Effect of propylene glycol on adipose tissue mobilization in postpartum over-conditioned Holstein cows

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ABSTRACT

Our objective was to investigate the quantitative and qualitative effects of propylene glycol (PG) allocation on postpartum adipose tissue mobilization in over-conditioned Holstein cows. Nine ruminally cannulated and arterially catheterized cows were, at parturition, randomly assigned to a ruminal pulse dose of either 500 g of tap water ($n = 4$) or 500 g of PG ($n = 5$) once a day. The PG was given with the morning feeding for 4 wk postpartum (treatment period), followed by a 4-wk follow-up period. All cows were fed the same prepartum and postpartum diets. At $-16 (\pm 3)$, $4 (\pm 0)$, $15 (\pm 1)$ and $29 (\pm 2)$ days in milk (DIM), body composition was determined using the deuterium oxide dilution technique, liver and subcutaneous adipose tissue biopsies were collected, and mammary gland nutrient uptake was measured. Weekly blood samples were obtained during the experiment and daily blood samples were taken from -7 to 7 DIM. Postpartum feed intake and milk yield was not affected by PG allocation. The body content of lipid was not affected by treatment, but tended to decrease from 4 to 29 DIM with both treatments. Except for the first week postpartum, no difference in plasma nonesterified fatty acids concentration was noted between treatments in the treatment period. Yet, PG allocation resulted in decreased plasma concentrations of β -hydroxybutyrate (BHB) and increased plasma concentrations of glucose. In the follow-up period, plasma concentrations of nonesterified fatty acids, glucose, and BHB did not differ between treatments. Additionally, the change in abundance of proteins in adipose tissue biopsies from prepartum to 4 DIM was not affected by treatment. In conclusion, the different variables to assess body fat mobilization were concur-

rent and showed that a 4-wk postpartum PG allocation had limited effect on adipose tissue mobilization. The main effect was an enhanced glucogenic status with PG. No carry-over effect of PG allocation was recorded for production or plasma metabolites, and, hence, a new period of metabolic adaption to lactation seemed to occur with PG treatment after ceasing PG allocation. Thus, PG seemed to induce a 2-step adaption to lactation, reducing the immediate postpartum nadir and peak of plasma concentration of glucose and BHB, respectively; which is beneficial for postpartum cows at high risk of lipid-related metabolic diseases.

Key words: dairy cow, propylene glycol, body composition, proteomics

INTRODUCTION

The highest occurrence of disease in dairy cows is observed within the first few weeks of lactation (Ingvarsen, 2006). A prerequisite for cows to stay healthy through this period is that the interaction between hormonal regulation, feed intake, milk yield, and physical condition of the cows is balanced. After parturition, cows have a natural genetic drive to mobilize adipose tissue, increasing with increasing BCS (Garnsworthy and Topps, 1982; Friggens et al., 2007). Thus, cows that are over-conditioned at parturition will be at greater risk for excessive mobilization of adipose tissue after parturition. Excessive mobilization of adipose tissue in cows has been revealed to be problematic and may result in hepatic lipidosis and ketosis (Grummer 1993; Ingvarsen, 2006). Hence, it is of particular interest to monitor postpartum adipose tissue mobilization in over-conditioned cows and prevent excessive mobilization.

Acute treatment and prophylactic treatment of ketosis by oral dosing with propylene glycol (PG) has been used since the 1950s (Nielsen and Ingvarsen, 2004). The administration method seems to be of major importance for the metabolic response of PG within the cow, as typically no metabolic responses have been observed when mixing PG into a TMR (Chibisa et al.,

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2008; Lomander et al., 2012); whereas responses have been observed when allocating PG as an oral drench or in separately fed concentrate (Pickett et al., 2003; Lien et al., 2010). This suggests that the metabolic effects of PG is established by a fast ruminal uptake of the PG dose that causes a prompt increase in plasma concentrations of PG and ruminally formed PG metabolites (Christensen et al., 1997; Kristensen and Raun, 2007). Results from periparturient cows dosed with PG show an increase in the glucogenic status during the allocation period, concurrent with a decrease in the plasma concentrations of BHB and NEFA (e.g., Formigoni et al., 1996; Butler et al., 2006; Lien et al., 2010). The reduction in plasma NEFA concentrations suggests a decreased adipose tissue mobilization. Despite the numerous PG studies in dairy cows, only a few studies have measured changes in fat mobilization. With PG allocation in TMR to periparturient dairy cows, Chibisa et al. (2008) did not observe effects of PG on body composition and blood metabolites.

Studying the abundance of proteins by proteome analysis might be useful to elucidate cellular mechanisms balancing adipose tissue mobilization and fat accretion. Proteome studies on subcutaneous adipose tissue of beef cattle have been related to fat accretion (e.g., Zhao et al., 2010; Romao et al., 2014), demonstrating differences in expression of proteins in animals with different subcutaneous fat thickness. Most recently, Zachut (2015) identified 143 adipose tissue proteins of multiparous dairy cows that were differentially expressed between d 17 prepartum and d 4 postpartum, and 3 of them were related to the process of lipolysis. Thus, proteome analysis seems to be suitable approach to study the influence of PG allocation adipose tissue protein abundance.

Carry-over effects on adipose tissue mobilization and metabolism have been indicated with allocation of PG in the first 2 or 3 d after parturition (Stokes and Goff, 2001; Pickett et al., 2003). Yet, PG is often allocated for several weeks postpartum in dairy livestock with automatic milking systems, and investigations of the carry-over effects on metabolism in cows allocated with PG for a longer period are lacking.

The hypothesis of the present experiment was that a daily intraruminal allocation of PG to over-conditioned postpartum cows would decrease mobilization of adipose tissue, thereby reducing the risk of ketosis and hepatic lipidosis, and that the decrease in mobilization would be reflected by altered protein abundance in adipose tissues. The objectives were to investigate both quantitative (whole-body pools of fat and protein, and net mammary nutrient uptake) and qualitative (plasma concentrations of metabolites and hormones, liver content of lipids, and protein abundance in subcu-

taneous adipose tissue) effects of PG on adipose tissue mobilization, and to delineate the metabolic effect of prophylactic use of PG in cows at high risk of lipid-related metabolic diseases.

MATERIALS AND METHODS

All procedures involving animals were evaluated and approved by the Danish Animal Experiments Inspectorate and complied with the Danish laws concerning animal experimentation and care of experimental animals.

