EFFECT OF ELEVATED LIPID MOBILIZATION ON GENE EXPRESSION AND FATTY ACID COMPOSITION OF CIRCULATING IMMUNE CELLS, MILK, AND BLOOD LIPID FRACTIONS OF PERIPARTURIENT DAIRY COWS

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INTRODUCTION

The energy requirements during early lactation are not met via diet, resulting in massive mobilization of adipose tissue. Mobilized adipose tissues release fatty acids (**FA**) into the bloodstream. The transport of FA is facilitated via circulation of various lipid fractions including neutral lipids (**NL**), phospholipids (**PL**) and non-esterified fatty acids (**NEFA**); NL and PL are carried by lipoproteins. The fatty acids are made available to cell metabolism by the action of lipoprotein lipases (Tall, 1995). Blood NEFA is mostly bound to albumin, but a small portion of NEFA is transported as unbound monomers in aqueous solution (Richieri and Kleinfeld, 1995).
Lipomibilization is a physiological adaptation by cows in response to an energy shortage. Lipomibilization affects not only the concentration of total plasma lipids and corresponding fractions; it also causes major shifts in FA composition (Douglas et al., 2007). With modifications in the plasma, the total lipid FA profile reflects the changes originating from the diet and increased rate of lipomobilization. However, the FA composition of total plasma lipids does not reflect the FA profile of plasma lipid fractions. This is due to the difference in FA distribution among lipid fractions. Neutral lipids such as triglycerides, diglycerides, monoglycerides, and cholesterol esters are intermediate molecules of lipid metabolism that can provide energy. However, PL provides lipid substrates for the biosynthesis of potent pro- or anti-inflammatory mediators, including the eicosanoids and platelet-activating factors (Henneberry et al., 2002). Therefore, NEFA is involved in intracellular signaling processes, modification of cellular functions, and as energy substrate.

Increased lipomobilization, which is evidenced by increases in blood NEFA, leads to impaired metabolic and immune cell functions (Mora and Pessing, 2002). Previous studies with dairy cows demonstrate that elevated NEFA largely affects the function of the immune cells during the periparturient period (Lacetera et al., 2004; Scalia at al., 2006). Plasma NEFA concentrations were positively correlated with altered peripheral blood mononuclear cells (PBMC) populations. During the periparturient period, the immune cells' functionality is affected not only by the quantity of plasma NEFA, but also by its FA profile.

Major FA of plasma NEFA and PL are palmitic, stearic, and oleic acids. During lipomobilization, hormone-sensitive lipases and triglyceride lipases release FA from triglyceride depots in the adipose tissue (Kershaw at al., 2006). The molecules are then released in circulation and transported bound to albumin, and during periods of negative energy balance, NEFA profiles may directly reflect the FA composition of adipose tissue. The FA profiles of subcutaneous adipose tissue in periparturient cows were found to be mainly palmitic, stearic, and oleic acids (Douglas et al., 2007).

The NEFA profile may reflect the FA content of plasma but also affect phospholipids fraction of peripheral blood mono-nuclear cells (**PBMC**). Saturated palimtic and stearic acid components of the PL fraction increased during weeks of intense lipomobilization (Contreras et al., 2010). The structures of plasma lipid fractions were reflected in the FA profile of the PL fraction of PBMC. Increased concentrations of saturated FA and reduced concentrations of polyunsaturated fatty acids (**PUFA**) in the PL fraction of PBMC could negatively affect the immune cell response during the periparturient period.

Elevated plasma NEFA-to-albumin ratio predisposes human subjects to immune-endothelial dysfunction diseases, such as pre-eclampsia (Endersen et al., 1992) and metabolic diseases such as type II diabetes (Cnop et al., 2001). Given the high prevalence of elevated plasma NEFA in periparturient cows, the altered NEFA-to-albumin ratio has certainly physiological outcomes that warrant investigation.

