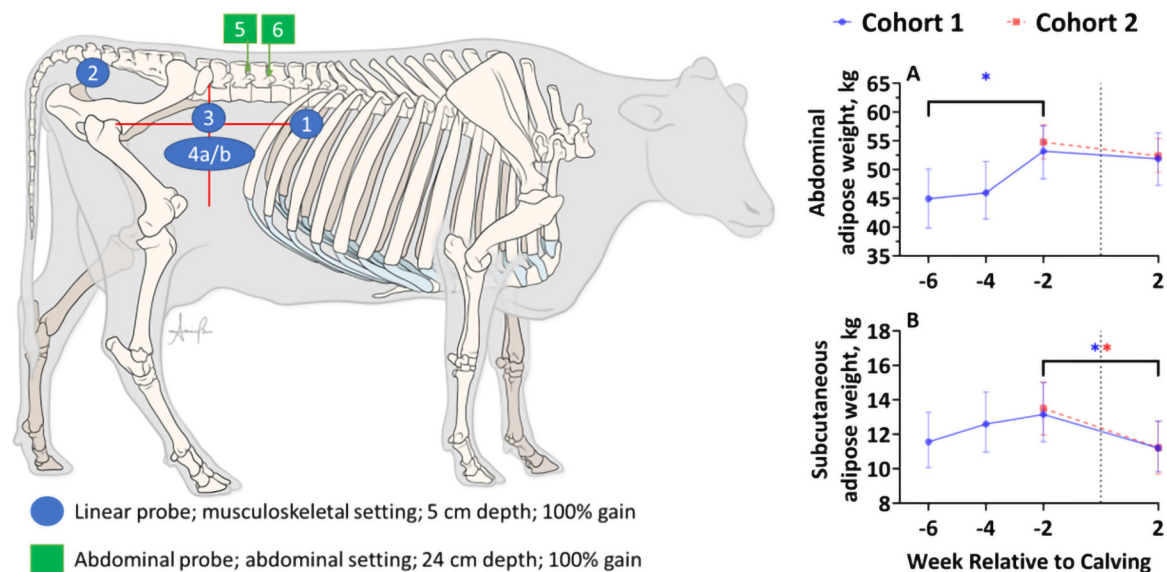


# The use of ultrasonography in the transition period to estimate adipose tissue depots and their association with risk of early postpartum hyperketonemia in Holstein dairy cattle

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## Graphical Abstract

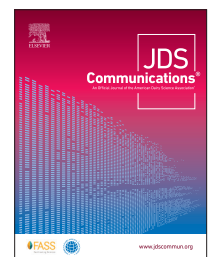


## Summary

Multiparous cows were examined via transcutaneous ultrasound to predict abdominal (A) and subcutaneous adipose tissue (AT; B) mass in 2 cohorts (cohort 1: n = 31; cohort 2: n = 28). Logistic regression and receiver operating characteristic curves were used to evaluate the association and predictive ability of adipose depots at –2 weeks relative to calving, the time of maximum estimated depot size, and elevated nonesterified fatty acid concentrations (NEFA  $\geq 720$   $\mu\text{Eq/L}$ ; NEFA<sub>H</sub>) or postpartum hyperketonemia ( $\beta$ -hydroxybutyrate  $\geq 1.2$  mmol/L; HYK) from 1 to 14 days in milk. Results show that abdominal and subcutaneous AT were not informative for the prediction of NEFA<sub>H</sub> (area under the curve [AUC]  $\leq 0.59$ ) or HYK (AUC  $\leq 0.56$ ), respectively.

## Highlights

- Peripartum change of adipose tissue can be monitored via ultrasonographic prediction.
- Abdominal adipose tissue increased from –6 to –2 weeks relative to calving.
- Subcutaneous adipose tissue decreased from –2 to 2 weeks relative to calving.
- Prepartum adipose tissue depots were noninformative for predicting NEFA<sub>H</sub> or HYK.