Animals, Diets, and Experimental Design

Ten ruminally cannulated and arterially catheterized over-conditioned Danish Holstein cows were used in a complete randomized design with repeated measurements. The experiment was subdivided into 3 periods: (1) prepartum, last 4 wk before expected calving, (2) treatment, wk 1 to 4 postpartum, and (3) follow-up, wk 5 to 8 postpartum (Figure 1A). Measurement of body composition and mammary gland nutrient uptake were conducted prepartum (-16 ± 3 d; mean $\pm$ SD), 4, 15, and 29 DIM. Cows were randomly assigned to ruminal infusion of either 500 g of tap water (**CON**; $n = 5$; 4 entering second and 1 entering third lactation), or 500 g of PG (**PPG**; Propylenglycol pcow aroma, Brenntag Nordic A/S, Hellerup, Denmark; $n = 5$; 4 entering second and 1 entering third lactation). Treatments were initiated at the day of calving (designated as 1 DIM) and were provided once a day during morning feeding (0800 h) until 29 DIM. Treatments were allocated using a ruminal cannula infusion device ending in the area between atrium and the ventral rumen sack at the sight of the cranial pillar, and regulated by a timer-controlled peristaltic pump (infusion rate: 3,000 g/h; Type 115/G42 with L-channels; Ole Dich Instrumentmakers ApS, Hvidovre, Denmark). The ruminal infusion device was made by drilling a hole in the cannula stopper in which a 24-cm long semifirm tube (internal diameter 14 mm; outer diameter: 24 mm) was attached. Within the tube, the infusion line (Silicone tube, 60 shore A, internal diameter: 6.0 mm; outer diameter: 9.0 mm, Ole Dich Instrumentmakers ApS) was secured. In case of calving between 0800 and 1700 h, a dose of water or PG was allocated manually just after calving. From 30 to 32 DIM, cows were gradually ceased from treatments (250 and 125 g of PG on d 30 and 31–32, respectively). Cows were subsequently followed until 57 DIM and slaughtered 17 to 19 wk postpartum.

The 10 over-conditioned cows used were selected from a group of 16 cows conditioned in the last 14 wk of the preceding lactation following the conditioning

From dry off, cows were housed in tiestalls bedded with rubber mats and wood shavings. For parturition cows were moved to an individual, straw-bedded maternity pen. All cows were offered the same prepartum or postpartum TMR diets (Table 1) and fed 3 times a day (0800, 1600, and 2400 h). Orts were registered and removed once a day before the morning feeding. The diets were allocated ad libitum (10% orts), except from the first 24 h postpartum (3×10.7 kg of lactation

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Table 1. Ingredients and nutrient composition of the prepartum and postpartum total mixed diets (g/kg of DM, unless otherwise noted)

Item	Prepartum	Postpartum
Ingredient		
Corn silage	455	350
Grass-clover silage	178	200
Barley, rolled		185
Barley straw	122	
Wheat, ground	119	
Soybean meal, 54% CP	99	85
Rapeseed meal		85
Sugar beet pulp, dried		50
Vegetable fat ¹	10	15
Sodium bicarbonate		12.5
Mineral/vitamin premix	12 ²	7.5 ³
Sodium chloride	5	
Limestone		5
Titanium(IV) oxide		2.5
Suplex D-alfa E-50000		1.5
Magnesium sulfate		1
Nutrient		
DM (g/kg)	398	430
Ash	59	70
CP	138	165
NDF	422	343
Starch	202	238
MP ⁴	75	98
MP _{Lys} (% of MP)	6.80	6.74
MP _{Met} (% of MP)	2.21	2.22
NE _L (MJ/kg of DM)	6.55	6.41

¹Palm oil FA distillate.

²Komix Gold D-alfa Bio org. Selen (Vitfoss A/S, Gråsten, Denmark) containing per kg: Ca, 20 g; P, 40 g; Mg, 192 g; Na, 36 g; S, 83 g; vitamin A, 1,091 kIU; vitamin D₃, 182 kIU; vitamin E, 18,182 mg; biotin, 91 mg; Mn, 5,455 mg; Cu, 1,455 mg; Co, 35 mg; Zn, 7,270 mg; I, 227 mg; Se, 25 mg.

³VM2 (Vitfoss A/S) containing per kg: Ca, 160 g; P, 50 g; Mg, 65g; Na, 90g; S, 2g; vitamin A, 600 kIU; vitamin D₃, 190 kIU; vitamin E, 4,000 mg; Mn, 4,000 mg; Cu, 1,500 mg; Co, 25 mg; Zn, 4,500 mg; I, 225 mg; Se, 50 mg.

⁴Calculated using the NorFor system (Volden, 2011).

diet). The cows were milked 3 times a day at 0515, 1315, and 2115 h.

Data Collection

Feed intake was recorded daily. Diets were sampled daily for DM determination, and the dried samples were pooled within diet and week for later chemical analyses. At every milking, milk yield was recorded using continuous flow sampling (TruTest HI, Tru-Test Scandinavia, Præstø, Denmark). Two days per week, milk was subsampled at each milking from the continuous flow sample into tubes precoated with bronopol (Eurofins Steins Laboratorium A/S, Vejen, Denmark), and refrigerated (~4°C) until analyzed.

During the 3 periods (prepartum, treatment, and follow-up), weekly blood samples were obtained between 1000 and 1100 h from the coccygeal vein by venipuncture into K₃EDTA and Na-heparin vacutainers (Vacu-

ette, Greiner Bio-One GmbH, Kremsmünster, Austria). Additionally, daily blood samples were obtained from the arterial catheter from -7 until 7 DIM. Two times the catheter volume was discarded before blood was obtained with new 20-mL syringes and stabilized in K₃EDTA and Na-heparin vacutainers (Vacuette, Greiner Bio-One GmbH). Samples were kept on ice until centrifugation (2,000 × *g* for 20 min at 4°C) and harvested plasma was stored at -20°C until analysis.

At 0900 h, on days of quantitative measurement of body composition and mammary nutrient uptakes (Figure 1B), cows were given an intravenous (vena subcutanea abdominis) injection of 453.6 g 10% deuterium oxide (D₂O; 99.9%; Sigma-Aldrich Co., St. Louis, MO) and saline solution. The D₂O injection was followed by an intraruminal dose of 442.4 g of CrEDTA [60.6 mmol/L of chromium (III) chloride hexahydrate 99.5%, Alfa Aesar GmbH & Co, Karlsruhe, Germany; EDTA, J. T. Baker Chemicals, Deventer, the Netherlands; Binnerts et al., 1968]. Thirteen sets of arterial blood and ventral rumen fluid samples were obtained at -1.5, -0.5, 0.25, 0.5, 0.75, 1.0, 1.5, 2.5, 3.5, 4.5, 5.5, 24, and 36 h (exact times recorded) relative to D₂O injection. Eight hourly mammary venous blood samples were obtained beginning -1.5 h relative to D₂O injection. Blood samples were collected using the same procedure as described above. Additionally, arterial and venous blood was sampled with isotonic heparinized 2-mL gas syringes (PICO50, Radiometer A/S, Copenhagen, Denmark). Ventral ruminal fluid was obtained by a 50-mL syringe attached to a suction strainer (#RT rumen fluid sampler tube, Bar Diamond Inc.) placed into the ventral rumen through the cannula. Arterial and venous blood samples were kept on ice and plasma was harvested after centrifugation (3,000 × *g* for 20 min at 4°C). Samples of rumen fluid and plasma were stored at -20°C until analysis. Liver biopsies were obtained after the 5.5-h sample following the procedure described by Bjerre-Harpøth et al. (2014), snap frozen in liquid nitrogen, and stored at -80°C until analysis. Subcutaneous adipose tissue biopsies were further taken from the incision for liver biopsies. The incision was prolonged and a piece of adipose tissue was positioned with a forceps, cut off with a scissor, snap frozen in liquid nitrogen, and stored at -80°C until analysis. Cows were weighed after obtaining biopsies. At slaughter, omasal and gastrointestinal (GI) contents were collected, weighed, and representative samples were obtained.

