Primary bovine hepatocyte lipolytic genes may respond differently to *LXRα* perturbation than murine

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BACKGROUND

- Fatty liver is a common metabolic disorder in dairy cows during the transition to lactation period
- Key genes and proteins involved in lipolysis have not been explored in bovine
  - May elucidate how fatty liver develops and how to mitigate it
- In other species
  - Patatin-like phospholipase domain-containing protein 3 (PNPLA3) may be regulated by sterol regulatory element-binding protein 1c (SREBP1c), a main driver of lipolysis in humans
  - Upon direct regulation of liver X receptor α (*LXRα*) in murine with 22(R)-hydroxycholesterol (AGO) and simvastatin (ANT), indirect regulation of SREBP1c has been seen
  - In humans, *LXRα* is highly expressed in liver and regulates inflammatory and lipolytic proteins such as SREBP1c
- An isoform of *LXRα* and less abundant in liver, *LXRβ* was studied to ensure AGO and ANT acted upon *LXRα*
- Involved in glycolysis, carbohydrate response element binding protein (*ChREBP*) competes with SREBP1c for substrates required for different fates
- Objective: To determine if PNPLA3 is regulated by SREBP1c in primary bovine hepatocytes through administration of AGO or ANT, as seen by *LXRα* regulation of murine models
- Hypothesis: Treatment of AGO will decrease SREBP1c and thus PNPLA3, while treatment of ANT will increase SREBP1c and thus PNPLA3

MATERIALS & METHODS

- Primary bovine hepatocytes were isolated from bull calves (n=3, < 7 d of age)
- About 2 million cells per 35 mm dish were cultured in monolayers for 24 hr in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% FBS, 1% antibiotic-antimycotic
  - Cells were at least 80% confluent before treatment
- Wells were randomly assigned treatment in triplicate with 0 or 100 µM of either AGO or ANT
  - Both the AGO and ANT were brought up in 1 N NaOH to reach a target stock concentration of 1 mM
- Primary bovine hepatocytes were harvested in TRIzol 24 hr after treatment
  - mRNA analysis
    - Gene expression using RT-qPCR with SsoAdvanced SYBR on a CFX-384; primers previously validated, all products exhibited a single melt curve
    - Standard curve method utilized
    - Arithmetic mean of reference genes used for normalization
  - Protein analysis
    - Protein abundance of 25 µg protein per lane
    - Stain-Free technology utilized for total lane protein normalization
    - In-house pool used to correct background and ensure blot quality
    - Samples made relative to control
- Data analyzed using PROC MIXED, SAS, 9.4, with fixed effect of treatment and random effect of calf
  - Data reported as LSM ± SEM with tendency (†) declared at $0.05 < P \leq 0.10$

RESULTS

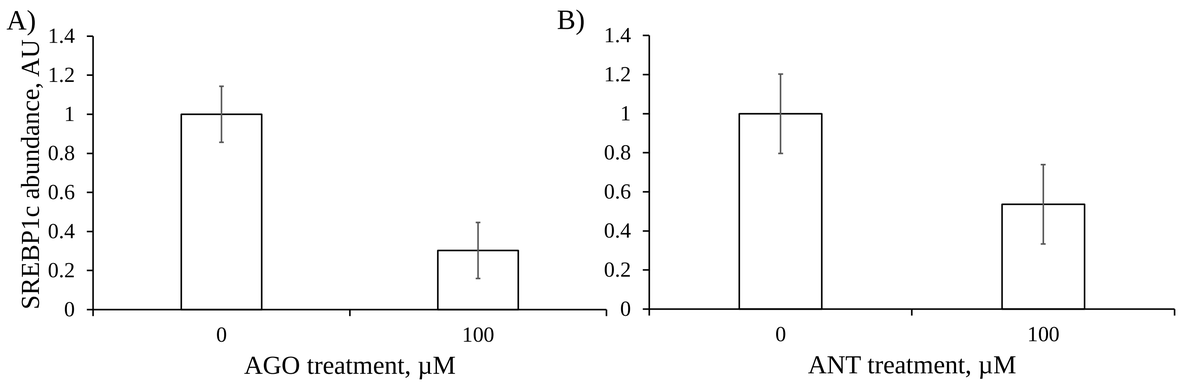

Figure 1. Protein abundance of sterol regulatory element-binding protein 1c (SREBP1c) with treatment of either A) 22(R)-hydroxycholesterol (AGO) or B) simvastatin (ANT) at either 0 µM or 100 µM. Panel A) SREBP1c abundance: 1.0 vs. 0.30 ± 0.14 arbitrary units (AU) (P=0.18); Panel B) SREBP1c abundance: 1.0 vs. 0.54 ± 0.20 AU (P=0.25).

