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Skeletal muscle and adipose tissue reserves and mobilisation in transition Holstein cows: Part 1 Biological variation and affecting factors

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ABSTRACT

Nutrient deficit during the periparturient period leads to mobilisation of body energy and protein reserves. Research regarding fat reserves and mobilisation is extensive, while, on the contrary, investigation of muscle mobilisation during the periparturient period is limited. The aim of this cohort study was to simultaneously investigate the biological variation of skeletal muscle and subcutaneous fat reserves together with their mobilisation in transition Holstein cows of different herds, using ultrasonography, and to assess potential affecting factors. For this purpose, ultrasound measurements of *longissimus dorsi* muscle thickness (**LDT**) and backfat thickness (**BFT**) from 238 multiparous cows of six dairy farms were obtained at six time points across the transition period (from 21 days pre- to 28 days postpartum). Concentrations of serum creatinine and non-esterified fatty acids were determined in order to confirm the loss of muscle mass and adipose tissue, respectively. Cases of clinical postparturient diseases and subclinical ketosis (**scKET**) during the first 28 days postcalving were recorded. Cows mobilised on average 32.8% and 37.3% of LDT and BFT reserves, respectively. Large between-cow variation was observed for both the onset and the degree of mobilisation. Time point, initial body condition score and parity were the most important predictors of LDT variation. Cows diagnosed with metritis (**MET**) had lower LDT postpartum and mobilised more muscle depth compared to cows not diagnosed with MET. Initial BCS, time point, initial BW (estimated by heart girth measurement) and parity were the most important predictors of BFT variation. Cows diagnosed with MET mobilised more backfat between −7d and 7d compared to cows not diagnosed with MET. Cows with scKET mobilised more backfat between 7- and 21 days postpartum compared to healthy ones. Variation of subcutaneous fat and skeletal muscle reserves during the transition period was large and affected by herd and several cow-level factors.

Implications

Cows mobilised significant amounts of muscle and fat reserves; mobilisation of both tissues started before calving. Herd, parity, body condition and postparturient disorders were associated with increased variation in both tissue reserve levels and mobilisation. Further research should focus on management practices that minimise excessive tissue mobilisation to improve cow health and welfare.

Introduction

Dairy cows undergo major metabolic adaptations when transitioning from late gestation to early lactation. High dietary requirements to meet glucose, amino acids and mineral demands for foetal growth, mammary gland development, colostrogenesis and gastrointestinal tissue remodelling, combined with reduced DM intake, often set cows in negative nutrient equilibrium even before parturition (Bell et al., 2000; Grummer et al., 2004). High genetic merit for milk yield further exacerbates the negative nutrient balance during early lactation, as milk yield increases at a higher rate than that of DM intake (Grummer et al., 2004). This nutrient deficit, along with an altered, genetically driven, hormonal tissue regulation, leads to mobilisation of body energy and protein reserves (Tamminga et al., 1997). Dairy cows were found to mobilise approximately 50 kg of adipose tissue and 20 kg of body protein during the first 4–5 weeks of lactation (Komaragiri et al., 1998). Research regarding energy balance and fat mobilisation in transition dairy cows is extensive; monitoring body condition score (**BCS**) and/or concentration of blood non-esterified fatty acids

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or β-hydroxybutyrate were the methods commonly used in such studies (McArt et al., 2013; Barletta et al., 2017; Gärtner et al., 2019). Ultrasonographic measurements of backfat thickness have also been employed (Strieder-Barboza et al., 2015; Mann et al., 2016; Szura et al., 2020) for this purpose.

Skeletal muscle tissue represents the most abundant reserve of readily available amino acids, which may contribute to glucose production, during periods of negative protein and/or energy balance (McCabe and Boerman, 2020). Muscle protein catabolism was shown to occur before parturition, regardless of the energy balance status of the cow (van der Drift et al., 2012). Research regarding skeletal muscle mobilisation in transition dairy cows is less extensive and has been done mostly as part of feeding trials (Doepel et al., 2002; Kokkonen et al., 2005; Chibisa et al., 2008). A limited number of observational cohort studies (van der Drift et al., 2012; Pires et al., 2013; Megahed et al., 2019) are also available; muscle protein mobilisation was estimated by means of ultrasonography and/or by measuring the serum concentration of creatinine and plasma concentration of 3-methylhistidine. Van der Drift et al. (2012) studied animals throughout their transition period and in weekly intervals, while Megahed et al. (2019) started measuring skeletal muscle tissue mobilisation at 3 days prepartum. However, a rather limited number of cows were used in the latter studies (32 Holstein-Friesian and crossbred Holstein-Friesian and 106 Holstein-Friesian cows, respectively), all from the same herd, in each case. Additionally, besides parity, the potential effect of factors, such as herd, BCS, BW, and incidence of postparturient diseases, on the onset and extent of both muscle and fat mobilisation, has not been investigated.

Hence, the aim of the present study was to investigate, using ultrasonography, the biological variation of skeletal muscle and subcutaneous fat reserves and mobilisation in transition Holstein cows raised in commercial herds and to assess potential affecting factors.

