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# Assessment of skeletal muscle dynamics and milk production across a 300-day lactation in multiparous dairy cattle

**Samantha L. Hanno, Theresa M. Casey, Hinayah Rojas de Oliveira, and Jacquelyn P. Boerman***
Department of Animal Sciences, Purdue University, West Lafayette, IN 47907

## ABSTRACT

The objective of this study was to evaluate changes in *longissimus dorsi* muscle depth (LDD) across lactation (0 to 300 DIM) and identify the impact of low versus high muscle reserves immediately after parturition on body weight and body reserve changes as well as production variables across a 300-d lactation. Forty multiparous cows were classified as high muscle (HM; LDD > 5.0 cm; n = 18) or low muscle (LM; LDD ≤ 5.0 cm; n = 22) based on LDD measurements collected within 24 h of parturition. Body weights (BW) and ultrasound scans to assess LDD and back fat depth (BFD) were collected monthly from parturition until 300 DIM. Ultrasound scans captured and measured using available software. Blood samples were taken at 7, 150 and 300 DIM, and plasma was analyzed for markers of metabolic status by measuring insulin, nonesterified fatty acids (NEFA), creatinine, and 3-methylhistidine (3-MH). Milk yield was recorded daily and milk components were analyzed monthly. Data analysis was performed and the statistical models included the fixed effect of muscle group, time, their interaction, and the random effect of cow nested within muscle group with repeated measures using a first-order autoregressive covariance structure. Muscle group was not related with BW or BFD for any of the time points measured. Cows lost BW from 0 to 60 DIM and gained weight from 60 to 300 DIM. Similarly, BFD decreased between 0 to 90 DIM and increased BFD after 90 DIM until 300 DIM. A muscle group by time interaction was observed for LDD. The HM cows had more muscle at 0 DIM, indicative of treatment assignment (1.34 cm more), and 300 DIM (0.78 cm more) and tended to have more muscle at 60 DIM (0.66 cm more) compared with LM. After 240 DIM, both muscle groups began net accretion of muscle reserves until 300 DIM. No differences were observed for blood metabolites measured based on muscle group. However, there were significant time effects for creatinine, 3-MH, and NEFA concentrations, which reflected the observed changes in BFD and LDD measured in ultrasound scans. For statistical analysis of daily milk production, observations were grouped into 3 stages of lactation, early (0–60 DIM), mid (60–240 DIM), and late lactation (240–300 DIM). There was a muscle group by stage of lactation interaction, where in early and mid-lactation, HM cows produced, on average, 1.9 kg more milk/d; however, in late lactation, LM cows produced 1.8 kg more milk/d. Our results indicate that muscle reserves are depleted in early lactation, and accreted in late lactation, whereas BW and BFD started to increase by 90 DIM. Data also supports that cows with more extensive muscle depletion in early lactation had greater milk production, however, substantial muscle accretion in late lactation may result in reduced milk production.
Keywords: muscle depletion, muscle accretion, *longissimus dorsi*, tissue reserves

## INTRODUCTION

Body tissue reserves, primarily adipose and skeletal muscle, are depleted and accreted by lactating dairy cows in response to the dynamic shifts in nutrient requirements and nutrient intake during lactation. These changes in tissue reserves are associated with different physiological states throughout lactation. In early lactation, dairy cattle experience a negative energy balance as feed intake does not meet energy requirements. This negative energy balance results in the mobilization of peripheral tissues to obtain gluconeogenic substrates to maintain glucose production, resulting in an overall loss in body weight and BCS (Pires et al., 2013; Larsen and Kristensen, 2013). Studies quantifying the dynamics of body tissues in dairy cattle have reported differing amounts of tissue depletion during early lactation. Research utilizing both direct and indirect measurement techniques for body composition has reported differences from prepartum to peak lactation of 30 to 100 kg of adipose tissue (Butler-Hogg et al., 1985; Tamminga et al., 1997) and 2.3 to 21 kg of muscle (Komagiri and Erdman, 1997; Tebbe and Weiss, 2021b).

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*Corresponding author: Jacquelyn P. Boerman, 270 S. Russell St., West Lafayette, IN 47907, (765) 496-6290, jboerma@purdue.edu

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

After peak lactation, energy and protein intake is able to meet the animal's milk and maintenance requirements. As energy and protein balances become positive, physiological changes such as increased insulin sensitivity result in reduced tissue depletion and stimulated tissue accretion (Chalmeh et al., 2015; Mann, 2016). It is established that adipose tissue accretion begins after peak lactation and continues throughout lactation (McNamara, 1991); however, there is limited research regarding skeletal muscle reserve changes after peak lactation.

In the past 2 decades, research about the importance of skeletal muscle in supporting metabolic demands of lactation in dairy cattle has increased, predominately during the periparturient period (Kuhla et al., 2011; van der Drift et al., 2012; Pires et al., 2013). Skeletal muscle reserves are mobilized before parturition to meet increased AA requirements necessary to support fetal growth, mammary development, and colostrum synthesis (Bell et al., 2000; van der Drift et al., 2012). Previous work has shown that cows with differing *longissimus dorsi* muscle depth (**LDD**) in late lactation maintain these differences in skeletal muscle reserves throughout the periparturient period. Cows with lower muscle reserves, assessed approximately one mo before calving, accreted LDD before calving compared with cows with higher muscle reserves where little change in LDD was observed (McCabe et al., 2021). However, little to no research has been conducted regarding the timing and extent of depletion and accretion of skeletal muscle reserves beyond the first 60 d of lactation. Understanding the dynamics of skeletal muscle reserves can provide greater insight into protein metabolism in lactating cows and enable more precise management such as grouping and breeding selection as well as amino acid supplementation throughout the stages of lactation.

Our objective was to evaluate changes in LDD across lactation to determine timing and magnitude of skeletal muscle depletion and accretion; and identify how these changes vary in cows of differing muscle phenotypes at the time of parturition. We hypothesized that cows would deplete skeletal muscle reserves during the first 30 DIM due to negative energy and protein balances. Subsequently, cows would accrete muscle reserves during mid-lactation as negative energy and protein balances would typically become positive (Bell et al., 2000; Grummer and Rastani, 2003). We also hypothesized that cows with greater skeletal muscle reserves at parturition would deplete and accrete muscle tissue to a greater extent than those with lesser muscle reserves.

