Tissue mRNA expression of branched-chain amino acid catabolism enzyme in early lactating dairy cows

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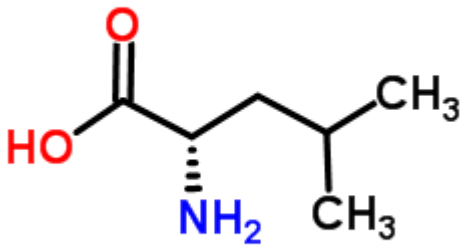
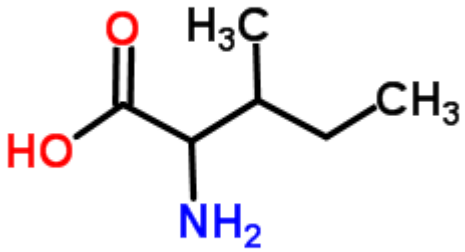
Introduction

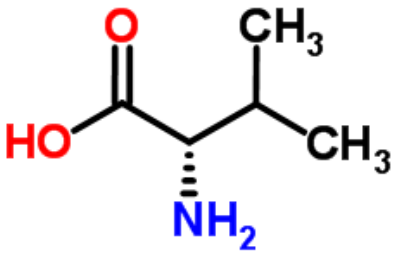
Branched-chain amino acids (BCAA)



Leucine



Isoleucine



Valine

Introduction

The BCAA are involved in numerous metabolic processes:

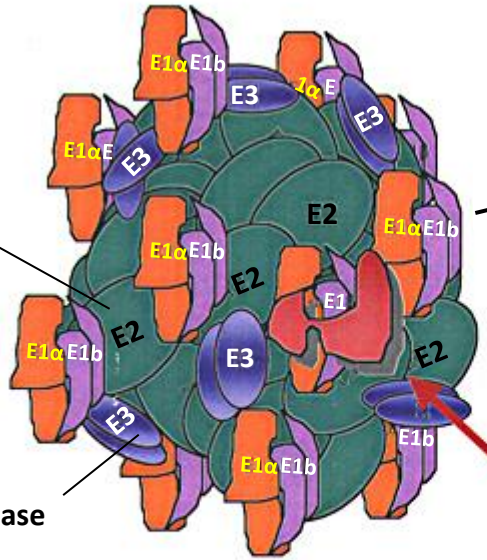
- as substrates for protein synthesis
- as energy substrates
- precursors for the synthesis of alanine and glutamine
- as a modulator of muscle protein synthesis via the insulin-signaling pathway and/or via insulin-independent manners (Macotela et al., 2011)

The BCAA catabolic pathway

Adopted from J. Nutr. 2006. 136: 207S–211S.

KIV = α-ketoisovalerate; **KMV** = α-keto-b-methylvalerate; **KIC** = alpha-ketoisocaproate
IB-CoA = isobutyryl-CoA; **MB-CoA** = α-methylbutyryl-CoA; **IV-CoA** = isovaleryl-CoA