Material and methods

Farms, animals and study design

The study was conducted from September 2016 to October 2019. A convenience sample of six commercial dairy farms in Central Macedonia, Greece, was selected based on: a) herd size of at least 100 milking Holstein cows, b) availability of headlocks at the feed bunk in both dry-cow and fresh-cow pens, c) use of a computerised herd management software with complete records of reproduction data, and d) milk records, either through individual digital milk recording at each milking, or participation in the official Greek Holstein Association milk recording scheme. Farms, designated as A, B, C, D, E and F, kept 110–360 milking cows with an average milk yield ranging from 9 000 kg to 12 000 kg per cow per lactation. Cows had a mean (±SD) dry period duration of 64.1 (±27) days. All farms, except for farm E, had two groups of dry cows (far-off and close-up pens). Cows were moved to close-up pens in groups of 3–5 cows. Dry cows were housed in open bedded packs, except for farms B (far-off) and E, where they were kept in free stalls. Calving pens were available only on farm C; cows were moved into these pens about 5–7 days before the expected calving date and remained for 3–4 days postcalving. During the postcalving period, cows on all farms, except for farm C, were housed in fresh-cow pens, for 10 days postcalving on farm A and for 21–28 days on all the rest. Fresh cows in all farms, except for farm A (bedded pack), were housed in 2- or 3-row free-stall barns and were milked twice (thrice in farm D) daily. Details regarding dietary management of transition cows are provided in "Supplementary Table S1".

From each farm, a number of multiparous cows representing at least 15% of the milking herd were randomly selected (farm A: n = 32; farm B: n = 39; farm C: n = 20; farm D: n = 41; farm E: n = 51 and farm F: n = 55), resulting in a total of 238 purebred clinically healthy (until calving) multiparous Holstein cows in different parities (2^{nd}: n = 101; 3^{rd}: n = 72, and $\geq 4^{th}$: n = 65). Farms were consecutively enrolled in the study and were visited by the first author (qualified veterinarian) three times per week, after the morning milking (0800–1000), for various measurements described below as well as clinical examination, and blood sampling. The study period was spread over winter months in farms A, C and E, and summer months in farms B, D and F.

Body condition score estimation, longissimus dorsi and backfat thickness measurements

All BCS and ultrasound measurements of *longissimus dorsi* muscle thickness (**LDT**) and backfat thickness (**BFT**) of each cow were assessed by the first author, at six time points relative to the day of calving (0d): −21d; −7d; 0d; 7d; 21d and 28d (± 2 days in all cases), resulting in a total of 1 345 records. Cows that calved > 5 days earlier than expected had no records for −7d. Twelve cows were involuntarily culled during the postpartum period; data prior to culling for these cows remained in the analysis.

Cows were minimally restrained with headlocks at the feed bunk for data collection. At first, cows were scored for BCS on a 5-point scale (1–5) with 0.25-unit increments (Ferguson et al., 1994). Live BW was estimated by measuring heart girth using the equation developed by Heinrichs et al. (1992). Subsequently, LDT and BFT were measured by real-time B-mode ultrasonography, using a portable 5.0–7.5 MHz linear transducer (ImaGo S, IMV imaging, GB) at 80–100 mm depth. Details regarding LDT and BFT measurements are presented in "Supplementary Material S1".

Blood sampling and analyses

A blood sample was collected from each cow at −21d, −7d, 7d, 21d and 28d into 10 ml sterile glass vacuum tubes without anticoagulant (BD Vacutainer®; Plymouth, UK). Samples were placed in a portable cooler immediately after collection. Serum was harvested by centrifugation (3 000*g* × 15 min) within 1–2 h of collection and stored at −40 °C pending analysis. Samples were analysed for determining the concentration of serum creatinine at −21d, −7d, 7d, 21d and 28d, the concentration of non-esterified fatty acids at −21d, −7d, 7d and 21d and the concentration of β-hydroxybutyrate at 7d and 21d with the Siemens ADVIA 1800 Chemistry System. Details regarding reagents and precision tests results are presented in "Supplementary Material S2". A total of 1 083 creatinine, 863 non-esterified fatty acids and 439 β-hydroxybutyrate measurements were available.

Disease definition

Parity distribution of cows and recorded events of postparturient diseases within each herd are presented in "Supplementary Table S2". Clinical cases of retained foetal membranes, milk fever, metritis (**MET**), mastitis, ketosis, left displacement of abomasum (**LDA**) and pneumonia, as well as subclinical ketosis (**scKET**) during the first 28 days postcalving were recorded. Diseases were diagnosed by the first author on visit days (three times per week); disease cases between visit days were either diagnosed by the farms' veterinarians or recorded by farm personnel using predefined protocols and then discussed with the first author to minimise interrater disagreement. Details regarding disease definitions are presented in "Supplementary Material S3". All diseased cows received treatment, based on the established farm treatment regimen.

Statistical analysis

All analyses were performed with IBM SPSS v.25 (Armonk, NY: IBM Corp.). Statistical significance level was set at $P < 0.05$.

Longissimus dorsi thickness, backfat thickness, serum creatinine and non-esterified fatty acids during the transition period

Variables used as potential predictors of LDT, BFT, serum creatinine and non-esterified fatty acids variation included time point relative to calving (six levels), herd, parity (three levels: 2nd; 3rd and ≥4th), initial BCS class (low: <3.00; medium: 3.00–3.50; high: >3.50), presence of each postparturient disease (each tested disease as a binary variable), dry period duration (continuous variable) and initial BW (continuous variable), as main effects and all 2-way interaction terms. The latter two covariates were assessed by building within-herd nested terms. Assessment was conducted with linear mixed models (**LMM**) using time points to specify within-subjects repeated observations, following a restricted estimation maximum likelihood function.

For consistency reasons between dependent variables, all independent variables were inserted in the models as fixed effects regardless of their statistical significance. The random effect of each cow nested within each herd was considered for repeated measurements. The first-order autoregressive covariance structure with heterogeneous variances was selected for all models. Assumptions of normality and homoscedasticity for the linear models were assessed with the visual observation of the Q-Q plots and the predicted values vs residuals plots, respectively ("Supplementary Material S4"). Both LDT and BFT measurements were ln-transformed to achieve normal distribution. Results are reported as back-transformed values. Multicollinearity was precluded by performing several diagnostic tests for regression models ("Supplementary Material S4"). At statistically significant F values, pairwise comparisons between the estimated marginal means were performed using the Bonferroni confidence interval adjustment.