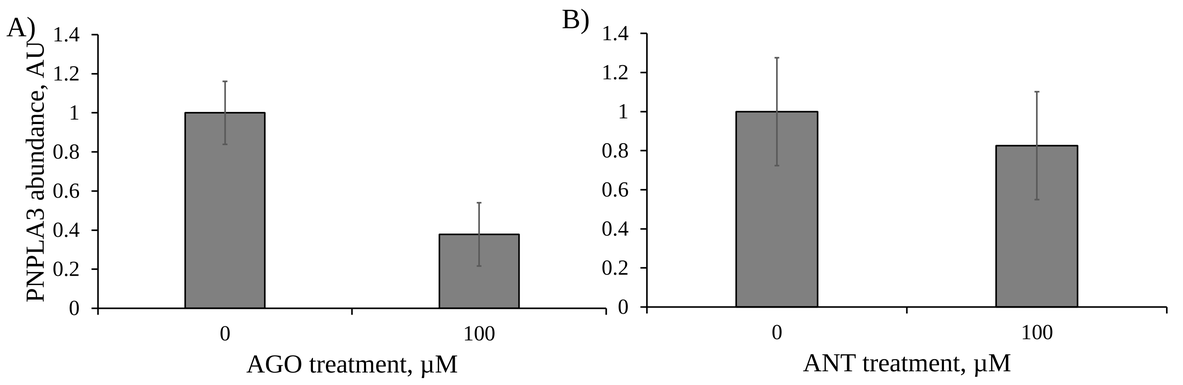

Figure 2. Protein abundance of patatin-like phospholipase domain-containing protein 3 (PNPLA3) with treatment of either A) 22(R)-hydroxycholesterol (AGO) or B) simvastatin (ANT) at either 0 µM or 100 µM. Panel A) PNPLA3 abundance: 1.0 vs. 0.38 ± 0.16 arbitrary units (AU) (P=0.23); Panel B) PNPLA3 abundance: 1.0 vs. 0.83 ± 0.28 AU (P=0.70).

RESULTS

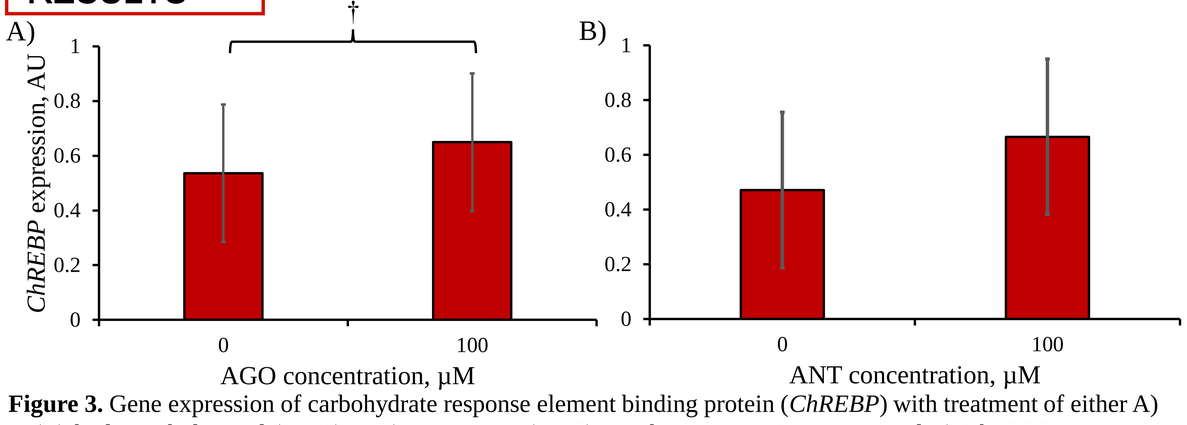

Figure 3. Gene expression of carbohydrate response element binding protein (*ChREBP*) with treatment of either A) 22(R)-hydroxycholesterol (AGO) or B) simvastatin (ANT) at either 0 µM or 100 µM. Panel A) *ChREBP* expression: 0.54 vs. 0.65 ± 0.26 arbitrary units (AU) (P=0.09); Panel B) *ChREBP* expression: 0.47 vs. 0.67 ± 0.28 AU (P=0.26).

