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Skeletal muscle and adipose tissue reserves and mobilisation in transition Holstein cows: Part 2 association with postpartum health, reproductive performance and milk production

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ABSTRACT

The aim of this study was, for the first time, to simultaneously assess the association of skeletal muscle and subcutaneous fat reserves and their mobilisation, measured by ultrasonography, with the incidence of specific postparturient health, reproduction, and milk production traits. For this purpose, ultrasound measurements of *longissimus dorsi* thickness (**LDT**) and backfat thickness (**BFT**) from 238 multiparous cows from 6 dairy farms were obtained at 6 time points during the transition period (from 21 days pre- to 28 days postpartum). In each case, LDT and BFT measurements at each time point and LDT and BFT mobilisation variables at each study period were assessed simultaneously. Cases of specific clinical postparturient diseases and subclinical ketosis were recorded. An additional disease trait was used, defined as the presence or absence of at least one clinical condition after calving (**CD_1-28**). The associated disease odds with LDT/BFT variables were assessed with binary logistic regression models. The associated hazard for 1st artificial insemination (**AI**) and for pregnancy by 150 days-in-milk (**PREG_150DIM**) was assessed with Cox proportional hazard models. Moreover, binary logistic models were used to assess the associated odds for pregnancy to 1stAI (**PREG_1stAI**). Finally, association with 30d, 100d and 305d milk yield was assessed with linear regression models. Increased muscle depth during transition was negatively associated with odds for metritis and CD_1-28, while associations with odds for subclinical ketosis were inconclusive. Moreover, increased LDT reserves were associated with greater hazard for 1st AI by 150 days-in-milk, but results were inconclusive regarding odds for PREG_1stAI. Increased LDT mobilisation was associated with increased odds for metritis. Increased BFT reserves were positively associated with odds for metritis, CD_1-28 and subclinical ketosis and with decreased hazard for PREG_150DIM. Increased BFT mobilisation was associated with increased odds for subclinical ketosis and with decreased odds for PREG_1stAI and decreased hazard for PREG_150DIM. Cows with moderate BFT reserves performed better. Finally, increased BFT mobilisation during −21d to −7d from parturition was associated with less milk by 30d and 100d. On the contrary, increased BFT mobilisation during −7d to 7d was associated with more milk by 305d. Metabolism of muscle and fat tissue during transition period was differently associated with different postparturient health, reproduction and milk production traits. In general, greater muscle mass and moderate fat reserves with limited muscle and fat mobilisation were associated with better performance.

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Implications

Cows with higher skeletal muscle and moderate subcutaneous fat reserves with controlled mobilisation of both tissues remained healthier after calving, were more fertile and produced more milk compared to those with lower muscle, higher fat reserves and increased mobilisation.

Introduction

Two decades after Drackley (1999) has highlighted the significance of understanding transition period biology as the final frontier to ameliorate cow health and productivity, transition-related

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health disorders and sub-optimal reproductive performance remain important concerns for the dairy industry (USDA, 2018). This period is characterised by reduced dry matter intake, intense metabolic adaptations and peripartal immunosuppression (Hayirli et al., 2002; Allen and Piantoni, 2013; Bradford et al., 2015). Most metabolic disorders and infectious diseases either occur or have their origin during this time frame.

So far, research has focused mostly on investigating the interactions of energy and macro-mineral dietary management with cow health and performance (Roche et al., 2013; Goff, 2018). Energy balance and fat metabolism during transition and associations with liver function, immune response, reproduction, and milk production have been studied extensively (Leroy et al., 2008; Roche et al., 2013; van Saun and Sniffen, 2014). Energy balance and fat metabolism have been assessed mainly by using indirect estimates (body condition score) and biochemical indices, such as blood non-esterified fatty acids and β-hydroxybutyrate concentrations.

Research regarding protein metabolism during transition is limited (Lean et al., 2013). Cows were found to mobilise about 21 kg of body protein along with 54 kg of adipose tissue 2 weeks before until 5 weeks after parturition (Komaragiri and Erdman, 1997). Cows experience a period of negative protein balance around calving, the degree of which appears to be independent of protein feeding levels prepartum (van Saun and Sniffen, 2014) and occurs even in the absence of negative energy balance (van der Drift et al., 2012). Skeletal muscle protein degradation is considered to occur during periods of negative protein balance to provide free amino acids either to cope with the negative energy balance, through direct oxidation or gluconeogenesis, and/or solely for protein synthesis to meet late gestation and lactation demands (Larsen and Kristensen, 2013).

Excessive fat mobilisation periparturiently has been associated with poor transition cow performance (Roche et al., 2013). On the other hand, the association of skeletal muscle tissue mobilisation during the transition period with health and productivity indices has not been investigated. Moreover, since muscle mobilisation occurs alongside fat mobilisation, either because of negative protein balance and/or as a consequence of negative energy balance, any associations would ideally be assessed in parallel with subcutaneous fat mobilisation, as both tissues contribute to body condition score (Lean et al., 2013). Ultrasonography is an easily applied and objective method to measure both fat and muscle reserves in transition cows, on-site (van der Drift et al., 2012; Megahed et al., 2019; McCabe et al., 2021).