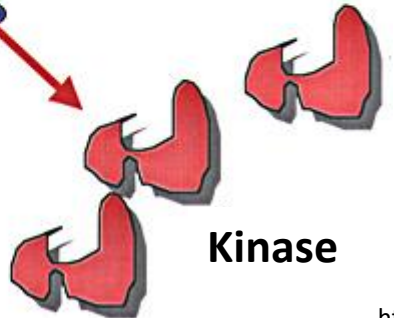
BCKD complex



BCKDHA gene

BCKDHB gene

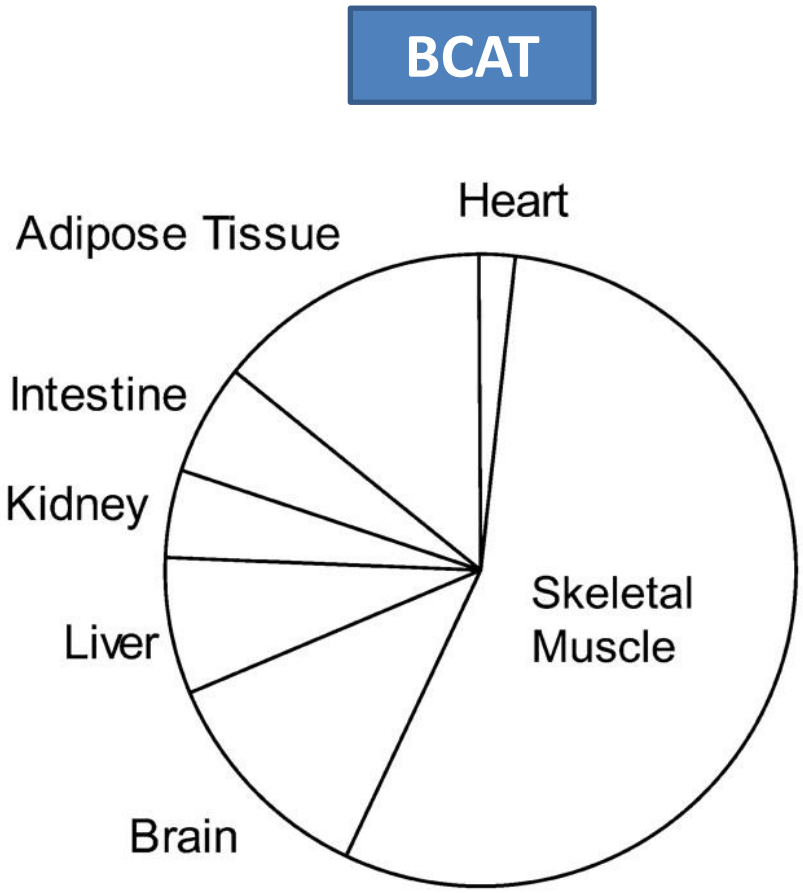
Branched-chain alpha-keto acid decarboxylase

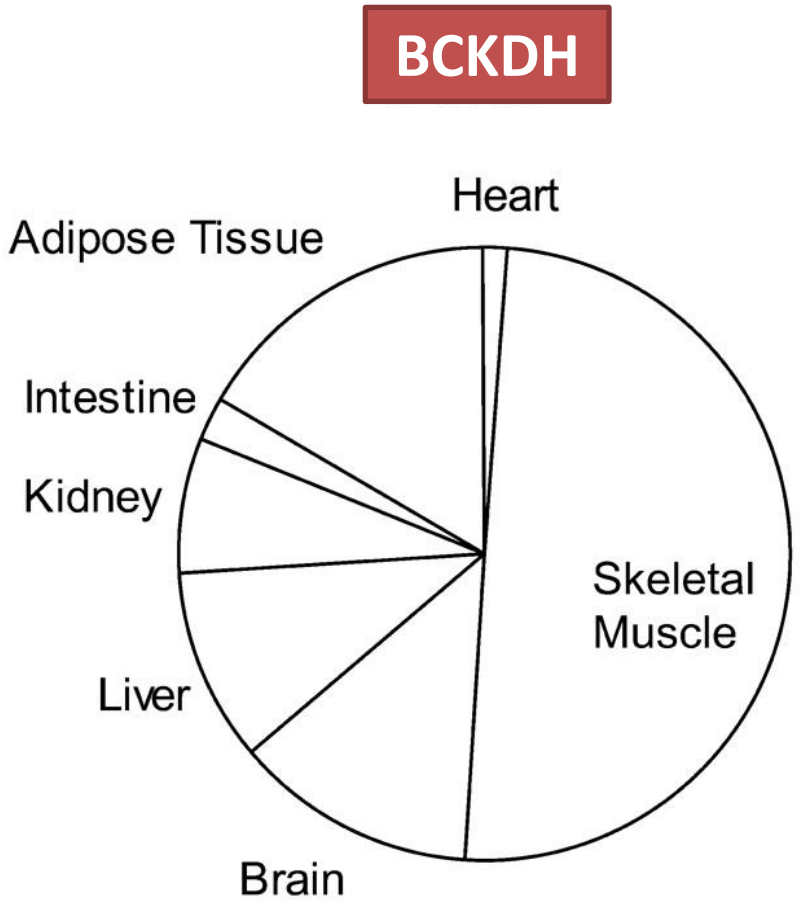


Kinase

https://www.bioscience.org/1998/v3/d/danner/fig2.jpg

Tissue distribution of BCAT and BCKD in human tissues (BW = 70 kg)





Adopted from J. Nutr. 2006. 136: 207S–211S.