## MATERIALS AND METHODS

### Animals

All protocols and procedures were approved by the Purdue University Institutional Animal Care and Use Committee (protocol #2109002197) before the start of the study. Data were collected from a convenience sample of the 40 multiparous Holstein dairy cows (n = 40) that calved from November 2022 to January 2023 at the Purdue Dairy Farm. Cows averaged 2.6 ± 0.87 (mean ± SD) lactations, 11,396 ± 1,338 kg for previous lactation milk yield, and 723 ± 75 kg body weight after parturition. The cows entered the study at calving (0 DIM) and ended at 300 ± 9.6 DIM. They were split into high muscle (**HM**; n = 18) and low muscle (**LM**; n = 22) groups based on LDD at calving. Cows with muscle depth greater than 5.0 cm were classified as HM (LDD 5.93 ± 0.14) and cows with muscle depth of 5.0 cm or less were classified as LM (LDD of 4.59 ± 0.13).

Throughout lactation, cows were fed a common diet (Table 1) once a day between 0500 and 0600 and milked twice per day at 0500 to 0800 and 1600 to 1900. Cows were housed in a tie-stall barn from calving to 28 DIM. After 28 DIM, they were moved to a free-stall barn and were housed in 2 pens with approximately 30–40 cows per pen for the duration of their lactation.

### Data collection

From 0 to 300 DIM, cows were weighed approximately every 30 d (30 ± 4.7 d) for a total of 11 time points per cow of 0 ± 0.6, 30 ± 6.0, 60 ± 9.8, 90 ± 10.4, 120 ± 8.6, 150 ± 8.0, 180 ± 10.0, 210 ± 10.1, 240 ± 11.0, 270 ± 10.7, 300 ± 9.6. Body weight (**BW**) in kg was collected as cows exited the milking parlor after the afternoon milking using a stationary scale.

From the day of parturition until 300 DIM, ultrasound images were collected approximately every 30 d (30 ± 4.7 d) after the afternoon milking for a total of 11 time points per cow (similar to BW time points). Images were taken of the *longissimus dorsi* muscle above the 12th intercostal space on the right side of each cow by one trained researcher with 3 images captured per cow per time point. An ALOKA SSD-500 ultrasound (Wallingford, CT) was used with a linear probe to capture images.

Blood samples were taken at 7 ± 2.3, 150 ± 8.0, and 300 ± 9.6 DIM from the coccygeal vessels using a 21-gauge needle (McKesson, Irving, TX) into 10-mL potassium EDTA tubes (Becton, Dickinson, and Company, Franklin Lakes, NJ). Samples were centrifuged at 4000 × *g* for 15 min. Separated plasma was aliquoted into 3 1.5-mL microcentrifuge tubes and stored at −20°C until further analysis.

Daily milk yield was collected using AfiMilk (Kibbutz Afikim, Israel). Test day components were collected monthly for concentrations of fat, protein, and somatic cell counts throughout lactation. Somatic cell counts were converted to linear somatic cell score (**SCS**) using the equation described by Wiggans and Shook (1987).

### Data analysis

Ultrasound images were saved and back fat depth (**BFD**) and LDD were measured in images using ImageJ software (NIH, Bethesda, MD). The BFD and LDD values were averaged across the 3 captured images per cow per time point. To evaluate differences among lactation stages, data was sectioned into stage of lactation groups of early lactation (0–60 DIM), mid-lactation (60–240 DIM), and late lactation (240–300 DIM). Fat and muscle depth values at the beginning and end of each stage of lactation were used to calculate absolute and percent change in BFD and LDD across lactation stages.

**Table 1.** Ingredient and nutrition composition of the lactating diet

| Item | % of DM unless stated otherwise |
|---|---|
| Ingredient | |
| Corn silage | 35.1 |
| Alfalfa silage | 13.5 |
| Small grain silage | 4.7 |
| Ground corn | 15 |
| Soybean meal | 8.4 |
| Soybean hulls | 4.6 |
| SoyPlus[1] | 3.5 |
| QLF 63/43[2] | 4.4 |
| Blood meal | 1.1 |
| Amino blend[3] | 5.2 |
| Lactating premix[4] | 3.7 |
| Spectrum Fusion[5] | 0.5 |
| EnerGII[6] | 0.3 |
| Nutrient Analysis | |
| DM (% of diet) | 49.2 |
| CP | 17.2 |
| ADF | 17.5 |
| $aNDF_{OM}$ | 26.8 |
| Ether extract | 4.1 |
| Starch | 28.3 |

[1]SoyPlus (Landus, Des Moines, IA).

[2]Mollases product (Quality Liquid Feeds, Dodgeville, WI)

[3]Amino blend contained 46.5% Alimet (Methionine analog 2-hydroxy-4-(methylthio) butanoate product; Novus International, St. Charles, MO), 25.9% Smartamine M (Rumen-protected methionine product; Adisseo, Alpharetta, GA), 25.5% ETX-5 (Anti-fungal agent; FeedworksUSA, Cincinnati, OH), and 2.2% Agolin Ruminant (Essential-oil blend product; Agolin, Biére, Switzerland).

[4]Lactating premix contained 30.2% calcium carbonate, 20.1% sodium bicarbonate, 10.7% salt, 8.0% urea, 6.7% calcium monophosphate, 6.7% magnesium oxide, 6.1% Diamond V XP (Diamond V, Cedar Rapids, IA), 5.3% porcine tallow, 3.3% DCAD Plus (Church and Dwight Co. Inc., Arm and Hammer Animal Nutrition, Ewing Township, NJ), 2.6% trace mineral vitamin premix #5390, and 0.3% vitamin E 20,000 IU

[5]Spectrum Fusion (Perdue Animal Nutrition, Salisbury, MD)

[6]EnergII (Virtus Nutrition, Corcoran, CA)

Blood plasma samples for 7, 150, and 300 DIM were analyzed for insulin and NEFA concentrations using their respective commercial kits: insulin kits from ALPCO (Salem, NH, USA), and NEFA kits from FUJIFILM Wako Chemicals (Richmond, VA, USA). Intra-plate coefficients of variance were 4.80% and 2.27% and inter-plate coefficients of variance were 8.10% and 4.72% for insulin and NEFA, respectively.