In the companion paper (Part 1 of this study), we described the biological variation of skeletal muscle and subcutaneous fat reserves and mobilisation in transition Holstein cows raised in commercial herds and we assessed potential affecting factors. The aim of the present study was to assess the simultaneous association of skeletal muscle and subcutaneous fat reserves, measured by ultrasonography, and their mobilisation, with the odds for postparturient health, as well as reproduction and milk production traits.

Material and methods

Farms, animals and study design

The study was conducted from September 2016 to October 2019. Two-hundred and thirty-eight purebred multiparous Holstein cows in different parities (2nd: n = 101; 3rd: n = 72, and ≥ 4th: n = 65) were used, from 6 commercial dairy farms in Central Macedonia, Greece (A: n = 32; B: n = 39; C: n = 20; D: n = 41; E: n = 51 and F: n = 55). Extensive information about cows, housing and dietary management during the transition period in the farms of this study are detailed in the companion paper (Part 1 of this study).

Body condition score estimation, longissimus dorsi and backfat thickness measurements

Body condition score was estimated, and ultrasound measurements of *longissimus dorsi* muscle thickness (**LDT**) and backfat thickness (**BFT**) were taken by the first author, at 6 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. Details regarding body condition score estimations and LDT and BFT measurements are presented in the companion paper (Part 1 of this study).

Postparturient diseases

Clinical cases of retained foetal membranes, milk fever, metritis, mastitis, ketosis, left displaced abomasum and pneumonia, as well as subclinical ketosis, during the first 28 days postcalving were recorded. A combined disease trait was defined as at least one clinical disease during the first 28 days postcalving (**CD_1-28**). Details about disease definition, β-hydroxybutyrate analysis and precision tests are presented in the companion paper (Part 1 of this study).

Reproduction traits

Cows in all farms were artificially inseminated (**AI**) after a minimum 50-day voluntary waiting period following visual oestrous detection (twice a day for 30 min). No routine oestrous synchronisation protocols were applied on any enrolled farm. Pregnancy diagnosis was conducted by the farm veterinarians; initial diagnosis was made 32–50 days post-AI by ultrasonography or rectal palpation, while pregnancy was confirmed 2–3 weeks after the initial positive diagnosis. All AI outcomes and pregnancy status by 150 days-in-milk were recorded and retrieved by the farms' records. Calving to 1stAI interval was available for 210 cows, while pregnancy to 1stAI (**PREG_1stAI**) and pregnancy by 150 days-in-milk (**PREG_150DIM**) were available for 204 cows.

Milk production traits

In 4 farms enrolled in this study, individual milk yield recordings at each milking were available, using digital milk meters in the parlour. The other 2 farms participated in the Greek Holstein Association milk recording scheme and had individual monthly recordings, performed by certified technicians. Only records consisting of at least 5 monthly test days were used. Complete milk yield records were available for 218 cows; the 30d, 100d and 305d milk production were calculated with Wood's formula (Wood, 1967).

Statistical analysis

All analyses were performed with IBM SPSS v.25 (Armonk, NY: IBM Corp.). Significance level was set at $P < 0.05$, and tendency was considered at $0.05 \leq P < 0.10$.

Parametrisation of longissimus dorsi and backfat thickness measurements

The association of skeletal muscle and subcutaneous fat with the odds for postparturient diseases, fertility and milk production traits was assessed considering both the reserves at each time point and their relative mobilisation during specific time periods. Separate models for each time point and each time period were used. Skeletal muscle and subcutaneous fat reserves were assessed using LDT and BFT measurements at each time point, respectively, as independent variables. At each time point, LDT/BFT variables were assessed as both continuous and categorical independent variables. For the latter approach, cows were classified into terciles, in order to identify potential non-linear associations. Muscle and fat mobilisation were assessed by creating five mobilisation variables (on a continuous scale) for each tissue: the total LDT and BFT loss during the entire study period and the changes in LDT and BFT (**Δ_LDT/Δ_BFT**) on four designated time periods: −21d to −7d, −7d to 7d, 7d to 21d and 7d to 28d.

To summarise, for the investigation of each health, reproduction and milk production trait, separate models were implemented with the simultaneous assessment of LDT/BFT independent variables as follows:

a) LDT and BFT reserves at each time point as continuous variables,
b) LDT and BFT reserves at each time point as terciles, and
c) LDT and BFT mobilisation parameters for each time period as continuous variables.

Descriptive statistics for LDT and BFT terciles at each time point and their corresponding body condition score estimates are given in "Supplementary Table S1".

Association of skeletal muscle and adipose tissue reserves and their mobilisation with postparturient diseases

Generalised linear models for a binary logistic response were fitted in order to assess the association of LDT/BFT variables with disease odds. Herd, parity and coexisting diseases were also entered as main effects, as were all 2-way interactions. Variables were initially screened with univariable binary logistic regression, and only those with a $P \leq 0.10$ were entered in the models. A backward likelihood ratio elimination method was used; it removed variables with a $P > 0.10$. Herd and parity were forced to remain in the models where significant associations of LDT/BFT variables were detected. The saved probabilities of each model were entered as test variable in a receiver operating characteristic analysis, and the area under the curve was calculated to assess the reliability of each model.