Ionophores alter transport of ions across the bacterial cell wall specifically inhibiting growth of Gram positive bacteria. Monensin is a carboxylic polyether ionophore that is naturally produced by *Streptomyces cinnamonensis* and fed to cattle in the form of sodium salt (Duffield and Bagg, 2000). The specific effect of monensin on rumen microbial populations results in improved efficiencies of energy metabolism, N metabolism, reduced bloat and lactic acidosis (Schelling, 1984; Duffield et al., 2008a). A meta-analysis of the impact of monensin on blood metabolites revealed that inclusion of monensin in dairy cattle diets results in significant reductions in blood nonesterified fatty acids (NEFA; by 7%), β-hydroxybutyrate (BHB; by 13%), and acetoacetate (by 14%), as well as reductions in short-chain fatty acids and stearic acid content of milk (Schelling, 1984; Duffield et al., 2008a). Overall, these changes are considered to be related to increased hepatic gluconeogenic activities. As a consequence, feeding monensin is associated with reduction in risk for energy-related disorders such as ketosis (Duffield et al., 2002 and 2008b).

An observational experiment (Scholte et al., 2014a) was conducted to determine the effect of subcutaneous fat stores on FA profile of plasma NEFA and PL lipid fractions as well as productive performance during the periparturient period. When periparturient cows monitored from d -28 (before expected parturition) through 28 d postpartum were retrospectively dichotomized into "over-conditioned (BCS $\geq$ 3.25) " as compared with "control (BCS $\leq$ 3.0)" groups before calving (d -7 to d -3), NEFA concentration and FA profile of plasma NEFA fraction differed between groups. As expected, serum NEFA concentration significantly differed between two groups (0.96 vs. 0.68 ± 0.06 mEq/L; P = 0.005; Figure 1).

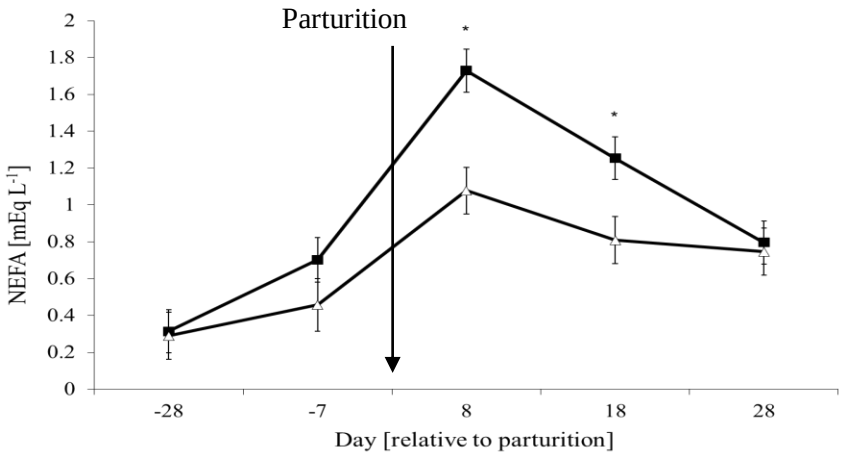

Figure 1. Serum nonesterified fatty acid concentrations (NEFA; Least Square Means ± SEM in mEq/L) obtained from dairy cows (n = 22) at various time points through the transition period that were retrospectively dichotomized by BCS at -5 d to -7 d before parturition. Closed squares present HIGH group (BCS ≥ 3.25) and open triangles present LOW group (BCS ≤ 3.0). *Represents that treatment means differed at those time-points specified, $P < 0.05$

The FA composition of the serum NEFA fraction differed by treatment for C14:1 and C18:0 as C14:1 concentration tended to increase in LOW as compared with HIGH treatment, whereas C18:0 significantly increased in HIGH. Phospholipids constitute a fraction of the serum lipids used primarily for cell-to-cell communications and lipid bi-layers, and function as mediators of inflammation. The serum PL fraction differed by treatment for several FA, where C16:0 (22.4 vs. 19.7 ± 1.0 g/100 g) and sum of C18:2 *cis* (28.7 vs. 26.0 ± 1.1 g/100 g) tended to increase in LOW as compared with HIGH cows. Conversely, C17:0 (0.51 vs. 0.81 ± 0.08 g/100 g) decreased and C20:2n6 (0.38 vs. 1.2 ± 0.3 g/100 g) tended to decrease in LOW as compared to HIGH treatments. The serum PL fraction differed by time as well (Table 1). For instance, C16:0 increased from ~18.6 to 22.3 g/100g over the time period tested (day -28 to day +28, relative to parturition).