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# The use of ultrasonography in the transition period to estimate adipose tissue depots and their association with risk of early postpartum hyperketonemia in Holstein dairy cattle

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**Abstract:** An elevated prepartum BCS is a risk factor for postpartum hyperketonemia (HYK) and elevated nonesterified fatty acid (NEFA) concentrations in dairy cattle. The association between different adipose tissue depots, such as subcutaneous (SCAT) as well as visceral adipose tissue (VAT) depots, and HYK and elevated NEFA concentrations remains unknown. The objective of this 2-part study was to describe SCAT and VAT depots using ultrasonography during the transition period and to associate them with metabolic markers of negative energy balance in early postpartum dairy cows. Multiparous Holstein cows were enrolled in a prospective observational cohort study with 2 cohorts in subsequent study years (cohort 1:  $n = 31$ ; cohort 2:  $n = 28$ ). At  $-6$ ,  $-4$ ,  $-2$ , and  $2$  wk relative to calving (cohort 1) or  $-2$  and  $2$  wk relative to calving (cohort 2) BCS was determined and cows were examined via transcutaneous ultrasound at 6 locations for prediction of adipose tissue mass in 5 depots: (1) abdominal adipose tissue (AAT), (2) retroperitoneal adipose tissue (RPAT), (3) omental adipose tissue (OMAT), (4) mesenteric adipose tissue (MAT), and (5) subcutaneous adipose tissue (SCAT). Postpartum serum NEFA and BHB concentrations were determined twice weekly from 1 to 14 DIM. Cows were categorized as having HYK or high NEFA concentrations (NEFA<sub>H</sub>) if  $\geq 1$  sample resulted in a BHB  $\geq 1.2$  mmol/L or NEFA  $\geq 720$   $\mu$ Eq/L, respectively. Critical thresholds associated with HYK or NEFA<sub>H</sub> for each depot at  $-2$  wk relative to calving and the time of maximum estimated depot size were evaluated using logistic regression and a receiver operator characteristic analysis. Abdominal AT increased from  $-6$  to  $-2$  wk relative to calving in cohort 1 but did not differ from  $-2$  to  $2$  wk relative to calving. Subcutaneous AT did not change from  $-6$  to  $-2$  wk but decreased from  $-2$  to  $2$  wk relative to calving in cohort 1 and 2. Omental AT accurately (area under the curve [AUC] = 0.77) predicted HYK in cohort 1 but was noninformative for the prediction of HYK in cohort 2 (AUC = 0.49). Predicted AT depots were not informative for the prediction of NEFA<sub>H</sub> (AUC  $\leq 0.59$ ) in either cohort. Results from this study suggest that AT can be monitored during the transition period using the described technique; however, estimated prepartum AT depots at  $-2$  wk relative to expected calving were inaccurate in distinguishing between postpartum NEFA<sub>H</sub> and non-NEFA<sub>H</sub> or HYK and non-HYK.

Lipid mobilization is a homeorhetic response to negative energy balance during the transition period (Bauman and Currie, 1980). During periods of negative energy balance, an increased flux of nonesterified fatty acids (NEFA) to the liver can be completely oxidized for energy, partially oxidized to synthesize ketone bodies, or converted to triacylglycerols for hepatic storage or export as very-low-density lipoproteins. While ketone production is physiological as an alternative energy source, hyperketonemia (HYK) is a common metabolic condition during early lactation and is commonly defined by increased concentrations of blood BHB (Mann et al., 2019). Elevated postpartum concentrations of NEFA or ketones have been associated with a greater risk for postpartum diseases (Ospina et al., 2010b; Bach et al., 2019), reduced reproductive success (Ospina et al., 2010a; Rutherford et al., 2016), and decreased milk production (Ospina et al., 2010a).