Analytical Procedures

Dry matter content in diets and digesta (omasum and GI tract) were determined by forced-air oven drying at 60°C for 48 h. The pooled diet samples were

analyzed for ash, CP, NDF, and starch as described by Kristensen et al. (2007). Concentrations of fat, CP, and lactose monohydrate in milk were analyzed using a CombiFoss 4000 (Foss Electric A/S, Hillerød, Denmark).

The weekly and daily plasma samples were analyzed for glucose, NEFA, BHB, and L-lactate as described by Bjerre-Harpøth et al. (2014). Insulin and glucagon concentrations were determined in all daily and the postpartum weekly samples using an AutoDELFIA Insulin kit (B080–101, PerkinElmer Inc., Waltham, MA; Løvendahl and Purup, 2002) and a glucagon RIA kit (GL-32K, Millipore, St. Charles, MO), respectively. The interassay coefficient of variation for insulin was 3.2%, and for glucagon the intra- and interassay coefficients of variation were 6.6 and 1.5%, respectively.

Arterial plasma samples from –0.5 h and 0.5 until 5.5 h relative to injections were analyzed for D₂O by isotope ratio MS as described by Storm et al. (2011), and ruminal samples from –0.5, 2.5 until 5.5, 24, and 36 h relative to injections were analyzed for CrEDTA by atom absorption spectrometry as described by Williams et al. (1962). The hourly sets of arterial and venous blood samples were analyzed for hematocrit, O₂, and CO₂, and the hourly sets of plasma samples were analyzed for glucose, L-lactate, BHB, and AA as described by Larsen et al. (2014a). Arterial plasma samples obtained 1 h after D₂O injection were analyzed for leptin concentrations using a Bovine Leptin ELISA kit (MBS743525, MyBioSource, San Diego, CA) according to standard procedures. Intraassay coefficient of variation was 16.3%.

Liver biopsies were extracted and analyzed for concentration of glucose, glycogen, triglyceride (**TAG**), and phospholipid as described by Bjerre-Harpøth et al. (2012). Adipose tissue biopsies were extracted for total protein in 200 μ L of 8 M urea, 50 mM Tris, 2% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (**CHAPS**), 40 mM dithiothreitol (**DTT**), and 0.5% immobilized pH gradient (**IPG**) buffer (GE Healthcare, Buckinghamshire, UK) as described by Schäff et al. (2012). A sample of 500 μ g of protein was added to 320 μ L of rehydration buffer (8 M urea, 2% CHAPS, 0.8% IPG buffer, and 18 mM DTT) and subsequently loaded to an 18-cm IPG strip (pH 3–10; GE Healthcare). Isoelectric focusing at 50 V for 12 h was followed by 500 V for 1 h, 1,000 V for 1 h, and 8,000 V for 4 h and 20 min using an Ettan IPGphor3 device (GE Healthcare). The IPG strips were equilibrated first with a buffer containing 50 mM Tris (pH 8.8), 30% glycerol, 6 M urea, 2% SDS, and 1% DTT, and subsequently with the same buffer except DTT was replaced with 2.5% iodoacetamide, each for 15 min. The IPG were transferred to 12.5% SDS-PAGE gels (20 $\times$ 20 $\times$

0.1 cm). Proteins were stained with colloidal Rotiblu (Roth, Karlsruhe, Germany) and scanned to quantify the abundance of individual protein spots. Spots which significantly differed between by $P < 0.05$ were identified on a 5800 MALDI TOF/TOF (matrix-assisted laser desorption/ionization tandem time-of-flight) Analyzer (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA) and a subsequent database search against NCBItr (http://www.ncbi.nlm.nih.gov/), as described earlier (Schäff et al., 2012).

Calculations and Statistical Procedures

Equations used for calculating body composition are displayed in Table 2. The calculation of body composition originates from the ability to determine the amount of empty body water and was calculated as the dilution space of D₂O (total body water) corrected for water content in forestomach and GI tract, fetal fluid, plasma, and milk yield at afternoon (1315 h) milking. Water content in the reticulo-rumen was determined by dilution of CrEDTA. Water content in omasum and GI tract was determined from slaughter data. Equilibration of D₂O and CrEDTA was defined as a one-compartment model, assuming mass action dilution. For one cow, omasal and GI content of water and DM was estimated from weight adjusted averages of the other cows, as the cow had to be euthanized 8 wk postpartum due to lameness.

Milk CP content was calculated using 6.38 as the nitrogen conversion factor. Mammary plasma flow was calculated described by Lapierre et al. (2012) using Phe + Tyr as internal markers and correcting for 5% NPN and 3.37% blood-borne proteins in milk CP. Mammary whole blood flow was calculated by relating plasma flow to hematocrit. The net mammary flux of nutrients was calculated as the arterial-venous concentration difference multiplied by the appropriate mammary plasma or blood flow. Milk secretion of Phe and Tyr was calculated using an average AA composition of milk true protein (Lapierre et al., 2012). The mammary uptake-to-output ratio in milk lactose of glucose was calculated by dividing mammary net flux by secretion in milk lactose assuming use of 2 glucose molecules for synthesis of one lactose molecule.

Data were subjected to ANOVA using the MIXED procedure in SAS (version 9.3, release TS1M0, SAS Institute Inc., Cary, NC). Data for DMI, milk yields, milk composition, and weekly and daily plasma were analyzed using a model including the fixed effects of treatment (**Trt**), period (**Per**; prepartum, treatment, and follow-up), time, and all interactions. Cow was considered as a random effect and time within cow and period was considered as a repeated measurement, al-

