Association of subclinical hypocalcemia dynamics with dry matter intake, milk yield, and blood minerals during the periparturient period

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ABSTRACT

Subclinical hypocalcemia (SCH) affects many high-producing dairy cows in the postpartum period. Recent work has shown that cows experiencing prolonged or delayed SCH are at increased risk for disease and produce less milk than cows experiencing a transient reduction in or normal concentrations of plasma Ca following parturition. Our objective was to determine the association between different postpartum SCH dynamics with pre- and postpartum dry matter intake (DMI), milk yield, and blood mineral concentrations. Data were retrospectively collected from multiparous Holstein cows (n = 78), and cows were classified into 1 of 4 SCH groups based on mean blood total Ca (tCa) concentrations at 1 and 4 d in milk (DIM): normocalcemic (NC; [tCa] >1.95 mmol/L at 1 DIM and >2.2 mmol/L at 4 DIM, n = 28); transient SCH (tSCH; [tCa] ≤1.95 mmol/L at 1 DIM and >2.2 mmol/L at 4 DIM, n = 27); delayed SCH (dSCH; [tCa] >1.95 mmol/L at 1 DIM and ≤2.2 mmol/L at 4 DIM, n = 6); and persistent SCH (pSCH; [tCa] ≤1.95 mmol at 1 DIM and ≤2.2 mmol/L at 4 DIM, n = 17). Linear mixed models were created to analyze the change in pre- and postpartum DMI, milk yield, and blood mineral concentrations over time as well as differences between SCH groups. Prepartum intake was similar between groups, but the NC and tSCH cows consumed more feed than the pSCH or dSCH cows during the first 3 wk of lactation. The tSCH cows produced more milk than the other 3 groups during the first 6 wk of lactation. Postpartum blood tCa and Mg were different between SCH groups and were highest in the NC cows and lowest in the pSCH cows. Our results suggest that the high level of DMI consumed by the NC and tSCH cows in the postpartum period supported an appropriate homeostatic response to the increased Ca demands of lactation, allowing for higher milk yield compared with their counterparts experiencing delayed or prolonged episodes of SCH.

Key words: subclinical hypocalcemia, dry matter intake, transition cow

INTRODUCTION

As the dairy cow transitions from late pregnancy to early lactation, she is faced with many metabolic challenges, one of which is a sudden increased demand for Ca. In a nonlactating state, a cow requires approximately 21 g of Ca/d (NRC, 2001) to maintain normal body functions; once lactating, a cow requires an additional 55 g of Ca/d to support colostrum and milk production (Goff et al., 2014). The sudden increase in Ca requirement is often not met by an equal increase in DMI in the immediate postpartum period, and appropriate maintenance of blood Ca falls heavily on metabolic homeostatic and homeorhetic regulation.

Following parturition, many cows are unable to adapt efficiently to the sudden increase in demand for Ca, resulting in blood Ca concentrations falling below normal physiological levels and cows succumbing to hypocalcemia. Over the last several decades, due to advancements in nutritional and management strategies, the incidence of clinical hypocalcemia has substantially declined, affecting only 5% of dairy cows (Goff, 2008). However, approximately 50% of early-lactation cows still experience subclinical hypocalcemia (**SCH**; Reinhardt et al., 2011), a decrease in blood Ca unaccompanied by signs of disease. The incidence of SCH is associated with increased risk for other negative health events such as metritis, hyperketonemia, displaced abomasum, and culling (Chapinal et al., 2012; Martinez et al., 2012) as well as decreased fertility and milk production (Ribeiro et al., 2016; McArt and Neves, 2020).

It is commonly thought that blood Ca concentrations reach a nadir 12 to 24 h after calving (Goff, 2008); however, work by Neves et al. (2018b) found that diagnosis of SCH based on a single blood sample collected within 12 h of calving is not a strong indicator of increased risk

for subsequent disease development. This observation was further supported by the findings of McArt and Neves (2020), who saw a positive association between the persistency of SCH beyond the first day in milk and an increased risk for subsequent disease development and decreased milk production. Furthermore, Caixeta et al. (2017) found that cows experiencing SCH for the first 3 DIM were at increased risk for disease and had reduced reproductive success compared with cows that were normocalcemic or hypocalcemic for only 1 d. These findings suggest that a transient drop in blood Ca following calving may be part of normal adaptation to the high demands of lactation, whereas persistent or delayed decreases in blood Ca may represent a larger metabolic disruption, leading to increased risk for disease and decreased productivity.

It is well known that voluntary DMI decreases in the immediate pre- and postpartum periods (Drackley, 1999), and work by Martinez et al. (2014) demonstrated that the induction of SCH resulted in a subsequent reduction in DMI compared with normocalcemic cows. Martinez et al. (2014) also observed a decrease in rumen contractions in cows with induced SCH, negatively affecting rumination and rate of passage. The decrease in digestive capacity observed in cows with SCH may explain the decrease in DMI; however, the relationship between DMI and the dynamics of SCH have not been investigated.

Thus, our objectives were to determine the associations between different SCH dynamics with pre- and postpartum DMI as well as milk yield and postpartum plasma mineral concentrations. We hypothesized that cows experiencing delayed or persistent SCH would have decreased DMI during the pre- and postpartum periods and produce less milk than their normocalcemic and transiently SCH counterparts.

MATERIALS AND METHODS

All animal protocols were approved by the Cornell University Institutional Animal Care and Use Committee (protocol no. 2012-0117, 2015-0038, and 2016-0063). Our analysis was conducted as a retrospective cohort design and included multiparous Holstein cows (n = 78) during the periparturient period from 3 previously reported studies (Leno et al., 2017a,b; Kerwin et al., 2019; studies A, B, and C, respectively) and was written following the STROBE-VET guidelines for strengthening the reporting of observational studies in epidemiology-veterinary extension. Cows were enrolled in their respective studies 38 to 28 d before expected calving, and enrollment occurred from March to August 2015 (study A; n = 29), May to July 2015 (study B; n = 20), and March to May 2017 (study C; n = 29).

All animals were housed in individual tiestalls at the Cornell University Ruminant Center (Harford, NY).