Aim of the study

To test the potential involvement of adipose tissue in BCAA metabolism in dairy cows during early lactation, through assaying mRNA expression of **BCKDHA** and **BCKDHB** in visceral and subcutaneous adipose tissue.

Materials and Methods

Primiparous German Holstein cows (n = 25)

Control (**CTR**) (n = 10)

100 g/d rumen-protected **fat preparation** (Silafat®); CLAs were substituted by stearic acid

Treatment (**CLA**) (n = 10)

100 g/d rumen-protected **CLA** preparation (Lutrell®). Contained **12%** each of ***c*-9, *t*-11** and ***t*-10, *c*-12**

Days in milk

1 — 42 — 105

No. of animals slaughtered

5 | CTR = 5, CLA = 5 | CTR = 5, CLA = 5

Sample collection

- **Skeletal Muscle** — ***M. semitendinosus***
- **Visceral adipose tissue**
  - **Mesenterial**
  - **Omental**
  - **Retroperitoneal**
- **Subcutaneous adipose tissue**
  - **Sternum**
  - **Tail head**
  - **Withers**

Materials and Methods

Gene Expression Analysis

- Using real-time RT-PCR in an Mx3000P cycler (Stratagene, Amsterdam, the Netherlands)
- Accordance with MIQE guidelines (Bustin et al., 2009)
- Data normalization: using the most stable reference genes (qBASEplus,Biogazelle, Ghent, Belgium)

Target Genes
BCKDHA
BCKDHB

Reference Genes	
Muscle	Fat depots
LRP10	EIF3K
EMD	LRP10
POLR2A	POLR2A
EIF3K	EMD
	MARVELD1
	HPCAL1