Association of skeletal muscle and adipose tissue reserves and their mobilisation with reproduction traits

Multivariable Cox proportional hazards models were performed to assess the association of LDT/BFT variables, with the hazard for 1^{st}AI and the hazard for PREG_150DIM. Six inseminated cows, out of 210, were culled before being diagnosed for pregnancy to 1^{st}AI and were excluded from the analysis for PREG_1^{st}AI and PREG_150DIM. There were 95 right-censored cases for PREG_150-DIM. Models included LDT/BFT variables, the effect of herd, parity, postparturient disease traits (each disease trait was considered separately) and all 2-way interaction terms. Moreover, in the assessment of hazard for PREG_150DIM, days-in-milk at 1^{st}AI were also entered as a continuous variable in each Cox model. All variables were initially assessed with univariate Cox regressions, and only those with a $P \leq 0.10$ were entered in the multivariable Cox models with a backward likelihood ratio elimination method. Herd and parity were forced to remain in the models where significant associations of LDT/BFT variables were detected. Assumption of proportional hazards for categorical variables was tested by visually inspecting the log-cumulative survival plots [log(-log (survival)) against log(time)]. Assumption was met when plotted lines were parallel (Supplementary material S1). Covariate-adjusted hazard curves were generated for significant categorical LDT/BFT variables.

Generalised linear models for a binary logistic response were performed to assess the simultaneous association of LDT/BFT variables with odds for PREG_1^{st}AI. Models included the effect of LDT/BFT variables, herd, parity, postparturient disease traits (each disease trait was considered separately) and all 2-way interaction terms. Variables were initially screened with univariable binary logistic regression and those with a $P \leq 0.10$ were entered in the final model, which was built following a backward likelihood ratio elimination method. Herd and parity were forced to remain in the models where significant associations of LDT/BFT variables were detected. The saved probabilities of each model were entered as test variable in receiver operating characteristic analysis, and the area under the curve was calculated to assess the reliability of each model.

Association of skeletal muscle and adipose tissue reserves and their mobilisation with milk production

Multivariable regression models using the general linear model procedure of SPSS were performed to assess the simultaneous association of LDT/BFT variables with 30d, 100d and 305d milk production. Models included the effect of LDT/BFT variables, herd, parity, postparturient disease traits (each disease trait was considered separately) and all 2-way interaction terms. Variables with a $P \leq 0.10$ on each milk production outcome at the initial screening were entered as independent variables in the models. Final models were built manually by eliminating variables with a $P > 0.10$. Herd and parity were forced to remain in the models where significant associations of LDT/BFT variables were detected. To achieve normal distribution for the linear models, values for 30d, 100d and 305d milk production were transformed into natural logarithms (Supplementary material S2). Results are reported as back-transformed estimates.

Results

The recorded postparturient disease incidence is reported in the companion paper (Part 1 of this study). Associations between LDT/BFT variables with postparturient diseases were statistically significant for metritis, CD_1-28 and subclinical ketosis.

Association with postparturient diseases

Metritis

All statistically significant covariate-adjusted associations of LDT/BFT variables with the odds of being diagnosed with metritis are presented as odds ratios (**ORs**) in Tables 1 and 2, and detailed in "Supplementary table S2". All models with statistically significant LDT/BFT associations yielded statistically significant area under the curve (ranging from 0.77 to 0.82). Herd and retained foetal membranes, but not parity, were statistically significant covariates; cows diagnosed with retained foetal membranes had almost 4 times higher odds ($P < 0.001$) for metritis, compared to healthy ones.

A 1 mm-higher LDT at −7d, 7d, 21d and 28d was statistically significantly associated with decreased odds of being diagnosed with metritis, which ranged from 6.8 % [OR = 0.93, 95 % confidence interval (**CI**): 0.87–1.00, $P = 0.025$] to 10.1 % (OR = 0.90, 95 % CI: 0.83–0.98, $P < 0.001$). Similarly, cows classified at the upper tercile at 7d, 21d and 28d had lower odds of being diagnosed with metritis by 66.6 % (OR = 0.33, 95 % CI: 0.12–0.92, $P = 0.033$) to 86.1 % (OR = 0.14, 95 % CI: 0.04–0.47, $P < 0.002$), compared to those classified at the lower tercile.

Over the entire study period, 1 mm-higher total LDT loss was associated with higher odds of being diagnosed with metritis (OR = 1.08, 95 % CI: 1.03–1.14, $P = 0.002$). Additionally, a 1 mm-

Table 1
Simultaneous associations of *longissimus dorsi* (LDT) and backfat thickness (BFT) variables in continuous scale with postparturient diseases and reproduction traits in 238 multiparous Holstein cows, as odds ratios (ORs) or hazard ratios (HRs) and 95 % confidence intervals (CIs). Each section represents a separate model with LDT/BFT parameters as independent variables, for a specific time point or time period. Only statistically significant associations (P < 0.05) are shown.