Longissimus dorsi and backfat mobilisation

The changes in LDT (**Δ_LDT**) and BFT (**Δ_BFT**) during the following five study periods: a) −21d to −7d; b) −7d to 0d; c) 0d to 7d, d) 7d to 21d, and e) 21d to 28d were calculated by subtracting the previous measurement from the latter. Variables used as potential predictors of Δ_LDT and Δ_BFT included time period (five levels), herd, parity (three levels: 2nd; 3rd and ≥4th), initial_BCS class (low: <3.00; medium: 3.00–3.50; high: >3.50), presence of each postparturient disease (each tested disease as a binary variable), dry period duration (continuous variable) and initial BW (continuous variable; covariate nested within herd), as main effects and all 2-way interaction terms. Assessment was conducted with LMMs using time periods to specify within-subjects repeated observations. To allow comparison between models, all independent variables were inserted in the models as fixed effects regardless of their statistical significance. The random effect of each cow nested within each herd was considered for repeated measurements. The interaction of time period × cow (with time period as a continuous variable) was also included in the random effect statement. Changes in BFT (Δ_BFT) were calculated following ln-transformation of BFT measurements in order to achieve normality of data distribution. Results are reported as back-transformed values. Model building, assessment of normality and homoscedasticity assumptions ("Supplementary Material S4") and pairwise comparisons for herd and parity effects were performed the same way as described in the previous section.

Results

Cows had a mean BCS (±SD) of 3.29 (±0.60) at the start of the study (−21d) and of 2.78 (±0.79) at the end of the study (28d). The percentages of cows mobilising LDT were 48.4% between −21d and −7d, 83.4% between −7d and 7d, 79.5% between 7d and 21d, and 59.6% between 21d and 28d. The percentages of cows mobilising BFT were 36.6% between −21d and −7d, 83.9% between −7d and 7d, 79.0% between 7d and 21d, and 54.6% between 21d and 28d. The percentages of cows mobilising LDT within those mobilising BFT were 60.9% between −21d and −7d, 85.8% between −7d and 7d, 83.0% between 7d and 21d, and 32.5% between 21d and 28d. The recorded postparturient disease incidence was as follows: retained foetal membranes: 13.9%, metritis: 29.0%, milk fever: 0.8%, mastitis: 5.5%, left displaced abomasum: 3.4%, ketosis: 2.9%, subclinical ketosis: 13.9% and pneumonia: 0.8%.

Longissimus dorsi thickness variation

Factors affecting LDT variation are presented in "Supplementary Table S3". Following a descending F-value order, time point, initial BCS, parity and MET were the most important statistically significant predictors. Moreover, herd and MET interacting with time points affected LDT variation statistically significantly. Random effects accounted for 17.6% of covariance, while residuals accounted for the rest.

Estimated marginal means for LDT and serum creatinine at each time point are depicted in Fig. 1A and 1B, respectively. Estimated marginal means for LDT were quite similar before calving, despite the numerical decrease after −7d. Cows mobilised on average 32.8% of maximum muscle depth until 28d. Similar to LDT, estimated marginal means for serum creatinine decreased by 31.8% between −7d (1.02 mg/dl, 95% CI: 0.93–1.10) and 21d (0.69 mg/dl, 95% CI: 0.63–0.76).

Estimated marginal means for LDT per parity and pairwise comparisons are presented in Table 1. Cows at 2nd parity had higher LDT than 3rd and ≥4th parity ones ($P < 0.05$) during the postpartum period. Cows at 2nd parity mobilised proportionally less muscle (28.7%) than 3rd and ≥4th parity ones (34.4% and 37.0%, respectively) during the study period and ended with greater muscle reserves at 28d.

Estimated marginal means for LDT per initial BCS class and pairwise comparisons are presented in Table 2. Cows at different BCS classes differed ($P < 0.001$) in their LDT measurements at each time point. All three BCS classes followed the same skeletal muscle depletion pattern. Estimated marginal means for high-, medium- and low-BCS cows decreased by 30.7%; 35.2% and 35.9%, respectively, during the whole study period.

Cows diagnosed with MET had statistically significantly lower LDT compared to healthy ones at 7d (25.9 mm, 95% CI: 23.6–28.4 vs 29.3 mm, 95% CI: 26.9–32.0; $P < 0.001$), 21d (22.4 mm, 95% CI: 20.4–24.7 vs 24.2 mm, 95% CI: 22.1–26.5; $P = 0.011$) and 28d (22.1 mm, 95% CI: 19.9–24.5 vs 25.0 mm, 95% CI: 21.8–26.5; $P = 0.006$).

Estimated marginal means for LDT per herd and pairwise comparisons are presented in "Supplementary Table S4". A statistically significantly large variation ($P < 0.05$) of LDT measurements among herds was observed at each time point. However, the trend across time points was similar in almost all herds; cows numerically increased their LDT between −21d and −7d, except for Farms A and B, where there was a numerical decrease. Mobilisation of LDT was intense between −7d and 21d and decelerated thereafter; in some cases, as on farms A and E, it even recovered, though only numerically.