Table 1. Least square means of fatty acid composition of nonesterified fatty acids (NEFA) fraction of serum lipids obtained from dairy cows (n = 22) at various time points throughout the transition period that were retrospectively dichotomized by body condition score at -5 d to -7 d before parturition.

	Day, relative to parturition						
Fatty acid[1] (g/100 g)	-28	-7	8	18	28	SEM[2]	*P*
C14:0	2.6^{a}	2.2^{bc}	2.3^{ab}	1.9^{c}	2.1^{bc}	0.2	0.01
C16:0	17.2^{a}	19.2^{ac}	21.8^{b}	21.5^{bc}	20.3^{bc}	1.0	0.01
C16:1	0.8^{a}	1.2^{ab}	2.1^{c}	2.2^{c}	1.5^{b}	0.2	0.001
C18:0	21.1	24.9	22.3	23.1	23.4	1.4	0.37
C18:1 *cis*	13.1	18.1^{a}	22.9^{ab}	27.0^{b}	21.8^{a}	2.1	0.001
C18:2 *trans*	15.4^{a}	11.0^{ab}	9.3^{b}	2.7^{c}	7.6^{bc}	2.2	0.01

C18:2 *cis*	6.7[a]	6.4[a]	4.6[b]	5.7[ab]	6.7[a]	0.5	0.02
C20:1	3.6[a]	2.7[ac]	2.4[ac]	0.9[b]	1.8[bc]	0.5	0.001
C20:3n3	0.65[ab]	0.76[a]	0.39[c]	0.48[bc]	0.58[ac]	0.09	0.03

[a, b, c, d] Means in a row without a common superscript differ, $P < 0.05$;
[1] C14:0 = myristic acid, C16:0 = palmitic acid, C16:1 = palmitoleic acid, C18:0 = stearic acid, C18:1 = oleic acid, C18:2 = linoleic acid, C20:1 = eicosenoic acid, C20:3n3 = eicosatrienoic acid;
[2] Largest SEM reported.

Productive performance such as feed intake was also altered because of the BCS status around parturition. During both prepartum and postpartum periods intake was reduced in over-conditioned cows as compared with control cows (P = 0.02 and 0.08 for pre- and postpartum periods, respectively; Figure 2). No significant difference however was detected in milk yield or composition.

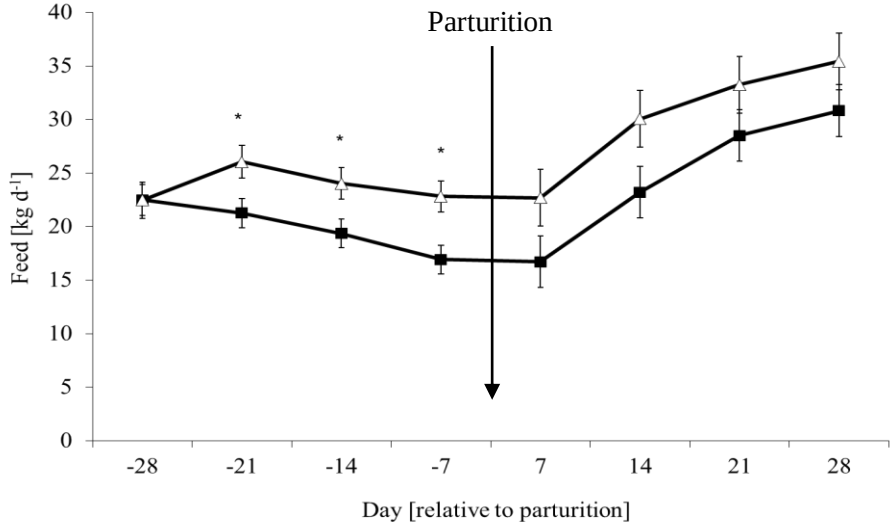

Figure 2. Pre- and postpartum mean weekly feed intakes (kg/d) obtained from dairy cows (n = 22) through the transition period that were retrospectively dichotomized by body condition score (BCS) at -5 d to -7 d before parturition. Closed squares present HIGH group ($BCS \geq 3.25$) and open triangles present LOW group ($BCS \leq 3.0$). * Represents that treatment means differed at those time-points specified, $P < 0.05$.

