HelmholtzZentrum münchen
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Muscle and serum acylcarnitine profiles in dairy cows during the periparturient period

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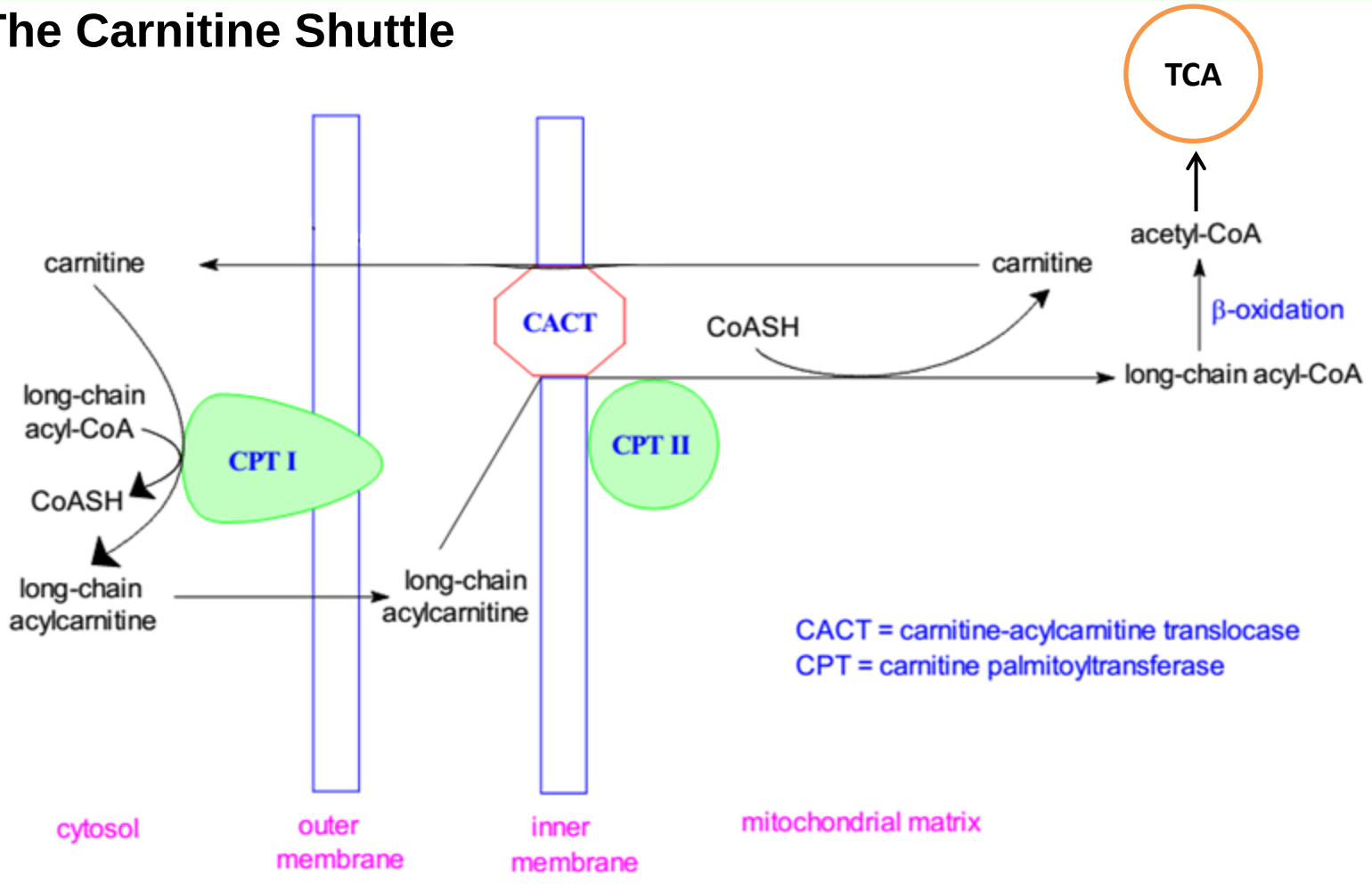
Introduction

- Negative energy balance and release of **free fatty acids** into the circulation during early lactation
- Turning **skeletal muscle** into the major site for use of fat-derived fuels
- Excess lipid supply and subsequent lipid accumulation in insulin-sensitive tissues (Van Epps-Fung et al., 1997; Pires et al., 2007; Schooneman et al., 2013)

The Carnitine Shuttle



Adapted from: http://lipidlibrary.aocs.org/Primer/content.cfm?ItemNumber=39299

Objective of the Study

To characterize the concentrations of **carnitine** and its acyl esters (acylcarnitines; **ACC**) both in **blood serum** and in **skeletal muscle** of dairy cows to address potential changes in the capacity for **mitochondrial β-oxidation of fatty acids** in skeletal muscle in context with the negative energy balance typical for early lactation.

Materials and Methods

- 11 Pluriparous German Holstein cows

- Biopsies from *M. semitendinosus* and blood sampling:

  day -21, 1, 21, and 70 relative to calving

- Muscle and serum ACC profiles:

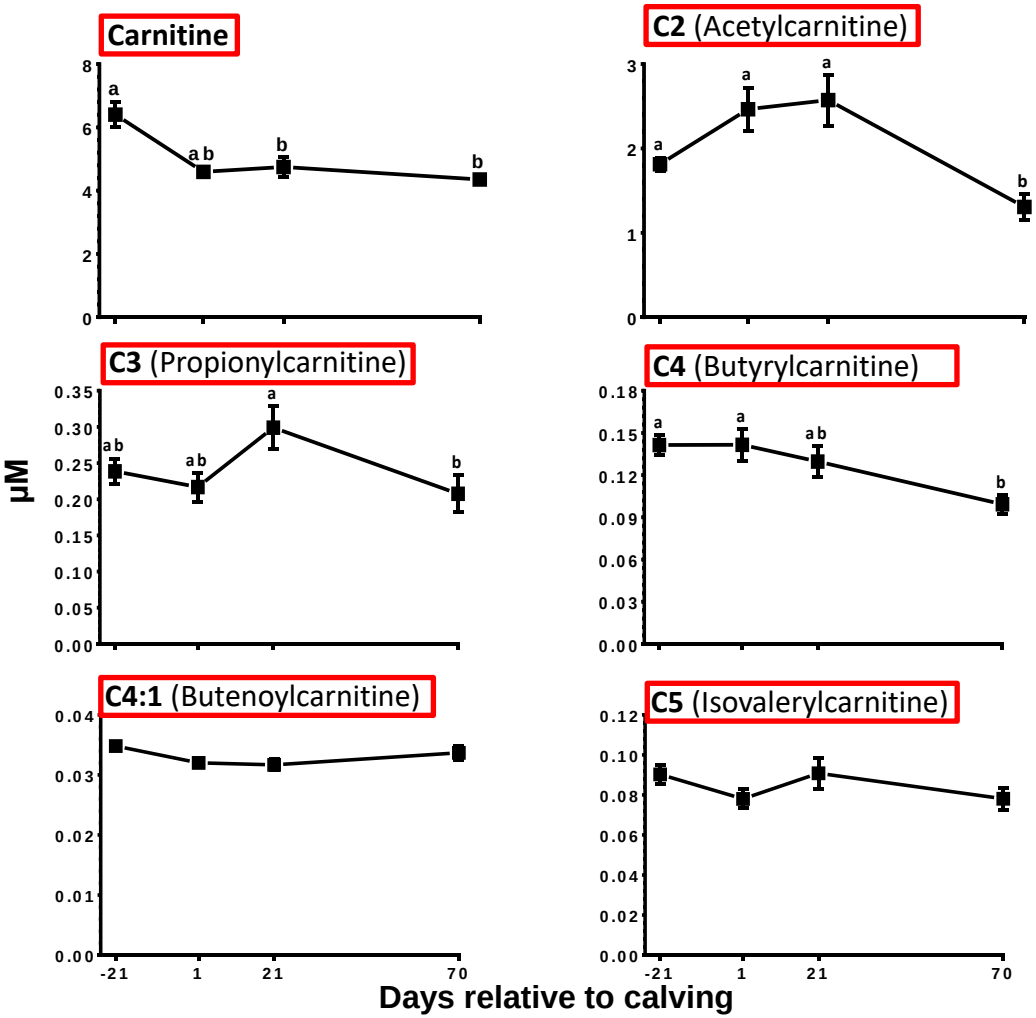
  by FIA-MS/MS profiling through **targeted metabolomics approach** (AbsoluteIDQ p180 Kit; Biocrates Life Sciences AG, Innsbruck, Austria).

Materials and Methods

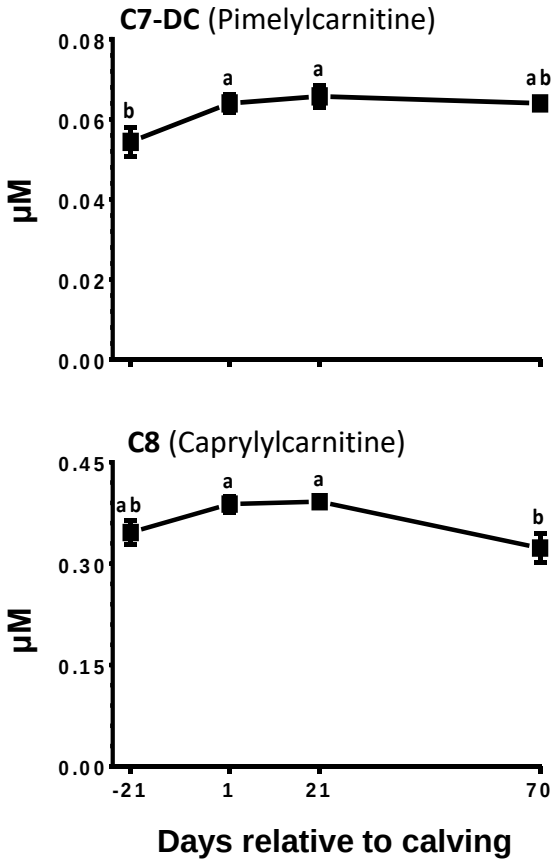
Statistical Analysis

- The PROC MIXED procedure of SAS (9.3) using repeated measures:
  - Fixed effect: Time (sampling day)
  - Random effect: Cow
- Correlation analysis using PROC CORR
- The threshold of significance: $P < 0.05$; Trends: $0.05 < P < 0.10$
- Data presented as means ± SEM