	Health traits			Reproduction traits		
Variable[1]	MET[2] OR (95 % CI)	CD_1-28[3] OR (95 % CI)	scKET[4] OR (95 % CI)	1[st]AI_150DIM[5] HR (95 % CI)	PREG_1[st]AI[6] OR (95 % CI)	PREG_150DIM[7] HR (95 % CI)
LDT −21d				1.03 (1.01–1.06)		
BFT −21d			1.13 (1.05–1.22)			0.97 (0.94–1.00)
LDT −7d	0.93 (0.87–1.00)			1.04 (1.01–1.06)		
BFT −7d			1.11 (1.03–1.18)			
LDT 0d				1.04 (1.02–1.05)		
BFT 0d			1.12 (1.04–1.21)			
LDT 7d	0.90 (0.83–0.98)	0.91 (0.86–0.97)		1.06 (1.03–1.08)		
BFT 7d	1.18 (1.09–1.27)		1.16 (1.07–1.26)			
LDT 21d	0.923 (0.86–0.97)	0.92 (0.87–0.99)		1.04 (1.02–1.06)		
BFT 21d						
LDT 28d	0.93 (0.86–0.98)	0.93 (0.88–0.99)		1.05 (1.03–1.07)		
BFT 28d						
Δ_LDT −21d to −7d						
Δ_BFT −21d to −7d						
Δ_LDT −7d to 7d	1.24 (1.06–1.45)					
Δ_BFT −7d to 7d	1.13 (1.04–1.22)					
Δ_LDT 7d to 21d	1.08 (1.00–1.16)					
Δ_BFT 7d to 21d			0.68 (0.56–0.83)			
Δ_LDT 7d to 28d						
Δ_BFT 7d to 28d						
Total LDT loss	1.08 (1.03–1.14)					
Total BFT loss			1.14 (1.00–1.29)		0.83 (0.74–0.94)	0.93 (0.87–0.97)

Abbreviations: Δ_LDT: changes in LDT; Δ_BFT: changes in BFT; AI: artificial insemination.
[1] variables in continuous scale (mm).
[3] MET: metritis.
[4] CD_1-28: at least one clinical disease diagnosed between 1d and 28d postpartum.
[5] scKET: subclinical ketosis.
[6] 1[st]AI_150DIM: hazard for 1[st]AI by 150 days-in-milk.
[7] PREG_1[st]AI: odds for pregnancy to 1[st]AI [8]PREG_150DIM: hazard for pregnancy by 150 days-in-milk.

higher LDT loss between −7d and 7d was associated with increased odds of being diagnosed with metritis by 23.7 % (OR = 1.24, 95 % CI: 1.06–1.45; P = 0.004).

A 1 mm-higher BFT at 7d was associated with increased odds of being diagnosed with metritis by 17.8 % (OR = 1.18, 95 % CI: 1.09–1.27; P = 0.013). Additionally, a 1 mm-higher BFT loss between −7d and 7d was associated with increased odds of being diagnosed with metritis by 12.7 % (OR = 1.13, 95 % CI: 1.04–1.22; P = 0.008).

Clinical diseases 1–28 days postcalving

All covariate-adjusted associations of LDT/BFT variables with the odds of being diagnosed with CD_1-28 are presented as OR in Tables 1 and 2, and detailed in "Supplementary Table S3". All models with statistically significant LDT/BFT associations yielded statistically significant area under the curve (ranging from 0.73 to 0.75). Herd, and in some cases parity, were identified as statistically significant covariates; $\geq 4^{th}$ parity cows had increased odds of being diagnosed with CD_1-28 by 60.2 % (P = 0.015) and 59.0 % (P = 0.026) compared to 2nd and 3rd parity cows, respectively.

A 1 mm-higher LDT at 7d, 21d and 28d was associated with decreased odds of being diagnosed with CD_1-28, which ranged from 6.7 % (OR = 0.93, 95 % CI: 0.88–0.99, P = 0.024) to 8.8 % (OR = 0.91, 95 % CI: 0.86–0.97, P = 0.002). Similarly, cows classified at the upper tercile at 21d and 28d were associated with lower odds of being diagnosed with CD_1-28 (OR = 0.34, 95 % CI: 0.12–0.94, P = 0.037 and OR = 0.34, 95 % CI: 0.12–0.91, P = 0.033, respectively), compared to those of the lower tercile.

On the contrary, cows classified at the intermediate upper BFT tercile at −21d and 7d were associated with lower odds of being diagnosed with CD_1-28 compared to those classified at the upper tercile (OR = 0.36, 95 % CI: 0.17–0.77, P = 0.013 and OR = 0.46, 95 % CI: 0.17–0.81, P = 0.013, respectively).

Subclinical ketosis

All covariate-adjusted associations of LDT/BFT variables with the odds of being diagnosed with subclinical ketosis are presented as OR in Tables 1 and 2, and detailed in "Supplementary table S4". All models with statistically significant LDT/BFT associations yielded statistically significant area under the curve (ranging from 0.77 to 0.82). Herd was consistently the only statistically significant covariate.

Associations of subclinical ketosis with LDT measurements at any time point were inconclusive. Regarding LDT mobilisation, over the entire study period, a 1 mm-higher total LDT loss was associated only with a tendency for higher odds of being diagnosed with subclinical ketosis (OR = 1.08, 95 % CI: 0.99–1.17, P = 0.078).

On the contrary, a 1 mm-higher BFT at −21d, −7d, 0d and 7d, was associated with increased odds of being diagnosed with subclinical ketosis, which ranged from 10.5 % (OR = 1.11, 95 % CI: 1.03–1.18, P = 0.005) to 16 % (OR = 1.16, 95 % CI: 1.07–1.26, P < 0.001). Similarly, cows classified at the lower and intermediate tercile at −21d, −7d, 0d, 7d, 21d and 28d were associated with decreased odds of being diagnosed with subclinical ketosis, which ranged from 69.5 % (OR = 0.31, 95 % CI: 0.10–0.96, P = 0.042) to 90.6 % (OR = 0.09, 95 % CI: 0.02–0.37, P < 0.001), compared to those classified at the upper tercile.