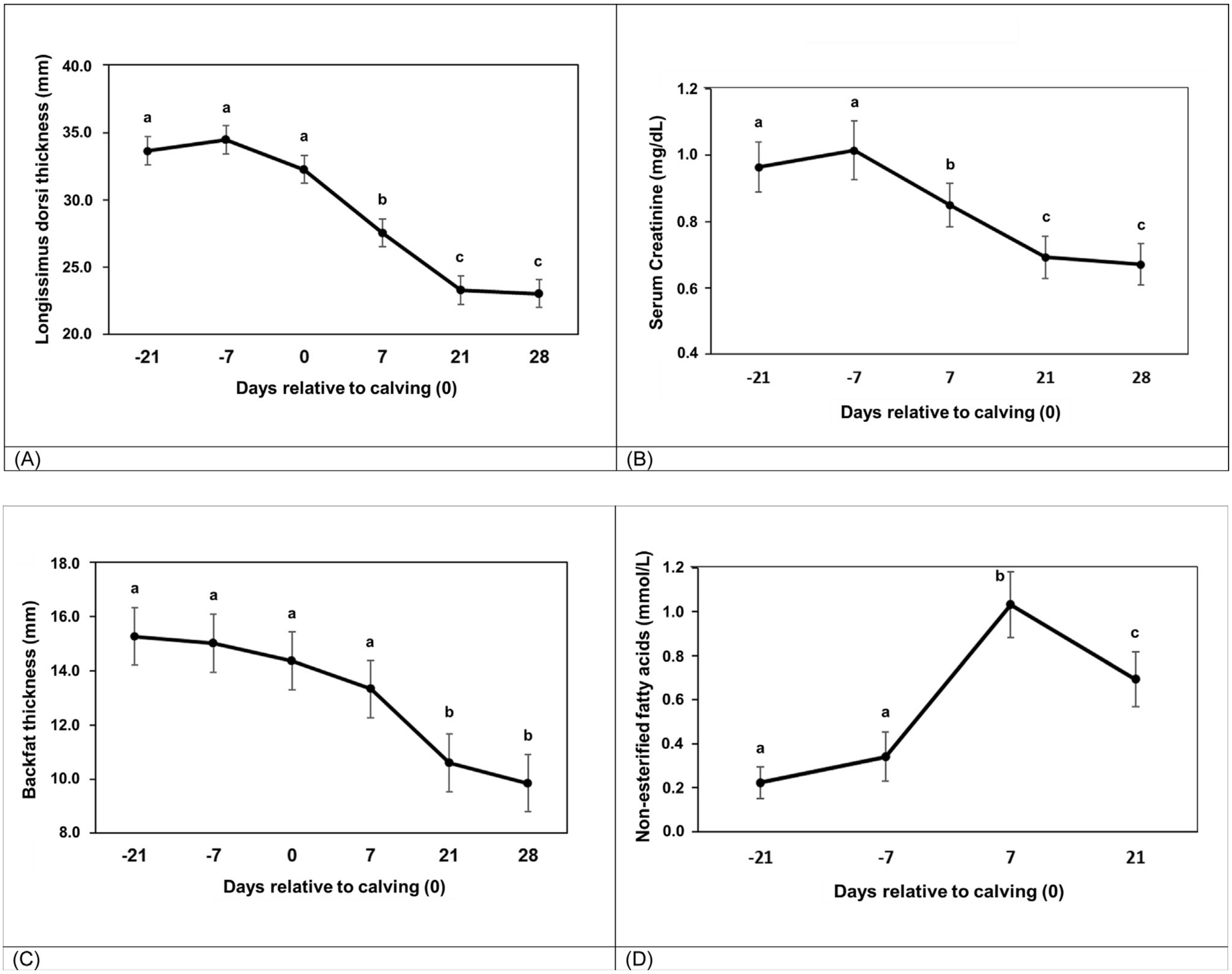

Fig. 1. Estimated marginal means (±95% confidence interval) derived from linear mixed models, showing: (A) the variation of longissimus dorsi thickness, (B) the variation of serum creatinine, (C) the variation of backfat thickness, and (D) the variation of non-esterified fatty acids, measured in 238 multiparous Holstein cows from 21 days prepartum to 28 days postpartum.$^{a\text{-}d}$ Different superscripts denote statistically significant differences at $P < 0.05$.

Table 1
Estimated marginal means (EMMs) showing the variation of longissimus dorsi muscle thickness (LDT) per parity, measured in 238 multiparous Holstein cows of six commercial dairy farms at six time points from 21 days prepartum to 28 days postpartum. Results are shown as back-transformed values from natural log transformation.

Time points	LDT (mm)					
	Parity 2 (n = 101)		Parity 3 (n = 72)		Parity ≥4 (n = 65)	
	EMM	95% CI	EMM	95% CI	EMM	95% CI
−21d	$35.52^{a,A}$	32.5–38.8	$33.82^{a,AB}$	31.1–36.8	$31.72^{a,B}$	28.7–35.1
−7d	$35.98^{a,A}$	32.8–39.4	$34.06^{a,A}$	31.2–37.2	$33.45^{a,A}$	30.1–37.2
0d	$34.74^{a,A}$	31.7–38.2	$31.69^{a,B}$	29.0–34.7	$30.45^{a,B}$	27.4–33.9
7d	$29.55^{bd,A}$	27.0–32.3	$26.87^{b,B}$	24.6–29.3	$26.36^{b,B}$	23.8–29.2
21d	$25.79^{c,A}$	23.4–28.4	$22.81^{c,B}$	20.8–25.0	$21.54^{c,B}$	19.3–24.0
28d	$26.00^{cd,A}$	23.5–28.8	$22.35^{c,B}$	20.2–24.7	$21.07^{c,B}$	18.8–23.6

Abbreviations: CI: Confidence Interval. EMMs: Estimated marginal means represent the mean response for each factor, adjusted for any other variables in the model.
$^{a\text{-}d}$ Different superscripts within the same column denote statistically significant differences among time points within parity at $P < 0.05$.
$^{A\text{-}B}$ Different superscripts within the same row denote statistically significant differences among parities within time point at $P < 0.05$.