Plasma samples concentrations of creatinine and 3-methylhistidine (**3-MH**) were measured using liquid chromatography tandem mass spectrometry. For analysis, 50 µL of plasma were extracted using acetonitrile. Samples were spiked with 5 µL of 100 ng/µL of $d_3$-creatinine with deuterated molecules on the tertiary amine (Toronto Research Chemical, North York, ON, Canada) and 5 µL deuterated $d_3$-3-MH (Sigma Aldrich, St. Louis, MO, USA) standards. Sample preparations were vortexed, centrifuged at 4000 × *g* for 8 min, and 75 µL of the supernatant was transferred to a liquid chromatography vial. Samples were quantified using an Agilent 1290 Infinity II liquid chromatography system coupled to an Agilent 6470 Triple Quadrupole mass spectrometer (Santa Clara, CA, USA). All data were analyzed using Agilent Masshunter Quantitative Analysis (v10.1).

Due to reading errors of transponders in the milking parlor, a portion of milk production data was not recorded (6.5% of data), thus, cumulative milk and daily milk production values could not be calculated using only the recorded milk weights. The lactcurves package (Strucken, 2021) available in RStudio (version 2023.12.1) was used to determine the best fit model to be used to predict the missing values. This package fits multiple pre-existing lactation curve models to the data provided and estimates various selection criteria for each model. On the basis of maximizing $R^2$ and minimizing AIC and BIC, the Khandekar model was selected to predict the missing data in this study. This model is described as (Guo and Swalve, 1995):

$$y = a + b \times DIM + c \times DIM^2 + d \times DIM^3 + f \times \ln(DIM),$$

where $y$ is the estimated milk weight in kg. The variables a, b, c, d, and f are the lactation curve parameters, and the variable DIM refers to days in milk. The MODEL procedure of SAS (version 9.4; SAS Institute, Cary, NC, USA) was then used with the Khandekar model for individual cows to determine parameter values and predict daily milk production for the days with missing data. These milk production estimates were used on days that milk yield was missing and allowed for cumulative milk production analysis.

### Statistical analysis

Data were analyzed using the MIXED procedure available in SAS (version 9.4; SAS Institute, Cary, NC, USA). The Shapiro-Wilk test was used to test normality and all data were normally distributed. For analysis of daily average milk yield by lactation stage, DIM was sectioned by similar stages of lactation as body reserve changes described previously, resulting in stages of lactation of 1 to 60, 61 to 240, and 241 to 300 DIM with milk weights averaged per stage of lactation. For analysis of milk components by lactation stage, DIM was similarly sectioned by stage of lactation of 0 to 60, 90 to 210, and 240 to 300 DIM with component concentrations averaged per stage of lactation. The model is described as:

$$Y_{ijkl} = \mu + M_i + T_j + MT_{ij} + C(M_i)_k + e_{ijkl},$$

where $\mu$ is the overall mean, $M_i$ represents the fixed effect of muscle group (i = high muscle vs. low muscle), $T_j$ represents the fixed effect of time (j = 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300 DIM; or 7, 150, 300 DIM; or stage of lactation), $MT_{ij}$ represents the interaction of muscle group and time, and $C(M_i)_k$ is the random effect of cow nested within muscle group. Repeated measures with cow as the subject were used with the first order autoregressive covariance structure to minimize BICC and AIC. Tukey adjustments were conducted on any variables with significant muscle group by time interaction to adjust for multiple comparisons. Group, time, and interaction terms at $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ were considered a tendency. LSM and SEM were reported for muscle group by time interactions, unless otherwise stated.

Early lactation mobilization of muscle and backfat was defined as muscle or backfat at 0 DIM minus muscle or backfat at 60 DIM, respectively. The CORR procedure of SAS was used to determine Pearson correlations between change in tissue depths from 0 to 60 DIM and initial tissue depths to determine relationships between amount of tissue at parturition and extent of tissue mobilization in early lactation.

## RESULTS

### Body weight and reserves

There was a time effect for BW ($P < 0.01$) with no muscle group effect ($P = 0.95$) or muscle group by time interaction ($P = 0.14$; Figure 1A). Cows from both muscle groups exhibited lower BW at 30 and 60 DIM compared with 0 DIM. After nadir, BW increased until 300 DIM, exhibiting an overall average increase in BW of 34 kg from parturition to 300 DIM.

When separated by lactation stage (Table 2), a time effect was observed for BW change ($P < 0.01$) with no muscle group nor muscle group by time interaction ($P > 0.05$). Cows experienced an early lactation loss of 58.6 kg, followed by a gain of 63.0 kg throughout mid-lactation, and a gain of 27.9 kg observed in late lactation. A time effect was also observed for percent BW change ($P < 0.01$). Cows from both muscle groups lost an average of 7.8% of their BW in the first 60 d of lactation. A gain in BW during mid-lactation was observed with cows demonstrating a 9.6% gain in BW followed by a 3.8% increase in BW during late lactation, regardless of muscle group.

A muscle group by time interaction was observed for LDD as HM cows demonstrated more muscle reserves at specific time points throughout their lactation compared with LM cows ($P < 0.01$; Figure 1B). High muscle cows had more muscle than LM cows at 0 DIM (1.34 cm more; $P < 0.01$) and 300 DIM (0.78 cm more; $P = 0.01$). At 60 DIM, HM cows tended to have 0.66 cm more muscle than LM cows ($P = 0.10$) with no significant difference in LDD at other time points measured. A muscle group effect was also observed with HM cows overall maintaining 0.53 cm more muscle than LM cows ($P < 0.01$).