Traditionally, BCS has been used as a method to predict risk of HYK or elevated NEFA concentrations as it has been shown that cattle with higher BCS during late gestation or at calving

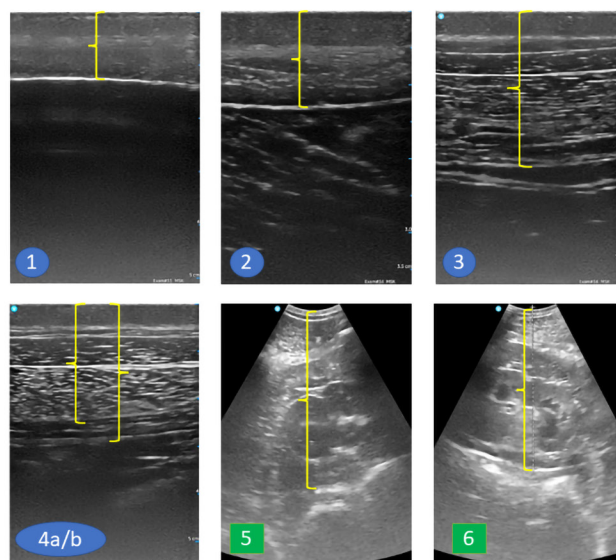
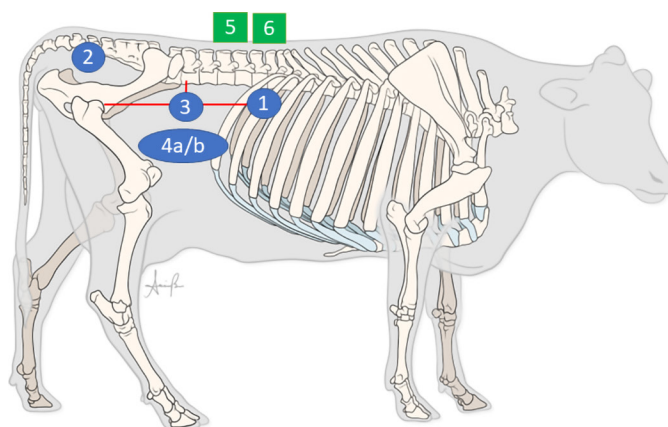
were at a greater risk for HYK (Gillund et al., 2001; Vanholder et al., 2015), elevated postpartum NEFA concentrations (Pires et al., 2013; Akbar et al., 2015), reduced DMI (Hayirli et al., 2002), and increased fat mobilization during early lactation (Pires et al., 2013). Although BCS has been a commonly accepted method to estimate subcutaneous adipose tissue (SCAT), variation has been reported in the accuracy and consistency of the system (Roche et al., 2009). Further, BCS might not be a reliable method to determine visceral adipose tissue (VAT; Mann, 2022); thus, research has been conducted to find measurements that allow for more accurate and precise determination of adipose tissue (AT) stores.

In a review by Schröder and Staufenbiel (2006), subcutaneous backfat thickness (BFT) determined by ultrasonography was shown to have higher accuracy in regard to predicting total body fat mass compared with BCS. It was estimated that 1 mm of subcutaneous BFT was equivalent to approximately 5 kg of total body fat (Schröder and Staufenbiel, 2006). Although BFT can be used to estimate total body AT, it does not describe the contribution of

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individual fat reserves in the cow, namely SCAT versus VAT. To extend the use of ultrasonography, a previous study by Raschka et al. (2016) used 16 ultrasound locations on the right side of the animal to measure tissue depth of the abdominal wall as well as accessible abdominal adipose tissue (AAT) around the left kidney to estimate different fat depots and SCAT. After measurements were taken, the 23 cows were euthanized and AT was dissected from the carcass, weighed, and used to create multivariable linear regression equations to estimate 5 adipose depots from the ultrasound measurements (Raschka et al., 2016). The authors determined that 7 ultrasound measurements taken from 6 different locations were needed for the prediction of SCAT, AAT, retroperitoneal adipose tissue (RPAT), omental adipose tissue (OMAT), and mesenteric adipose tissue (MAT) in test cows with an  $R^2$  of 0.84 to 0.96 (Raschka et al., 2016). This novel ultrasound technique to estimate VAT could reflect a noninvasive, economical, and simple tool for the prediction of HYK or elevated NEFA concentrations. As such, we investigated the association between prepartum VAT depots and metabolic indicators of negative energy balance in Holstein dairy cows. We hypothesized that the mass of VAT was associated with HYK and elevated NEFA concentrations. The objective of this study was to describe VAT using ultrasonography during the transition period and to associate VAT depots with the HYK status and NEFA concentrations of postpartum dairy cows.