Table 2. Equations for calculation of body composition

Body composition parameter	Equation	No.
Empty body water (EBW), kg	Total body water (TBW; kg) – [water content in forestomach + gastrointestinal tract (kg)] – fetal fluid (kg) – milk (kg) at 1315 h milking – plasma (kg)	[1]
Empty body protein (EBP), kg	Prepartum: EBW/3.50 ¹ ; 4 DIM: EBW/3.36 ² ; 15 DIM: EBW/3.47 ² ; 29 DIM: EBW/3.39 ²	[2]
Empty body ash (EBA), kg	Prepartum: EBW/12.8 ¹ ; 4 DIM: EBW/12.7 ² ; 15 DIM: EBW/13.4 ² ; 29 DIM: EBW/12.1 ²	[3]
Empty body lipid (EBL), kg	BW (kg) – TBW (kg) – EBP (kg) – EBA (kg) – [forestomach + gastrointestinal DM (kg)] – fetal DM (kg)	[4]
Preceding equations		
TBW was calculated as the D ₂ O dilution space, ³ kg	D ₂ O infused/(D ₂ O concentration at time 0 – D ₂ O concentration of plasma background)	[5]
Water content in forestomach + gastrointestinal-tract, kg	Reticulo-rumen water (calculated from CrEDTA dilution of the reticulo-rumen; kg) + omasum water (slaughter data; kg) + gastric and intestine water (slaughter data; kg)	[6]
Fetal fluid, ⁴ kg	–28.885 + 0.182 × gestation day	[7]
Plasma volume, ⁵ kg	Prepartum: 60 mL/kg of BW; 4 DIM 40 mL/kg of BW; 15 and 29 DIM: 50 mL/kg of BW	[8]
Forestomach + gastrointestinal DM, kg	Reticulo-rumen DM ⁶ (kg) + omasum DM (slaughter data; kg) + gastric and intestine DM (slaughter data; kg)	[9]
Reticulo-rumen DM, kg	[Reticulo-rumen water (kg) / (100 – DM % in reticulo-rumen)] × DM % in reticulo-rumen	[10]
Fetal DM, ¹ kg	Fetus (kg) × 0.241	[11]
Fetus, ⁴ kg	0.00138 × e ^{(0.06801 – 0.00011 × gestation day) × gestation day}	[12]

¹Andrew et al. (1995).²Gibb et al. (1992).³D₂O concentration at time 0 was estimated as the intercept assuming mass action dilution of D₂O into the total body water pool. D₂O concentration of plasma background equals the concentration at first sample before the D₂O infusion. Based on the equality between medial rumen fluid and plasma for D₂O, the equilibration time for D₂O was set to 2.5 h. Data used in the first order kinetic model were D₂O plasma concentration between 2.5 and 5.5 h after the D₂O infusion.⁴House and Bell (1993).⁵Larsen et al. (2014b).⁶A. C. Storm (unpublished data). Dry matter content in reticulo-rumen: 127.5 g/kg in the dry period and 147.5 g/kg in the early lactation period.

lowing the covariance to vary by period and thereby accounting for eventual heterogeneity among periods. Within each period, time was defined as d 1 to 28 for DMI and milk yields, sample 1 to 8 for milk composition, wk 1 to 4 for weekly plasma samples, and d 1 to 7 for daily plasma samples (Figure 1A). Data for body composition, liver and adipose biopsies, BW, BCS, and mammary gland nutrient uptake postpartum were analyzed using a mixed model with fixed effects of Trt, DIM, and the interaction where DIM within cow was considered as a repeated measurement. The prepartum observations were subsequently included to test the prepartum to 4 DIM difference × treatment interaction ($P_{\text{Transition}} \times \text{Trt}$) using Fisher's LSD test. Treatment means within period or DIM were separated using protected Fisher's LSD test.

Covariance structures used were autoregressive order 1 or heterogeneous autoregressive order 1 depending on Akaike's information criteria. Denominator degrees of freedom were calculated using the Kenward-Roger method. Least significant means are presented due to missing observations: one cow with CON was excluded

from the experiment due to severe ketosis and diarrhea at 5 DIM. Significance was declared at $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$.

RESULTS

No calving problems were observed and only few illnesses occurred in the 8-wk postpartum period (Table 3). One cow with CON suffered from both mastitis and lameness, and one cow with PPG suffered from both metritis and mastitis.

Feed Intake and Milk Production

The DMI was not affected by treatment in the 2 periods after parturition, but was greater for CON in the prepartum period compared with PPG ($P_{\text{Trt} \times \text{Per}} < 0.01$; Table 4). Milk yield was not affected by treatment ($P_{\text{Trt}} = 0.59$) and averaged 44.9 ± 2.3 and 43.1 ± 2.1 kg/d ($\pm$ SE) with CON and PPG, respectively, during the entire postpartum period. However, milk yield increased as lactation progressed ($P_{\text{Per}} < 0.01$) with

Table 3. Body weight and BCS of cows at dry off, calf birth weights (mean $\pm$ SD), and treated illness during the experiment

Item	Treatment ¹	
	CON	PPG
No. of animals	4	5
BW at dry off (kg)	685 $\pm$ 72	678 $\pm$ 44
BCS at dry off ² (1–5 scale)	3.9 $\pm$ 0.4	3.8 $\pm$ 0.3
Calf birth weights (kg)	43.6 $\pm$ 3.3	45.5 $\pm$ 3.8
Treated illnesses ³ (n)		
Calving problems	0	0
Metritis	0	1
Mastitis	3	2
Lameness	1	0

¹Treatments were ruminal infusion of 500 g of tap water (CON) or 500 g of propylene glycol (PPG) at each morning feeding from 1 to 29 DIM.

²Ferguson et al. (1994).

³Definitions: calving problems = help provided by barn staff or veterinarian; metritis = foul smell and discharge from vagina, enlarged uterus, and fever; mastitis = change in normal milk appearance verified by a California Mastitis Test (Bovivet, Kruuse A/S, Langeskov, Denmark), and fever; lameness = difficulty when standing up, discomfort when standing, swelling around joints, and fever.

both treatments. The milk fat content showed different profiles for PPG compared with CON during the treatment and follow-up periods ($P_{\text{Trt} \times \text{Per} \times \text{Time}} = 0.04$). Milk protein content was greater for CON compared with PPG in the first week postpartum, after which milk protein content did not differ between treatments ($P_{\text{Trt} \times \text{Per}} = 0.03$).

Metabolic Status

The weekly and daily plasma concentrations of measured metabolites and hormones did not differ between treatments in the prepartum period (Table 4). Postpartum, plasma glucose concentrations decreased more for CON in the treatment period compared with PPG; however, the difference between treatments disappeared during the follow-up period, as the plasma glucose concentration decreased with PPG after cease of PG allocation ($P_{\text{Trt} \times \text{Per}} < 0.01$; Table 4; Figure 2). The concentration of plasma BHB showed an inverse pattern of the plasma glucose concentrations. Compared with the prepartum period, the plasma BHB con-

Table 4. Dry matter intake, milk production, and plasma concentration of metabolites and hormones¹