Change in LDD across the 3 stages of lactation exhibited a muscle group by time interaction with HM cows losing more LDD in early lactation and gaining more in late lactation compared with LM cows ($P < 0.01$; Table 2). Conversely, LM cows gained more LDD during mid-lactation than HM cows, as HM had very little change in LDD during this time. A time effect was also observed with cows expressing a negative change in LDD in early lactation, little to no gain in mid-lactation, and greater LDD gain in late lactation ($P < 0.01$). A muscle group by time interaction was observed for percent change in LDD with LM cows demonstrating muscle accretion of 13.9% during mid-lactation while HM cows exhibited a 2.8% increase in LDD until late lactation ($P < 0.01$). High muscle cows exhibited a 26.7% gain in LDD during the last 60 d of lactation where LM gained 15.2%. A time effect was also observed with cows from both muscle groups losing 32.9% of LDD during early lactation ($P < 0.01$). By the end of lactation, neither muscle group reestablished the skeletal muscle reserves present at parturition. HM cows exhibited an overall net loss of 1.02 cm of LDD whereas net loss of LDD in LM cows was 0.48 cm from parturition to 300 DIM ($P = 0.04$).

A negative correlation was observed between LDD at parturition and the change in LDD from 0 to 60 DIM ($R^2 = -0.63$; $P < 0.01$; Figure 2A). Cows with more muscle at parturition exhibited a more negative change in LDD within the first 60 d of lactation, equating to a greater extent of muscle depletion.

There were no differences between muscle groups for BFD throughout lactation ($P = 0.15$; Figure 1C). However, there was a time effect across the 11 time points ($P < 0.01$). Cows mobilized fat reserves after calving until nadir was reached at 60 DIM. After nadir was reached, fat depth increased consistently as cows gained an average of 0.18 cm of BFD between 60 and 300 DIM. A time effect was observed for absolute change in BFD across stages of lactation ($P < 0.01$) with cows losing BFD in early lactation and gaining fat for the duration of their lactation. Cows mobilized an average of 0.12 cm of BFD during early lactation (Table 2). This loss was restored with an average of 0.14 cm gain in BFD during mid-lactation followed by a gain of 0.05 cm in late lactation. A time effect was also observed for percent change in BFD ($P < 0.01$) with no differences demonstrated between muscle groups ($P > 0.05$). As a percentage, cows lost 28.4% of BFD during early lactation with a subsequent increase of 40.5% during mid-lactation and an additional 11% of BFD was gained in late lactation.

A strong negative correlation was observed between BFD at parturition and the change in BFD in early lactation ($R^2 = -0.81$; $P < 0.01$; Figure 2B). Cows with more BFD at parturition exhibited a more negative change in fat depth in the first 60 DIM, indicating a greater extent of fat mobilization.

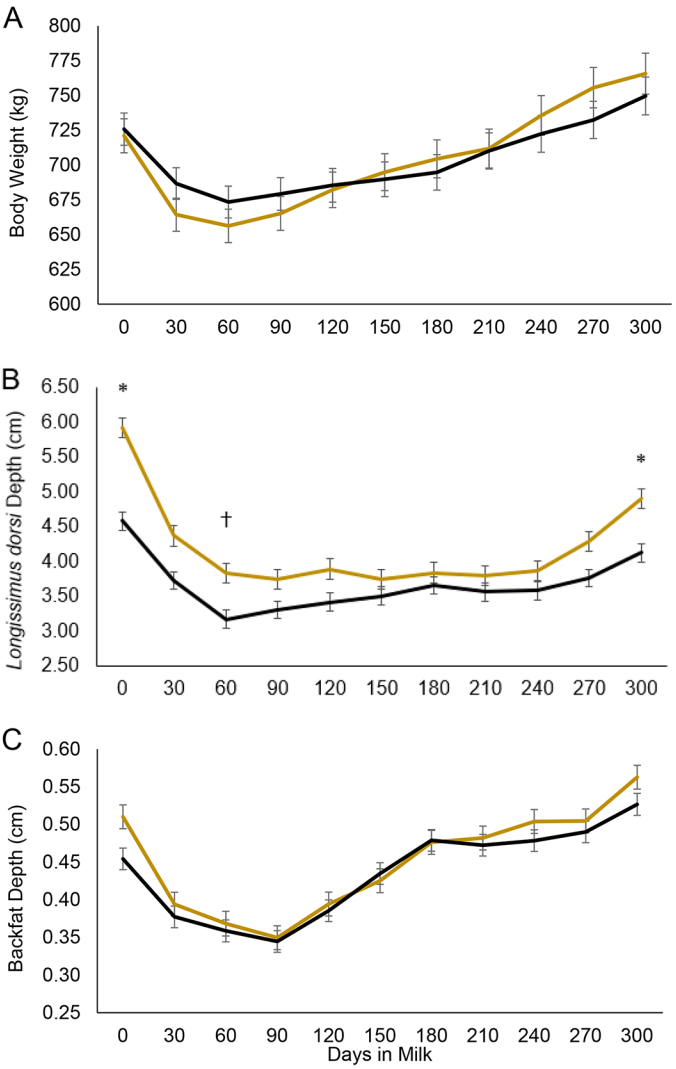


**Figure 1.** Body weight (kg), *longissimus dorsi* depth (cm), and backfat depth (cm) across lactation by muscle group. Gold lines indicate high muscle cows (HM; *longissimus dorsi* depth > 5.0 cm at parturition) and black lines indicate low muscle cows (LM; *longissimus dorsi* depth ≤ 5.0 cm at parturition). Significant group differences within a timepoint are indicated by * (B). Tendencies for group differences within a timepoint are indicated by † (B). (A) Muscle group: $P$=0.95; Time: $P$<0.01; Muscle group × Time: $P$=0.14. (B) Muscle group: $P$<0.01; Time: $P$<0.01; Muscle group × Time: $P$<0.01. (C) Muscle group: $P$=0.15; Time: $P$<0.01; Muscle group × Time: $P$=0.62. Values are least squares means ± standard error of the mean.