A convenience sample of multiparous Holstein cows housed at the Cornell University College of Veterinary Medicine Teaching Dairy were enrolled in a prospective observational cohort study between June and September 2022 ( $n = 31$ ; cohort 1) and June and September 2023 ( $n = 28$ ; cohort 2). At  $-6$ ,  $-4$ ,  $-2$ , and  $2$  wk relative to calving (cohort 1) or  $-2$  and  $2$  wk relative to calving (cohort 2), BCS was determined using a 1- to 5-point scale with 0.25 point increments (Edmonson et al., 1989) and cows were scanned via ultrasound at 6 locations (1, 2, 3, 4a, 4b, 5, and 6; corresponding to Raschka et al., 2016, R12, BFT, AW1b, AW3b, AW3c, KD2c, and KD3b, respectively; Figure 1) by trained personnel to estimate AT depots according to the methods and equations described previously in detail (Raschka et al., 2016). In brief, all measurements were performed on the right side of the animal using a curved (2–5 MHz) and linear (3–12 MHz) array dual-probe hand-held ultrasound (Vscan Air CL, GE HealthCare). The skin at the location of each scan was brushed, hair was clipped to the size of the ultrasound probe, and 70% alcohol was applied as a coupling agent while avoiding pressure. Measurements were made from frozen images using the ultrasound calipers, repeated 3 times, and the average was used for analysis. Subsequent measurements were made in the same location where hair had been clipped. Location 1 (L1) measured subcutaneous fat on the twelfth rib in line with the greater trochanter. Location 2 (L2; BFT) was measured approximately 10 cm caudal of the tuber coxae and determined the distance between the profound fascia above the gluteus medius and the surface, according to the methods described in Staufienbiel (1992). Location 3 (L3) measured the distance from the skin to the muscle margin away from the transducer at the intercept of the last lumbar vertebra with the greater trochanter. Locations 4a and 4b were in the center of the paralumbar fossa and determined the distance from the skin to the muscle margin away from the probe (L4a) and from the skin to the peritoneum (L4b) in the same image, respectively. Location 5 (L5) determined the distance from the skin to the peritoneum, avoiding the kidney, at the lumbar intertransverse space cranial to the one where the caudal kidney pole was visible. Location 6 (L6) measured from the skin to the kidney margin away from the probe at the lumbar intertransverse space cranial to L5.



- Linear probe; musculoskeletal setting; 5 cm depth; 100% gain
- Abdominal probe; abdominal setting; 24 cm depth; 100% gain

**Figure 1.** Anatomic locations as well as ultrasonographic image examples for locations used for the prediction of adipose depots in peripartum Holstein cows. For explanation of landmarks and schematics explaining tissue details to correctly position the ultrasound, please see Raschka et al. (2016).

tertransverse space cranial to the one where the caudal kidney pole was visible. Location 6 (L6) measured from the skin to the kidney margin away from the probe at the lumbar intertransverse space cranial to L5.

The averages of the 3 ultrasonographic measurements from each location were used to estimate AT depots (kg) according to the regression equations previously described by Raschka et al. (2016; Tables 7 and 8):  $AAT = -39.5 + 1.02(L1) + 0.92(L3) + 0.25(L5)$ ;  $RPAT = -9.55 + 0.62(L1) + 0.06(L6)$ ;  $OMAT = -2.32 + 0.55(L2) + 0.37(L4a)$ ;  $MAT = -12.8 + 0.38(L3) + 1.73(L4a) - 1.45(L4b) + 0.07(L5)$ ; and  $SCAT = -6.66 + 0.72(L1) + 0.31(L4b)$ , where  $L =$  location.