Feeding Management, Sampling, and Analysis

Cows in all studies were fed rations formulated to meet or exceed the requirements of high-producing dairy cattle in their respective physiological states with slight dietary differences between studies. For all studies, the prepartum forages used were based on corn silage and wheat straw. During the prepartum period, cows in study A were fed a low-K ration with no anion supplementation. Cows in study B were fed the common source prepartum ration and 1 of 2 rations postpartum: either a ration formulated to meet NRC (2001) suggested Mg requirements (B-low, Mg = 0.30% of DM, n = 10) or exceed Mg requirements (B-high; Mg = 0.45% of DM, n = 10). Only cows not receiving a zeolite supplement in the prepartum period from study C were included in this analysis and were fed rations during the pre- and postpartum periods representative of those fed to high-producing dairy cattle in the Northeast and Upper Midwest United States. Ingredient composition of all prepartum and postpartum diets is shown in Table 1.

For all studies, cows were fed once daily at 0700 h for lactating cows and 0900 h for dry cows into individual feed bins. Orts were collected, weighed, and recorded daily, and daily intake was calculated by subtracting daily orts from feed offered the day prior. The amount of feed delivered was adjusted daily to target a refusal rate of 10% to allow for ad libitum intake. Rations were formulated using the Cornell Net Carbohydrate and Protein System (CNCPS, versions 6.1 and 6.5; Cornell University, Ithaca, NY).

Samples of TMR, forages, and grain mixes were collected weekly, and DM was determined by drying in a forced-air oven at 40°C for 96 h. Dried samples were composited at 4-wk intervals and composites were ground through a 2-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). Four-week composite samples of the TMR and a single composite of forages and grain mixes were analyzed by a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) using wet chemistry methods for DM at 135°C (method 930.15; AOAC International, 2000), CP (method 990.03; AOAC International, 2000), ADF (method 973.18; AOAC International, 2000), NDF (Van Soest et al., 1991), starch (Hall et al., 2015), sugar (Dubois et al., 1956), ether extract (method 2003.05; AOAC International, 2000), minerals (method 985.01; AOAC International, 2000), chloride (silver nitrate titration after extraction with 0.5% nitric acid using a Brinkman Metrohm 848 Titrino Plus, Brinkmann In-

Table 1. Composition (% of DM unless otherwise noted) of diets fed to multiparous Holstein cows (n = 78) during the periparturient period from 3 different experiments[1]

Item	Prepartum			Postpartum			
	A	B	C	A	B-low	B-high	C
Ingredient							
Corn silage	44.8	37.6	40.1	36.3	38.1	38.1	40.3
Wheat straw	28.0	23.2	33.3	7.9	6.2	6.2	4.6
Haylage	—	—	—	8.8	—	—	—
Alfalfa silage	—	—	—	—	—	—	10.7
Alfalfa hay	—	—	—	—	7.6	7.6	3.7
Concentrates[2]	27.2	39.2	26.6	47.0	48.1	48.1	40.7
Analyzed nutrient composition							
DM, %	45.3 ± 1.5	43.0 ± 1.8	46.9 ± 2.2	44.9 ± 2.2	43.0 ± 1	43.2 ± 1.1	45.5 ± 2.2
CP	13.0 ± 0.3	14.3 ± 0.4	13.6 ± 1.0	15.7 ± 0.2	14.9 ± 0.2	15.0 ± 0.3	16.4 ± 0.4
ADF	30.2 ± 0.6	28.1 ± 0.9	29.8 ± 1.1	20.6 ± 0.8	20.9 ± 0.2	21.5 ± 0.6	18.8 ± 1.3
NDF	44.3 ± 1.1	43.4 ± 0.8	46.4 ± 1.4	31.1 ± 0.9	32.5 ± 0.2	33.0 ± 0.3	30.6 ± 2.8
Starch	17.0 ± 0.4	15.8 ± 0.8	16.8 ± 1.7	26.0 ± 0.7	25.5 ± 0.9	25.3 ± 0.6	26.1 ± 1.6
NFC	33.6 ± 0.8	33.0 ± 1.2	32.9 ± 1.4	45.8 ± 1.1	45.2 ± 0.7	43.7 ± 0.3	43.3 ± 2.6
Fat	1.90 ± 0.50	2.17 ± 0.08	2.24 ± 0.13	3.0 ± 0.2	3.25 ± 0.3	3.1 ± 0.09	2.64 ± 0.24
Ca	1.54 ± 0.1	1.44 ± 0	0.68 ± 0.05	0.95 ± 0.03	1.21 ± 0.08	1.13 ± 0.06	1.0 ± 0.07
P	0.44 ± 0	0.35 ± 0	0.39 ± 0.03	0.41 ± 0.02	0.36 ± 0.01	0.34 ± 0.01	0.38 ± 0.01
Mg	0.47 ± 1.28	0.49 ± 0.02	0.42 ± 0.05	0.44 ± 0.02	0.35 ± 0.02	0.40 ± 0.01	0.51 ± 0.04
K	1.28 ± 0.06	1.08 ± 0.02	1.14 ± 0.06	1.37 ± 0.1	1.0 ± 0.03	0.98 ± 0.03	1.91 ± 0.11
S	0.20 ± 0	0.45 ± 0.01	0.25 ± 0	0.29 ± 0.01	0.32 ± 0.01	0.33 ± 0.02	0.46 ± 0.04
Na	0.13 ± 0.01	0.26 ± 0.01	0.14 ± 0.03	0.44 ± 0.02	0.42 ± 0.01	0.42 ± 0	0.82 ± 0.04
Cl	0.27 ± 0.02	0.79 ± 0.1	0.31 ± 0.03	0.40 ± 0.02	0.53 ± 0.01	0.53 ± 0.02	0.53 ± 0.03
DCAD, mEq/100 g of DM	18.3 ± 0.7	−11.2 ± 1.0	11.03 ± 2.06	25.0 ± 1.4	8.70 ± 1.1	7.70 ± 1.2	40.75 ± 2.5

[1]Study A: Leno et al. (2017a); study B: Leno et al. (2017b); study C: Kerwin et al. (2019). In study B, cows were fed postpartum rations with 2 different levels of Mg, low (B-low; Mg = 0.30% of DM) and high (B-high; Mg = 0.45% of DM).