Materials and Methods

Statistical Analysis

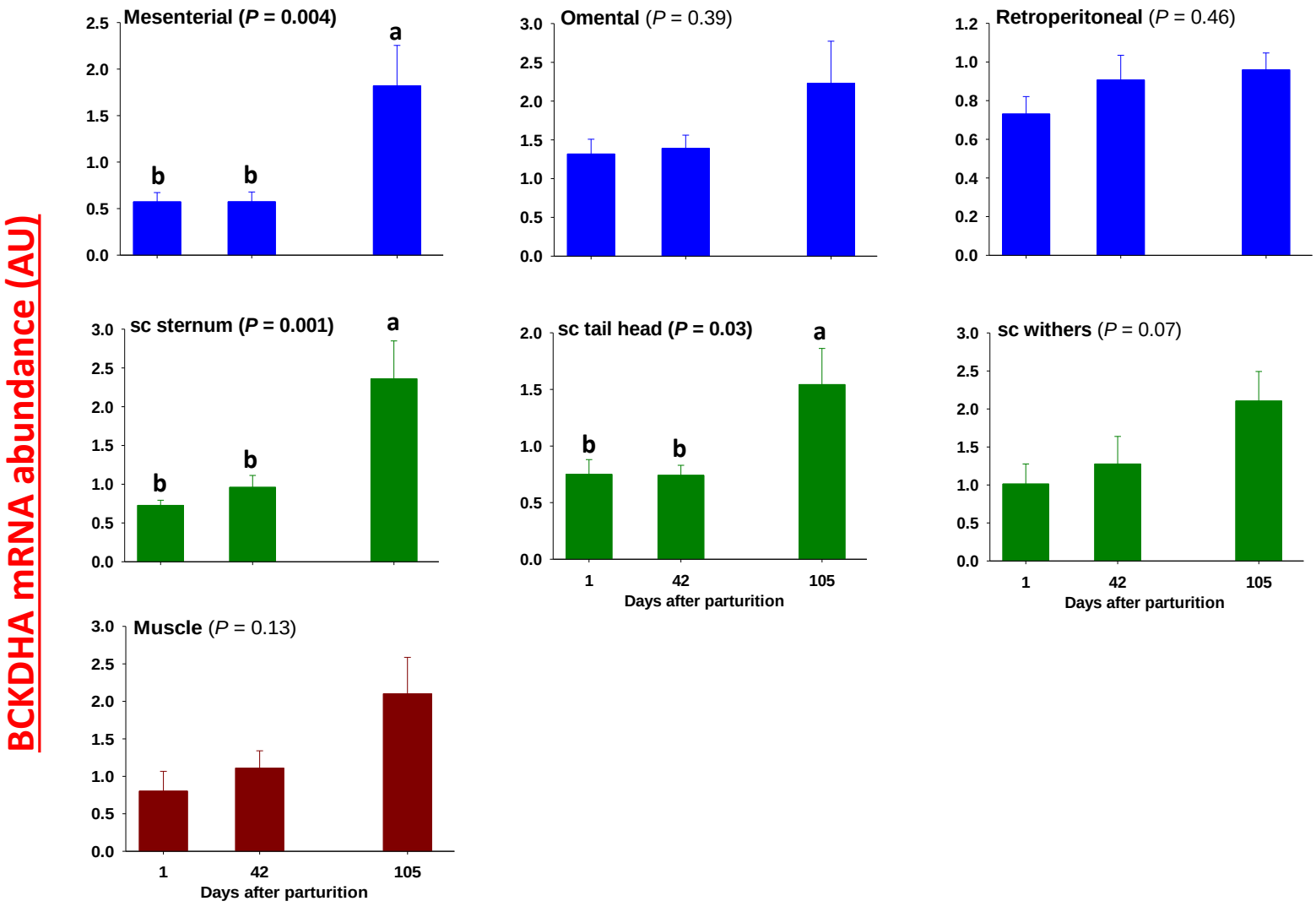
Preliminary statistical evaluation: no CLA effect on the tested variables