Blood samples were taken from the coccygeal vessels into plain evacuated collection tubes twice weekly from 1 to 14 DIM. Serum was harvested by centrifugation at  $1,900 \times g$  at room temperature

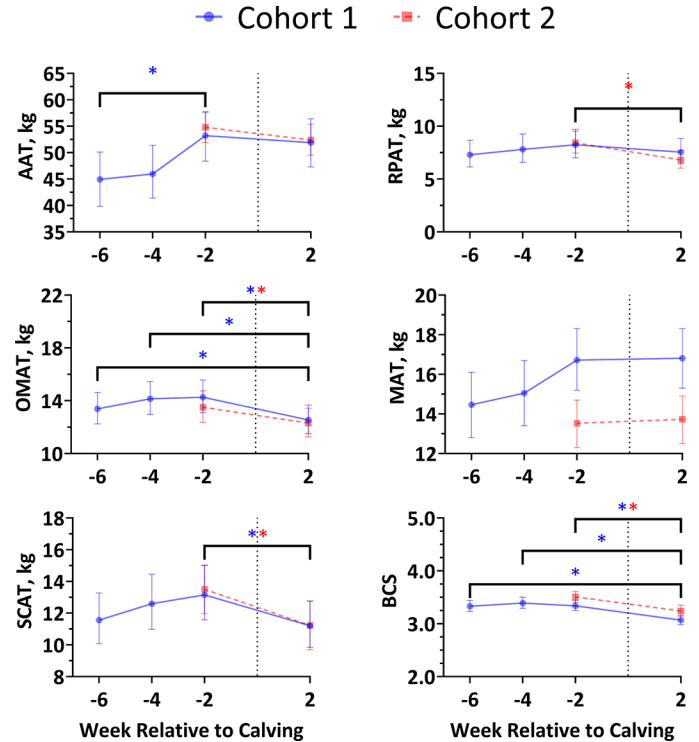


for 15 min and frozen at  $-20^{\circ}\text{C}$  until analysis. Serum concentrations of BHB and NEFA were determined using a Precision Xtra point-of-care device (Abbott) in samples warmed to  $37^{\circ}\text{C}$  (Leal Yepes et al., 2018), and by enzymatic colorimetric analysis (HR Series NEFA-HR (2), Wako Life Sciences), respectively, as previously described (Mann et al., 2016a).

A convenience sample size was used to describe VAT during the transition period and associate AT depots with the HYK status and NEFA concentrations of postpartum dairy cows. Cohort 1 was examined 3 times in the prepartum period to determine the time point at which estimated AT depots reached maximal size during the prepartum period. Cohort 2 was only examined at  $-2$  wk relative to expected calving based on data from cohort 1 showing the greatest AT depots at this time. The change in adipose depots over time was therefore analyzed separately for cohort 1 and 2 using repeated measures ANOVA with the effects of time point and parity group (2 vs.  $\geq 3$ ) in JMP v. 17.0 (SAS Institute Inc.). To visually meet the model assumptions of normality and homoscedasticity of the residuals, RPAT and OMAT were transformed using the natural logarithm. Tukey's post hoc test was used to adjust for multiple comparisons. Cows were categorized as having HYK or high circulating NEFA concentrations ( $\text{NEFA}_H$ ) if  $\geq 1$  sample resulted in a BHB  $\geq 1.2$  mmol/L (McArt et al., 2013) or NEFA  $\geq 720$   $\mu\text{Eq/L}$  (Ospina et al., 2010a), respectively. Each adipose depot at  $-2$  wk relative to expected calving was separately assessed in cohort 1 and 2 for the threshold associated with predicting HYK and  $\text{NEFA}_H$  using JMP v. 17.0. Receiver operating characteristic curves were generated from binary logistic regression models to identify the optimum threshold defined by the point with the highest combined sensitivity (Se) and specificity (Sp). The HYK or  $\text{NEFA}_H$  status were used as the dependent variables and the adipose depots at  $-2$  wk relative to calving were used as the independent variables. Sensitivity and specificity with 95% CI at the defined threshold were determined using MedCalc Statistical Software (MedCalc Software Ltd.). Area under the curve (AUC)  $0.70 \leq \text{AUC} < 0.90$  was considered accurate and  $\text{AUC} \geq 0.90$  was considered highly accurate (Swets, 1988). Sensitivity was defined as the proportion of animals with HYK or  $\text{NEFA}_H$  that were above the identified adipose depot cut-point. Specificity was defined as the proportion of animals without HYK or  $\text{NEFA}_H$  that were below the identified cut-point.

