Physiological concentrations of fatty acids impact lipolytic genes in primary bovine hepatocytes

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BACKGROUND

- During the periparturient period, dairy cows undergo metabolic stress
  - Adipose tissue mobilizes fatty acids (FA) into the bloodstream
  - The main fates of these circulating FA in the liver are
    - β-oxidation (TCA cycle)
    - Ketogenesis
    - Storage as triglycerides
  - As a result, fluctuations in gene expression and protein abundance are apparent as the cow adjusts to demands brought on by lactation
- Circulating FA proportions are dynamic
  - May regulate gene expression or protein abundance differentially in dairy cows
- Other species—human and rodents—have shown FA also have a regulatory effect on lipolytic genes and proteins either directly or indirectly
  - Human: *SREBP1c* mRNA abundance is reduced with C20:4 (HepG2)
  - Mouse: *SREBP1c* mRNA abundance is reduced with C16:1
  - Rat: SREBP1c protein abundance is reduced with C18:2, C20:5, and C22:6
- Species-specific knowledge of FA regulation is required
- Objective: To determine the effect of FA found in circulating bovine serum during liver lipid recovery on both gene expression and protein abundance of lipid-related genes and proteins
- Hypothesis: Physiologically relevant doses of circulating individual FA found in dairy cows will affect both gene expression and protein abundance of primary bovine hepatocytes

MATERIALS & METHODS

- Primary bovine hepatocytes were isolated from bull calves (n=4; < 7 d of age)
- About 2 million cells per 35 mm dish were cultured in monolayers fo 24 hr in Dulbecco's Modified Eagles Medium, 10% FBS, 1% antibiotic-antimycotic
- Wells at least 80% confluent were randomly assigned FA treatment of C14:0, C16:0, C18:0, C18:1, C18:2, and C22:6 at either
  - 0.25 mM
  - Physiologically relevant concentrations (PRC) observed in vivo FA profiles during liver lipid recovery (Table 1)
- Individual FA were bound to bovine serum albumin (BSA) at a final concentration of 8 mM; a 1% bovine serum albumin control (CTL) was used for normalization
- Primary bovine hepatocytes were harvested 24 h after treatment in TRIzol for subsequent
  - RNA analysis
    - Gene expression using RT-qPCR with SsoAdvanced SYBR on a CFX-384; previously validated primers exhibiting one melt curve
    - Standard curve method utilized; reference genes used for normalization
  - Protein analysis
    - Protein abundance using 25 µg protein per lane; stain-Free technology utilized for total lane protein normalization and log transformed for normality
    - In-house pool used to correct background and ensure blot quality
- Data was analyzed using PROC MIXED, SAS, 9.4, with fixed effect of FA and random effect of calf and preplanned contrasts
- Data are reported as LSM ± SEM; tendency (†) declared at $0.05 < P \leq 0.10$ and significance (*) declared at $P \leq 0.05$