Item	Prepartum		Postpartum treatment		Postpartum follow-up			P-value ²			
	CON	PPG	CON	PPG	CON	PPG	SEM	Trt	Per	Trt $\times$ Per	Trt $\times$ Per $\times$ Time
DMI (kg/d)	12.8	10.6	19.0	17.7	22.1	22.9	1.2	0.58	<0.01	<0.01	0.81
Milk yield (kg/d)											
Milk	—	—	42.5	40.3	47.4	45.9	2.3	0.59	<0.01	0.42	0.94
Fat	—	—	1.98	1.62	1.98	1.87	0.16	0.31	0.03	0.03	0.19
Protein	—	—	1.50	1.34	1.39	1.35	0.06	0.26	0.23	0.09	0.78
Lactose	—	—	1.97	1.91	2.22	2.19	0.11	0.77	<0.01	0.55	0.69
Milk composition (%)											
Fat	—	—	4.63	4.11	4.18	4.07	0.24	0.37	0.02	0.01	0.04
Protein	—	—	3.58	3.44	2.94	2.96	0.06	0.48	<0.01	0.03	0.88
Lactose	—	—	4.66	4.81	4.70	4.79	0.05	0.12	0.91	0.36	0.77
Plasma weekly											
Glucose (mM)	3.87	3.90	2.58	3.57*	2.91	3.11	0.09	<0.01	<0.01	<0.01	0.28
BHB ³ (mM)	0.53	0.55	2.07	0.47*	1.44	1.25	0.21	<0.01	<0.01	<0.01	0.05
NEFA (μ M)	59	106	351	277	231	250	41	0.95	<0.01	0.41	0.94
L-Lactate ³ (mM)	0.42	0.53	0.25	0.37†	0.30	0.34	0.06	0.25	0.05	0.15	0.95
Insulin (pM)	—	—	16	24	29	23	3.1	0.83	0.09	0.04	0.31
Glucagon (pg/mL)	—	—	102	85	119	101	12	0.35	<0.01	0.90	0.64
Plasma daily											
Glucose (mM)	3.94	3.93	2.93	3.55*	—	—	0.08	0.01	<0.01	<0.01	0.15
BHB ³ (mM)	0.73	0.64	1.12	0.57*	—	—	0.04	<0.01	0.02	<0.01	0.02
NEFA ³ (μ M)	143	187	499	321*	—	—	56	0.92	<0.01	<0.01	0.95
L-Lactate (mM)	0.29	0.29	0.31	0.44*	—	—	0.02	0.01	<0.01	0.02	0.28
Insulin ³ (pM)	61	51	12	17	—	—	7.8	0.66	<0.01	0.14	0.86
Glucagon (pg/mL)	58	59	76	63	—	—	7.9	0.54	<0.01	0.02	0.02

¹Treatments (Trt) were ruminal infusion of 500 g of tap water (CON) or 500 g of propylene glycol (PPG) at each morning feeding in the first 4 wk after calving. The prepartum period (Per) represent wk -4 to -1, postpartum treatment period wk +1 to +4, and postpartum follow-up period wk +5 to +8.

²Fixed effects of treatment (Trt), period (Per), and time where time represent d 1–28 for DMI and milk yields, sample 1–8 for milk composition, wk 1–4 for weekly plasma variables, and d 1–7 for daily plasma variables (see Figure 1A).

³P-values are from ln-transformed data.

* $P \leq 0.05$, † $P \leq 0.10$; symbols signify difference between LS means within period and rows.

centration increased for CON in the treatment period, whereas it was unchanged with PPG. The difference between treatments disappeared during the follow-up period, as the plasma BHB concentration increased with PPG after cease of PG allocation ($P_{\text{Trt} \times \text{Per} \times \text{Time}} = 0.05$). Plasma NEFA concentrations were lower in the prepartum period compared with the 2 postpartum periods ($P_{\text{Per}} < 0.01$), but were not affected by treatment ($P_{\text{Trt}} = 0.95$). No difference in plasma L-lactate concentration was noted between treatments ($P_{\text{Trt}} = 0.25$). Concentrations of glucose, BHB, NEFA, and lactate in daily plasma samples reflected largely the patterns observed in weekly samples; however, in the first week postpartum a greater increase in plasma NEFA concentration was observed for CON compared with PPG ($P_{\text{Trt} \times \text{Per}} < 0.01$; Table 4 and Figure 2). The plasma L-lactate concentration was greater for PPG compared with CON first week after parturition ($P_{\text{Trt} \times \text{Per}} = 0.02$).

The daily plasma concentration of insulin decreased for both treatments from the last week prepartum to the first week postpartum ($P_{\text{Per}} < 0.01$; Figure 3). From the treatment period until the follow-up period, the plasma insulin concentration increased with CON but did not increase with PPG ($P_{\text{Trt} \times \text{Per}} = 0.04$). The daily plasma concentration of glucagon did not differ between treatments in the last week prepartum. In the first week postpartum, plasma glucagon concentrations increased for CON, whereas they did not change for PPG ($P_{\text{Trt} \times \text{Per} \times \text{Time}} = 0.02$; Figure 3); hereafter, the concentrations did not differ between treatments ($P_{\text{Trt}} = 0.35$). In both treatments, the plasma glucagon increased postpartum ($P_{\text{Per}} < 0.01$).

Body Fat Status

For BW and BCS, the change from prepartum to 4 DIM was not affected by treatment ($P_{\text{Transition} \times \text{Trt}} \geq 0.17$; Table 5). The BW was unaffected for PPG after parturition, whereas BW decreased from 4 to 29 DIM for CON ($P_{\text{Trt} \times \text{DIM}} = 0.05$). The BCS was not affected by treatment ($P_{\text{Trt}} = 0.49$) and tended to decrease with progressing lactation for both treatments ($P_{\text{DIM}} = 0.06$). The whole-body lipid content was not affected by treatment ($P_{\text{Trt}} = 0.61$), but tended to decrease from 4 to 29 DIM with both treatments ($P_{\text{DIM}} = 0.06$). The body protein content was not affected by treatment ($P_{\text{Trt}} = 0.78$), but was greater at 4 DIM compared with 15 and 29 DIM for both treatments ($P_{\text{DIM}} < 0.01$). The decrease in plasma leptin concentration from prepartum to 4 DIM was not affected by treatment ($P_{\text{Transition} \times \text{Trt}} = 0.82$). Postpartum, plasma leptin was not affected by treatment ($P_{\text{Trt}} = 0.48$) but increased from 4 to 29 DIM with both treatments ($P_{\text{DIM}} = 0.01$).

Liver Biopsies

The liver tissue content of glucose tended to decrease with both treatments as lactation progressed ($P_{\text{DIM}} = 0.08$; Table 5). The liver content of glycogen decreased less from prepartum to 4 DIM for PPG compared with CON ($P_{\text{Transition} \times \text{Trt}} < 0.01$). Postpartum, the liver glycogen content was higher for PPG compared with CON ($P_{\text{Trt}} = 0.01$) and increased from 4 to 29 DIM with both treatments ($P_{\text{DIM}} < 0.01$). From prepartum to 4 DIM, the liver phospholipid content decreased with PPG but did not change with CON ($P_{\text{Transition} \times \text{Trt}} = 0.05$). Postpartum, the liver content of phospholipids were not affected by treatment or DIM. The liver content of TAG tended to be affected by treatment and DIM ($P_{\text{Trt} \times \text{DIM}} = 0.10$). However the large standard error for this variable showed large variation between cows at the different days of measurements.