[2]Refer to Leno et al. (2017a,b) and Kerwin et al. (2019) for complete descriptions of concentrates.

struments Inc., Westbury, NY), and sulfur (Leco Corp., 2008).

Animal Sampling and Analysis

Blood samples were collected from a coccygeal vessel at 0630 h following the morning milking and before feed delivery. Blood was obtained daily for the first 6 DIM and again at 10 DIM. Samples from studies A and B were collected using 10-mL sodium heparin evacuated tubes (Becton Dickinson, Franklin Lakes, NJ) and 20-G Vacutainer needles (Becton Dickinson) and immediately placed on ice. Plasma was harvested after centrifugation at $2,000 \times g$ for 20 min at 4°C. Samples from study C were collected using 10-mL evacuated tubes with no anticoagulant (Becton Dickinson) and 20-G Vacutainer needles (Becton Dickinson). Samples were left to clot for at least 30 min at room temperature before centrifugation at $2,000 \times g$ for 15 min at 20°C for the separation of serum. Plasma and serum samples were aliquoted into 1.7-mL microfuge tubes, snap frozen in liquid N_2, and stored at −20°C until analysis. Samples were analyzed for total Ca (**tCa**), Mg, and P using commercially available kits (tCa: Calcium Gen.2, Roche Diagnostics, Indianapolis, IN; Mg: Magnesium Gen.2, Roche Diagnostics; P: Inorganic Phosphorous, Roche Diagnostics) at the New York State Animal Health Diagnostic Center (Ithaca, NY) on an automated analyzer (Hitachi Modular P800, Roche Diagnostics). Coefficients of variation between and within assays were all less than 2%.

Lactating cows were milked thrice daily at 0600, 1400, and 2200 h. Daily milk weights were recorded, and daily milk yield was calculated as the sum of yields at all 3 milkings throughout each day. Average weekly yield was calculated for analysis by summing daily yields for the week and dividing by 7. Cows were weighed once weekly following the 1400 h milking on the same day of the week for the first 3 wk of lactation. For a complete description of the individual studies included in our analysis, refer to Leno et al. (2017a,b) and Kerwin et al. (2019).

Statistical Analysis

Descriptive statistics were generated using the FREQ procedure of SAS software (v. 9.4, SAS Institute Inc., Cary, NC). Before statistical analysis of DMI, milk yield, and blood minerals, cows were retrospectively classified into 1 of 4 SCH groups based on blood tCa concentrations at 1 and 4 DIM. The cut points for defining the SCH groups were based on the mean tCa concentrations at 1 and 4 DIM for all cows. The SCH groups were as follows: normocalcemic (**NC**; [tCa] >1.95 mmol/L at 1 DIM and >2.2 mmol/L at 4 DIM, n = 28); transient SCH (**tSCH**; [tCa] ≤1.95 mmol/L at 1 DIM and >2.2 mmol/L at 4 DIM, n = 27); delayed SCH (**dSCH**; [tCa] >1.95 mmol/L at 1 DIM and ≤2.2 mmol/L at 4 DIM, n = 6); and persistent SCH (**pSCH**; [tCa] ≤1.95 mmol at 1 DIM and ≤2.2 mmol/L at 4 DIM, n = 17).

A post hoc power calculation was conducted using G*Power (v. 3.1.9.7; Faul et al., 2007). Allowing for a 5% risk of conducting a type I error, our sample size yielded 14.7% power to detect a 1 kg/d difference in prepartum DMI between SCH groups given a standard deviation (**SD**) of 2.5 kg/d and 41% power to detect a 4.0 kg/d difference in postpartum DMI between SCH groups given an SD in DMI of 3.5 kg/d. Our sample size yielded 81% power to detect a 4 kg/d difference in milk yield between SCH groups given an SD in milk yield of 8 kg/d. Similarly, this sample size provided 90% power to detect a 0.2 mmol/L difference in tCa given an SD of 0.1 mmol/L difference between SCH groups, 91% power to detect a 0.05 mmol/L difference in Mg between SCH groups given an SD in Mg of 0.02 mmol/L, and 11.7% power to detect a 0.04 mmol/L difference in P between SCH groups given an SD in P of 0.06 mmol/L.

Dry matter intake, milk yield, and blood mineral concentrations over time were analyzed using generalized linear mixed models created through the MIXED procedure of SAS. Models were developed to assess changes over time and differences between SCH groups for each of the following: prepartum DMI, postpartum DMI, postpartum BW, milk yield, tCa, P, and Mg. Multiple measurements over time were analyzed using the REPEATED statement. Models included the random effect of cow and the fixed effects of SCH group, time, study, and their respective 2- and 3-way interactions. Variables and interactions were removed in a backward stepwise manner if $P > 0.10$. The 2-way interaction between time and study was removed in the final models analyzing prepartum DMI, postpartum DMI, postpartum BW, tCa, and Mg as $P > 0.10$. The effect of time, SCH group, and their respective 2-way interaction was kept in all models regardless of significance. To improve the normality of residuals, the $\log_{10}$ transformation of blood P was made. The final models for all outcomes of interest are as follows:

$$\text{Prepartum DMI} = \text{study} + \text{SCH group} + \text{DRTC} + \text{group} \times \text{DRTC} + \varepsilon,$$

$$\text{Postpartum DMI} = \text{study} + \text{SCH group} + \text{DRTC} + \text{group} \times \text{DRTC} + \varepsilon,$$

$$\text{Milk yield} = \text{study} + \text{SCH group} + \text{week of lactation}$$
$$+ \text{SCH group} \times \text{week of lactation} + \text{study}$$
$$\times \text{week of lactation} + \varepsilon,$$

$$\text{Ca} = \text{study} + \text{SCH group} + \text{DRTC} + \text{group}$$
$$\times \text{DRTC} + \varepsilon,$$

$$\text{Mg} = \text{study} + \text{SCH group} + \text{DRTC}$$
$$+ \text{group} \times \text{DRTC} + \varepsilon,$$

$$\text{Log}_{10}(\text{P}) = \text{study} + \text{SCH group} + \text{DRTC}$$
$$+ \text{SCH group} \times \text{DRTC} + \text{study} \times \text{DRTC} + \varepsilon, \text{ and}$$

$$\text{BW} = \text{study} + \text{SCH group} + \text{week of lactation}$$
$$+ \text{SCH group} \times \text{week of lactation} + \varepsilon,$$

where DRTC (days relative to calving) is the time variable and ε is the residual error of the model.

