Responses of lipid associated proteins to FA treatment in bovine primary hepatocytes

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BACKGROUND

- Negative energy balance, subsequent rapid mobilization of triglycerides (TG), and accumulation of excess TG within the liver are characteristic of the transition to lactation period in dairy cattle
- Rapid TG mobilization can exceed liver oxidation and secretion capacity, resulting in liver lipid accumulation
- Up to 50% of dairy cows may have fatty liver
- Hepatic lipid associated proteins (HLAPs) may allow for dynamic storage or utilization of liver triglyceride (lvTG)
- Findings in human lipase studies suggest lipases may be transcriptionally and post transcriptionally regulated by fatty acids (FA) and the fed or fasted status
- Hypothesis: Varied fatty acid composition and concentration applied to hepatocytes in cell culture will result in differential expression of HLAPs
- Objective: Determine the abundance of HLAPs in cultured bovine primary hepatocytes subjected to fatty acid treatment

MATERIALS & METHODS

- Primary hepatocytes isolated from 4 Holstein calves were maintained as monolayer cultures for 24 h before treatment
- Fatty acid treatments are visualized in table 1
- Cells were harvested in RIPA buffer
- Protein analysis
  - 25 ug of protein per sample was run on a 4-20% Criterion™ TGX™ Precast Mini Protein Gel
  - A quality control pool was included on each gel
    - intra-blot coefficient of variations were calculated and minimized through picture selection and analysis to ensure blot quality
  - Protein abundance was quantified and normalized to the associated total lane protein
- HLAPs of interest are shown in table 2
- HLAPs abundances were analyzed using the GLIMMIX procedure of SAS 9.4
  - The linear predictors for HLAPs abundances included FA trt, concentration, FA trt X concentration and the random effect of calf
  - Linear, quadratic, and cubic contrasts were tested
  - Studentized residuals were visually assessed to ensure model assumptions were reasonably met
  - Data are presented as lsmeans ± standard error of the mean
  - Effects were considered significant when $P \leq 0.05$
  - Lsmeans differences were considered significant when Tukey-Kramer adjusted $P \leq 0.10$

MATERIALS & METHODS

Table 1. Fatty acid treatments applied to hepatocytes.

	Fatty Acid Type			
Concentration	Palmitic acid (16:0, PA)	Oleic Acid (18:1n6, OA)	α-linolenic acid (C18:3n3, ALA)	Fatty Acid[1] Cocktail (FAC)
0 mM	0 mM Fatty Acids			
0.25 mM	0.25 mM PA	0.25 mM OA	0.25 mM ALA	0.25 mM FAC
0.5 mM	0.5 mM PA	0.5 mM OA	0.5 mM ALA	0.5 mM FAC
0.75 mM	0.75 mM PA	0.75 mM OA	0.75 mM ALA	0.75 mM FAC
1.0 mM	1.0 mM PA	1.0 mM OA	1.0 mM ALA	1.0 mM FAC

[1] Fatty acid cocktail with a profile of FA reflective of plasma FA at parturition (3% C14:0, 27% C16:0, 23% C18:0, 31% C18:1n6, 8% C18:2 n6, and 8% C18:3n3)

Table 1. HLAPs and antibodies used in western blotting.

HLAP	Primary Antibody (dilution, time/temperature of incubation)	Secondary Antibody (dilution)
ABHD5[2]	ab59488; Abcam, Cambridge, MA (1:500, 1 hour at RT[1])	ab6741; Abcam (1:20,000)
HSL[3]	4107S; Cell Signaling, Danvers, MA (1: 1,000, ~12 hours at 4°C)	ab97051; Abcam (1:5,000)
PHSL	4139S; Cell Signaling (1: 1,000, ~12 hours at 4°C)	ab97080; Abcam (1:10,000)
PLIN/PPLIN[4]	ab10200; EMD Millipore Sigma, Billerica, MA (1:3,000, 1 hour at RT)	ab97080; Abcam (1:5,000)
PNPLA2[5]	ab99532; Abcam (1:3,000, ~12 hours at 4°C)	ab97051; Abcam (1:5,000)
PNPLA3[6]	ab81874; Abcam (1:500, 1 hour at RT)	ab97080; Abcam (1:5,000)