Mean (SD) coefficient of variation was 4.2% (3.6%), 3.0% (2.5%), 4.6% (4.4%), 4.2% (3.4%), 4.2% (3.0%), 3.3% (2.4%), and 3.0% (2.2%) for cohort 1 and 3.2% (2.0%), 2.8% (2.0%), 3.3% (2.6%), 3.5% (2.5%), 3.8% (3.3%), 2.5% (1.6%), and 2.5% (2.3%) for cohort 2 ultrasound locations L1, L2, L3, L4a, L4b, L5, and L6, respectively. The median (range) NEFA concentrations were 399 (103 to 1,146) and 592 (95 to 1,947)  $\mu\text{Eq/L}$  in cohort 1 and 2, respectively. Median (range) BHB concentrations in cohorts 1 and 2 were 0.8 (0.4 to 5.5) and 0.8 (0.2 to 3.9) mmol/L, respectively. The prevalence of cows classified as HYK and  $\text{NEFA}_H$  was 10 (32.3%) and 14 (45.2%) cows in cohort 1 and 17 (60.7%) and 19 (67.9%) cows in cohort 2, respectively.

The change in adipose depots and BCS over time is shown in Figure 2. Abdominal adipose tissue increased from  $-6$  to  $-2$  wk relative to calving ( $P = 0.03$ ). Retroperitoneal AT decreased from  $-2$  to 2 wk relative to calving in cohort 2 (8.4 [7.5–9.5] to 6.8 [6.0–7.7] kg;  $P < 0.01$ ) but did not differ in cohort 1 ( $P = 0.50$ ). Omental adipose tissue was lower at 2 wk compared with  $-6$ ,  $-4$ ,



**Figure 2.** Ultrasonographic predictions of abdominal (AAT), retroperitoneal (RPAT), omental (OMAT), mesenteric (MAT), and subcutaneous (SCAT) adipose weight as well as BCS from transition dairy cows (cohort 1:  $n = 31$ ; cohort 2:  $n = 28$ ). A blue asterisk denotes a difference in cohort 1 ( $P \leq 0.05$ ; Tukey's test); a red asterisk denotes a difference in cohort 2 ( $P \leq 0.05$ ). Error bars are 95% CI.

and  $-2$  wk relative to calving ( $P \leq 0.01$ ). Mesenteric adipose tissue mass did not differ by time in either cohort ( $P \geq 0.11$ ). Subcutaneous adipose mass decreased from  $-2$  to 2 wk relative to calving in both cohorts ( $P \leq 0.05$ ). Body condition score was lower at 2 wk compared with  $-6$ ,  $-4$ , and  $-2$  wk relative to calving ( $P \leq 0.01$ ).

Critical thresholds to predict HYK or  $\text{NEFA}_H$  for each adipose depot are shown in Table 1. In cohort 1,  $\text{OMAT} \geq 12.9$  kg at  $-2$  wk relative to calving was accurate ( $\text{AUC} = 0.77$ ;  $\text{Se} = 100.0$ ;  $\text{Sp} = 55.0$ ) for the prediction of HYK. However,  $\text{OMAT}$  was no longer informative for prediction of HYK in cohort 2 ( $\text{AUC} = 0.49$ ). Abdominal AT, RPAT, OMAT, MAT, and SCAT at  $-2$  wk relative to calving were not sufficiently accurate to distinguish between HYK and non-HYK, as well as  $\text{NEFA}_H$  and non- $\text{NEFA}_H$  cows, respectively ( $\text{AUC} \leq 0.59$ ).