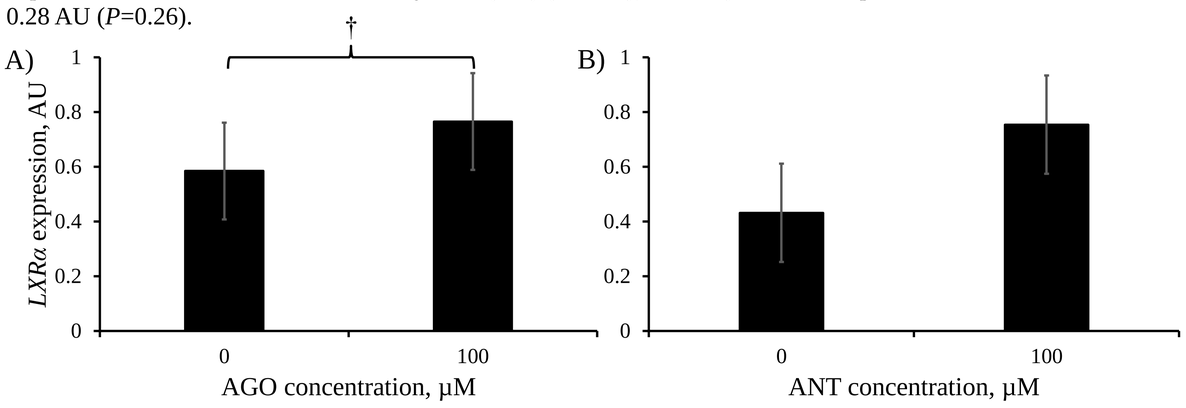

Figure 4. Gene expression of liver X receptor α (*LXRα*) with treatment of either A) 22(R)-hydroxycholesterol (AGO) or B) simvastatin (ANT) at either 0 µM or 100 µM. Panel A) *LXRα* expression: 0.58 vs. 0.77 ± 0.18 arbitrary units (AU) (P=0.07); Panel B) *LXRα* expression: 0.43 vs. 0.75 ± 0.18 AU (P=0.16).

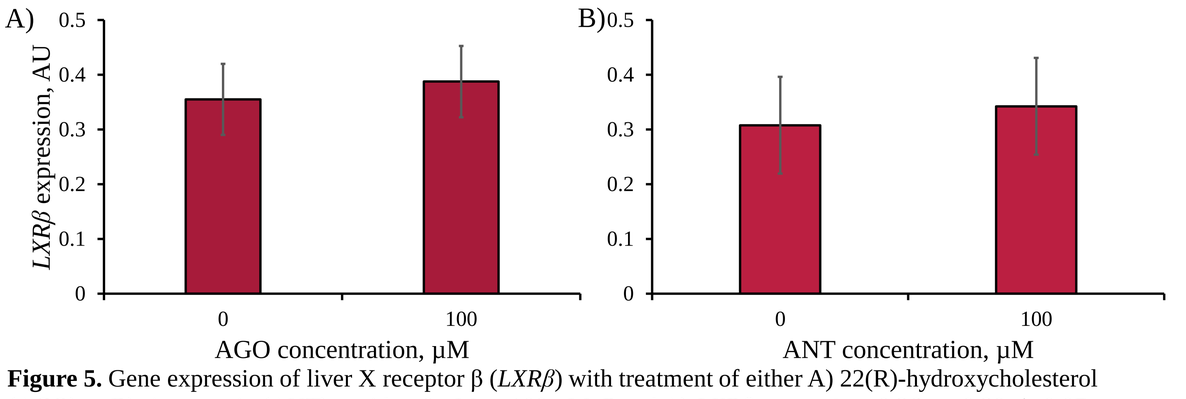

Figure 5. Gene expression of liver X receptor β (*LXRβ*) with treatment of either A) 22(R)-hydroxycholesterol (AGO) or B) simvastatin (ANT) at either 0 µM or 100 µM. Panel A) *LXRβ* expression: 0.36 vs. 0.39 ± 0.07 arbitrary units (AU) (P=0.30); Panel B) *LXRβ* expression: 0.31 vs. 0.34 ± 0.09 AU (P=0.80).

