Evaluating the role of dietary polyunsaturated fatty acids on long-chain acyl-CoA synthetase isoform 4 (ACSL4) expression

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Abstract

Long-chain acyl-CoA synthetases (ACSLs) are a family of enzymes that catalyze the rate-limiting thioesterification of a fatty acid and coenzyme A into a fatty acyl-CoA, which can then be further metabolized. ACSL isoform 4 (ACSL4) has been shown to play an important role in beta-cell glucose-stimulated insulin secretion (GSIS); this isoform also specifically favors polyunsaturated fatty acids (PUFAs, linoleic acid (18:2) and arachidonic acid (20:4)) as substrates. We have previously observed that exposing beta-cells to PUFAs, specifically arachidonic and linoleic acids, specifically reduced ACSL4 mRNA expression and total acyl-CoA synthetase (ACS) activity. We hypothesize that a mixture of dietary fatty acids that contain PUFAs would result in similar reductions in ACSL4 expression and total ACS activity. Using a rat insulinoma cell line (INS 832/13), we examined the effects of different fatty acid exposures on ACSL4 expression by quantitative real time RT-PCR and western blot analysis, and total ACS activity. We found that after exposing INS 832/13 cells to arachidonic acid for 48 hours, there was a statistically significant reduction in ACSL4 mRNA expression. Though not statistically significant, exposure to linoleic acid by itself as well as exposure to combinations of dietary fatty acids that contain PUFAs for 48 hours also reduced ACSL4 mRNA expression. However, Western blot analysis showed increased relative ACSL4 protein expression with all fatty acid exposures. Total ACS activity was reduced across all individual fatty acid exposures, but contrary to our expectations, ACS activity was actually slightly increased with exposure to the combinations of dietary fatty acids with PUFAs. Ultimately, the data did not support our hypothesis that a mixture of dietary fatty acids with PUFAs would reduce ACSL4 expression and total ACS activity. Due to some findings inconsistent with previous studies, there is a need for further investigation.

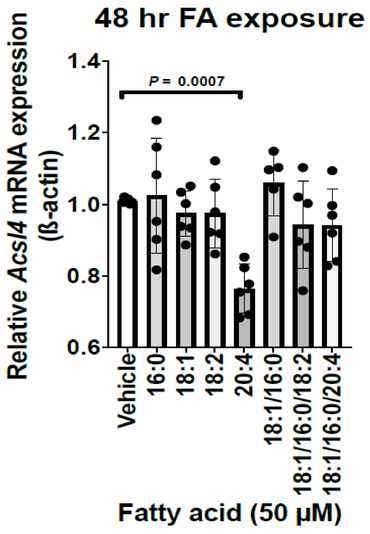
Background

- Fatty acids (FAs) are essential for beta-cell glucose-stimulated insulin secretion (GSIS)[1–5].
- Acyl-CoA synthetases (ACSLs) convert FA to FA-CoAs[6].
- ACSL4 has been shown to be important for beta-cell GSIS and specifically favors polyunsaturated fatty acids (PUFAs)[7,8].
- ω-6 PUFAs, linoleate (18:2) and arachidonate (20:4), reduce ACSL4 mRNA and protein expression[7,9].
- Hypothesis: combinations of dietary fatty acids containing PUFAs reduce ACSL4 expression and total acyl-CoA synthetase activity.

Design

- Cell culture: A rat insulinoma cell line (INS 832/13) was cultured and exposed to 8 different groups of exposures – vehicle, oleic acid (OA), palmitic acid (PA), arachidonic acid (AA), linoleic acid (LA), OA/PA, OA/PA/AA, OA/PA/LA
- Quantitative real time RT-PCR: total RNA was extracted from INS 832/13 cells and reverse transcribed to cDNA, which was amplified by real time PCR.
- Western blot analysis: cells were lysed and protein was loaded on SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The blots were probed with antibodies against GAPDH for control and ACSL4.
- ACS activity assay: ACS activity was measured with [1-^{14}C]palmitic acid and [1-^{14}C]oleic acid as substrates.

Results



Exposure to ω-6 PUFA and dietary fatty acid/PUFA combinations for 48 hours reduces ACSL4 mRNA expression in INS 832/13 cells by 4 to 26 %

Figure 1- *Acsl4* mRNA expression is significantly reduced in INS 832/13 cells exposed to 50 µM arachidonate (20:4) for 48 hours (p = 0.0007 vs vehicle). Although not statistically significant, exposure to linoleate (18:2) and combinations of dietary fatty acids with PUFAs also reduced mRNA expression. Data are means of n = 6 experiments; 1-way ANOVA with Dunnett's multiple comparisons test.