Raschka et al. (2016) observed that AAT, RPAT, OMAT, and MAT increased from  $-42$  to 3 d relative to calving and decreased from 3 to 21 DIM, whereas SCAT did not change from  $-42$  to 3 d relative to calving and decreased from 3 to 21 DIM. In contrast, we did not observe statistically significant increases in RPAT, MAT, or OMAT from  $-6$  to  $-2$  wk relative to expected calving, but total abdominal AT increased from  $-6$  to  $-2$  wk relative to calving and SCAT decreased from  $-2$  to 2 wk relative to calving. Differences in DMI, milk production, and diet composition that affect energy balance as well as differences in the time points used might, in part, explain the differences between the current and the aforementioned

**Table 1.** Test characteristics for prediction of elevated fatty acid (NEFA<sub>H</sub>; NEFA  $\geq$  720  $\mu$ Eq/L) or BHB (HYK; BHB  $\geq$  1.2 mmol/L) concentrations in early-lactation dairy cows (1–14 DIM) with ultrasonographic estimated adipose tissue depots at –2 wk before expected calving

Variable <sup>1</sup>	Threshold <sup>2</sup>	Sensitivity	95% CI	Specificity	95% CI	AUC <sup>3</sup>
Cohort 1 (n = 31)						
NEFA <sub>H</sub>						
AAT, kg	39.5	100.0	76.8–100.0	25.0	7.3–52.4	0.59
RPAT, kg	5.9	100.0	76.8–100.0	31.3	11.0–58.7	0.58
OMAT, kg	17.9	92.9	66.1–99.8	37.5	15.2–64.6	0.48
MAT, kg	18.7	35.7	12.8–64.9	87.5	61.7–98.5	0.55
SCAT, kg	9.9	100.0	76.8–100.0	31.3	11.0–58.7	0.59
HYK						
AAT, kg	45.5	90.0	55.5–99.8	30.0	11.9–54.3	0.56
RPAT, kg	6.9	100.0	69.2–100.0	40.0	19.1–64.0	0.57
OMAT, kg	12.9	100.0	69.2–100.0	55.0	31.5–76.9	0.77
MAT, kg	21.5	30.0	6.7–65.3	95.0	75.1–99.9	0.56
SCAT, kg	10.2	100.0	69.2–100.0	30.0	11.9–54.3	0.53
Cohort 2 (n = 28)						
NEFA <sub>H</sub>						
AAT, kg	50.7	26.3	9.2–51.2	88.9	51.8–99.7	0.43
RPAT, kg	6.6	21.1	6.1–45.6	100.0	66.4–100.0	0.53
OMAT, kg	15.3	36.8	16.3–61.6	88.9	51.8–99.7	0.52
MAT, kg	15.8	31.6	12.6–56.6	88.9	51.8–99.7	0.53
SCAT, kg	12.3	36.8	16.3–61.6	77.8	40.0–97.2	0.47
HYK						
AAT, kg	46.1	11.8	1.5–36.4	100.0	71.5–100.0	0.44
RPAT, kg	8.0	52.9	27.8–77.0	72.7	39.0–94.0	0.56
OMAT, kg	17.8	94.1	91.3–99.9	18.2	2.3–51.8	0.49
MAT, kg	12.2	70.6	44.0–89.7	54.6	23.4–83.3	0.52
SCAT, kg	12.4	47.1	23.0–72.2	72.7	39.0–94.0	0.51

<sup>1</sup>AAT = abdominal adipose tissue; RPAT = retroperitoneal adipose tissue; OMAT = omental adipose tissue; MAT = mesenteric adipose tissue; SCAT = subcutaneous adipose tissue. Adipose depots determined using equations previously described (Raschka et al., 2016).

<sup>2</sup>Highest combined sensitivity and specificity.

<sup>3</sup>Area under the curve.

study. Although internal AT depots were not measured, SCAT increased during the prepartum period and decreased until 42 DIM to a greater extent in cows fed a prepartum diet formulated to supply 150% of estimated ME requirement compared with cows fed a controlled energy diet formulated to supply 100% of estimated ME requirements (Mann et al., 2016b), suggestive of an influence of diet composition on AT accumulation and mobilization. This was further demonstrated in nonpregnant, nonlactating cows fed a high energy diet that had a greater total abdominal AT, but BCS did not differ compared with cows fed a low energy diet (Drackley et al., 2014).