Means were considered different when $P \leq 0.05$ with marginal evidence for difference when $0.05 < P \leq 0.10$. When main effects and least squares means were different between groups, Tukey-Kramer studentized adjust-ments were used to account for multiple comparisons. Results are reported as least squares means and 95% confidence intervals. Figures were created using Microsoft Excel (2018; Microsoft Corp., Redmond, WA).

RESULTS

A total of 78 cows were included in our analysis entering median parity of 3; 36% (n = 28) of the cows were classified as NC, 34% (n = 27) were tSCH, 22% (n = 17) were pSCH, and 8% (n = 6) were dSCH. Study A contributed 29 cows, of which 9 were classified as NC, 11 were tSCH, 7 were pSCH, and 2 were dSCH. Study B contributed 20 cows, of which 12 were NC, 6 were tSCH, and 2 were dSCH. Study C contributed 29 cows, of which 7 were NC, 10 were tSCH, 10 were pSCH, and 2 were dSCH.

DMI, Milk Yield, and BW

Prepartum DMI decreased as calving approached ($P < 0.001$) but was not different by SCH group ($P = 0.6$). Intake in the postpartum period increased over time and was different by SCH group ($P < 0.001$), with pSCH cows consuming less than both the NC and
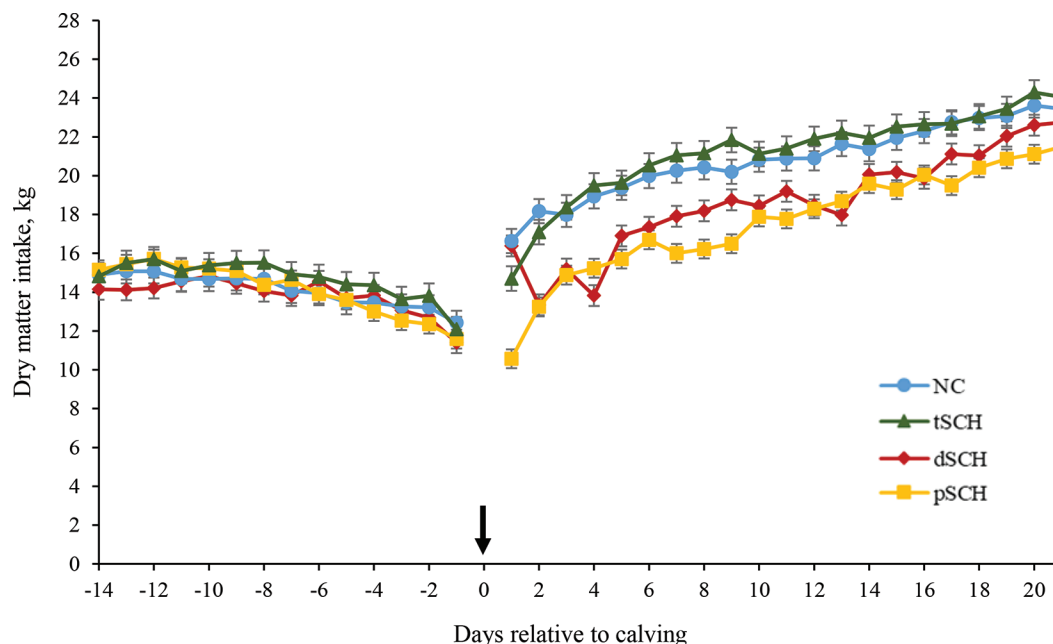
Figure 1. Dry matter intake for multiparous Holstein cows (n = 78) from 3 different experiments (Leno et al., 2017a,b; Kerwin et al., 2019) during the periparturient period. Cows were retrospectively classified into 1 of 4 subclinical hypocalcemia (SCH) groups based on mean plasma total Ca (tCa) concentration at 1 and 4 DIM: normocalcemic (NC; [tCa] >1.95 mmol/L at 1 DIM and >2.2 mmol/L at 4 DIM, n = 28, blue circles); transient SCH (tSCH; [tCa] ≤1.95 mmol/L at 1 DIM and >2.2 mmol/L at 4 DIM, n = 27, green triangles); delayed SCH (dSCH; [tCa] >1.95 mmol/L at 1 DIM and ≤2.2 mmol/L at 4 DIM, n = 6, red diamonds); and persistent SCH (pSCH; [tCa] ≤1.95 mmol/L at 1 DIM and ≤2.2 mmol/L at 4 DIM, n = 17, yellow squares). The final prepartum model included time ($P < 0.001$), SCH group ($P = 0.6$), and time × SCH group ($P = 0.8$). The final postpartum model included time ($P < 0.001$), SCH group ($P = 0.01$), and time × SCH group ($P = 0.001$). The black arrow indicates time of calving, and error bars represent SE.

tSCH cows ($P < 0.001$). Dry matter intake during the prepartum and postpartum periods by SCH group are summarized in Figure 1 and Table 2.