## Milk production

A muscle group by stage of lactation effect was observed for daily milk production when sectioned into lactation stages ($P < 0.01$). Differences in daily yield between HM and LM cows by lactation stage were observed with HM cows producing 2.5 kg/d more milk compared with LM in early lactation. This difference continued through mid-lactation as HM cows produce 1.4 kg/d more milk. However, in late lactation, HM cows produced 1.8 kg/d less milk, compared with LM cows

No muscle group effects were observed for cumulative milk production with cows from both groups exhibiting a cumulative 300-d production of 11,206 kg ± 341 ($P > 0.05$; data not shown). There was a muscle group effect on milk fat concentration with HM cows demonstrating higher concentrations of milk fat compared with LM cows (0.15 percentage units; $P = 0.05$). A muscle group effect was also observed for SCS ($P < 0.01$) with HM cows exhibiting a higher SCS compared with LM cows. No muscle group effect was observed for milk protein ($P > 0.05$). A stage of lactation effect was observed for milk protein and SCS (all $P < 0.01$). Cows from both muscle groups increased milk protein and SCS as lactation progressed.

Milk components for each month exhibited a time effect with milk fat concentration at 120 DIM ranging from 0.22 to 0.47 percentage units greater than any other time point ($P = 0.03$; data not shown). Milk protein concentration was highest at 0 DIM, exhibited a 0.67 percentage unit decrease from d 7 to 30 DIM and steadily increased until 300 DIM ($P < 0.01$; data not shown).

### Blood Analytes

Circulating concentrations of 3-MH, creatinine, NEFA, and insulin did not differ between muscle groups ($P > 0.05$; Table 3). However, a time effect was observed for all analytes ($P < 0.01$). Cows exhibited greater creatinine concentrations at 7 DIM compared with 150 DIM, indicating a reduction in overall muscle mass from early to mid-lactation. Creatinine concentration returned to post-calving concentrations by 300 DIM. Concentrations of 3-MH were similar between d 7 and 150, in early and mid-lactation. Plasma 3-MH concentrations declined from 150 to 300 DIM. The ratio of 3-MH to creatinine was 0.342 at 7 DIM. Because of the consistency of 3-MH concentrations and the decrease in creatinine at 150 DIM, the 3MH:creatinine ratio was increased at 150 DIM. At 300 DIM, the ratio considerably decreased as 3-MH concentrations declined and creatinine concentrations increased. At 7 DIM, plasma NEFA concentrations were elevated relative to 150 DIM and remained low through 300 DIM. Plasma insulin was similar between 7 and 150 DIM and then significantly elevated by 300 DIM.

## DISCUSSION

Cows in the present study depleted over 30% of skeletal muscle reserves present at parturition within the first 60 d of lactation and substantial restoration of these reserves did not occur until late lactation. This finding was contrary to the proposed hypothesis that muscle accretion would occur in mid-lactation. Depletion and accretion patterns of muscle over the dairy cows' production cycle differed from subcutaneous fat, which was depleted from parturition through early lactation but began to increase after nadir was reached at 90 DIM. The observed changes in body tissues were supported by changes in plasma analytes. The differences in patterns of LDD depletion and accretion between HM and LM cows reflected differences in milk production at different stages of lactation. In early and mid-lactation, the greater depletion of HM skeletal muscle reserves translated to greater kg/d of milk production. In late lactation, HM cows accreted more muscle reserves than LM cows, and, relative to LM, produced less milk yield.

Cows in the present study depleted both adipose and skeletal muscle reserves after parturition through early lactation, however, the timing of these reserve changes differed. Adipose tissue was mobilized until 90 DIM while muscle was depleted until 60 DIM which aligns with previous literature demonstrating that fat stores are mobilized over a longer period compared with muscle (Komagiri and Erdman, 1997). Previous research indicated that skeletal muscle reserves are mobilized over the first 4 to 5 wk of lactation (Tamminga et al., 1997; van der Drift et al., 2012), whereas muscle depletion was observed through 60 DIM in the present study; although notably, LDD measures were only captured monthly. Others reported a decrease in body protein across the

**Table 2.** Milk production, milk composition, and change in body weight, muscle depth, and backfat depth during three stages of lactation[1] for cow with high muscle depth (HM)[2] and cows with low muscle depth (LM)[2] at parturition

| | 0–60 DIM | | 60–240 DIM | | 240–300 DIM | | | *P*-values[3] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable[4] | HM | LM | HM | LM | HM | LM | SEM[5] | Muscle Group | Time | Muscle Group × Time |
| Milk Production | | | | | | | | | | |
| Milk Yield (kg/d) | 43.5 | 41.0 | 39.1 | 37.7 | 28.8 | 30.6 | 1.75 | <0.01 | <0.01 | <0.01 |
| Milk Fat (%) | 4.15 | 3.84 | 4.21 | 4.00 | 4.04 | 4.11 | 0.11 | 0.05 | 0.50 | 0.12 |
| Milk Protein (%) | 3.03 | 3.09 | 3.29 | 3.31 | 3.48 | 3.52 | 0.05 | 0.23 | <0.01 | 0.89 |
| Somatic Cell Score | 1.59 | 0.91 | 2.41 | 1.40 | 2.94 | 1.36 | 0.30 | <0.01 | <0.01 | 0.58 |
| Absolute Change | | | | | | | | | | |
| Δ Body Weight (kg) | −71.4 | −45.8 | 65.1 | 61.5 | 26.3 | 29.5 | 8.70 | 0.20 | <0.01 | 0.18 |
| Δ Muscle Depth[6] (cm) | −2.12 | −1.42 | 0.01 | 0.41 | 1.04 | 0.54 | 0.16 | 0.09 | <0.01 | <0.01 |
| Δ Backfat Depth[6] (cm) | −0.15 | −0.10 | 0.15 | 0.12 | 0.05 | 0.04 | 0.03 | 0.97 | <0.01 | 0.17 |
| Percent Change | | | | | | | | | | |
| Δ Body Weight (%) | −9.0 | −6.5 | 9.5 | 9.8 | 3.4 | 4.2 | 1.21 | 0.20 | <0.01 | 0.60 |
| Δ Muscle Depth[6] (%) | −35.1 | −30.8 | 2.8 | 13.9 | 26.7 | 15.2 | 3.85 | 0.65 | <0.01 | 0.01 |
| Δ Backfat Depth[6] (%) | −28.4 | −21.3 | 45.3 | 35.6 | 12.8 | 9.0 | 5.58 | 0.61 | <0.01 | 0.27 |

[1]Stages of lactation of early lactation (0–60 DIM), mid-lactation (60–240 DIM), and late lactation (240–300 DIM).