Exposure to ω-6 PUFA and dietary fatty acid and PUFA combinations for 48 hours increases ACSL4 protein expression in INS 832/13 cells by 38 to 224 %

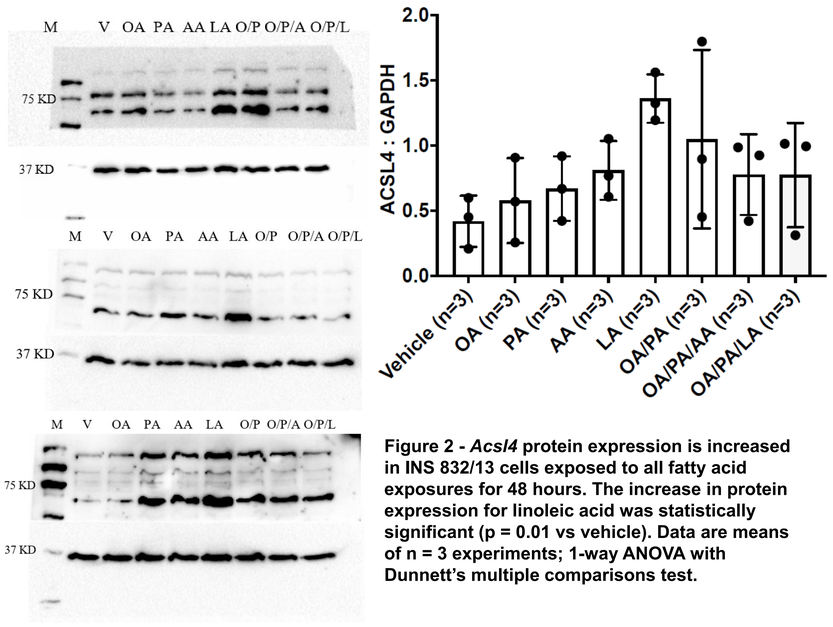


Figure 2 - *Acsl4* protein expression is increased in INS 832/13 cells exposed to all fatty acid exposures for 48 hours. The increase in protein expression for linoleic acid was statistically significant (p = 0.01 vs vehicle). Data are means of n = 3 experiments; 1-way ANOVA with Dunnett's multiple comparisons test.

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Acknowledgements

- This work is supported by NIH/NIDDK grant DK107481 (to ELK)
- A special thanks goes to the Klett lab and my mentors Dr. Eric Klett and Shufen Chen

Exposure to combinations of dietary fatty acids with PUFAs for 48 hours increases ACS activity in INS 832/13 cells by 5 to 10 %

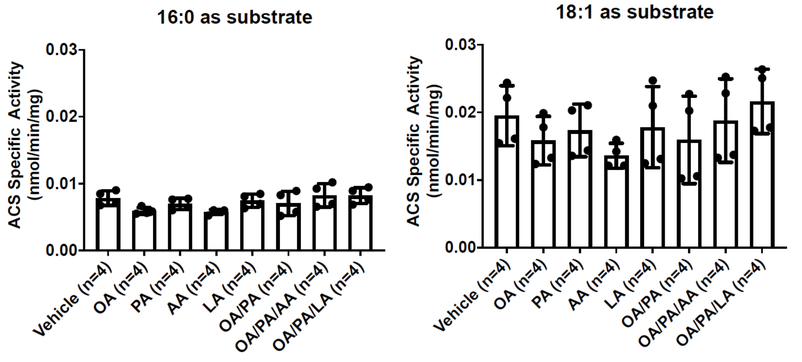

Figure 3 – ACS activity is increased in INS 832/13 cells exposed to combinations of dietary fatty acids with PUFAs for 48 hours and decreased across all other exposures, although there was no statistical significance. ACS activity with palmitic acid (16:0) as substrate is much lower relative to using oleic acid (18:1) as substrate. Data are means of n = 4 experiments; 1-way ANOVA with Dunnett's multiple comparisons test

Summary

- ω-6 PUFA exposure for 48 hours reduces ACSL4 mRNA expression in INS 832/13 cells
- Combinations of dietary fatty acids with PUFAs reduces ACSL4 mRNA expression in INS 832/13 cells
- All fatty acid exposures increase relative ACSL4 protein expression
- ω-6 PUFA exposure reduces total ACS activity
- Combinations of dietary fatty acids with PUFAs increases total ACS activity

Conclusions

- There was a lack of significant reduction in mRNA for combination of dietary fatty acids compared to individual PUFA – it is possible that in combination, the saturated and monounsaturated fatty acids overwhelmed the effects of the PUFAs.
- In comparing the western blot analysis to mRNA, there is no reduction – this could potentially be due to a shorter half life for mRNA compared to protein.
- The quantification of western blot protein concentration is crude, the total ACS activity measured is more important.
- Total ACS activity was reduced with arachidonic acid but ACSL4 protein expression was not altered – since the enzyme assay is measuring all ACS activity and not specific to ACSL4, there is a possibility that other proteins may be contributing to total ACS activity.
- The substrates used in the ACS activity assays were palmitic acid (the most prominent saturated dietary FA) and oleic acid (the most prominent monounsaturated dietary FA) – future research could investigate the effects of using PUFAs as the substrate instead.
- These combinations of dietary fatty acids with PUFAs were meant to mimic a Western diet – the lack of significant reduction in ACS activity and ACSL4 mRNA expression for combinations of PUFAs versus individual PUFAs could indicate that the oleic and palmitic acids negate the effects of PUFAs.