Milk yield increased as lactation progressed ($P < 0.001$). There was also an effect of SCH group on milk production ($P = 0.01$), with tSCH cows producing more milk than dSCH cows ($P = 0.02$) and pSCH cows ($P = 0.08$). Additionally, NC cows tended to produce more milk than dSCH cows ($P = 0.07$). Milk yield is summarized in Figure 2 and Table 2. Average BW were similar between SCH groups ($P = 0.13$) and between studies ($P = 0.31$).
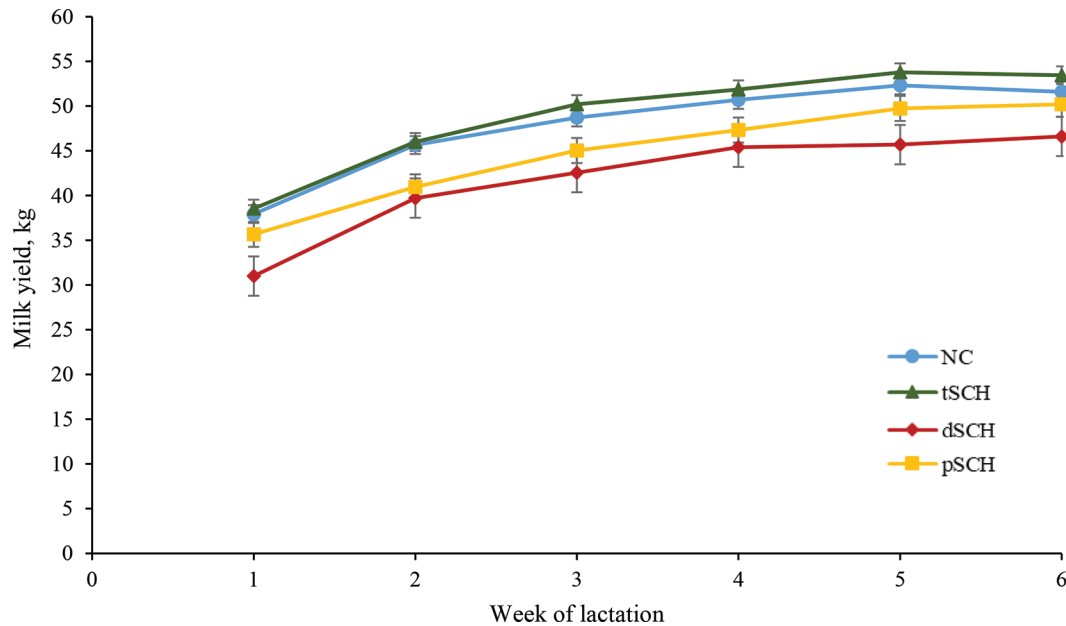
Blood Minerals

Postpartum tCa changed over time, with concentrations increasing daily from 1 to 4 DIM (1.92 and 2.25 ± 0.02 mmol/L, respectively). Additionally, tCa was different by SCH group ($P < 0.001$), with NC cows having higher concentrations of tCa than the other 3 groups ($P = 0.02$) and pSCH cows experiencing lower concentrations of tCa than all other groups ($P = 0.03$). Phosphorus concentrations were not affected by time or SCH group ($P = 0.2$ and 0.3, respectively); however, pSCH cows had numerically lower concentrations of P than the other 3 groups. Concentrations of Mg changed over time ($P < 0.001$) and were different by SCH group ($P = 0.03$). Normocalcemic cows had higher concentrations of Mg than pSCH cows ($P = 0.02$). Changes in blood minerals over time and by SCH group are shown in Figure 3 and Table 2.

DISCUSSION

The objective of our study was to determine the association between SCH dynamics and DMI in the pre- and postpartum periods. We also sought to analyze the effect of SCH dynamics on milk yield and blood mineral concentrations in the postpartum period.

In the 2 wk leading up to calving, DMI steadily decreased and all cows, regardless of SCH group, consumed similar amounts of feed. The few studies that have examined prepartum DMI as it relates to SCH development have reported varying results. Jawor et al. (2012) saw increased intake during the 3 wk leading up to calving in cows that were diagnosed as SCH within the first 24 h of calving. Similar to our results, Goff et al. (2020) saw comparable levels of intake between cows that would remain normocalcemic or develop SCH after calving. Goff et al. (2020) also saw little correlation between prepartum rumination activity and plasma Ca concentrations in the first 12 h after calving. However, our study was underpowered to detect a difference in prepartum intake between SCH groups. Perhaps with

Table 2. Dry matter intake, milk yield, BW, and blood mineral concentrations (means, with 95% CI in parentheses) for multiparous Holstein cows (n = 78) from 3 different experiments[1] during the periparturient period

Item	Group[2]				P-value			
	NC	tSCH	dSCH	pSCH	Time	Trial	Group	Time × group
Prepartum DMI,[3] kg	14.1 (13.4, 14.8)	14.7 (13.9, 15.4)	13.8 (12.3, 15.4)	14.1 (13.1, 15.1)	<0.001	0.002	0.6	0.8
Postpartum DMI,[4] kg	20.8[a] (20, 21.7)	21.2[a] (20.3, 22.1)	18.6[ab] (16.8, 20.5)	17.6[b] (16.4, 18.8)	<0.001	0.01	<0.001	0.001
Milk yield,[5] kg	47.8[ab] (45.8, 49.8)	49.0[a] (46.9, 51)	41.8[b] (37.5, 46.1)	44.8[ab] (42.1, 47.6)	<0.001	<0.001	0.01	0.6
BW,[6] kg	702.6 (680.2, 725.0)	734.5 (710.6, 758.4)	678.8 (628.1, 729.5)	720.6 (690.5, 750.8)	<0.001	0.25	0.13	0.16
Blood minerals, mmol/L								
tCa	2.36[a] (2.32, 2.40)	2.24[b] (2.20, 2.28)	2.16[bc] (2.07, 2.24)	2.03[c] (1.98, 2.09)	<0.001	0.01	<0.001	<0.001
P	1.43 (1.39, 1.46)	1.43 (1.39, 1.47)	1.43 (1.35, 1.52)	1.29 (1.24, 1.33)	0.32	0.03	0.19	0.1
Mg	0.73[a] (0.70, 0.76)	0.71[ab] (0.68, 0.73)	0.67[ab] (0.62, 0.73)	0.66[b] (0.62, 0.69)	<0.001	0.02	0.03	0.03

[a-c] Different superscripts represent differences ($P < 0.05$) in means separated by Tukey's studentized adjustments to account for multiple comparisons.

[1] Leno et al. (2017a,b) and Kerwin et al. (2019).