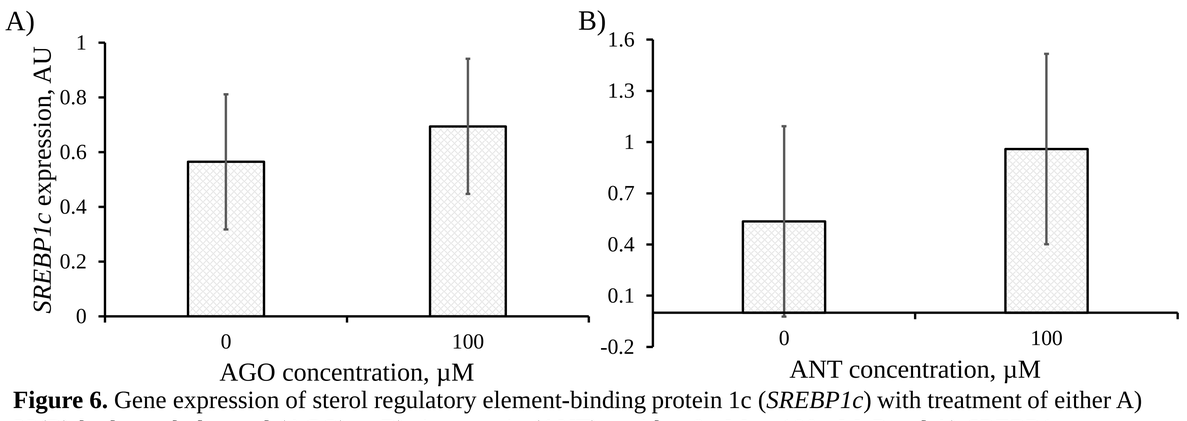

Figure 6. Gene expression of sterol regulatory element-binding protein 1c (*SREBP1c*) with treatment of either A) 22(R)-hydroxycholesterol (AGO) or B) simvastatin (ANT) at either 0 µM or 100 µM. Panel A) *SREBP1c* expression: 0.56 vs. 0.69 ± 0.25 arbitrary units (AU) (P=0.45); Panel B) *SREBP1c* expression: 0.54 vs. 0.96 ± 0.56 AU (P=0.47).

SUMMARY

- Genes involved in both glycolysis and lipolysis tended to have increased expression with treatment of 100 µM AGO
  - *ChREBP*
  - *LXRα*
- Other genes involved in lipolysis had no evidence of expression change with treatment of 100 µM AGO
  - *LXRβ*
  - *SREBP1c*
- Treatment of ANT at 100 µM had no evidence of expression change on any lipolytic genes analyzed
- Protein abundance had no evidence of change with treatment of either 100 µM AGO or ANT

CONCLUSIONS

- Treatment of AGO, a direct agonist of *LXRα*, tended to increase expression as expected
- Treatment of ANT, a direct antagonist of *LXRα*, did not seem to impact on gene expression
  - Bovine *LXRα* may not behave similarly as murine does to ANT
- Expression of *ChREBP*, involved in glycolysis in other species, tended to increase with addition of AGO
  - This may suggest differential regulation of AGO in bovine
  - The function of *ChREBP* within
- There was no evidence that either *SREBP1c* gene expression or SREBP1c protein abundance were affected by either AGO or ANT
  - This may reflect a difference in SREBP1c responsiveness to *LXRα* activation in bovine or may reflect a lack of responsiveness of bovine *LXRα* to the slected agents and concentrations for AGO and ANT treatment
- No evidence was found for any treatment effect on protein abundance of PNPLA3
- Further research should examine
  - The relationship between gene expression and protein abundance of SREBP1c and PNPLA3 is warranted
  - If bovine PNPLA3 is regulated by SREBP1c
- In regards to treatment with ANT
  - Research drawing conclusions from other species, especially murine models, must be critically analyzed when applying to bovine
- In regards to treatment with AGO
  - Other sterols may be better suited in regulation of bovine lipolytic gene expression and protein abundance

ACKNOWLEDGEMENTS

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