Adipose Tissue Biopsies

Out of 807 adipose tissue proteins detected on the two-dimensional gel, spots where the abundance significantly differed by $P < 0.05$ ($n = 52$) were selected. From these, 16 different annotated proteins were identified by mass spectrometry and database search (Table 6). Besides 8 extracellular proteins, also known to be part of blood, 8 cellular proteins are considered of depot-specific origin. Among these, the abundance of collagen, cytokeratin, annexin A2, UMP-CMP kinase, glutathione S-transferase, zinc finger protein 34, and acetyl-Coenzyme A acetyltransferase 2 (**ACAT2**) differed between PPG and CON groups ($P_{\text{Trt}} < 0.05$); however, significant changes attributable the PG effect in the postpartum period could not be observed ($P_{\text{Transition} \times \text{Trt}} \geq 0.24$). Moreover, the abundance of collagen, annexin A5, and ACAT2 changed with DIM ($P_{\text{DIM}} < 0.01$).

Mammary Gland Fluxes

Mammary plasma flow was not affected by treatment or DIM ($P_{\text{Trt}} = 0.85$; Table 5); however, when related to milk yield, mammary plasma flow decreased from 619 to 453 ± 47 L/h/kg milk ($\pm$ SE; $P_{\text{DIM}} < 0.01$) between 4 and 29 DIM, respectively. The net mammary uptake of BHB was greater for CON compared with PPG ($P_{\text{Trt}} = 0.05$), whereas net uptake of glucose and L-lactate was not affected by treatment ($P_{\text{Trt}} \geq 0.21$). Mammary net uptake of glucose, L-lactate, and BHB increased from 4 to 29 DIM ($P_{\text{DIM}} < 0.01$). The mammary uptake to output ratio of glucose equivalents was numerically greater for PPG compared with CON ($P_{\text{Trt}} = 0.14$).

Figure 2. Weekly and daily plasma concentrations of glucose, BHB, and NEFA in periparturient dairy cows ruminally infused with either 500 g of tap water (CON, ○; n = 4) or 500 g of propylene glycol (PPG, ●; n = 5) from 1 to 29 DIM. Each data point is the mean $\pm$ SE and dashed lines indicate separation of experimental periods.

Figure 3. Daily and weekly plasma concentrations of insulin and glucagon in periparturient dairy cows ruminally infused with either 500 g of tap water (CON, ○; $n = 4$) or 500 g of propylene glycol (PPG, ●; $n = 5$) from 1 to 29 DIM. Each data point is the mean $\pm$ SE and dashed lines indicate separation of experimental periods.

DISCUSSION

Traditionally, effects of PG treatment on subacute or acute ketosis have been explained by reduced adipose tissue mobilization leading to less presence of NEFA in the liver, leading to reduced formation of ketone bodies (Nielsen and Ingvarsen, 2004). However, the current experiment showed a distinctly decreased concentration of plasma BHB with PG allocation despite limited effects on mobilization of adipose tissue. Additionally, plasma concentrations of glucose were markedly greater with PG allocation, indication that glucogenic status played the largest role in regulating the ketogenesis.

The ad libitum feed allocation in the prepartum period resulted in greater DMI for CON compared with PPG. The ad libitum prepartum feed allocation of a

relatively high-energy density diet was chosen to ensure a high BCS at parturition and to avoid priming the liver for high postpartum metabolism of NEFA (Friggens et al., 2004; Bjerre-Harpøth et al., 2014). Plasma concentrations of glucose, BHB, and NEFA in the prepartum period did not differ between treatment groups indicating limited metabolic effect of the greater DMI with CON. Therefore, it was assumed that cows had not been primed for postpartum metabolism of NEFA during the prepartum period.

Effect on Body Fat Mobilization

The BCS, which gives a rough evaluation of adipose tissue depots, was not affected by the PG allocation, and the more refined D₂O technique for determination

Table 5. Body fat status, liver variables, mammary gland plasma flow, and net uptakes¹

Item	Prepartum		4 DIM			15 DIM			29 DIM			P-value ²			Trt × DIM	P-value ³ Transition × Trt
	CON	PPG	CON	PPG		CON	PPG		CON	PPG		SEM	Trt	DIM		
DMI (kg/d)	12.3	10.5	16.8	14.1		18.9	17.3		20.9	20.4		1.3	0.34	<0.01	0.63	0.62
Milk yield (kg/d)	—	—	37.1	33.4		43.4	42.3		46.4	45.8		2.5	0.56	<0.01	0.60	—
Body status																
BW (kg)	723	689	691	625		652	607		620	609		29	0.35	<0.01	0.05	0.17
BCS ⁴	3.8	3.8	3.8	3.5		3.3	3.3		3.3	3.1		0.2	0.49	0.06	0.42	0.31
Lipid pool (kg)	119	109	104	91		93	110		82	75		14	0.98	0.06	0.27	0.90
Protein pool (kg)	89	88	97	89		84	73		80	79		4	0.28	<0.01	0.07	0.05
Water pool/BW (%)	64.8	65.0	65.7	65.6		67.2	64.4		67.9	68.5		1.4	0.62	0.06	0.23	0.90
Lipid pool/BW (%)	16.2	15.7	14.6	14.6		14.3	18.0		13.0	12.3		1.8	0.61	0.06	0.25	0.79
Protein pool/BW (%)	12.3	12.8	14.1	14.2		12.9	12.2		12.8	13.0		0.4	0.78	<0.01	0.45	0.46
Leptin (ng/mL)	18.8	17.9	6.5	4.0		12.1	9.4		16.7	10.1		4.1	0.48	0.01	0.59	0.82
Liver																
Glucose (μmol/g)	25.9	29.5	17.8	19.0		13.3	11.5		15.9	16.2		2.3	0.97	0.08	0.82	0.69
Glycogen (μmol/g)	231	189	63	116†		64	123*		102	153*		12	0.01	<0.01	0.65	<0.01
Phospholipid (μmol/g)	22.2	22.8	20.8	16.4†		18.7	17.1		17.7	18.8		1.1	0.16	0.89	0.28	0.05
TAG ⁵ (μmol/g)	5.7	6.6	24	96		125	64		68	105		33	0.56	0.31	0.10	0.16
Mammary gland																
Mammary plasma flow (L/h)	—	—	941	873		885	955		861	906		95	0.85	0.91	0.73	—
Hematocrit (%)	—	—	31.2	31.8		28.1	28.5		26.9	27.0		0.9	0.77	<0.01	0.88	—
Net mammary plasma flux																
BHB (mmol/h)	—	—	209	129		367	235*		332	264		34	0.05	<0.01	0.15	—
Glucose (mmol/h)	—	—	502	530		605	721†		697	759		43	0.21	<0.01	0.24	—
Uptake to output ratio of glucose	—	—	1.32	1.52		1.28	1.51		1.37	1.44		0.08	0.14	0.89	0.18	—
L-Lactate (mmol/h)	—	—	−2.4	8.0		38	27		31	42		12	0.79	<0.01	0.31	—
Net mammary blood flux	—	—	1,920	1,799		1,998	2,114		2,162	2,140		182	0.96	0.22	0.68	—
Oxygen (mmol/h)	—	—	−2,885	−2,604		−2,609	−2,877		−2,905	−2,816		284	0.90	0.90	0.54	—
Carbon dioxide (mmol/h)	—	—														—

¹Treatments (Trt) were ruminal infusion of 500 g of tap water (CON) or 500 g of propylene glycol (PPG) at each morning feeding from 1 to 29 DIM.²Fixed effects when prepartum observations are excluded from data set.³Fisher's LSD test for treatment effect on difference between prepartum and 4 DIM.⁴The BCS recorded closest to the date of DIM was used in the analyses.⁵P-values are from ln-transformed data.* $P \leq 0.05$, † $P \leq 0.10$; symbols signify difference between LS means within DIM and rows.