Serum short chain ACC

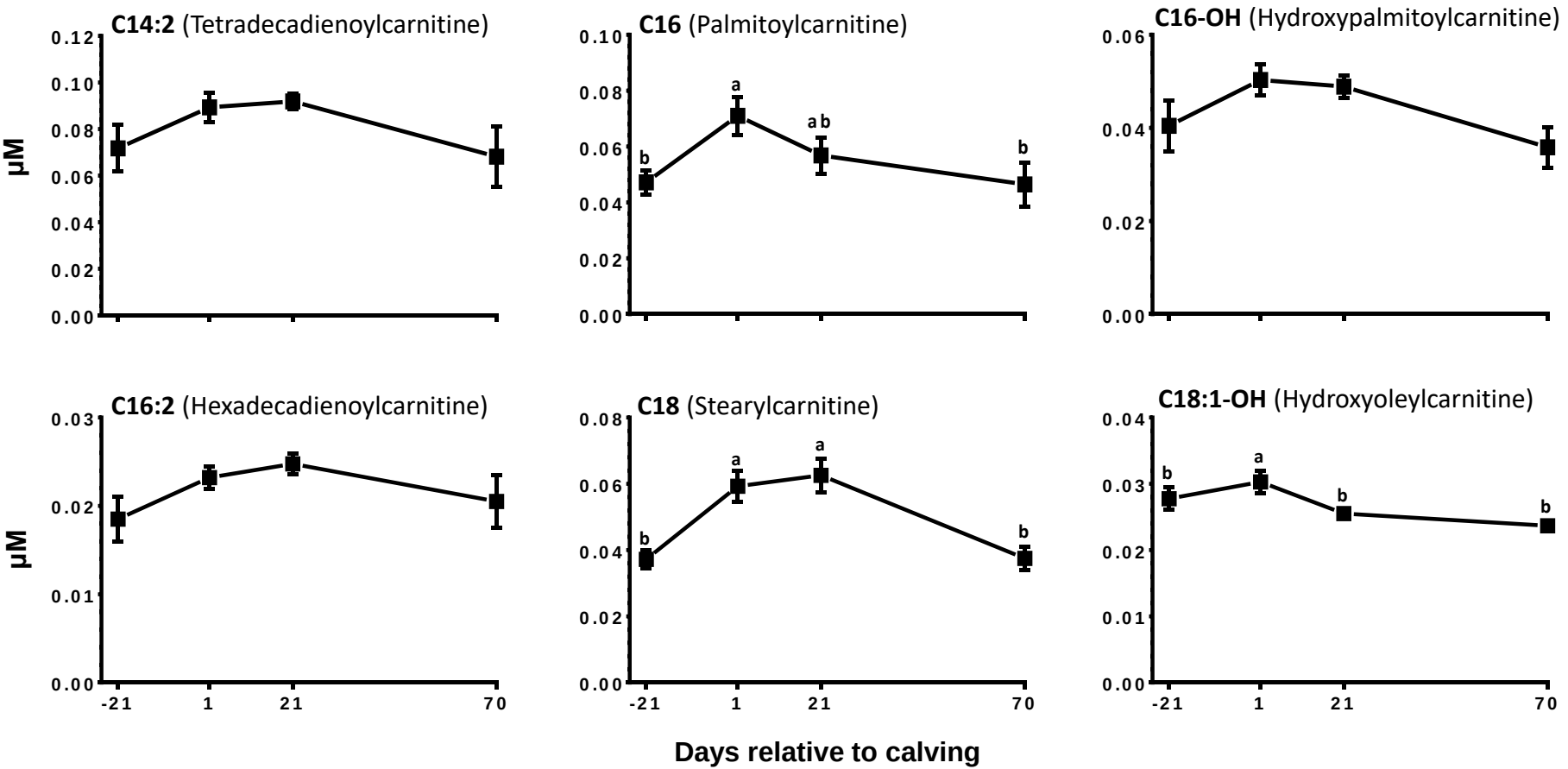


Serum medium chain ACC



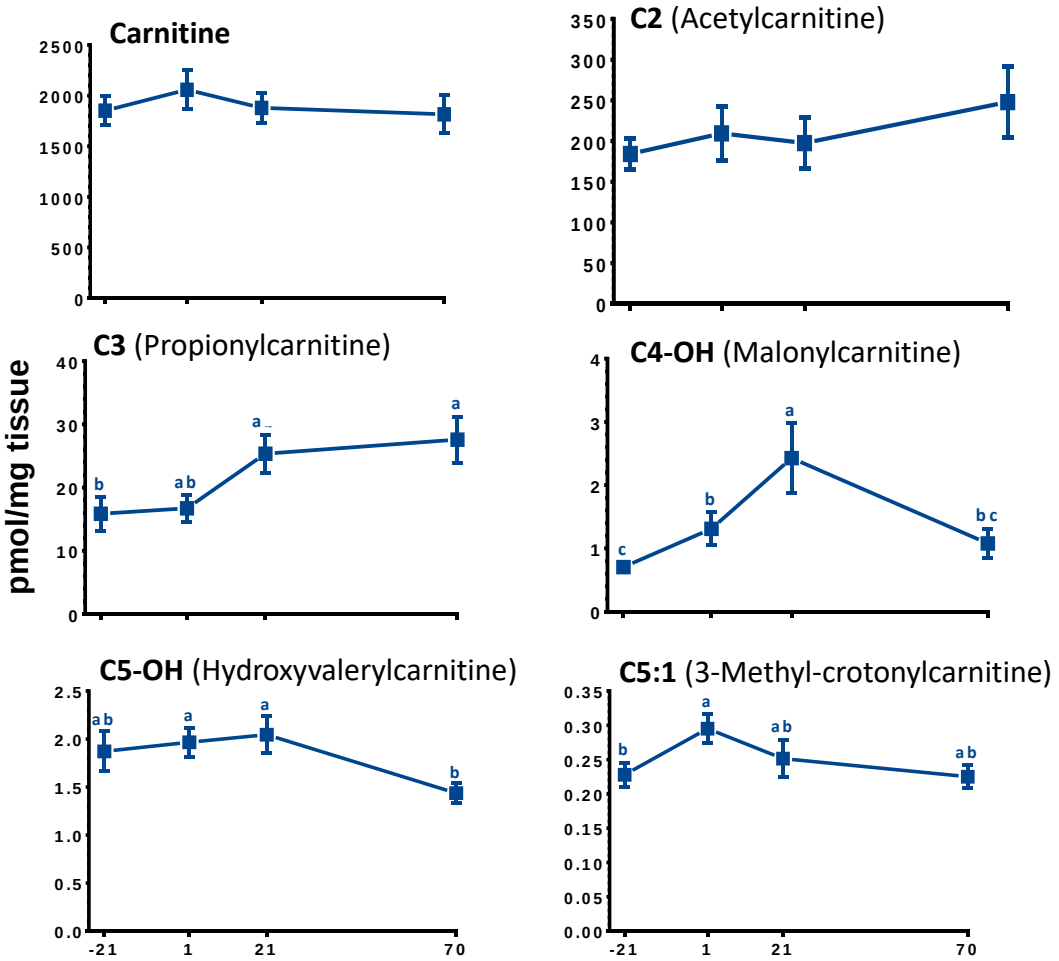
$P < 0.05$; except C4:1 and C5 ($P < 0.10$)