Backfat thickness variation

Factors affecting BFT variation during the periparturient period are presented in "Supplementary Table S3". Following a descending F-value order, initial BCS, time point, initial BW and parity were the most important statistically significant predictors. Moreover, herd, MET and scKET interacting with time points affected BFT variation statistically significantly. Random effects accounted for 18.4% of covariance, while residuals accounted for the rest.

Table 2

Estimated marginal means (EMMs) showing the variation of longissimus dorsi muscle thickness (LDT) per initial (at 21 days prepartum) BCS classification, measured in 238 multiparous Holstein cows of six commercial dairy farms at six time points from 21 days prepartum to 28 days postpartum. Results are shown as back-transformed values from natural log transformation.

Time points	LDT (mm)					
	Initial BCS <3.00 (n = 65)		Initial BCS 3.00–3.50 (n = 112)		Initial BCS >3.50 (n = 61)	
	EMM	95% CI	EMM	95% CI	EMM	95% CI
−21d	28.90^{a,A}	26.3–31.7	33.41^{a,B}	30.6–36.5	39.45^{a,C}	35.8–43.4
−7d	29.20^{a,A}	26.5–32.2	35.09^{a,B}	32.0–38.4	40.00^{a,C}	36.2–44.1
0d	28.28^{a,A}	25.6–31.2	31.94^{a,B}	29.1–35.0	37.15^{a,C}	33.6–41.1
7d	23.69^{bd,A}	21.5–26.1	27.91^{b,B}	25.5–30.5	31.66^{b,C}	28.7–34.9
21d	20.25^{c,A}	18.3–22.5	23.52^{c,B}	21.4–25.9	26.60^{c,C}	24.0–29.5
28d	21.01^{cd,A}	18.8–23.4	22.74^{c,A}	20.5–25.2	25.66^{c,C}	23.0–28.6

Abbreviations: CI: Confidence Interval; BCS: Body Condition Score. EMMs: Estimated marginal means represent the mean response for each factor, adjusted for any other variables in the model.

$^{a-d}$ Different superscripts within the same column denote statistically significant differences among time points within initial BCS class at $P < 0.05$.

$^{A-C}$ Different superscripts within the same row denote statistically significant differences among initial BCS classes within time point at $P < 0.05$.

Estimated marginal means for BFT and non-esterified fatty acids at each time point are shown in Fig. 1C and 1D, respectively. Overall, BFT followed a decreasing trend prepartum. Mobilisation mostly started after the first week postpartum. Cows mobilised on average 35.6% of maximum BFT until the end of the study period (28d). Estimated marginal means for non-esterified fatty acids increased statistically significantly at 7d (1.03 mmol/l, 95% CI: 0.88–1.18), coinciding with the beginning of the most intense BFT decrease, and declined thereafter.

Estimated marginal means for BFT per parity and pairwise comparisons are presented in Table 3. Cows at ≥4th parity had constantly the lowest ($P < 0.05$) BFT measurements during the postpartum period. Cows at 2nd parity mobilised the least amount of subcutaneous fat (29.8%), while cows at ≥4th parity mobilised the most (40.5%).

Estimated marginal means for BFT per initial BCS class and pairwise comparisons are presented in Table 4. All BCS classes differed statistically significantly ($P < 0.05$) in their BFT measurements at each time point. However, all three BCS classes followed the same evolution pattern. Decline in BFT mostly started at −7d and was more intense for high-BCS cows. Estimated marginal means for high-, medium- and low-BCS cows decreased by 30.6%; 37.5% and 38.0%, respectively, during the whole study period.

Estimated marginal means for BFT per herd and pairwise comparisons are presented in "Supplementary Table S5". Among herds, cows differed in their BFT measurements at each time point ($P < 0.05$). However, the evolution trend across time points was similar in all herds; a continuous decrease was recorded from −7d until 28d.

Longissimus dorsi muscle mobilisation

Factors affecting LDT mobilisation are shown in "Supplementary Table S6". The model was adjusted for covariates at the following values: dry period duration = 63.4 days and initial_BW = 662 kg. Time period, and interactions of MET, LDA and herd by time period, were statistically significant predictors of Δ_LDT. Random effects accounted for 26.5% of covariance, while residuals accounted for the rest.

Loss of LDT was notable from −7d to 21d, with the most of tissue depth being mobilised during 0d to 7d (Fig. 2A). Daily rate of LDT mobilisation was higher during −7d to 0d and decreased gradually during 0d to 7d and 7d to 21d (Fig. 2B).

Cows diagnosed with MET mobilised 2.75 mm (95% CI: 1.36–4.14, $P < 0.001$) and 1.64 mm (95% CI: 0.17–3.12, $P = 0.030$) more LDT than healthy ones during 0d to 7d and 7d to 21d, respectively. Additionally, cows diagnosed with LDA mobilised 4.04 mm (95% CI: 0.59–7.49 $P = 0.022$) more LDT than healthy ones during 0d to 7d.

Backfat mobilisation

Factors affecting BFT mobilisation are shown in "Supplementary Table S6". The model was adjusted for covariates at the following values: dry period duration = 63.4 days and initial_BW = 662 kg. Time period, parity and interactions of herd, MET and scKET by time period were statistically significant predictors of Δ_BFT. Random effects accounted for 17.9% of covariance, while residuals accounted for the rest.

Table 3

Estimated marginal means (EMMs) showing the variation of backfat thickness (BFT) per parity, measured in 238 multiparous Holstein cows of six commercial dairy farms at six time points from 21 days prepartum to 28 days postpartum. Results are shown as back-transformed values from natural log transformation.