Over the entire study period, 1 mm-higher total BFT loss was associated with higher odds of being diagnosed with subclinical ketosis (OR = 1.14, 95 % CI: 1.00–1.29, P = 0.044). Between 7d and 21d, a 1 mm-lower BFT loss was associated with decreased

Table 2
Simultaneous associations of *longissimus dorsi* (LDT) and backfat thickness (BFT) variables in categorical scale with postparturient diseases and reproduction traits in 238 multiparous Holstein cows, as odds ratios (ORs) or hazard ratios (HRs) and 95 % confidence intervals (CIs). Each section represents a separate model with LDT/BFT parameters as independent variables, for a specific time point. Only statistically significant associations (*P* < 0.05) are shown.

Terciles[1]	Health traits			Reproduction traits		
	MET[2] OR (95 % CI)	CD_1-28[3] OR (95 % CI)	scKET[4] OR (95 % CI)	1stAI_150DIM[5] HR (95 % CI)	PREG_1stAI[6] OR (95 % CI)	PREG_150DIM[7] HR (95 % CI)
LDT −21d (L)				Ref		
(I)						
(U)				1.71 (1.20–2.42)		
BFT −21d (L)			0.15 (0.04–0.51)			1.74 (1.08–2.81)
(I)		0.36 (0.17–0.77)	0.26 (0.09–0.80)			1.90 (1.17–3.08)
(U)		Ref	Ref			Ref
LDT −7d (L)				Ref		
(I)						
(U)				1.85 (1.25–2.72)		
BFT −7d (L)			0.11 (0.03–0.44)			
(I)			0.29 (0.09–0.91)			
(U)			Ref			
LDT 0d (L)				Ref		
(I)						
(U)				2.05 (1.44–2.91)		
BFT 0d (L)			0.11 (0.03–0.41)			
(I)			0.31 (0.10–0.96)			
(U)			Ref			
LDT 7d (L)	Ref			Ref		
(I)	0.30 (0.12–0.74)					
(U)	0.14 (0.04–0.47)			2.01 (1.42–2.85)		
BFT 7d (L)			0.09 (0.02–0.37)			
(I)		0.46 (0.17–0.81)	0.26 (0.08–0.83)			
(U)		Ref	Ref			
LDT 21d (L)	Ref	Ref		Ref		
(I)	0.30 (0.12–0.75)	0.41 (0.18–0.93)				
(U)	0.33 (0.12–0.92)	0.34 (0.12–0.94)		1.57 (1.24–2.48)		
BFT 21d (L)			0.20 (0.06–0.68)			
(I)						
(U)			Ref			
LDT 28d (L)	Ref	Ref		Ref		
(I)	0.31 (0.11–0.84)	0.32 (0.13–0.89)		1.82 (1.28–2.58)		
(U)	0.23 (0.09–0.62)	0.34 (0.12–0.91)		1.90 (1.35–2.68)		
BFT 28d (L)			0.24 (0.07–0.76)			
(I)			0.26 (0.08–0.83)			
(U)			Ref			

Abbreviations: AI: artificial insemination; Ref: reference category.
1 terciles: variables in categorical scale, classified into terciles (U, I and L: Upper, Intermediate and Lower tercile).
2 MET: metritis.
3 CD_1-28: at least one clinical disease diagnosed between 1d and 28d postpartum.
4 scKET: subclinical ketosis.
5 1st AI_150DIM: hazard for 1stAI by 150 days-in-milk.
6 PREG_1stAI: odds for pregnancy to 1stAI.
7 PREG_150DIM: hazard for pregnancy by 150 days-in-milk.

odds of being diagnosed with subclinical ketosis (OR = 0.68, 95 % CI: 0.56–0.83, *P* < 0.001).

Association with reproductive traits

Hazard ratios for 1st artificial insemination by 150 days-in-milk

All covariate-adjusted associations of LDT/BFT variables with the proportional hazard for 1stAI by 150 days-in-milk are presented as hazard ratios (**HRs**) in Tables 1 and 2, and detailed in "Supplementary table S5". Subclinical ketosis was the only statistically significant covariate; cows diagnosed with subclinical ketosis were associated with lower proportional hazard for 1stAI by 150 days-in-milk, by about 40 % (*P* < 0.05).

A 1 mm-higher LDT at −21d, −7d, 0d, 7d, 21d and 28d was associated with higher proportional hazard for 1stAI by 150 days-in-milk, which ranged from 3.0 % (HR = 1.03, 95 % CI: 1.01–1.06; *P* = 0.014) to 5.9 % (HR = 1.06, 95 % CI: 1.03–1.08; *P* < 0.001). Similarly, cows classified at the upper tercile at −21d, −7d, 0d, 7d, 21d and 28d were associated with higher proportional hazard for 1stAI by 150 days-in-milk, which ranged from 1.70 times (HR = 1.71, 95 % CI: 1.20–2.72, *P* = 0.003) to 2.05 times (HR = 2.05, 95 % CI: 1.44–2.91, *P* < 0.001, Fig. 1), compared to those of the lower tercile. Associations between BFT variables and hazard for 1stAI were inconclusive.