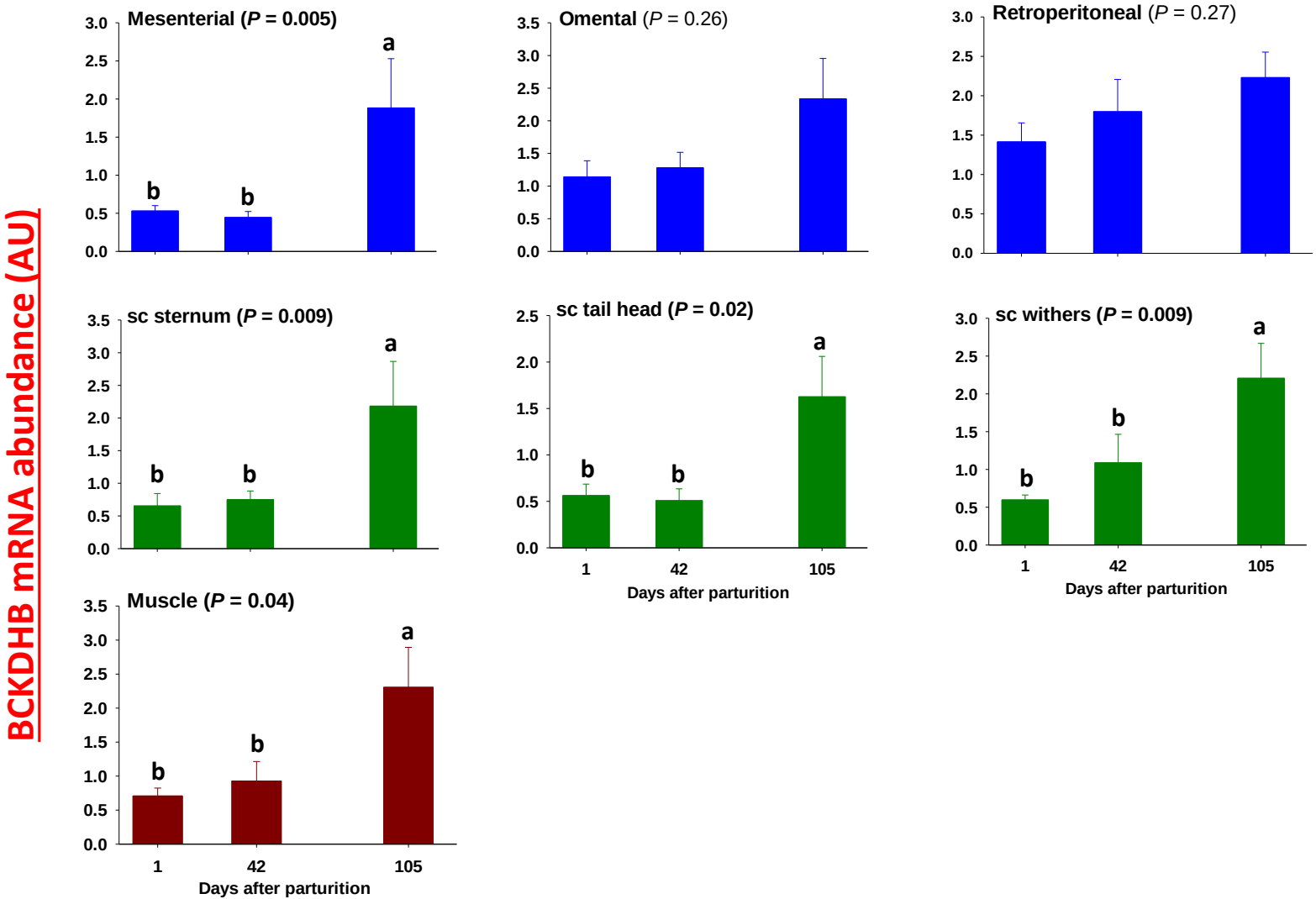
- The PROC MIXED procedure of SAS (9.3) using repeated measures:
  - Fixed effect: Time (sampling day)
  - Random effect: Cow

- The threshold of significance: $P < 0.05$; Trends: $0.05 < P < 0.10$.

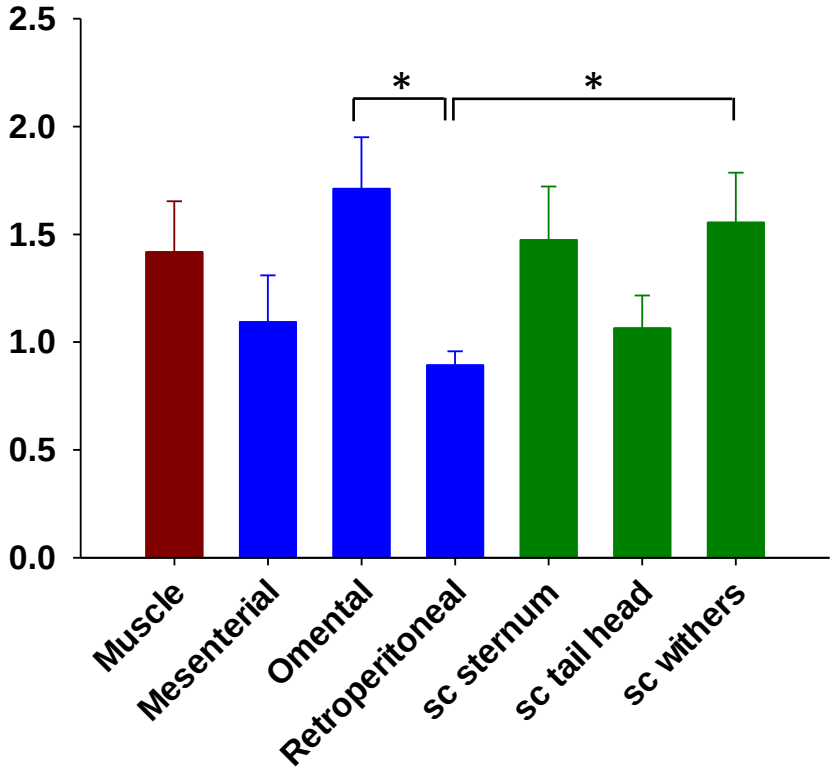
- Data are presented as means ± SEM.