[2] Cows were retrospectively classified into 1 of 4 subclinical hypocalcemia (SCH) groups based on mean plasma total Ca (tCa) concentration at 1 and 4 DIM: normocalcemic (NC; [tCa] >1.95 mmol/L at 1 DIM and >2.2 mmol/L at 4 DIM, n = 28); transient SCH (tSCH; [tCa] ≤1.95 mmol/L at 1 DIM and >2.2 mmol/L at 4 DIM, n = 27); delayed SCH (dSCH; [tCa] >1.95 mmol/L at 1 DIM and ≤2.2 mmol/L at 4 DIM, n = 6); and persistent SCH (pSCH; [tCa] ≤1.95 mmol/L at 1 DIM and ≤2.2 mmol/L at 4 DIM, n = 17).

[3] Dry matter intake from −14 to −1 d relative to calving.

[4] Dry matter intake from 1 to 21 DIM.

[5] Average weekly milk yield for the first 6 wk of lactation.

[6] Weekly BW from 1 to 21 DIM.

Figure 2. Average milk yield of multiparous Holstein cows (n = 78) from 3 different experiments (Leno et al., 2017a,b; Kerwin et al., 2019) for the first 6 wk of lactation. Cows were classified into 1 of 4 subclinical hypocalcemia (SCH) groups based on mean blood total Ca (tCa) concentrations at 1 and 4 DIM: normocalcemic (NC; [total Ca (tCa)] >1.95 mmol/L at 1 DIM and >2.2 mmol/L at 4 DIM, n = 28, blue circles); transient SCH (tSCH; [tCa] ≤1.95 mmol/L at 1 DIM and >2.2 mmol/L at 4 DIM, n = 27, green triangles); delayed SCH (dSCH; [tCa] >1.95 mmol/L at 1 DIM and ≤2.2 mmol/L at 4 DIM, n = 6, red diamonds); and persistent SCH (pSCH; [tCa] ≤1.95 mmol/L at 1 DIM and ≤2.2 mmol/L at 4 DIM, n = 17, yellow squares). Time $P < 0.001$, SCH group $P = 0.01$, time × SCH group $P = 0.6$. Error bars represent SE.

an increase in sample size, we would see a difference similar to that of Jawor et al. (2012), with the tSCH cows consuming more feed in the prepartum period than the other SCH groups.

Of the 3 studies included in our analysis, 2 studies fed a positive DCAD ration during the prepartum period and 1 fed a negative DCAD ration prepartum. It is of note that cows retrospectively classified as pSCH were all fed rations with a positive DCAD in the prepartum period. Negative DCAD rations have been shown to improve blood Ca in the postpartum period and reduce the incidence of clinical hypocalcemia (Lean et al., 2006). Similarly, positive DCAD rations fed prepartum are associated with lower concentrations of tCa after calving (Santos et al., 2019). Taken together, it could be hypothesized that cows reaping the acidifying benefits of the negative DCAD ration were better primed for the metabolic challenges of lactation, thereby minimizing the duration of low blood Ca following parturition. This could potentially explain why pSCH cows came only from studies feeding positive DCAD rations prepartum; however, this observation warrants further exploration.

Following calving, we saw differences in DMI between SCH groups. At 1 DIM, intake was already lower for the pSCH cows than for the other 3 groups. At 2 DIM, intake began to increase for the NC and tSCH cows,

whereas dSCH cows experienced a drop in DMI, falling to levels similar to the pSCH cows. For the remainder of the study period, while DMI steadily rose for all groups, pSCH and dSCH cows continued to consume less than NC and tSCH cows. Our observations support previous work that saw an immediate reduction in DMI in cows induced with SCH (Hansen et al., 2003; Martinez et al., 2014). Similarly, our observations support the work of McArt and Neves (2020), concluding that although the tSCH cows experienced low plasma Ca at 1 DIM, they were adequately prepared for the demands of lactation as their DMI was high.