Odds ratios for pregnancy to 1st artificial insemination

Covariate-adjusted associations of LDT/BFT variables with odds for PREG_1stAI are presented as OR in Table 1, and detailed in "Supplementary table S6". The models with statistically significant LDT/BFT associations yielded statistically significant area under the curve (0.75). Associations between LDT variables and PREG_1stAI were inconclusive. Over the entire study period, a 1 mm-higher total BFT loss was associated with lower odds for PREG_1stAI (OR = 0.83, 95 % CI: 0.74–0.94; *P* = 0.002).

Hazard ratios for pregnancy by 150 days-in-milk

All associations of LDT/BFT variables with the proportional hazard for PREG_150DIM, adjusted for covariates, are presented as HR

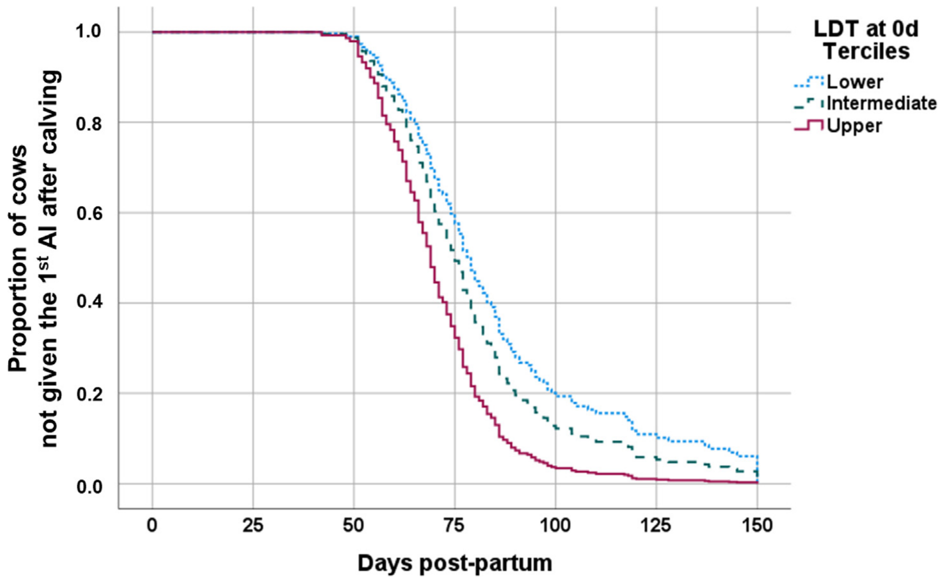

Fig. 1. Covariate-adjusted Cox hazard curves showing the proportional hazard for 1st artificial insemination (AI) by 150 days-in-milk among terciles of *longissimus dorsi* thickness (LDT) at calving (0d). Cows classified at the upper tercile were associated with higher hazard for 1stAI by 150 days-in-milk by 2.05 times (hazard ratio = 2.05; 95 % confidence interval: 1.44–2.91, P < 0.001) compared to those classified at the lower tercile.

in Table 1, and detailed in "Supplementary table S7". Days-in-milk at 1stAI was consistently associated with hazard for PREG_150DIM; a 1 day-higher days-in-milk at 1stAI was associated with lower hazard for PREG_150DIM by approximately 3.5 % (P < 0.001). Subclinical ketosis was also identified as a statistically significant covariate. Cows diagnosed with subclinical ketosis were associated with lower proportional hazard for PREG_150DIM, by 46 % (P = 0.023).

Cows classified at the upper tercile at 28d were associated with higher hazard for PREG_150DIM (HR = 1.84, 95 % CI: 1.56–3.18; P = 0.029) compared to those classified at the lower tercile.

On the contrary, a 1 mm-higher BFT at −21d was associated with lower hazard for PREG_150DIM (HR = 0.97, 95 % CI: 0.94–1.00; P = 0.044). Similarly, cows classified at the intermediate tercile at −21d and 28d were associated with increased hazard for PREG_150DIM compared to those classified at the upper tercile (HR = 1.90, 95 % CI: 1.17–3.08; P = 0.010, and HR = 1.61, 95 % CI: 1.01–2.57; P = 0.046, respectively).

Over the entire study period, a 1 mm-higher total BFT loss was associated with lower hazard for PREG_150DIM (HR = 0.93, 95 % CI: 0.87–0.97; P = 0.004).

Association with milk production traits

All covariate-adjusted associations of LDT/BFT variables with milk production traits are presented as ln-transformed estimates in "Supplementary Table S8". Below, results are reported as back-transformed values.

A 1 mm-higher LDT at 0d was associated with 2.9 L more milk by 30d (95 % CI: 0.0–6.4; P = 0.037). Cows classified at the upper BFT tercile at −21d were associated with 1 550 L more milk by 305d (95 % CI: 49–2 866; P = 0.043) compared to those classified in the lower tercile. Between −21d and −7d, a 1 mm-higher BFT loss was associated with 8.6 L less milk by 30d (95 % CI: 1.3–15.9; P = 0.045) and 32.5 L less milk by 100d (95 % CI: 0.0–64.6; P = 0.047). On the contrary, between −7d and 7d, a 1 mm-higher BFT loss was associated with 257 L more milk by 305d (95 % CI: 39–484; P = 0.021).