Serum long chain ACC

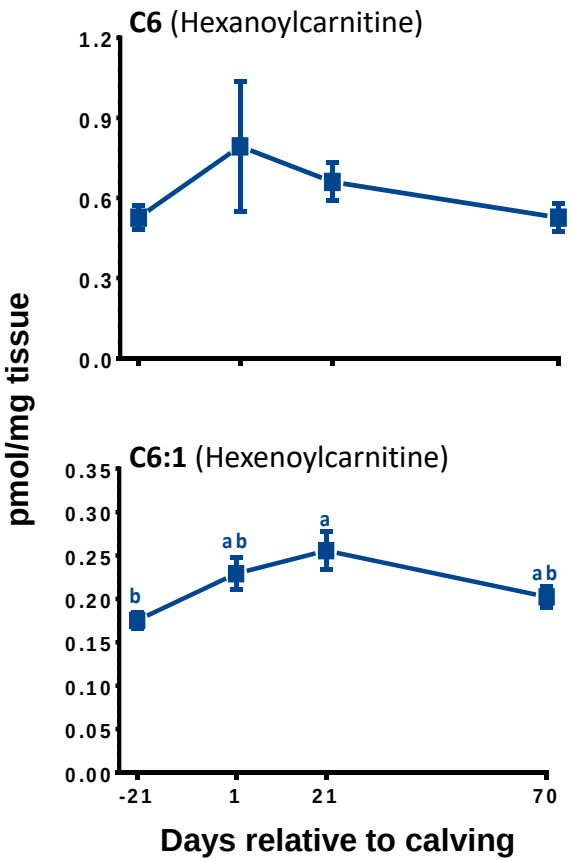


***P* < 0.02; except C14:2, C16-OH, and C16:2 (*P* < 0.10)**

Muscle short chain ACC

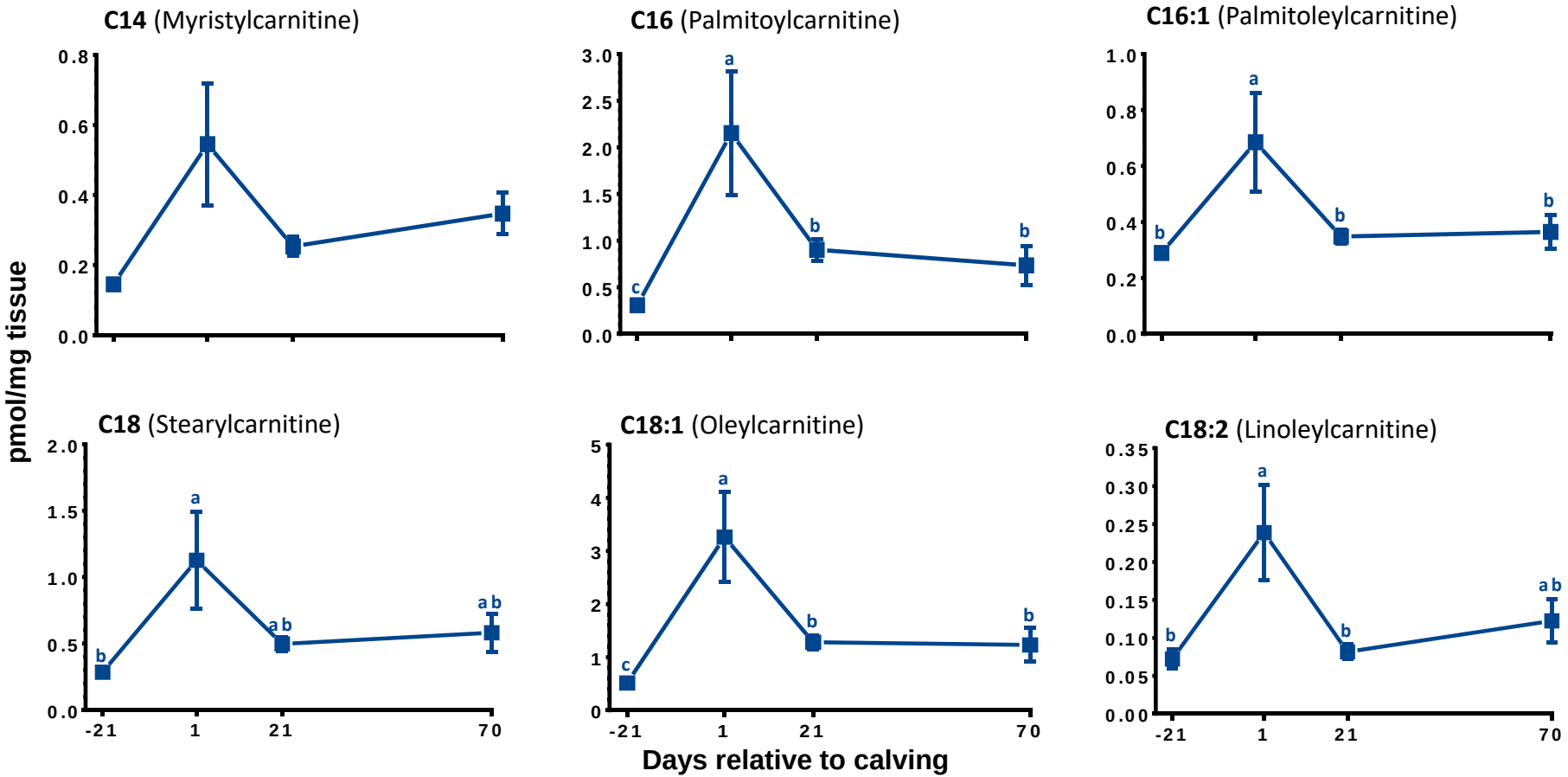


Muscle medium chain ACC



***P* < 0.03; except carnitine, C2, and C6: non significant**

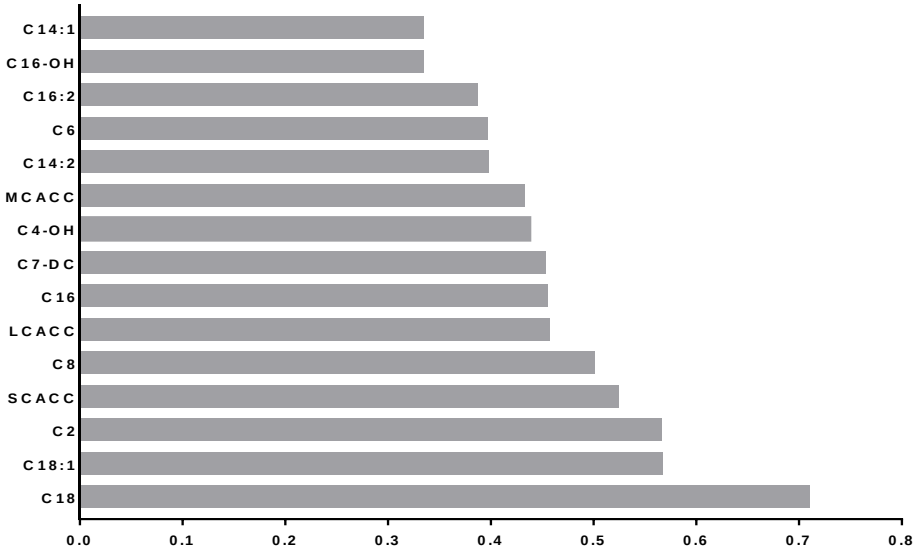
Muscle long chain ACC



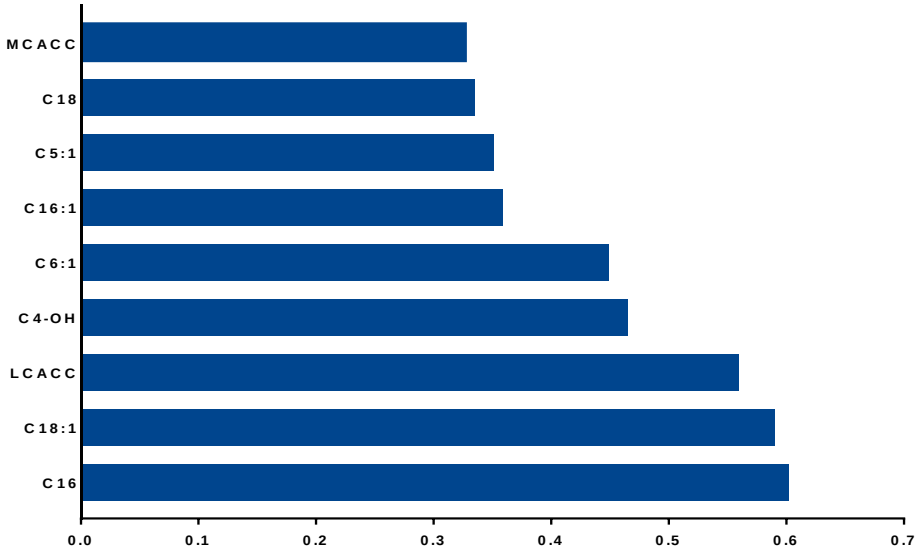