[1] Room temperature, [2]Abhydrolase domain containing 5, [3]Hormone sensitive lipase, [4]Perilipin 1, [5] Patatin like phospholipase domain containing 2, [6] Patatin like phospholipase domain containing 3

RESULTS

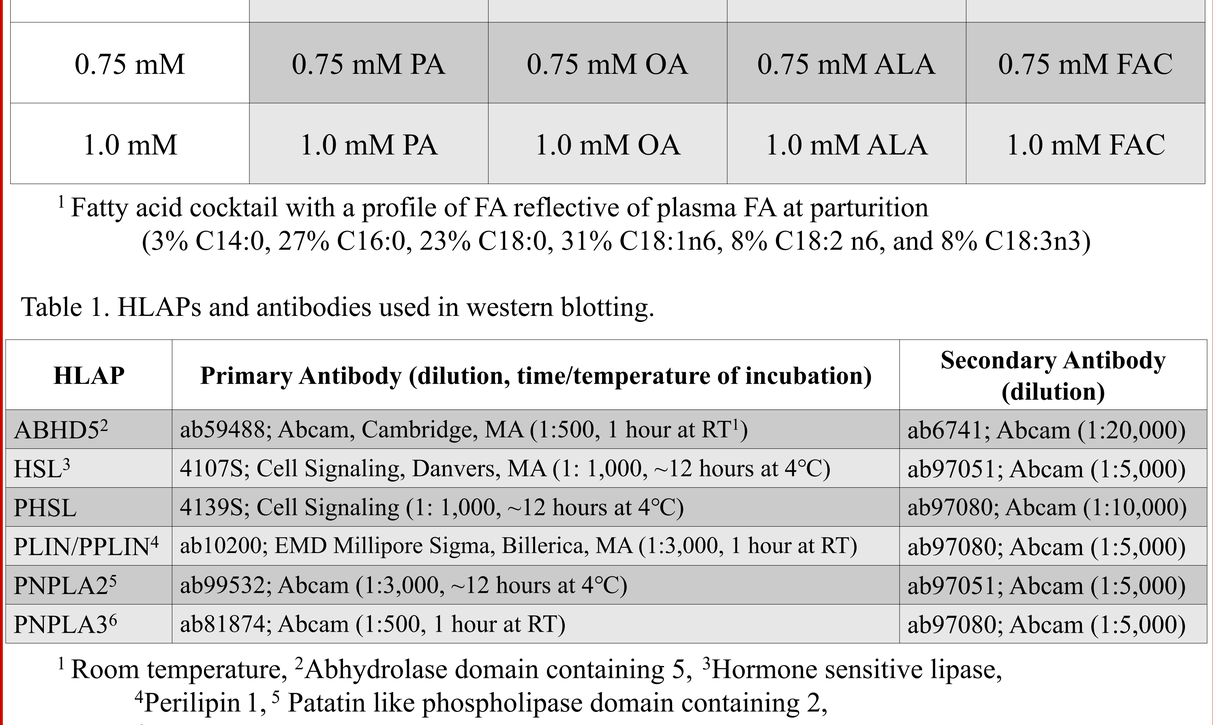

Figure 1. Mean abundance (arbitrary units, AU) of hepatic HSL, PPLIN, PLIN, and PNPLA3 normalized to total protein, and transformed as natural log(abundance) in hepatocytes across fatty acid treatments. Differences denoted by different subscripts with main effect of FA treatment *p*-value given.