[2]Muscle groups consisted of high muscle (HM; n = 18) with *longissimus dorsi* depth of > 5.0 cm at parturition and low muscle (LM; n = 22) with *longissimus dorsi* depth of ≤ 5.0 cm at parturition.

[3]*P*-values associated with muscle group (HM vs. LM), time effects (0–60 DIM, 60–240 DIM, 240–300 DIM), and the interaction between muscle group and time.

[4]Least squares means are reported for each variable.

[5]Largest standard error of the mean reported.

[6]Muscle and fat depth changes were based on differences between measurements of ultrasounds above the *longissimus dorsi*.

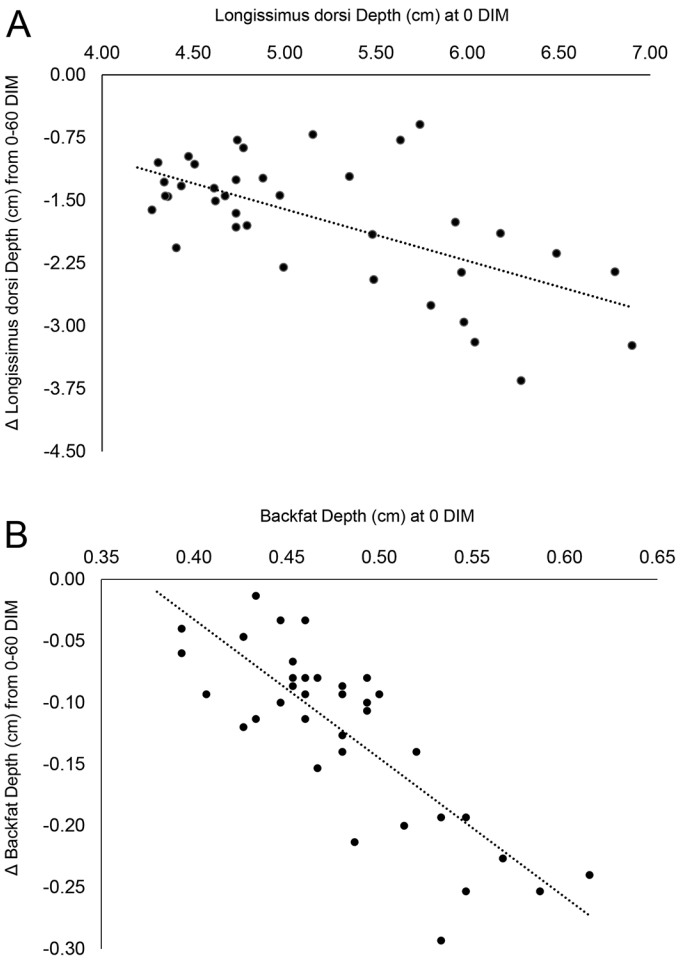


**Figure 2.** Relationships between initial body tissue reserves and the change in tissue reserves from 0 to 60 DIM. (A) Linear correlation analysis of *longissimus dorsi* depth at parturition and the change in *longissimus dorsi* depth from 0 to 60 DIM; $R^2 = -0.63$; $P < 0.01$. (B) Linear correlation analysis of backfat depth at parturition and the change in backfat depth from 0 to 60 DIM; $R^2 = -0.81$; $P < 0.01$

first 2 mo of lactation, which more closely aligns with our findings (Chilliard et al., 1991; Ryder et al., 2023). Because samples in the current study were taken monthly, it is possible that skeletal muscle nadir was reached between 30 and 60 DIM. Slaughter studies comparing body composition of cows across different physiological states found no changes in body protein, which may appear contrary to the present study as muscle reserves were extensively mobilized (Gibb et al., 1992; Andrew et al., 1994). However, Andrew et al. (1994) observed a difference in the proportion of protein across different tissues with early lactation cows exhibiting less carcass protein and more protein in the digestive tract and mammary gland, demonstrating that skeletal muscle is depleted but the AA released are potentially redistributed into tissues required to sustain milk production.

Studies that analyzed adipose tissue as empty body fat, typically regarded as total body lipids excluding digesta, urine, and fetal tissue, has demonstrated a greater extent of depletion compared with muscle which has been previously reported as empty body protein (Komagiri and Erdman, 1997; Tamminga et al., 1997). Empty body protein consists of body protein excluding that associated with digesta, urine, and fetal tissue. A study by van der Drift et al. (2012) found that using ultrasound measurements of backfat and muscle in dairy cows, a greater percent of fat was lost compared with muscle, with cows depleting 35% of their backfat and 18% of muscle in the first 4 wk of lactation. In the current study cows lost more muscle than fat, with cows depleting 26% of their backfat and 32% of muscle. Positive associations between the amount of tissue reserves present and the amount of tissue mobilized have been reported for adipose and muscle (van der Drift et al., 2012; McCabe et al., 2021). This is similar to the association observed in the present study with more BFD and LDD at parturition resulting in a more negative

**Table 3.** Plasma 3-methylhistidine, creatinine, 3-methylhistidine:creatinine ratio, nonesterified fatty acids, and insulin concentrations at three stages of lactation for cows with high muscle depth (HM)[1] and cows with low muscle depth (LM)[1] at parturition

| | 7 DIM | | 150 DIM | | 300 DIM | | | *P*-values[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable[3] | HM | LM | HM | LM | HM | LM | SEM[4] | Muscle Group | Time | Muscle Group × Time |
| 3-MH (ng/μL)[5] | 2.09 | 1.86 | 2.16 | 1.78 | 0.998 | 0.973 | 0.178 | 0.19 | <0.01 | 0.54 |
| Creatinine (ng/μL) | 5.95 | 5.75 | 4.43 | 4.43 | 5.97 | 5.80 | 0.273 | 0.60 | <0.01 | 0.91 |
| 3-MH: Creatinine | 0.353 | 0.330 | 0.493 | 0.457 | 0.169 | 0.171 | 0.044 | 0.60 | <0.01 | 0.89 |
| NEFA (mmol/L)[6] | 0.539 | 0.543 | 0.125 | 0.124 | 0.114 | 0.115 | 0.041 | 0.97 | <0.01 | 0.99 |
| Insulin (μIU/mL) | 0.392 | 0.368 | 0.402 | 0.348 | 0.640 | 0.616 | 0.079 | 0.52 | <0.01 | 0.97 |

[1]Muscle groups consisted of high muscle (HM; n = 18) with *longissimus dorsi* depth > 5.0 cm after parturition and low muscle (LM; n = 22) with *longissimus dorsi* depth ≤ 5.0 cm after parturition.