P < 0.001; except C14 (*P* < 0.09)

Metabolites correlating with NEFA (across all time points)

Serum



Muscle



Pearson correlation coefficients

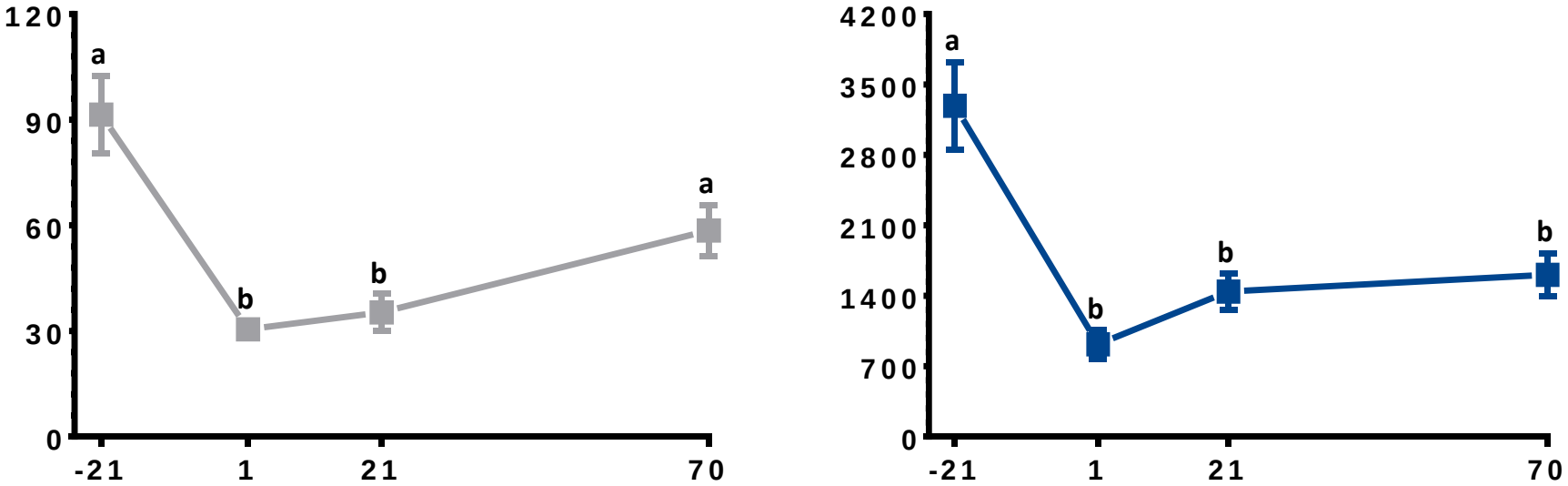
***P* < 0.04; except Serum C16-OH (*P* < 0.07)**

CPT-I Ratio (carnitine/C16+C18)*

Serum

$P \leq 0.0001$

Muscle



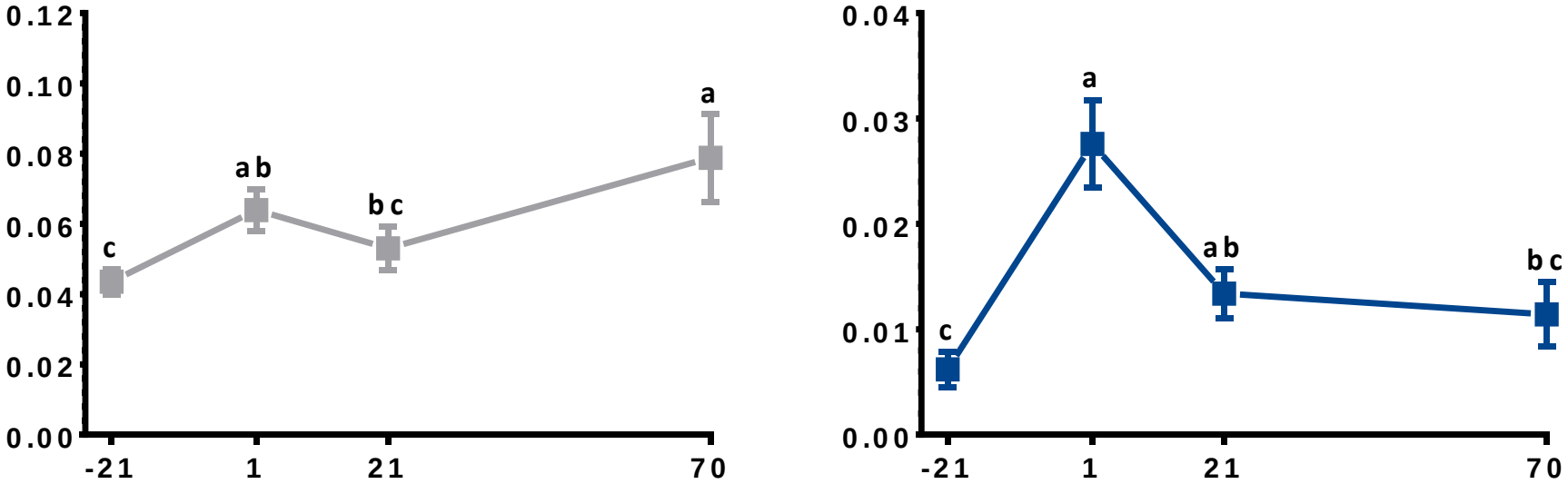
*Paapstel et al. (2016). IJC Metabolic & Endocrine. 11:13 –18.