RESULTS

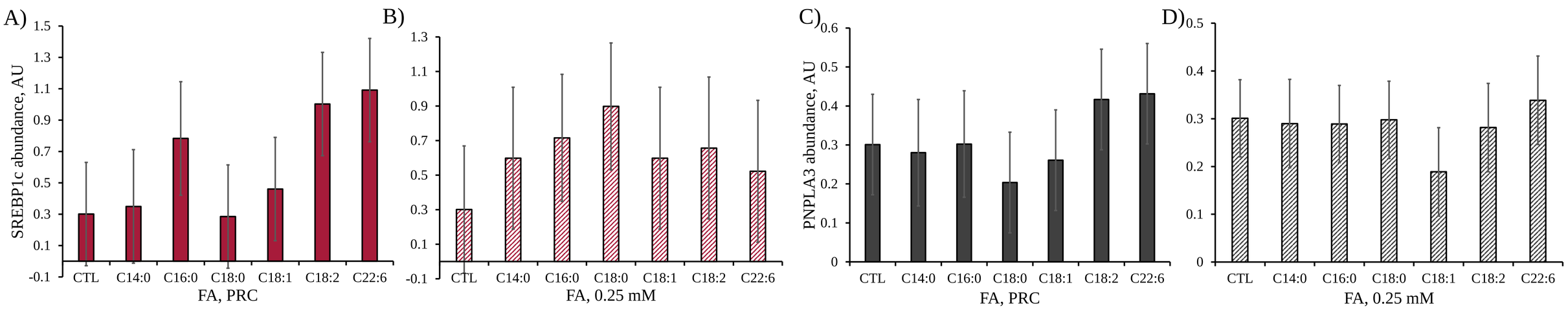

Figure 1. Protein abundance changes of sterol regulatory element binding protein 1c (SREBP1c) in response to fatty acids (FA) with treatment of either A/C) physiologically relevant concentrations (PRC) or B/D) 0.25 mM of control (CTL; 1% BSA), C14:0 (myristic), C16:0 (palmitic), C18:0 (stearic), C18:1 (oleic), C18:2 (linoleic), or C22:6 (docosahexaenoic) acids. Panel A) SREBP1c abundance: 0.30 vs. 0.35 vs. 0.78 vs. 0.29 vs. 0.46 vs. 1.00 vs. 1.09 ± 0.34 arbitrary units (AU) ($P > 0.30$); Panel B) SREBP1c abundance: 0.30 vs. 0.60 vs. 0.72 vs. 0.90 vs. 0.60 vs. 0.66 vs. 0.52 ± 0.39 AU ($P > 0.83$). Protein abundance changes of patatin-like phospholipase domain-containing protein 3 (PNPLA3) in response to fatty acids (FA) with treatment of either C) physiologically relevant concentrations (PRC) or D) 0.25 mM of control (CTL; 1% BSA), C14:0 (myristic), C16:0 (palmitic), C18:0 (stearic), C18:1 (oleic), C18:2 (linoleic), or C22:6 (docosahexaenoic) acids. Panel C) PNPLA3 abundance: 0.30 vs. 0.28 vs. 0.30 vs. 0.20 vs. 0.26 vs. 0.42 vs. 0.43 ± 0.13 AU ($P \geq 0.85$); Panel D) PNPLA3 abundance: 0.30 vs. 0.29 vs. 0.29 vs. 0.30 vs. 0.19 vs. 0.28 vs. 0.34 ± 0.09 AU ($P > 0.97$).