In a follow up study in our laboratory (Scholte et al., 2014b) periparturient Holstein dairy cows were blocked by party and randomly assigned to either receive dry cow ration plus 10 kg/d per head additional corn or dry cow ration (no additional corn) plus 400 mg/d monensin per head during the prepartum period whereas all cows received a common lactation ration. Three days after parturition, treatment cows were fasted for 8 h. Serum samples were collected on -28, -7, +1, +6, +15, and +21 d for FA analysis of the specific lipid fractions. Milk samples were obtained on +1, +3, +6, +15, and +21 d for composition, yield and FA analyses. Real-time q-PCR gene analysis for intercellular adhesion molecule 1 (*ICAM-1*), interleukin (*IL*) 1β and 6, and tumor necrosis factor-α (*TNF-α*) was performed on PBMC collected on days -28, +3, +12, and

+21. Data were analyzed as repeated measures ANOVA using mixed model procedures in SAS and significance was declared at $P \leq 0.05$.

Preliminary results show that within serum NEFA fraction, C16:1, sum of C18:1 *trans*, and C18:3n3 were greater in control than in treatment prepartum, but no significance detected in postpartum (Table 2). In contrast, sum of C18:1 *trans* was greater in treatment compared with that of control during postpartum period. Serum concentration of C20:4n6 tended to be greater in treatment compared with that in control (1.12 vs 0.86 ± 0.11%; $P = 0.06$).

Table 2. Least square means of serum NEFA fatty acids of dairy cows that received either the treatment of an additional10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 (relative to parturition) or control of 400 mg of monensin/hd per day prepartum

	Treatment		Control			P - value			
Fatty Acid (g / 100g)	Multiparous	Primiparous	Multiparous	Primiparous	SEM[2]	Treatment	Treatment × Time	Treatment × Parity	Treatment × Parity × Time
C 16:0	28.0	27.4	27.6	27.6	0.7	0.89	0.70	0.76	0.04
C 18:0	23.0	24.8	24.4	25.0	1.0	0.41	0.22	0.48	0.10
Σ C 18:1 cis	22.7	21.8	21.9	19.0	1.3	0.13	0.41	0.42	0.0001
Σ C 18:2 trans	0.07	0.08	0.08	0.08	0.02	0.81	0.26	0.89	0.07
Σ C 18:2 cis	7.9	7.4	7.6	6.9	0.6	0.42	0.30	0.91	0.01
C 20:3n3	0.18	0.19	0.26	0.30	0.09	0.19	0.52	0.78	0.002
Σ saturated	58.1	58.6	59.2	60.7	1.4	0.19	0.93	0.66	0.19
Σ unsaturated	41.9	41.4	40.8	39.3	1.4	0.19	0.93	0.66	0.19
Σ MUFA	30.1	30.2	29.9	27.7	1.7	0.37	0.64	0.45	0.0003
Σ PUFA	11.8	11.2	10.9	11.6	0.9	0.75	0.87	0.44	0.0009
Σ n-6	8.8	8.5	8.4	8.1	0.6	0.50	0.29	0.95	0.06
Σ n-3	1.6	1.7	1.6	2.5	0.4	0.25	0.53	0.21	0.18
n-6:n-3	6.8	6.1	5.9	4.5	0.5	0.01	0.11	0.42	0.82
saturated: unsaturated	1.5	1.5	1.5	1.6	0.1	0.15	0.95	0.54	-

In milk fat, C16:1 and C18:2 *cis* were greater for treatment and C15:0, C18:3n3 and C22:2 were lower for treatment compared with those in control.