[2]*P*-values associated with muscle group (HM vs. LM), time effects (7, 150, 300 DIM), and the interaction between parity and time.

[3]Least squares means are reported for each variable.

[4]Largest standard error of the mean reported.

[5]3-MH = 3-methylhistidine.

[6]NEFA = nonesterified fatty acids.

change in these reserves during the first 60 DIM. van der Drift et al. (2012) reported a greater amount of fat and lesser amount of muscle, measured weekly from 4 to one wk prepartum, compared with our observed values, which may explain the differences in the observed depletion of body tissue reserves. Previous studies have also highlighted the association between lesser fat reserves, as measured by BCS, and greater depletion of muscle reserves to acquire energetic substrates (Pires et al., 2013; Ryder et al., 2023). While we did not measure this specific correlation as BCS was not measured in the present study, it helps to support the conclusion that these associations imply that lactating dairy cattle will use what tissue reserves they have in greater abundance.

The presence of 3-MH in the blood is a product of the breakdown of the actin and myosin microfilaments that make up skeletal muscle tissue and is unable to be reused by the body and is hence excreted via urine (Asatoor and Armstrong, 1967). As a by-product of muscle breakdown, circulating 3-MH concentrations are measured to determine the extent of muscle breakdown. Previous literature reports an increase in circulating 3-MH concentrations during early lactation compared with the prepartum period, indicating an increase in muscle breakdown at the onset of lactation (Blum et al., 1985; Pires et al., 2013). Plasma concentrations of 3-MH range from 9 to 15 μM after parturition (van der Drift et al., 2012; Pires et al., 2013; Yang et al., 2020). In the present study, cows from both muscle groups demonstrated an average plasma 3-MH concentration of 1.97 ng/μL at 7 DIM, equivalent to 11.65 μM, indicating elevated concentrations during this time that is comparable to other studies. Current literature reports a decrease in plasma 3-MH concentrations by 4 wk into lactation compared with the wk before and after parturition (van der Drift et al., 2012; Pires et al., 2013; Tebbe and Weiss, 2021a). Interestingly, 150 DIM samples were also 1.97 ng/μL (equivalent to 11.64 μM) which is contrary to others that report decreased circulating 3-MH concentrations of by 25 DIM (Tebbe and Weiss, 2021a). Our results indicate continued elevated muscle breakdown throughout mid-lactation, as evidenced by the highest 3-MH:creatinine, which accounts for muscle breakdown per unit of muscle mass, occurring at 150 DIM. Despite this, the amount of LDD did not change. This may be due to the anabolism and catabolism pathways for muscle protein turnover occurring at a similar rate, resulting in no net change (Schiaffino et al., 2013).

### *Regardless of muscle phenotype, cows did not recover the total muscle reserves present at*

parturition by 300 DIM and, instead, demonstrated a net loss in LDD from 0 to 300 DIM. This may be due to the lack of substantial muscle accretion until 240 DIM. These results imply that available AA were not utilized at a rate sufficient for considerable muscle gain, but instead were utilized for milk protein and, to a lesser extent, fetal growth (Bell et al., 2000; Kuhla et al., 2011). However, LM cows demonstrated a minor accretion of skeletal muscle after nadir was reached but plateaued by 180 DIM before a greater accretion after 240 DIM.

While no reproductive factors were reported in the current study, most animals were successfully bred during the trial. Models for gravid uterine gain utilize the weight of the uterus at parturition which is directly associated with calf birth weight (NASEM, 2021). High muscle cows have demonstrated greater calf birth weights (McCabe et al., 2021; Beckett et al., 2024), suggesting that HM cows may have greater energy and protein requirements for gestation compared with LM cows. This could potentially lead to a lack of muscle accretion to prioritize nutrients toward the developing fetus; however, protein and energy requirements are low until 200 d into gestation but may differ based on muscle phenotype (NASEM, 2021). Evidence from previous work indicates muscle reserves will continue to accrete through the dry period, implying that these reserves may be restored by the next lactation (Mann et al., 2016; McCabe et al., 2021).

Body tissue accretion is regulated by hormonal factors. In early lactation, dairy cows exhibit low plasma insulin concentrations and insulin resistance in peripheral tissues to shunt glucose to the mammary gland for milk production, resulting in reduced circulating insulin (Koster and Opsomer, 2013). Decreased milk production in late lactation allows for an increase in circulating glucose that can be utilized by peripheral tissues which is mainly an insulin-dependent process, thus, increasing circulating insulin for utilization (Sartin et al., 1988). In the current study, insulin concentrations were low in early and mid-lactation but demonstrated a significant increase in late lactation. These elevated insulin concentrations likely allowed for the net accretion of skeletal muscle observed in late lactation by stimulating protein synthesis and downregulating protein breakdown (Dimitriadis et al., 2011). These findings are similar to the ones reported by Accorsi et al. (2005), as insulin remained low until a substantial increase after 210 DIM. Plasma insulin did not differ between muscle phenotypes, which was similarly observed by McCabe et al. (2021).