Discussion

In the present study, we investigated the association of peri-parturient skeletal muscle and subcutaneous fat reserves and their mobilisation with the odds for postparturient diseases, reproduction, and milk production in dairy cows of 6 commercial farms. So far, research on muscle metabolism has been conducted in a limited number of cows always from one farm. Our approach has a series of novelties; the association of muscle reserves and mobilisation with cow health and performance has never been assessed before. Moreover, at each time point and study period, relevant LDT and BFT measurements and changes, respectively, were assessed simultaneously to provide an integrated assessment. The inclusion of cows from multiple farms applying different nutritional programmes broadens the range of biological variation in our sample and renders results relevant to the wider Holstein population. Muscle and backfat reserves and their mobilisation were found to be differently associated with odds for diseases, reproduction and milk production outcomes.

Association with odds for postparturient diseases

Skeletal muscle and adipose reserves during transition were contrariwise associated with odds of being diagnosed with metritis. Odds for metritis decreased at higher LDT and lower BFT reserves. During the prepartum period, significant associations were obtained for LDT at −7d, while a tendency was observed for measurements at −21d and 0d. The association of increased muscle mass prepartum with metritis could be due to an indirect effect of optimal dry matter intake and nutrient balance before calving, reflecting greater lean body mass, and/or a direct benefit of high labile protein reserves on efficiently supporting the innate immune response and eliminating the bacterial challenge. Bacteria are found in the uterine lumen of almost all cows after calving; in most cases, cows manage to eliminate them within 2 weeks (Sheldon et al., 2006). Pathogens activate innate immune defences, including toll-like receptors, acute phase proteins, antimicrobial peptides, cytokines and chemokines, thus eliciting the migration of neutrophils and macrophages (Sheldon et al., 2009). Recruitment and proliferation of immune cells and the production of molecules such as cytokines and chemokines require energy and amino acids (Ji and Dann, 2013). An acute immune response to induced endotoxemia after lipopolysaccharide administration has been estimated to consume more than 1 kg of glucose within 12 h postinfection (Kvidera et al., 2017). Unfortunately, the protein requirements of a fully activated immune system of dairy cows have not been determined, yet. One can assume that longer inflammatory stimuli require even more fuel, as well as amino acids, to efficiently cope with the challenge.

Cows with metritis spend less time at the feed bunk and consume less feed prepartum than those that remain healthy (Huzzey et al., 2007). The association of higher LDT reserves prepartum with lower odds for metritis may result from increased body reserves enabling cows better to respond to an infection challenge postpartum. Moreover, the association of intense muscle mobilisation with metritis between −7d and 7d, e.g. the period that precedes its diagnosis, represents the increased inflammation-induced metabolic demands. On the other hand, the association detected between LDT at 21d and 28d and the odds of being diagnosed with metritis is most probably the direct result of intense muscle mobilisation postchallenge. Uterine inflammation increases cow nutrient requirements to support the immune system while causing a reduction in dry matter intake (Bradford et al., 2015). During disease, muscle protein degradation releases amino acids, which are used by the liver and the immune system (Karinch et al., 2001). In severe cases, when infection progresses into sepsis, a dramatic reduction in protein synthesis rate and severe muscle atrophy can be induced in just a few days (Cooney et al., 1999). The association of intense LDT mobilisation with metritis, between −7d and 7d, which coincides with the actual bacterial challenge, corroborates the notion that muscle reserves are indeed used to support the immune system and, therefore, they should be plentiful prepartum. Further research is needed to define whether this association is due to a causal relationship between the degree of muscle catabolism and immune efficiency of peri-parturient cows.

Excessive negative energy balance and fat mobilisation have been associated with increased odds for metritis and impaired polymorphonuclear cell function (Hammon et al., 2006; Ospina et al., 2010; Giuliodori et al., 2013). The simultaneous assessment of muscle and fat in our study revealed the opposite association of each tissue's reserves with odds for metritis. Although mobilisation of both LDT and BFT between −7d and 7d was associated with increased odds for metritis, associations of fat reserves and fat mobilisation during the succeeding period with metritis were inconclusive, while those of LDT were statistically significant. This finding suggests that inflammation and immune system activation probably affects more severely skeletal muscle metabolism rather than that of adipose tissue. Therefore, fat and muscle mobilisation are concurrently happening metabolic events in transition cows but should be measured separately.

Similarly to results obtained for metritis, higher skeletal muscle reserves during the postpartum period were associated with decreased odds for CD_1-28. Despite the fact that CD_1-28 is a variable pooling infectious and metabolic clinical conditions involving different pathophysiologic mechanisms, it seems that cows with higher muscle mass periparturiently may successfully overcome inflammatory challenges and muscle loss postpartum is probably the result of increasing nutrient demands in support of lactation and following activation of the immune system. As anticipated, cows with moderate fat reserves prepartum had lower odds for CD_1-28 compared to over-conditioned ones. As already reported, over-conditioned cows are probably experiencing substantial reduction in DM intake both prepartum and postpartum, failing to meet energy requirements (Ingvartsen and Andersen, 2000; Hayirli et al., 2002).