CPT-II Ratio (C16+C18:1/C2)*

Serum

$P < 0.003$

Muscle



*Gempel et al., 2002. J. Inherit. Metab. Dis. 25:17-27.

Conclusions

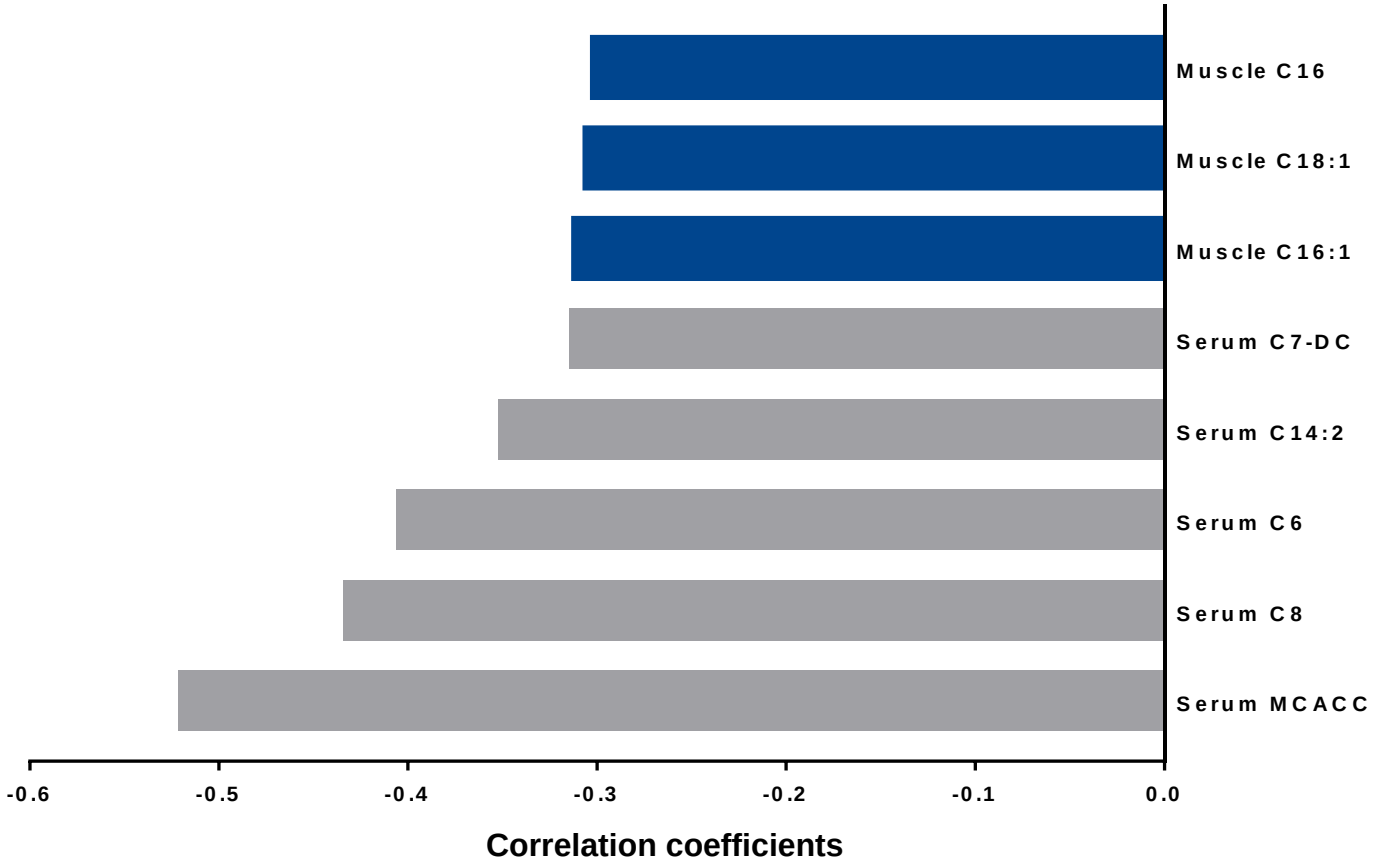
- Serum concentrations of carnitine decreased with the onset of lactation, probably due to increased carnitine excretion in milk and increased carnitine uptake by the muscle to maintain the intracellular concentrations.

- ACC profiles demonstrated increased lipid flux and consequently incomplete or overloaded fatty acids oxidation in the skeletal muscle around calving.