Results

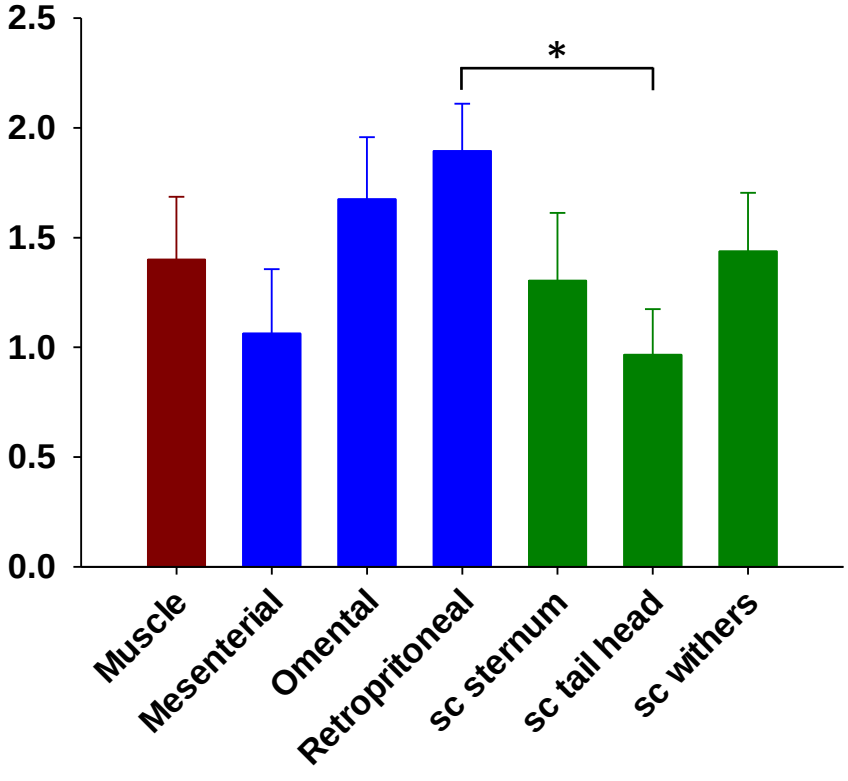

BCKDHA mRNA abundance (AU)
Mesenterial (P = 0.004)
a
b
b
Omental (P = 0.39)
Retroperitoneal (P = 0.46)
sc sternum (P = 0.001)
a
b
b
sc tail head (P = 0.03)
a
b
b
sc withers (P = 0.07)
Muscle (P = 0.13)
1
42
105
Days after parturition


BCKDHB mRNA abundance (AU)
Mesenterial (P = 0.005)
Omental (P = 0.26)
Retroperitoneal (P = 0.27)
sc sternum (P = 0.009)
sc tail head (P = 0.02)
sc withers (P = 0.009)
Muscle (P = 0.04)
a
b
1
42
105
Days after parturition

BCKDHA mRNA abundance (AU)



BCKDHB mRNA abundance (AU)



Conclusion

- The BCKDHA and BCKDHB mRNA expression were detectable in different fat depots in a comparable abundance with that of muscle.

- The reduced abundance of **BCKDHA/B** mRNA on days 1 and 42 compared to day 105, may suggest an attenuation of BCAA oxidation in the studied tissues shortly after parturition. This would favor sparing of BCAA for milk protein synthesis and protein synthesis in other organs.

- Further studies that include **protein expression** and **activity** of this enzyme may provide additional clues to the involvement of adipose tissue in whole body BCAA metabolism in dairy cows.

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Thank you for your attention