Given differences in the patterns of adipose tissue accumulation and mobilization between depots, this study aimed to determine the utility of prepartum ultrasonographic prediction of AT depots for the prediction of postpartum HYK or NEFA<sub>H</sub>. The mass of AAT was approximately 4 times greater than SCAT at –2 wk relative to calving in the current study and was in line with the previously reported 2 to 6 times greater difference between AAT and SCAT (von Soosten et al., 2011; Ruda et al., 2019; Szura et al., 2020). In addition to larger quantities of AT in the abdomen compared with SCAT, differences in mobilization rate between depots have been demonstrated. In a study by Ruda et al. (2019), the relative average daily growth did not differ between AAT and SCAT during the dry period; however, AAT showed a greater relative and quantitative average daily mobilization from 3 to 21 DIM compared with SCAT. Further, RPAT showed greater time-dependent changes

in adipocyte size compared with SCAT such that retroperitoneal adipocytes increased in size from –42 to 1 d relative to calving and decreased to a greater extent from 1 to 100 DIM (Kenéz et al., 2015). Larger adipocytes were associated with a greater basal activity and a greater sensitivity to lipolytic signals (De Koster et al., 2016). Differences in lipolytic potential in RPAT was further supported by an increased hormone sensitive lipase expression on 21 DIM in RPAT compared with SCAT (Locher et al., 2011).

Despite these documented differences in metabolic activity among AT depots, all estimated adipose depots at –2 wk relative to calving resulted in low predictive ability for HYK and NEFA<sub>H</sub>. In contrast to the current study that did not find prepartum AT depots sufficiently accurate to distinguish between NEFA<sub>H</sub> and non-NEFA<sub>H</sub> cows, a negative correlation was observed between the average daily change in SCAT, AAT, RPAT, and OMAT from 7 to 28 DIM with NEFA concentration at 7 DIM ( $r = -0.38$  to  $-0.53$ ; Szura et al., 2020). Further, in Strieder-Barboza et al. (2015), reductions in backfat >50% (AUC: 0.70; Se: 0.67; Sp: 0.77) and RPAT thickness >75% (AUC: 0.59; Se: 0.29; Sp: 0.86) between  $-28 \pm 5$  and  $8 \pm 3$  d relative to calving were reported for the prediction of NEFA concentrations  $\geq 720$   $\mu$ Eq/L at  $8 \pm 3$  d. Compared with a single prepartum time point used in the current study, use of 2 time points, as done in the aforementioned studies, might better reflect AT mobilization. Inclusion of a small sample size with animals from one herd remains a limitation of the current study. However, the lack of association between HYK as well as NEFA<sub>H</sub> and prepartum AT

depots in the 2 cohorts differing in their prevalence suggests that AT mass itself is not sufficiently predictive of the risk for these metabolic outcomes. Further, the degree of bias in Se and Sp has been shown to be related to sample size when optimal cutpoints were selected using the Youden index (Leeflang et al., 2008); thus, the small sample size in this study might overestimate Se and Sp.

We further acknowledge that ultrasound technique, transducers, and cow size might have differed between this study and the study by Raschka et al. (2016) on which the equations were based, and might have decreased accuracy of predicting AT depots. This might partially explain why estimated prepartum AT depots at -2 wk relative to expected calving were noninformative for the prediction of HYK and NEFA<sub>H</sub> in postpartum Holstein cows. However, results from this study show that ultrasonographic prediction of AT depots could be used as a noninvasive method to monitor accumulation and mobilization of AT during the transition period.

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## Notes

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**Nonstandard abbreviations used:** AAT = abdominal adipose tissue; AT = adipose tissue; AUC = area under the curve; BFT = backfat thickness; HYK = hyperketonemia; L# = location number; MAT = mesenteric adipose tissue; NEFA = nonesterified fatty acids; NEFA<sub>H</sub> = NEFA  $\geq$  720  $\mu$ Eq/L; OMAT = omental adipose tissue; RPAT = retroperitoneal adipose tissue; SCAT = subcutaneous adipose tissue; Se = sensitivity; Sp = specificity; VAT = visceral adipose tissue.