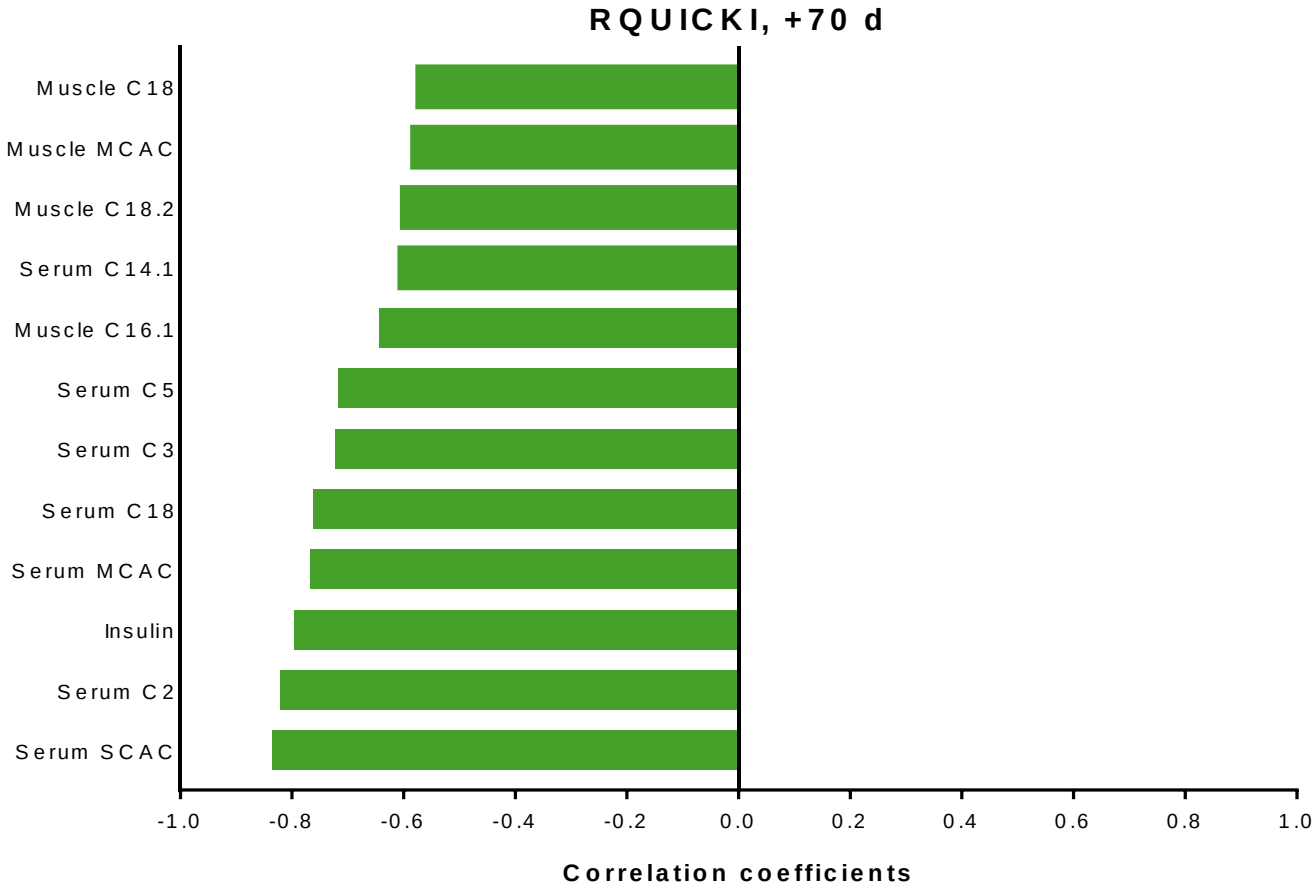
- These data suggest that post-CPT1 events including deficiency or impaired CPT2 function and/or depletion of several TCA cycle intermediates are more probable factors causing accumulation of long chain ACC in the muscle around parturition.

Thank you for your attention

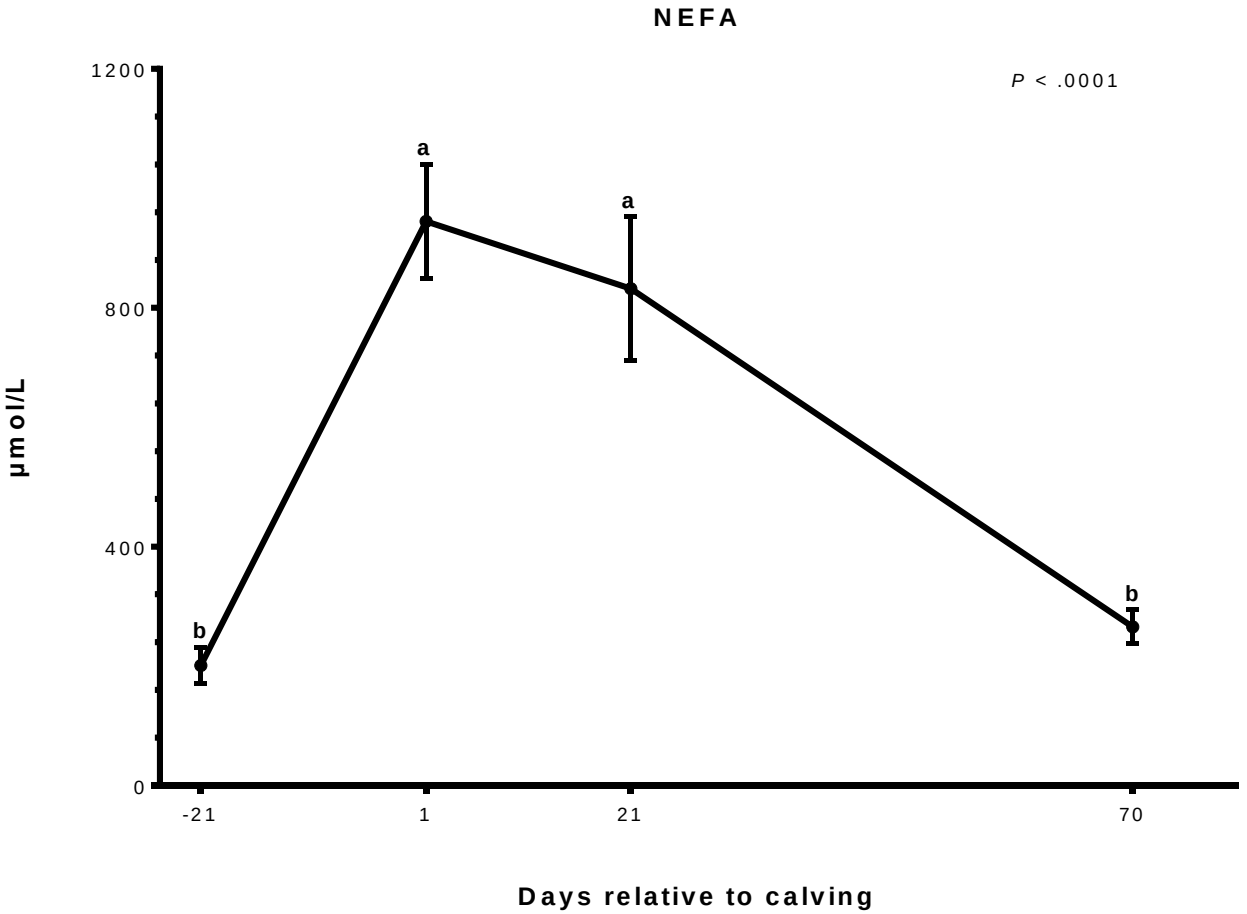
Metabolites correlating with RQUICKI (across all time points)