The role of Ca on smooth muscle contraction might explain the reduction in DMI following prolonged episodes of SCH. Upon the stimuli initiating muscle contraction, Ca channels in the smooth muscle fibers open, allowing Ca to enter the cytosol. Calcium then binds to calmodulin, a Ca-binding messenger protein, which, once activated, binds to myosin light chain kinase to initiate muscle contraction. Hypocalcemia reduces both the strength and speed of smooth muscle contraction (Webb, 2003) as action potentials in motor neurons are reduced when plasma Ca falls below physiologically normal levels. This is important because rumen motility is an involuntary aspect of rumination that involves contraction of smooth muscles. When blood ionized Ca falls below 1.3 mmol/L, contractions of ru-
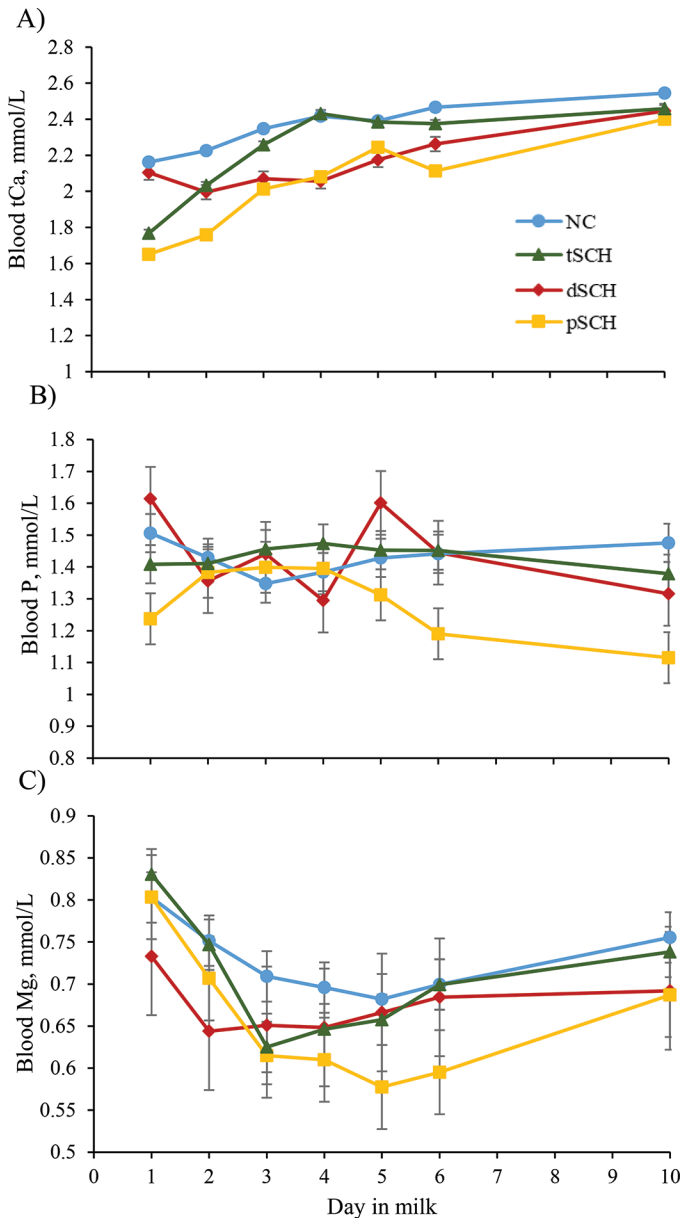
Figure 3. Blood mineral concentrations of multiparous Holstein cows (n = 78) from 3 different experiments (Leno et al., 2017a,b; Kerwin et al., 2019) during the first 10 DIM. Cows were classified into 1 of 4 subclinical hypocalcemia (SCH) groups based on mean blood total Ca (tCa) concentrations at 1 and 4 DIM: normocalcemic (NC; [total Ca (tCa)] >1.95 mmol/L at 1 DIM and >2.2 mmol/L at 4 DIM, n = 28, blue circles); transient SCH (tSCH; [tCa] ≤1.95 mmol/L at 1 DIM and >2.2 mmol/L at 4 DIM, n = 27, green triangles); delayed SCH (dSCH; [tCa] >1.95 mmol/L at 1 DIM and ≤2.2 mmol/L at 4 DIM, n = 6, red diamonds); and persistent SCH (pSCH; [tCa] ≤1.95 mmol/L at 1 DIM and ≤2.2 mmol/L at 4 DIM, n = 17, yellow squares). (A) Blood tCa: time $P < 0.001$, SCH group $P < 0.001$, time × SCH group $P < 0.001$. (B) Blood P: time $P = 0.3$, SCH group $P = 0.2$, time × SCH group $P = 0.2$. (C) Blood Mg: time $P < 0.001$, SCH group $P = 0.03$, time × SCH group $P = 0.03$. Error bars represent SE.

men smooth muscles cease (Jørgensen et al., 1998). A great deal of evidence supports the positive correlation between blood Ca concentrations and rumen motility and rumination. Cows with induced SCH observed an almost 2-fold reduction in the number of rumen contractions per minute compared with normocalcemic cows (Hansen et al., 2003; Martinez et al., 2014). Goff et al. (2020) also observed a strong positive correlation between rumination time during the first 2 d following parturition and plasma tCa concentrations analyzed immediately after calving. Interestingly, Goff et al. (2020) saw a weak correlation between plasma tCa at 1.5 DIM and rumination time at 2 DIM.

Additionally, when ruminal contractions cease, the absorption of Ca by rumen epithelium also decreases (Hyde et al., 2019). When Hyde et al. (2019) compared rumen absorptive capacity between cows with and without parturient paresis, they saw similar Ca absorptive capacity in the weeks leading up to calving between the 2 groups. At the onset of parturient paresis, rumen contractions ceased and ruminal Ca absorption significantly declined compared with the healthy animals. However, following treatment with intravenous calcium borogluconate, the ruminal absorptive capacity of the paretic cows was restored to levels similar to their healthy counterparts. Although cows in the present study did not experience parturient paresis, the lower level DMI and reduction in tCa witnessed in the pSCH and dSCH cows suggest that rumination and Ca absorption by ruminal epithelium was disrupted, perpetuating the disruption in Ca homeostasis in these cows.

The effect of blood Ca concentrations on rumination might also explain the decline in DMI we observed in dSCH cows at 2 DIM. At 1 DIM, the dSCH cows had tCa concentrations >1.95 mmol/L, comparable with the NC cows, and the 2 groups consumed similar amounts of feed. However, tCa and DMI in the dSCH cows began to decrease at 2 DIM, supporting previous work that saw a depression in intake following the reduction in plasma tCa (Hansen et al., 2003; Martinez et al., 2014). Similarly, the pSCH cows consumed the least amount of feed in the postpartum period and had continuously depressed concentrations of blood tCa. The results of our study and others suggest that concentrations of tCa experienced in the first several days after calving may have persistent effects on DMI and rumination. Furthermore, the sustained reduction in DMI consumed by the dSCH and pSCH cows suggests that these cows failed to properly adapt to the demands of lactation and that the delayed or persistently decreased concentrations of tCa may be associated with prolonged metabolic disruptions leading to a reduction in intake.

Milk yield was also affected by the dynamics of SCH. Milk production was similar between NC and tSCH cows but was higher than the dSCH and pSCH cows. When McArt and Neves (2020) retrospectively classified cows based on plasma Ca dynamics during the first 4 DIM using a classification scheme comparable with ours, they saw similar trends in milk production, with tSCH cows producing the most milk and dSCH cows producing the least amount of milk for the first 10 wk of lactation. Similar to our tSCH cows, Neves et al. (2018b) saw greater milk yield from cows with tCa ≤1.95 mmol/L at 1 DIM compared with cows with tCa >1.95 mmol/L. Alternatively, when Martinez et al. (2012) classified cows as SCH when plasma tCa concentrations were <2.14 mmol/L at least once in the first 3 DIM, they saw no difference in milk production between normocalcemic and SCH cows. The agreement between our study and McArt and Neves (2020) as well as the results of Neves et al. (2018b) and Martinez et al. (2012) suggest that accounting for the dynamics of blood Ca in the days following parturition more accurately captures the effect of blood Ca concentrations on milk production.