Table 6. Relative abundance of identified proteins differentially expressed with time or between groups¹

Protein ²	Prepartum			4 DIM			15 DIM			29 DIM			P-value ³			P-value ⁴	
	CON	PPG	CON	CON	PPG	CON	CON	PPG	CON	PPG	SEM	Trt	DIM	Trt	Transition × Trt		
Extracellular																	
Apolipoprotein A-I	1.251	1.829	1.393	2.079	2.146	2.802	2.531	0.128	0.25	0.05	0.35	0.32					
Apolipoprotein A-IV	0.176	0.182	0.222	0.296	0.452	0.383	0.346	0.020	0.92	0.01	0.72	0.68					
FGG protein	0.030	0.039	0.070	0.062	0.044	0.046	0.037	0.003	0.55	<0.01	0.07	0.79					
Fibrinogen, β chain	0.544	0.536	0.579	0.405	0.643	0.459	1.063	0.041	0.01	0.69	0.12	0.25					
Alpha-2-macroglobulin	0.024	0.017	0.018	0.023	0.021	0.006	0.031	0.002	0.72	<0.01	0.72	0.67					
Serum albumin	0.001	0.007	0.003	0.012	0.005	0.012	0.004	0.001	0.05	0.52	0.77	0.98					
Hemoglobin, subunit α	0.006	0.014	0.006	0.028	0.028	0.040	0.004	0.018	0.05	0.09	0.31	0.79					
Hemoglobin, subunit β	0.015	0.049	0.030	0.053	0.016	0.037	0.018	0.050	0.02	0.10	0.94	0.99					
Cellular																	
Collagen α-1(I) chain	0.026	0.006	0.013	0.007	0.020	0.008	0.040	0.011	<0.01	<0.01	0.02	0.35					
Keratin, type II	0.008	0.050	0.033	0.026	0.016	0.047	0.000	0.004	0.04	0.15	0.14	0.67					
Annexin A2	0.045	0.014	0.035	0.027	0.036	0.025	0.056	0.023	0.01	0.88	0.12	0.75					
Annexin A5	0.042	0.038	0.023	0.011	0.014	0.016	0.035	0.074	0.50	0.05	0.03	0.29					
UMP-CMP kinase	0.005	0.002	0.003	0.004	0.018	0.003	0.005	0.003	0.01	0.48	0.16	0.24					
Glutathione S-transferase	0.036	0.013	0.016	0.018	0.041	0.005	0.018	0.013	0.03	0.68	0.25	0.43					
Zinc finger protein 34	0.015	0.012	0.020	0.006	0.016	0.006	0.012	0.006	0.02	0.44	0.31	0.34					
Acetyl-Coenzyme A acetyltransferase 2	0.012	0.002	0.007	0.003	0.014	0.006	0.031	0.007	0.01	<0.01	<0.01	0.49					

¹Treatments (Trt) were ruminal infusion of 500 g of tap water (CON) or 500 g of propylene glycol (PPG) at each morning feeding from 1 to 29 DIM.

²Eight different proteins were identified as uncharacterized proteins and are not listed in this table.

³Fixed effects when prepartum observations are excluded from data set.

⁴Fisher's LSD test for treatment effect on difference between prepartum and 4 DIM.

of whole body lipid content revealed corresponding results. The extent of postpartum body fat mobilization determined in the current experiment was similar to previous determinations when expressed in both absolute terms (Chilliard et al., 1984; Chibisa et al., 2008) and changes in relative body composition (Andrew et al., 1995). In the present experiment, the body lipid loss averaged ($\pm$ SE) 18.9 ± 8.7 kg from 4 to 29 DIM. Chilliard et al. (1984) observed that cows lost 15 to 40 kg adipose tissue from wk 1 to 8 postpartum, and Chibisa et al. (2008) observed the body lipid pool to decrease by 19 kg from -14 to 38 DIM. Andrew et al. (1995) observed the body lipid content to 15.4% at 7 d prepartum and to 8.5% at 63 DIM.

In PG studies with demonstrated differences in plasma NEFA concentrations in early lactation, PG had been allocated periparturient (Formigoni et al., 1996; Butler et al., 2006; Lien et al., 2010) and exclusively postpartum (Sauer et al., 1973; Pickett et al., 2003). Studer et al. (1993) showed that PG allocation exclusively prepartum tended to affect the postpartum plasma NEFA concentration. However, in the present experiment the concentration of plasma NEFA complied with the described limited PG influence on lipid metabolism. As a consequence, the plasma NEFA concentrations only differed between treatments in the first week after parturition.

The proteome analysis further supports the limited influence of postpartum PG allocation on adipose tissue mobilization. Although 7 adipose tissue-specific proteins were differentially expressed between PPG and CON animals, these differences were not evident when evaluated for the allocation period only. However, annexin A5, collagen, and ACAT2 differed with DIM. The expression changes with DIM might be attributed to changes in back fat thickness. Recent proteome studies reported that the expression of annexin 1 is closely linked to fat accretion, back fat thickness, and adiposity in bovine species (Zhao et al., 2010; Romao et al., 2014; Zachut, 2015). Proteins of the annexin family act as phospholipase inhibitors, regulators of exocytosis, and linkage of membrane-associated complexes to the cytoskeleton. In annexin A1-null mice, it was recently shown that annexin A1 alters the sensitivity of adipose tissue toward lipolytic stimuli, such as catecholamines and glucocorticoids, and that annexin A1 modulates lipolysis and adipose tissue mass accretion (Warne et al., 2006). In periparturient cows, the expression of annexin A1 in adipose tissue was also lowered postpartum as compared with prepartum (Zachut, 2015). Thus, the altered abundance of annexin A1 with increasing DIM indicates its potential involvement in the regulation of exocytosis, lipolysis, and fat accretion (Warne et al., 2006; Zhao et al., 2010; Romao et al., 2014) during

the periparturient period. Moreover, ACAT2, which is involved in β -oxidation, and thus in the degradation of long-chain acyl-CoA, was increasingly expressed during the progression of lactation in both groups. The resulting intensified degradation of long-chain acyl-CoA during early lactation would ultimately reduce acyl-CoA-mediated inhibition of triglyceride lipase and thus contribute to support lipolysis (Nagy et al., 2014). However, we did not observe alterations in the abundance of triglyceride lipase or any other lipase such as hormone-sensitive lipase, which is differentially phosphorylated in periparturient cows (Locher et al., 2011). The activity of hormone-sensitive lipase is likely determined by its phosphorylation status rather than by its mere abundance. However, periparturient changes in the abundance of hormone-sensitive lipase can only be observed in cows with insulin-resistant but not insulin-sensitive adipose tissue (Zachut, 2015). Nevertheless, our proteome results highlight new candidate proteins earlier known to be involved in the regulation of lipid degradation, exocytosis, and lipolysis to play a role in adipose tissue metabolism during the transition period of dairy cows.