Time points	BFT (mm)					
	Parity 2 (n = 101)		Parity 3 (n = 72)		Parity ≥4 (n = 65)	
	EMM	95% CI	EMM	95% CI	EMM	95% CI
−21d	15.09^{A}	13.3–17.2	15.99^{a,A}	14.1–18.1	14.76^{a,A}	12.7–17.1
−7d	15.21^{a,A}	13.4–17.3	15.61^{a,A}	13.8–17.7	14.25^{a,A}	12.3–16.5
0d	14.73^{a,A}	12.9–16.8	15.07^{b,A}	13.3–17.1	13.34^{b,A}	11.5–15.5
7d	13.85^{b,A}	12.2–15.8	14.10^{c,A}	12.4–16.0	12.12^{c,B}	10.4–14.1
21d	11.21^{c,A}	9.8–12.8	11.28^{d,A}	9.9–12.9	9.39^{d,B}	8.0–11.0
28d	10.68^{c,A}	9.4–12.2	10.22^{e,AB}	8.9–11.6	8.78^{d,B}	7.5–10.2

Abbreviations: CI: Confidence Interval. EMMs: Estimated marginal means represent the mean response for each factor, adjusted for any other variables in the model.

$^{a-e}$ Different superscripts within the same column denote statistically significant differences among time points within parity at $P < 0.05$.

$^{A-B}$ Different superscripts within the same row denote statistically significant differences among parities within time point at $P < 0.05$.

Table 4

Estimated marginal means (EMMs) showing the variation of backfat thickness (BFT) per initial (at 21 days prepartum) BCS classification, measured in 238 multiparous Holstein cows of six commercial dairy farms at six time points from 21 days prepartum to 28 days postpartum. Results are shown as back-transformed values from natural log transformation.

Time points	BFT (mm)					
	Initial BCS <3.00 (n = 65)		Initial BCS 3.00–3.50 (n = 112)		Initial BCS >3.50 (n = 61)	
	EMM	95% CI	EMM	95% CI	EMM	95% CI
−21d	$11.17^{a,A}$	9.7–12.8	$15.77^{a,B}$	13.9–17.9	$20.23^{a,C}$	17.5–23.3
−7d	$11.16^{a,A}$	9.7–12.8	$15.33^{a,B}$	13.5–17.4	$19.79^{a,C}$	17.2–22.8
0d	$10.87^{a,A}$	9.5–12.5	$14.50^{b,B}$	12.7–16.5	$18.80^{b,C}$	16.3–21.7
7d	$9.92^{a,A}$	8.6–11.4	$13.71^{c,B}$	12.0–15.6	$17.39^{c,C}$	15.1–20.1
21d	$8.18^{b,A}$	7.1–9.5	$10.55^{d,B}$	9.2–12.1	$13.75^{d,C}$	11.9–15.9
28d	$7.75^{b,A}$	6.7–8.9	$9.85^{d,B}$	8.6–11.2	$12.54^{e,C}$	10.8–14.5

Abbreviations: CI: Confidence Interval; BCS: Body Condition Score

EMMs: Estimated marginal means represent the mean response for each factor, adjusted for any other variables in the model.

$^{a-e}$ Different superscripts within the same column denote statistically significant differences among time points within initial BCS classification at $P < 0.05$.

$^{A-C}$ Different superscripts within the same row denote statistically significant differences among initial BCS classes within time point at $P < 0.05$.

Loss of BFT was notable from 7d to 21d (Fig. 2A). However, the daily rate of BFT mobilisation was constant during −7d to 21d (Fig. 2B).

Cows diagnosed with MET mobilised 1.07 mm (95% CI: 1.02–1.12, $P = 0.011$) and 1.08 mm (95% CI: 1.02–1.14, $P = 0.005$) more BFT than healthy ones during −7d to 0d and 0d to 7d, respectively. Cows diagnosed with scKET mobilised 1.11 mm (95% CI: 1.01–1.21, $P = 0.027$) more BFT than healthy ones during and 7d to 21d.

Discussion

We investigated the variation of LDT and BFT during the transition period in 238 multiparous Holstein cows raised in six commercial herds with different management and housing strategies. Skeletal muscle mobilisation was confirmed by decreasing LDT and serum creatinine values. Creatinine is considered an indirect indicator of total muscle mass in euhydrated animals with normal renal function (Russell and Roussel, 2007). Accordingly, adipose tissue mobilisation was confirmed by decreasing BFT and increasing non-esterified fatty acid values. We assessed several potential factors associated with LDT and BFT reserves and mobilisation, such as parity, BCS and selected postparturient diseases, during the whole study period and between specific time points, adjusting for animal variability. Accounting for the proportion of total variance due to animal variation renders the estimation of tested fixed factors and covariates unbiased and more accurate.

In contrast to most other studies, van der Drift et al. (2012) reported that fat mobilisation started after parturition, while muscle tissue mobilisation started before parturition. In our study, several cows started mobilising both muscle and fat tissue before parturition, presenting a large variation between time points. Interestingly, while most cows mobilised both muscle and fat between −7d and 0d, several cows mobilised only muscle between −21d and −7d and at the same time period increased their fat reserves. Moreover, several cows were still mobilising fat between 21d and 28d, while at the same time, they had stopped mobilising muscle; all these suggest that the metabolic state of muscle and adipose tissue do not necessarily coincide.