Our results were inconclusive regarding an association of muscle reserves with odds for subclinical ketosis, despite a tendency for an association with muscle mobilisation during the entire study period. Van der Drift et al. (2012) found a negative association between the 3-methylhistidine plasma concentration and β-hydroxybutyrate serum concentration in cows, after excluding 3 cows, with severe hyperketonemia, from the analysis. They suggested that muscle protein degradation during periods of negative energy balance may restrict β-hydroxybutyrate production by providing glucogenic precursors. Whether higher muscle protein degradation provides additional carbon chains from amino acids as precursors for gluconeogenesis is highly debatable (Larsen and Kristensen, 2013).

Subcutaneous fat reserves and mobilisation were the main variables of interest associated with subclinical ketosis odds. As anticipated, cows with low and moderate fat reserves prepartum and during the entire study period had lower odds for subclinical ketosis than over-conditioned ones. Hyperketonemia reflects excessive liver fat accumulation from non-esterified fatty acids entering hepatocytes, beyond liver's capacity to secrete triglycerides (re-esterified fatty acids) as very low-density lipoproteins into bloodstream (Drackley et al., 2001). Our findings corroborate current knowledge, while quantifying effects in tissue depth (mm) with the use of ultrasound measurements, instead of body condition score estimations.

Association with reproduction

In our analysis, postparturient diseases were included in the models as potential predictors, but only subclinical ketosis was found to be negatively associated with reproductive outcomes, even though metritis has been constantly associated with impaired fertility (Dubuc et al., 2011; Giuliodori et al., 2013; Mahnani et al., 2015). This may be due to type II error; our study was not specifically designed to detect such differences. Moreover, since cows were closely monitored, all cases were diagnosed early and treated properly, thus reducing the prospective negative effect on fertility.

Any association of skeletal muscle reserves and mobilisation during the transition period with reproduction indices has not been published before, to the best of our knowledge. After adjusting for herd and parity effects, cows with greater muscle mass during the whole transition period had a higher hazard for 1^{st}AI by 150 days-in-milk, while the contribution of fat reserves was inconclusive. On the other hand, odds for PREG_1^{st}AI and hazard for PREG_150DIM were found to be associated with fat reserves and fat mobilisation; the greater the fat reserves and mobilisation periparturiently, the lower the odds and hazard, respectively. This interesting disparity probably indicates different effects of each tissue's degree of mobilisation or levels on physiological mechanisms associated with cow fertility. The effects of energy status and negative energy balance on the hypothalamus-pituitary-ovary-uterus axis have been investigated and described extensively; they are mainly associated with low glucose, insulin and insulin-like growth factor-I concentrations, delayed first ovulation, impaired oocyte quality and corpus luteum function (Lucy, 2001; Leroy et al., 2008).

The effects of fat reserves (moderate BFT was best) and mobilisation on reproduction (odds for PREG_1^{st}AI and hazard for PREG_150DIM) were well in line with current knowledge (López-Gatius et al., 2003; Cardoso et al., 2013). However, the use of ultrasound measurements allowed us to quantify the effects in mm of tissue depth; to the best of our knowledge, there are no similar published data in the literature, yet. Overall, results of the present study indicate that resumption of ovarian cyclicity and oestrous behaviour seem associated mainly with muscle reserves, while conception rate following 1st AI and pregnancy by 150 days-in-milk seems associated mainly with fat reserves.

Association with milk production

Associations of LDT variables with overall milk production were either minimal or inconclusive. Recently, McCabe et al. (2021) reported that cows with higher LDT reserves prepartum (-35d) had a lower milk production during the first 60 days-in-milk compared to cows with lower LDT reserves at the same time point. This was not found in our study. On the other hand, we confirmed the positive correlation between mobilisation of fat reserves and milk production in modern high-yielding cows (Roche et al., 2013). The physiological mechanisms (low blood insulin and low tissue insulin sensitivity) have been described (De Koster and Opsomer, 2013). The more fat mobilised after −7d, the more milk was produced during early and the whole lactation. However, this was also associated with higher odds for disease and impaired reproductive performance. Moreover, fat mobilisation between −21d and −7d was negatively associated with milk production during early lactation and yielded higher odds for clinical disease and impaired reproduction; management practices focusing in avoiding this development must be adopted.

As already mentioned in the companion paper, the lack of different "levels" of the variable "season" within each farm, due to study design, did not allow us to adjust for any potential season effect on muscle and backfat mobilisation. This is a limitation of the present study. Moreover, reported associations with odds for metritis may be underestimated, since diagnosis was performed by rectal palpation only and some cases may have been undiagnosed, despite the close monitoring of cows.

Based on our results, skeletal muscle tissue reserves and their mobilisation during the transition period were associated with odds for postparturient disease and with reproductive performance. Muscle and fat metabolism during transition are differently associated with disease odds. Moreover, muscle metabolism appears associated with calving to first oestrous interval, while fat metabolism is associated with pregnancy odds. Optimal management for a successful transition should focus on maintaining cows with increased muscle mass and moderate fat reserves prepartum, preventing negative nutrient balance between −21d and −7d, and minimising both muscle and fat loss around calving and thereafter.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100626.

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

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Declaration of interest

None.

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