The concentration of leptin was initially expected to be higher for PPG compared with CON due to expected lower mobilization of adipose tissue in PPG (Delavaud et al., 2002) and due to expected higher plasma insulin concentrations in PPG (Leury et al., 2003; Thorn et al., 2008). Still, neither of the previously mentioned body fat variables nor the plasma insulin concentrations showed differences between treatments. As a consequence, the concentration of leptin in plasma was not different between groups, but the observed periparturient pattern concurred with previous observations (Block et al., 2001).

Overall, the present quantitative observations of body composition in combination with the qualitative observations of plasma metabolites and hormones, as well as abundance of adipose tissue proteins, indicate that postpartum allocation of PG had limited effect on the extent of postpartum adipose tissue mobilization, despite large influences on glucose and BHB plasma concentrations. These results underline the strength of the genetic drive of lactating dairy cows to mobilize adipose tissue after parturition (Friggens et al., 2007; Roche et al., 2009).

Effect on Metabolism

Even though the mode of action of PG is not fully described, it is generally accepted that PG has a glucogenic effect (Grummer et al., 1994; Nielsen and Ingvarsen, 2004; Chung et al., 2009). The glucogenic status of the cow has an influence on the liver metabolism of

NEFA and, thereby, of the regulation of ketogenesis. Adequate glucose availability causes an increase in the concentration of malonyl-CoA in the liver. Malonyl-CoA suppresses the carnitine palmitoyl transferase I activity, which leads to a decrease transport of NEFA into the mitochondria (Heitmann et al., 1987; Herdt, 2000). Thus, the low glucose availability seen in postpartum dairy cows results in an increased amount of NEFA (in form of acetyl-CoA) within the mitochondria available for oxidation. The partitioning of formed acetyl-CoA between the Krebs cycle and ketogenesis is not fully understood; however, a relatively high NADH-to-NAD ratio within ruminant mitochondria may promote ketogenesis (Zammit, 1990).

The influence of the glucogenic status of the cows on liver metabolism was demonstrated in the present experiment, as cows with PPG had a significantly lower plasma BHB concentration compared with cows with CON. In addition, it seems as though the higher plasma glucose concentration for PPG had a parsimonious effect on the liver glycogen storage in the beginning of lactation compared with CON. Despite the metabolic changes caused by the differences in glucogenic status, liver TAG concentration was unaffected by PG. Even though the liver TAG concentrations in CON were in agreement with the general knowledge of liver TAG profiles in early lactation (Drackley et al., 2005), with the largest concentration of liver TAG at 15 DIM, the relatively high variation observed for this variable indicates that the TAG results should be interpreted with caution.

The effect of PG on plasma insulin concentration seems to be dependent on physiological stage of the cows, as no effect of treatment on plasma insulin concentration was seen in the current experiment, despite the difference in plasma glucose concentrations. Similar trends were seen in other studies with periparturient cows (Butler et al., 2006; Lien et al., 2010), whereas studies with cows in other stages of lactation have shown increased plasma insulin concentrations in response to PG allocation (Grummer et al. 1994; Christensen et al. 1997; Kristensen and Raun, 2007). These results showing different effects of PG on insulin depending on stage of lactation may suggest that insulin in the periparturient period largely plays a homeorhetic role, over time changing to homeostatic control.

Regardless of the state of the cow, PG studies generally suggest that the metabolic effects of PG is established when the PG dose undergoes a fast ruminal uptake that results in a prompt increase in the concentrations of PG and the ruminally formed PG metabolites within the blood (Christensen et al., 1997; Kristensen and Raun, 2007). In the present experiment, intraruminal allocation was chosen to ensure that no

PG is spilt during allocation, as may happen with oral drenching; yet, the allocation method ensured the PG dose was delivered in the ruminal water phase, thereby securing fast absorption of PG.

The large effect of postpartum PG allocation on circulating levels of glucose and BHB was reflected in mammary uptake and metabolism, as BHB uptake was greater with CON when BHB plasma concentrations were greater. Though mammary glucose uptake was unaffected by the greater plasma glucose concentration with PPG, the numerically greater uptake-to-output ratio of glucose equivalents indicate that the greater glucogenic status increased the intramammary utilization of glucose for energetic purposes.

In studies with prepartum (Studer et al., 1993) or 2 to 3 d postpartum allocation of PG (Stokes and Goff, 2001; Pickett et al., 2003), carry-over effects in plasma NEFA concentrations were observed; no carry-over effect on plasma concentrations of glucose and BHB were noted, however. In the current experiment, the cessation of PG allocation caused a rapid decrease in plasma glucose concentration coinciding with increasing plasma BHB, whereas the plasma concentration of glucose and BHB for PPG did not differ from CON from the second week of the follow-up period. Therefore, the present experiment suggests that longer postpartum allocation of PG does not induce a carry-over effect. Meaning PG allocation can be used to obtain a 2-step adaptation to lactation in cows with high risk of lipid-related metabolic disease (e.g., over-conditioned at parturition). This implies that the first step of adaptation occurs at parturition and the second step of adaptation can be imposed upon cows when the largest increments in milk yield and feed intake have occurred (e.g., around 3 wk postpartum). As seen with PPG, this will reduce the nadir and peak in plasma concentration of glucose and BHB, respectively; however, this needs further investigation.

CONCLUSIONS

The traditional explanation of the effect of PG, related to decreased mobilization of adipose tissue, was not clearly observed in the present experiment. The different variables to measure body fat and its mobilization (body composition, plasma NEFA, liver TAG, and adipose protein abundance) were concurrent and showed that PG had limited influence on mobilization from adipose tissue in the first few weeks after parturition. Thus, the risk of hepatic liposis in over-conditioned postpartum cows was likely even between treatments. Rather, the main effect of PG was related to glucose metabolism, increasing the glucogenic status in the PG-allocated cows. As a consequence, the con-

centration of plasma BHB was lowered with PPG and these cows had a decreased risk of developing ketosis. The experiment showed no carry-over effects of PG allocation in postpartum dairy cows identified to be at risk of lipid-related metabolic diseases, thus indicating a possibility to induce a 2-step adaption to lactation with PG; this needs further investigation, however.

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