Large variation in both LDT and BFT measurements and their progression during the study period was observed among herds. Nutritional management, in terms of nutrient supply, also differed. For instance, dietary CP content for close-up and fresh cows ranged from 11.0% to 12.5% and from 14.6% to 17.6%, respectively, while dietary metabolisable protein (protein truly digestible in the small intestine, Institut National de la Recherche Agronomique (INRA), 2007) ranged from 6.8% to 9.6% and from 9.5% to 10.9%, respectively. While generally, LDT decreased from −7d to 0d, LDT in cows from farms feeding low CP close-up diets decreased numerically from −21d. During the postpartum period, a common pattern was observed among farms, regardless of the protein feeding plane. However, we did not include any dietary factor in our analysis, since the number of farms was small and feed intake data were not available. Moreover, published research questions the effect of prepartum dietary protein levels on the predicted nitrogen balance and muscle protein mobilisation during the transition period (Overton and Burhans, 2013). Further investigation under well-designed feeding trials is needed to investigate the potential effects of dietary nutrient supply on body reserves mobilisation. Under controlled environmental and dietary conditions, evidence for genetically driven body energy mobilisation during early lactation has been reported (Friggens, et al., 2007); a similar genetic effect on skeletal muscle mobilisation has not been documented, yet.

Megahed et al. (2019) found that primiparous cows have constantly higher LDT compared to multiparous ones, while no differences in BFT were detected during the postpartum period. However, to our knowledge, there are no published data regarding comparisons of BFT and LDT among different parities of multiparous transition cows. In our study, 2nd parity cows had higher LDT than 3rd and ≥4th parity ones during the postpartum period. Additionally, ≥4th parity cows had lower BFT than 2nd and 3rd parity ones. Cows at ≥4th parity also mobilised, proportionally, the most LDT and BFT compared to younger cows. Differences regarding changes in body energy reserves during early lactation among parities have been reported (Friggens et al., 2007). These findings corroborate common clinical experience that older cows face greatest challenges during transition (Vergara et al., 2014). Moreover, it seems that LDT stabilises as cows reach maturity, within the parity range examined in this study. On the other hand, ≥4th parity cows had consistently lower BFT than 3rd parity ones. This phenomenon has never been described, to the best of our knowledge, and needs further investigation.

In the present study, high-BCS cows faced a more intense fat mobilisation compared to medium- and low-BCS ones. However, muscle tissue mobilisation was similar among BCS classes. The latter finding opposes Pires et al. (2013), who reported a higher 3-methylhistidine: creatinine ratio for low-BCS cows at calving compared to medium- and high-BCS ones, suggesting a more intense muscle protein degradation in lean cows. Direct comparisons between the two studies are difficult due to different study design (ultrasound measurements vs blood biomarkers, different BCS classification at different time point) and the small number of cows per BCS group (n = 9–10) in the latter study. The different classification of low-BCS cows (≤2.75) in our study may have masked any negative effects appearing at lower scores. Moreover, the recently reported wide LDT range within each BCS estimate (Siachos et al., 2021) further aggravates comprehension of this issue.