P < 0.04; except serum C7-DC and muscle C16, C18:1, and C16:1 (*P* < 0.09)



$P \leq 0.04$, except Muscle C16.1, C18.2, MCAC, C18, and Serum C14.1 ($P = 0.0853$, 0.0653, 0.0987, 0.0822, and 0.0629, respectively)


NEFA
P < .0001
1200
800
400
0
µmol/L
a
a
b
b
-21
1
21
70
Days relative to calving

Acylcarnitine

BC code	Analyte
C0	Carnitine (free)
C2	Acetylcarnitine
C3	Propionylcarnitine
C3:1	Propenoylcarnitine
C3-OH	Hydroxypropionylcarnitine
C4	Butyrylcarnitine / Isobutyrylcarnitine
C4:1	Butenoylcarnitine
C4-OH (C3-DC)	Hydroxybutyrylcarnitine (Malonylcarnitine)
C5	Isovalerylcarnitine / 2-Methylbutyrylcarnitine / Valerylcarnitine
C5:1	Tiglylcarnitine / 3-Methyl-crotonylcarnitine
C5:1-DC	Glutaconylcarnitine / Mesaconylcarnitine
C5-DC (C6-OH)	Glutarylcarnitine (Hydroxyhexanoylcarnitine [= Hydroxycaproylcarnitine])
C5-M-DC	Methylglutarylcarnitine
C5-OH (C3-DC-M)	Hydroxyisovalerylcarnitine / Hydroxy-2-methylbutyryl / Hydroxyvalerylcarnitine (Methylmalonylcarnitine)
C6 (C4:1-DC)	Hexanoylcarnitine [= Caproylcarnitine] (Fumarylcarnitine)
C6:1	Hexenoylcarnitine
C7-DC	Pimelylcarnitine
C8	Octanoylcarnitine [= Caprylylcarnitine]
C9	Nonanoylcarnitine [= Pelargonylcarnitine]
C10	Decanoylcarnitine [= Caprylcarnitine]
C10:1	Decenoylcarnitine
C10:2	Decadienoylcarnitine
C12	Dodecanoylcarnitine [= Laurylcarnitine]
C12:1	Dodecenoylcarnitine
C12-DC	Dodecanedioylcarnitine
C14	Tetradecanoylcarnitine [= Myristylcarnitine]
C14:1	Tetradecenoylcarnitine [= Myristoleylcarnitine]
C14:1-OH	Hydroxytetradecenoylcarnitine [= Hydroxymyristoleylcarnitine]
C14:2	Tetradecadienoylcarnitine
C14:2-OH	Hydroxytetradecadienoylcarnitine
C16	Hexadecanoylcarnitine [= Palmitoylcarnitine]
C16:1	Hexadecenoylcarnitine [= Palmitoleylcarnitine]
C16:1-OH	Hydroxyhexadecenoylcarnitine [= Hydroxypalmitoleylcarnitine]
C16:2	Hexadecadienoylcarnitine
C16:2-OH	Hydroxyhexadecadienoylcarnitine
C16-OH	Hydroxyhexadecanolycarnitine [= Hydroxypalmitoylcarnitine]
C18	Octadecanoylcarnitine [= Stearylcarnitine]
C18:1	Octadecenoylcarnitine [= Oleylcarnitine]
C18:1-OH	Hydroxyoctadecenoylcarnitine [= Hydroxyoleylcarnitine]
C18:2	Octadecadienoylcarnitine [= Linoleylcarnitine]

Muscle Acylcarnitines	Valid data (%)	Serum Acylcarnitines	Valid data (%)
C0	95.5%	C0	52%
C2	95.5%	C2	100%
C3	95.5%	C3	100%
C5-OH (C3-DC-M)	95.5%	C5-OH (C3-DC-M)	0%
C3-OH	0.0%	C3-OH	55%
C3:1	0.0%	C3:1	0%
C4	95.5%	C4	100%
C3-DC (C4-OH)	95.5%	C3-DC (C4-OH)	61%
C4:1	25.0%	**C4:1**	**43%**
C5	95.5%	C5	100%
C5-DC (C6-OH)	43.2%	C5-DC (C6-OH)	0%
C5-M-DC	0.0%	C5-M-DC	0%
C5:1	86.4%	C5:1	84%
C5:1-DC	34.1%	C5:1-DC	77%
C6 (C4:1-DC)	90.9%	C6 (C4:1-DC)	80%
C6:1	86.4%	C6:1	30%
C7-DC	22.7%	C7-DC	75%
C8	2.3%	C8	80%
C9	0.0%	C9	25%
C10	2.3%	C10	57%
C10:1	0.0%	C10:1	0%
C10:2	0.0%	C10:2	0%
C12	6.8%	C12	9%
C12-DC	0.0%	C12-DC	0%
C12:1	0.0%	C12:1	0%
C14	65.9%	C14	50%
C14:1	22.7%	C14:1	98%
C14:1-OH	25.0%	C14:1-OH	64%
C14:2	2.3%	C14:2	80%
C14:2-OH	2.3%	C14:2-OH	27%
C16	95.5%	C16	89%
C16-OH	2.3%	C16-OH	75%
C16:1	81.8%	C16:1	66%
C16:1-OH	27.3%	C16:1-OH	64%
C16:2	11.4%	C16:2	77%
C16:2-OH	4.5%	C16:2-OH	48%
C18	95.5%	C18	95%
C18:1	93.2%	C18:1	70%
C18:1-OH	2.3%	C18:1-OH	50%
C18:2	93.2%	C18:2	50%