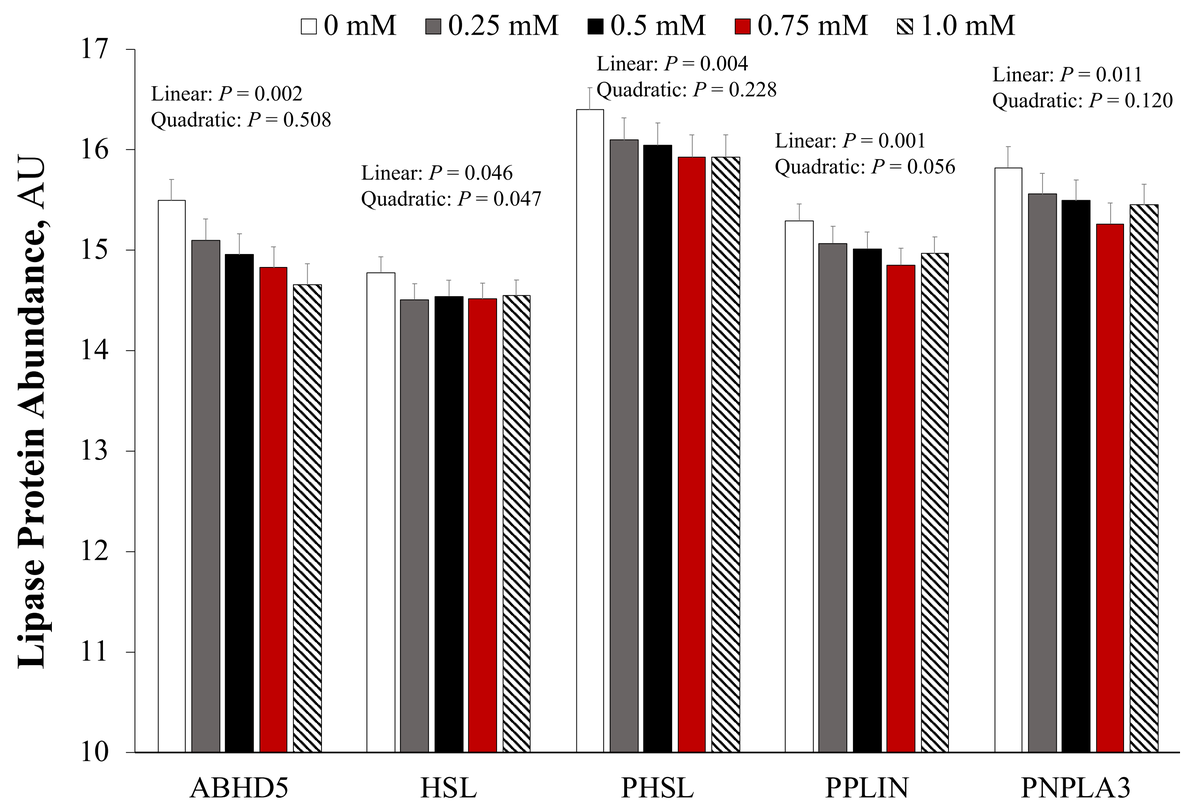

Figure 2. Mean abundance (arbitrary units, AU) of hepatic ABHD5, HSL, PHSL, PPLIN, and PNPLA3 normalized to total protein, and transformed as natural log(abundance) in hepatocytes across fatty acid concentrations. Linear and quadratic contrasts given, no cubic contrasts were found to be significant.

SUMMARY

- Treatment with ALA and FAC increased HSL but decreased PPLIN, compared to PA and OA
- PLIN was decreased by FAC while PNPLA3 was increased by ALA, compared to OA and PA
- Abundance of ABHD5, PPLIN, PHSL, PNPLA3 were all decreased linearly by increasing FAC
- Increasing FA concentration quadratically affected HSL abundance
- No interactions of FA trt x concentration were detected

CONCLUSIONS

- In addition to FA's role as a metabolite, the ability to alter abundance of HLAPs suggests a regulatory role
- The responsiveness of HLAPs to changes in fatty acid concentrations may play a role in peripartum regulation
  - The reduction in HLAP abundance with increasing FA suggests a coordination of increased FA supply with a decrease in the cells ability to remobilize stored FA
- Metabolites related to hepatic fatty acid metabolism should be evaluated for potential roles in HLAP regulation
- Responses to ALA were similar to FAC responses, whereas responses to other individual FA were of an opposing pattern
- Given that the FAC contained ALA, responses to FAC may have been mediated through ALA presence and should be further examined

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