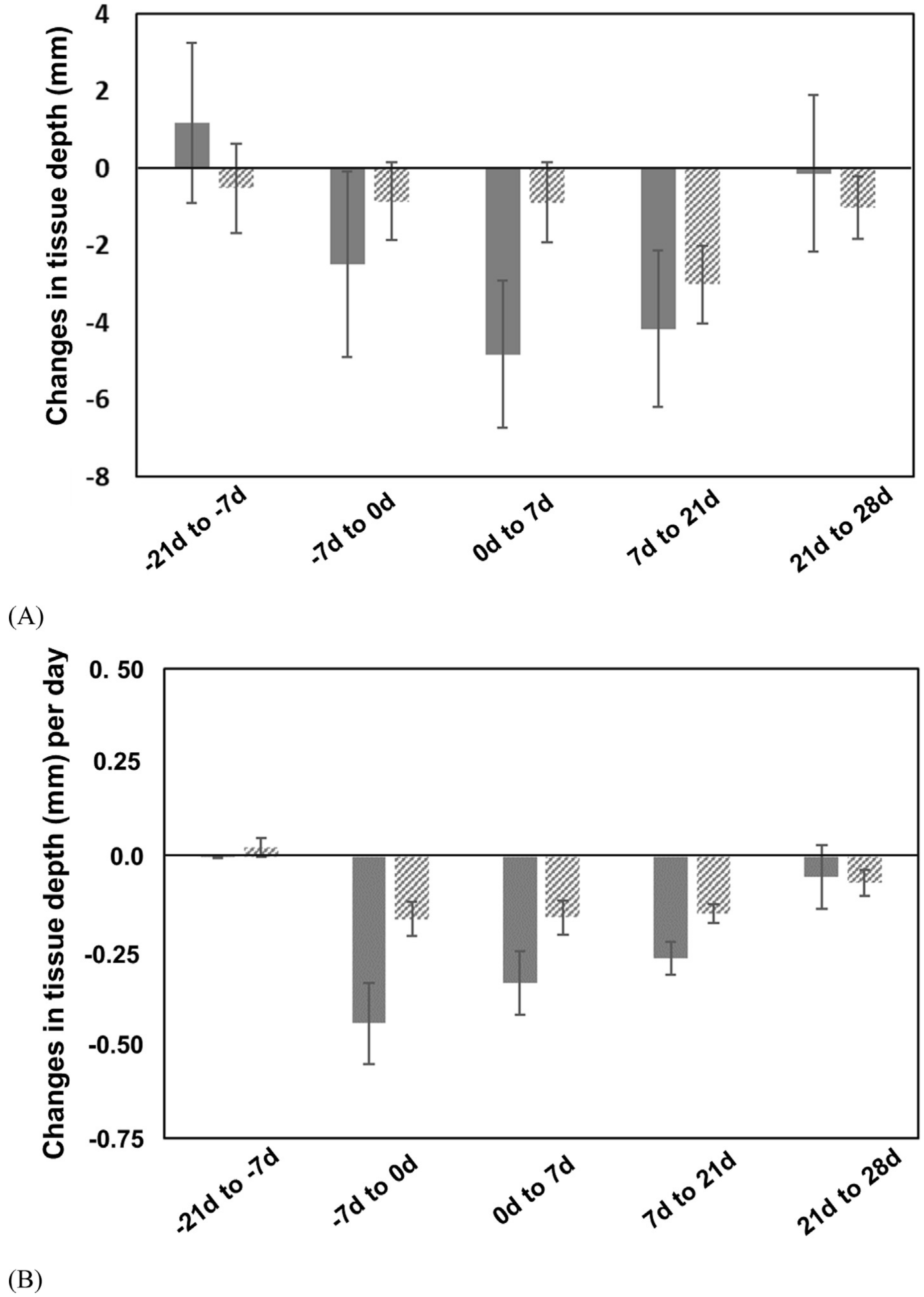

Fig. 2. Estimated marginal means (±95% confidence interval) derived from linear mixed models, showing: A) the changes in longissimus dorsi thickness (Δ_LDT, solid bars) and backfat thickness (Δ_BFT, dash bars), and B) the daily rate of LDT (Δ_LDT, solid bars) and BFT (Δ_BFT, dash bars) changes in 238 multiparous Holstein cows during five time periods from 21 days prepartum to 28 days postpartum. Abbreviations Δ DT: changes in longissimus dorsi thickness; Δ BFT: changes in backfat thickness

Another novelty of the present study is the inclusion of common postparturient diseases in models predicting variation and mobilisation of both LDT and BFT. Postparturient diseases assessed in the present study are either directly or indirectly related to feeding behaviour, nutrient balance and body tissue reserves and their mobilisation during the whole transition period time frame (Grummer et al., 2004; Huzzey et al., 2007). Therefore, any attempt of modelling BFT and LDT in transition dairy cows should consider the associated metabolical or infectious diseases in order to obtain useful results.

Metritis was associated with both BFT and LDT reserves and mobilisation during the transition period. Cows diagnosed with MET mobilised more BFT around parturition compared to healthy ones. Risk for MET has already been statistically associated with negative energy balance and excessive body fat mobilisation (Giuliodori et al., 2013). Moreover, cows diagnosed with MET had lower LDT postpartum and mobilised more LDT between 0d and 21d compared to healthy ones. Skeletal muscle mobilisation postpartum is considered to provide amino acids for hepatic synthesis of acute phase proteins and the overall immune activation and function (Ji and Dann, 2013). Moreover, MET is accompanied by decreased feed intake (Wittrock et al., 2011) and increased intestinal permeability (Horst et al., 2021), exacerbating negative protein balance and aggravating muscle protein breakdown.

Subclinical ketosis was associated only with fat mobilisation. Cows diagnosed with scKET mobilised more BFT between 7d and 21d. This finding was rather anticipated, as hyperketonemia reflects excessive fat mobilisation and the magnitude of negative energy balance during the transition period (McArt et al., 2013). Association between BFT mobilisation and hyperketonemia has also been reported by van der Drift et al. (2012). Analyses of interrelations between subclinical ketosis and LDT or LDT change at any time period were inconclusive. The association of LDT and BFT reserves and mobilisation with the risk for MET, scKET and other postparturient diseases is further investigated in the companion paper (Part 2 of this 2-article study).

The present study has some limitations that should be discussed. Due to logistical/time management, farms were enrolled consecutively in the study. As a result, there were not different "levels" of the variable "season" within each farm; therefore, any potential season effect on muscle and backfat mobilisation could not be distinguished from farm effects. Heat stress aggravates body condition loss during early lactation, not only by decreasing feed intake but also by altering nutrient metabolism towards thermoregulation instead of milk production (Baumgard and Rhoads, 2013). To our knowledge, the possible association of high ambient temperature with muscle protein mobilisation has not been assessed with sufficient sample size (Kamiya et al., 2006; Roths et al., 2020). Moreover, any linkage between milk yield potential and LDT and BFT during the transition period was not assessed in the present study, since neither standardised lactation recordings for previous lactations, nor genetic indices were available for some of the enrolled farms. On the other hand, the main strength of this study was that cows of all farms were closely monitored during the whole transition period by the same person, thus precluding any inter-evaluator bias in the obtained measurements.

Our results showed that a large variation in skeletal muscle and subcutaneous fat tissue reserves and their mobilisation during the transition period was observed in dairy cows. Farm and several cow-level factors, such as parity, BCS and postparturient diseases, were identified as statistically significant predictors. In most cases, mobilisation of both tissues started before parturition, with muscle mobilisation starting at −21d and ending at 21d, while that of backfat started at −7d and continued until the end of the study, at 28d. However, a large among-cows variation was also observed regarding the metabolic state of each tissue at each time period. Further investigation and quantification of the effects of different farm management practices and genetics on muscle tissue reserves and mobilisation could lead to improvements in cow health and welfare during the transition period.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100627.

Ethics approval

The study was conducted in compliance with ethical and institutional guidelines set by the Research Committee of the Aristotle University of Thessaloniki, Greece (approval protocol number 62/15-12-2015).

Data and model availability statement

None of the data were deposited in an official repository. The data/models that support the study findings are available to reviewers.

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Author contribution

N. Siachos: Conceptualisation, Methodology, Investigation, Formal analysis, Writing- Original draft preparation. **G. Oikonomou**: Conceptualisation, Methodology, Software, Writing- Reviewing and Editing. **N. Panousis**: Methodology, Writing- Reviewing and Editing. **V. Tsiamadis**: Software, Writing- Reviewing and Editing. **G. Banos**: Software, Writing- Reviewing and Editing. **G. Arsenos**: Resources, Writing- Reviewing and Editing. **G. Valergakis**: Conceptualisation, Methodology, Investigation, Formal analysis, Writing- Original draft preparation, Supervision.

Declaration of interest

None.

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