Table 3. Least square means of milk fatty acids of dairy cows that received either the treatment of an additional 10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 (relative to parturition) or control of 400 mg of monensin/hd per day prepartum

	Treatment		Control			P - value			
Fatty Acid (g / 100g)	Multiparous	Primiparous	Multiparous	Primiparous	SEM[1]	Treatment	Treatment × Time	Treatment × Parity	Treatment × Parity × Time
C 16:0	31.9	31.1	31.6	31.9	0.6	0.65	0.87	0.37	0.02
C 16:1	2.4	1.7	2.1	1.6	0.1	0.06	0.27	0.39	0.56
C 17:0	0.69	0.94	0.79	0.97	0.05	0.10	0.45	0.36	0.02
Σ C 18:2 cis	3.0	2.7	2.9	2.4	0.1	0.03	0.0009	0.14	0.06
C 20:1	0.43	0.52	0.40	0.53	0.03	0.67	0.82	0.41	0.04
C 20:3n3	0.24	0.23	0.25	0.20	0.02	0.57	0.56	0.14	0.0004
C 22:2	0.04	0.06	0.05	0.09	0.01	0.004	0.21	0.16	0.65
Σ saturated	56.2	58.8	56.9	61.0	1.2	0.17	0.99	0.50	0.22
Σ unsaturated	43.7	41.1	43.0	38.9	1.2	0.18	0.89	0.49	0.22
Σ MUFA	39.9	37.4	39.1	35.4	1.3	0.22	0.89	0.58	0.21
Σ PUFA	3.8	3.7	3.8	3.5	0.1	0.32	0.03	0.29	-
Σ n-6	3.1	2.8	3.0	2.5	0.1	0.04	0.002	0.18	0.08
Σ n-3	0.58	0.70	0.66	0.76	0.03	0.01	0.44	0.66	-
saturated: unsaturated	1.4	1.5	1.4	1.7	0.1	0.22	0.84	0.20	-

Gene expression for *IL-1β* in PBMC was greater in control than treatment, whereas *ICAM-1*, *IL-1β*, *IL-6*, and *TNF-α* were greater in primiparous than multiparous cows, without a detectable treatment effect. Prepartum, control cows consumed more feed than treatment. Postpartum intake tended to differ by a treatment × time interaction with intake wavering until d +11 and treatment consuming more thereafter.

Table 3. Least square means of delta Ct of peripheral blood mononuclearcytes of dairy cows that received either the treatment of an additional10 kg of corn/hd per day prepartum and were fasted for 8 h on d +3 (relative to parturition) or control of 400 mg of monensin/hd per day prepartum

	Treatment		Control			P - value	
Gene	Multiparous	Primiparous	Multiparous	Primiparous	SEM[1]	Treatment	Parity
ICAM	8.44	7.32	8.65	7.19	0.38	0.91	0.0004
IL-1β	5.69	3.69	4.67	3.11	0.43	0.04	0.0001
IL-6	13.6	11.8	13.3	11.5	0.5	0.55	0.0009
TNF-α	4.83	3.61	4.32	3.51	0.40	0.39	0.0070

In summary, Serum NEFA C18:3n3 and n-6 to n-3 ratio differed by treatment while C20:4n6 tended to differ by treatment. Milk C18:2 *cis*, C18:3n3, total n-6, n-3, and n-6 to n-3 ratio differed by treatment. IL-1β differed by treatment and parity, whereas ICAM, IL-6, and TNF-α only differed by parity.

CONCLUSIONS

Lipid mobilization affects all lactating dairy cows as they go through the periparturient period. During this time period, cows with greater BCS mobilize more lipid than cows with lower BCS. Small changes in prepartum BCS altered the FA profile of serum NEFA and PL fractions during the periparturient period. Some FA profile changes are to be expected as predominant FA in the circulating NEFA fraction provide energy to meet the energy deficit caused by the onset of lactation and insufficient DMI. Changes in the FA profile of the PL fraction however, could have greater consequences as PL provide substrates for synthesis of potent pro- and anti-inflammatory mediators and therefore can alter immune cell responsiveness.

These results support the hypothesis that cows with increased subcutaneous fat stores around time of parturition have different circulating NEFA and PL fatty acid profiles and consume less dry matter than cows with decreased subcutaneous fat. Further research is needed to understand the direct effects of these fat stores on the change in FA profile of circulating NEFA and PL fractions and consequently on immune cell FA profiles and their responsiveness to pathogens.

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