; n = 6) for 6 h. *P ≤ 0.05. Data presented as LSM ± SEM.
decreased PBMC proliferation and cytokine secretion when NEB and high NEFA concentrations were induced by feed restriction (Ollier et al., 2014; Ollier et al., 2016). Again, these results support the hypothesis that NEFA are immunosuppressive in dairy cows and that this effect is independent from the NEB or physiological changes. Although we cannot rule out that trauma-induced systemic inflammation is immunosuppressive, its contribution is likely small, because the removal of the mammary gland considerably reduced the effect of calving on the number of immune cells and their associated functions (Kimura et al., 1999, 2002; Nonnecke et al., 2003). Nevertheless, metabolic stress in the postpartum period is associated with an upregulation of inflammatory pathways (Mann et al., 2019). These authors also reported that calving and metabolic stress were associated with an upregulation of toll-like receptor signaling, including TLR4 signaling in particular. Therefore, systemic inflammation may indeed be an important immunosuppressive factor, but this may be more of a response to high NEFA levels than to traumas.

In conclusion, the results of this study support the hypothesis that NEFA induces immune cell dysfunction independently of energy balance and other changes occurring during the transition period. They also suggest that high blood lipid concentration causes insulin resistance. Therefore, strategies that limit the rise of the NEFA concentration during the transition period might be helpful to limit immune dysfunctions and insulin resistance.

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