Muscle phenotype was related to milk production as HM cows produced more milk in early and mid-lactation compared with LM cows. This is contrary to previous research in which cows with lesser muscle reserves produced more milk during early lactation (McCabe et al., 2021). Greater depletion of muscle reserves may allow for greater milk production, as increased abundance of AA and glycogen from muscle depletion can be used as

substrates for gluconeogenesis or directed toward the mammary gland (Kuhla et al., 2011). In early lactation, the nutrient requirements of the mammary gland are increased several-fold, resulting in metabolic adaptations to prioritize mammary uptake of energy, AA, and fatty acids (Bell, 1995). Cows with higher muscle at parturition exhibited significantly greater muscle loss and numerically greater BW loss in early lactation that likely contributed more energy toward milk synthesis. The conversion of metabolizable energy to $NE_L$ is 66% efficient whereas retained energy is converted to $NE_L$ at 89% efficiency, leading to the conclusion that mobilized tissues can be more efficiently partitioned to the mammary gland compared with fed energy (NASEM, 2021). Additionally, the conversion of metabolizable energy to retained energy is 74% efficient (NASEM, 2021). Using these coefficients, the efficiency of converting metabolizable energy to retained energy that is then converted to $NE_L$ is 0.66, similar to that of metabolizable energy to $NE_L$. This suggests that there is no energy cost to the cow in storing energy for later use in lactation. This difference in efficiency may partially explain why HM cows produced more milk in early and mid-lactation. In late lactation, as cows were accreting muscle reserves, HM cows produced less milk than LM cows as they accreted more muscle. This leads to the notion that muscle accretion may be at the cost of milk production. Metabolizable energy is converted to retained energy more efficiently than $NE_L$ (NASEM, 2021). The accretion of muscle stores may be prioritized in late lactation and because energy can be more readily stored than used for milk production, therefore energy is partitioned away from the mammary gland. However, NASEM (2021) does not differentiate the efficiencies of energy partitioning between adipose and muscle tissue and, therefore, more research is needed to determine if the relationship between muscle depletion and milk production.

The contribution of AA toward gluconeogenesis in early lactation is relatively low compared with other glucose precursors (Reynolds et al., 2003; Larsen and Kristensen, 2013) and the AA released from muscle depletion is more likely transported to the mammary gland for milk protein synthesis (Kuhla et al., 2011). Despite this, no differences were observed in milk protein concentration between muscle phenotypes. These findings are similar to the ones reported by McCabe et al. (2021). Tebbe and Weiss (2021a) also reported no effects on milk protein when cows were fed diets adequate in metabolizable protein or balanced for limiting AA. However, HM cows demonstrated more milk fat across lactation, similar to McCabe et al. (2021). While not significant, HM cows did have numerically greater fat reserves at parturition, which, when extensively mobilized, may contribute to greater milk production and milk fat concentration (Nogalski et al., 2012; Siachos et al., 2022). High muscled cows exhibited a greater extent of muscle mobilization, however, a portion of this decrease measured via ultrasonography may be due to intramuscular fat rather than muscle depletion (Megahed et al., 2019). Supporting that intramuscular fat is mobilized with skeletal muscle, HM cows had elevated milk fat concentration, especially in early lactation. As adipose tissue is mobilized, NEFA are released and transported to the liver for gluconeogenesis (Larsen and Kristensen, 2013) or directed toward the mammary gland for milk fat synthesis (Miller et al., 1991). Nonesterified fatty acid concentrations and the change in BFD are similar in both muscle groups, indicating similar extents of fat mobilization and, therefore, similar amounts of milk fat precursors. However, the substrates supplied by muscle depletion may have contributed to gluconeogenesis and allowed more NEFA to be transported to the mammary gland for milk fat synthesis. As previously stated, AA minimally participate in gluconeogenesis, however, muscle glycogen from depleted reserves can be converted to lactate and used for glucose production via the Cori cycle (Kuhla et al., 2011; Larsen and Kristensen, 2013). These contributions may have allowed for greater transport of NEFA to the mammary gland rather than the liver in HM cows.

In the current study, we observed distinct muscle phenotypes that responded differently to similar conditions throughout lactation. Research focused on how nutrition impacts muscle phenotypes and muscle reserve changes is limited and is an area of opportunity. Cows were housed in a freestall barn, which precluded measuring individual intake and is a limitation of this study. More frequent and extensive analysis of blood and milk samples for metabolites and components may have strengthened the study and increased knowledge of metabolic changes and how partitioning of nutrients varied by lactation stage and muscle group. Blood collection monthly would have provided greater insight into the fluctuations in the plasma analytes. Following cows through the dry period would have been of value to evaluate muscle reserves through the entire dry period and the extent of a full lactation to determine if muscle reserves are completely reestablished by the beginning of the subsequent lactation. The main purpose of this study was to observe the changes in the LDD across the length of lactation, therefore, the samples collected were focused around quantification and timing of reserve changes across a long period of time. No reproductive data was evaluated in the present study and is an area of opportunity in future research. Cows throughout the study developed lameness and mastitis at similar rates compared with the rest of the herd, however, it is known that disease states increase glucose requirements which can be met through the depletion of body tissues (Habel and Sundrum, 2020). These cases were not

extreme and therefore cows were not excluded from this study; however, the potential impact that diseased states may have effected muscle reserves.

## CONCLUSION

Cows deplete 30 – 35% of their *longissimus dorsi* muscle reserves in early lactation and appreciable muscle accretion begins in late lactation. Patterns of depletion and accretion of LDD differed between cows with HM and LM, but fat depletion and accretion were similar between the 2 groups. High muscled cows depleted more skeletal muscle reserves in early lactation, likely to support the greater milk production observed in early and mid-lactation compared with low muscled cows. However, HM cows experienced decreased milk production in late lactation compared with low muscle cows, likely due to greater muscle accretion. Ultrasound imaging observations were supported by circulating 3-MH and creatinine concentrations, indicating skeletal muscle was not accreted in mid-lactation, with constant protein breakdown likely occurring at a similar rate as protein synthesis, resulting in no net change. Different muscle phenotypes exhibit differing muscle change dynamics as well as differences in milk production.

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## ORCIDS

Samantha L. Hanno https://orcid.org/0009-0008-2652-6437
Theresa M. Casey https://orcid.org/0000-0002-8835-3550
Hinayah Rojas de Oliveira https://orcid.org/0000-0002-0355-8902
Jacquelyn P. Boerman https://orcid.org/0000-0002-0336-8295