When Neves et al. (2018a) analyzed the association of plasma tCa concentrations at 1 and 4 DIM with milk production, they saw an association between depressed plasma Ca concentrations at 1 DIM and increased milk production for the first 15 wk of lactation, whereas lower concentrations of plasma Ca at 4 DIM were associated with decreased milk production. Although Neves et al. (2018a) did not explicitly classify cows as SCH based on the dynamics of plasma Ca, their sampling scheme further supports our observation that the tSCH cows produced more milk, supporting the idea that these cows were unconstrained to the metabolic demands of lactation and that a transient drop in blood Ca was part of the normal adaption to prepare for high milk production. Similarly, the lower yield achieved by the dSCH and pSCH cows as well as decreased DMI suggest that these animals experienced a disruption in the homeostatic response as they transitioned to lactation, leading to delayed or persistently reduced blood Ca and negatively affecting their production potential.

When a cow experiences a decrease in blood Ca, parathyroid hormone (**PTH**) secretion from the parathyroid gland is increased. Parathyroid hormone works to restore blood Ca concentrations by stimulating the release of Ca from bone, reducing Ca excretion through urine, and increasing Ca resorption in the intestines. The sensitivity of PTH to decreased blood Ca concentrations has been shown to be blunted by hypomagnesemia, as the activation of the PTH receptor requires Mg (Goff, 2000). The relationship between concentrations of Mg and Ca in blood is made evident by the

differences in Mg concentrations observed in our study between SCH groups, with NC cows having the greatest average Mg concentrations and pSCH cows having the lowest Mg concentrations. The sustained reduction in blood Mg evident in the pSCH cows could be affecting the actions of PTH required to increase blood Ca, partially explaining the persistently low concentrations of tCa in this group. The prolonged reduction in blood Mg of the pSCH cows could potentially be due to the lower DMI consumed by these cows compared with the NC and tSCH cows. The primary site of Mg absorption occurs in the rumen, and the maintenance of normomagnesia is almost entirely dependent on the influx of Mg from the diet (Goff, 2006).

Increased concentrations of Mg have been associated with increased PTH action, thus allowing for the maintenance of optimal blood Ca concentrations. For that reason, feeding prepartum rations high in dietary Mg has become a strategy to mitigate SCH (Lean et al., 2006). Increasing dietary concentrations of Mg has had variable effects on blood Mg concentrations, though, leading to increases in plasma Mg in some cases (Greene et al., 1983), but having no apparent affect in others (Jittakhot et al., 2004). We analyzed cows from 3 previously reported studies (Leno et al., 2017a,b; Kerwin et al., 2019) in which cows were all fed rations balanced to meet their energetic needs but formulated to address the objectives of the respective studies. Leno et al. (2017b) fed a common prepartum ration but had 2 postpartum rations, one meeting the NRC (2001) recommended level of dietary Mg at 0.30% of DM and a second exceeding recommended levels at 0.45% of DM. Although we did see an effect of study on blood Mg concentrations in our analysis, it is unlikely solely due to the 2 levels of Mg fed in study B because blood Mg was lower in the cows from study C and similar between cows in studies A and B. The effect could perhaps be an artifact of the differences in DMI and altered homeostatic and metabolic regulation of Ca and Mg metabolism.

We saw no difference in concentrations of blood P between SCH groups; however, due to our relatively small sample size and lack of power, the likelihood of committing a type II error may have limited our ability to detect a difference in P concentrations. We did see numerically lower concentrations of P in the pSCH cows at 1 and 10 DIM compared with the other groups. Goff et al. (2020) saw a positive relationship between plasma Ca and P concentrations. The relationship between plasma P and Ca might be explained by the increased salivary secretion and renal excretion of P in response to elevated PTH concentrations (Wright et al., 1984). In a normocalcemic state, P secreted in saliva can be recovered in the rumen; however, when Ca

concentrations are decreased, leading to reduced rumen motility, the rumen will sequester P, thereby further reducing blood P concentrations (Goff, 2000). Despite the lack of difference in blood P concentrations between SCH groups in our study, work by Rodney et al. (2018, 2019) suggests that the regulation of blood Ca and P is correlated through the metabolism of 1,25-dihydroxyvitamin D_3, which increases intestinal absorption of Ca. Increases in circulating P have been previously demonstrated to stimulate the production of fibroblast growth factor 23 by osteoclasts and osteoblasts, which inhibits 1α-hydroxylase, a key factor in the conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3 (Bikle, 2014).

The higher levels of intake that we observed in the NC and tSCH cows, paired with greater milk yield and elevated concentrations of tCa, Mg, and P, suggest that these cows were efficiently able to adapt to the metabolic challenges of lactation. Conversely, the dSCH and pSCH cows likely experienced metabolic disruption that delayed or perpetuated the reduction in tCa, negatively affecting milk yield and intake for weeks to come; however, the results of our study cannot fully explain this homeostatic disruption. Additionally, our analysis represents cows that were housed in individual tiestalls with ad libitum access to feed, where 58/79 cows in our study population were offered a positive DCAD ration in the prepartum period, an environment that may not be entirely representative of modern-day dairy management practices. Furthermore, our sample size was small, limiting the number of cows represented in each SCH group and restricting our power to detect differences in certain outcomes between SCH groups. Therefore, further studies are needed to explore the effects of SCH dynamics on PTH as this relationship may provide insight into the metabolic adaptation occurring within the respective SCH groups.

CONCLUSIONS

Our results demonstrate that SCH dynamics are associated with both DMI and milk yield. Cows that remained normocalcemic or experienced only a transient reduction in blood tCa at 1 DIM consumed more feed and produced more milk than cows that had persistent or delayed reductions in blood tCa. Blood mineral status was also affected by SCH dynamics, and cows experiencing prolonged episodes of SCH had reduced concentrations of blood Mg and P. The dichotomy of blood mineral status between the different SCH groups combined with differences in intake and milk production suggest that cows experiencing persistent or delayed SCH were faced with a homeostatic disruption and un-

able to efficiently adapt to the metabolic challenges of the transition to lactation.

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