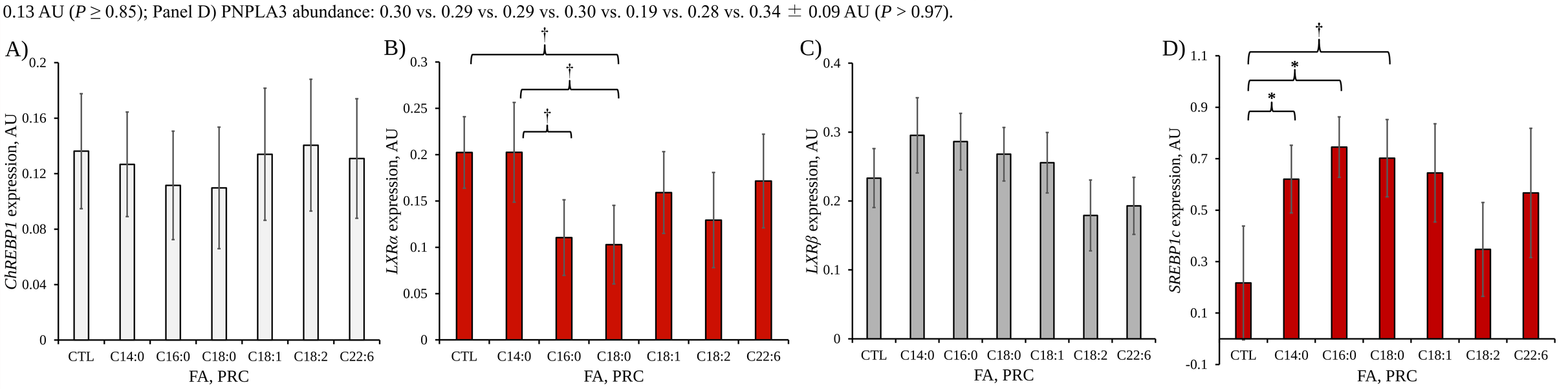

Figure 2. Gene expression changes of carbohydrate regulatory element binding protein 1 (*ChREBP1*), liver X receptor (*LXR*) *α* and *β*, and sterol *REBP1c* (*SREBP1c*) in response to fatty acids (FA) with treatment of physiologically relevant concentrations (PRC) of control (CTL; 1% BSA), C14:0 (myristic), C16:0 (palmitic), C18:0 (stearic), C18:1 (oleic), C18:2 (linoleic), or C22:6 (docosahexaenoic) acids. Panel E) *ChREBP1* expression: 0.14 vs. 0.13 vs. 0.11 vs. 0.11 vs. 0.13 vs. 0.14 vs. 0.13 ± 0.04 arbitrary units (AU) ($P > 0.45$). Panel F) *LXRα* expression: 0.20 vs. 0.20 vs. 0.11 vs. 0.10 vs. 0.16 vs. 0.13 vs. 0.17 ± 0.05 AU ($P > 0.12$); CTL vs. C18:0 ($P = 0.07$); C14:0 vs C16:0 ($P = 0.10$); C14:0 vs. C18:0 ($P = 0.06$). Panel G) *LXRβ* expression: 0.23 vs. 0.30 vs. 0.29 vs. 0.27 vs. 0.26 vs. 0.18 vs. 0.19 ±0.04 AU ($P > 0.21$). Panel H) *SREBP1c* expression: 0.22 vs. 0.62 vs. 0.75 vs. 0.70 vs. 0.64 vs. 0.65 vs. 0.57 ±0.18 AU ($P > 0.14$); CTL vs. C14:0 ($P = 0.05$); CTL vs. C16:0 ($P = 0.05$); CTL vs C18:0 ($P = 0.09$).

SUPPLEMENT

Composition		Treatments, mM	
FA[1]	RECOV[2], %	0.25	PRC[3]
Myristic C14:0	1.81	0.25	0.009
Palmitic C16:0	34.93	0.25	0.175
Stearic C18:0	43.39	0.25	0.279
Oleic C18:1	4.2	0.25	0.021
Linoleic C18:2	0.95	0.25	0.005
DHA[4] C22:6	2.26	0.25	0.0113

Table 1. Composition of circulating fatty acid (FA) profile found in dairy cows during the transition to lactation period, specifically the liver lipid recovery phase.
[1]FA = fatty acid; name of individual FA with carbon bonds listed
[2]RECOV = circulating serum FA profile at the time of liver lipid recovery after parturition in dairy cows
[3]PRC = physiologically relevant concentration of treated FA
[4]DHA = docosahexaenoic acid

SUMMARY & CONCLUSION

- Slight changes in FA profile throughout the transition period impact lipolytic gene expression, but not necessarily protein abundance
  - No evidence of protein abundance was altered by any FA at either treatment for SREBP1c nor PNPLA3
- It appears gene expression and protein abundance of the gene encoding a protein are not regulated in the same way
  - E.g., *SREBP1c* gene expression was altered by C14:0, C:160, and tended to change with C18:0; yet SREBP1c protein abundance did not seem to be
- Gene expression of *LXRα* tended to decrease with treatment of C16:0 and C18:0 at PRC
- Dairy cows may have a different regulation method on lipolytic proteins than other species
  - Rat models decreased SREBP1c abundance with administration of C18:2, whereas bovine had no detectable effect
- This research indicates that individual FA, even at PRC, are not sufficient to regulate SREBP1c and PNPLA3 protein abundance

ACKNOWLEDGEMENTS

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