REGULATION OF MARBLING DEVELOPMENT BY FATTY ACIDS

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Funded by The Beef Checkoff

Pasture feeding depresses marbling.

Pasture-fed for 8 mo

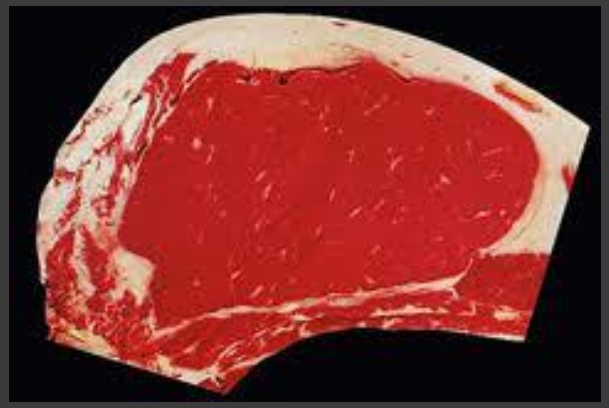
USDA Select

Wagyu Corn-fed for 16 mo

Super Prime

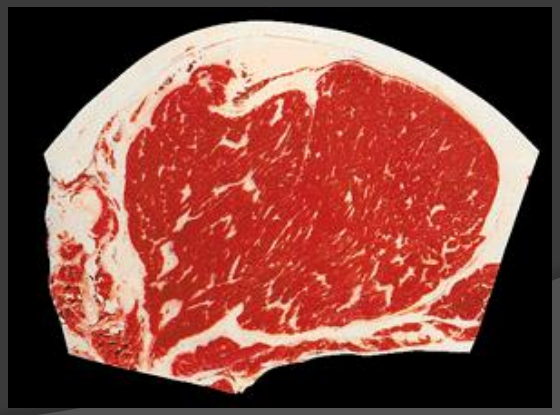
Corn feeding promotes marbling.

Corn-fed for 6 mo



USDA Choice

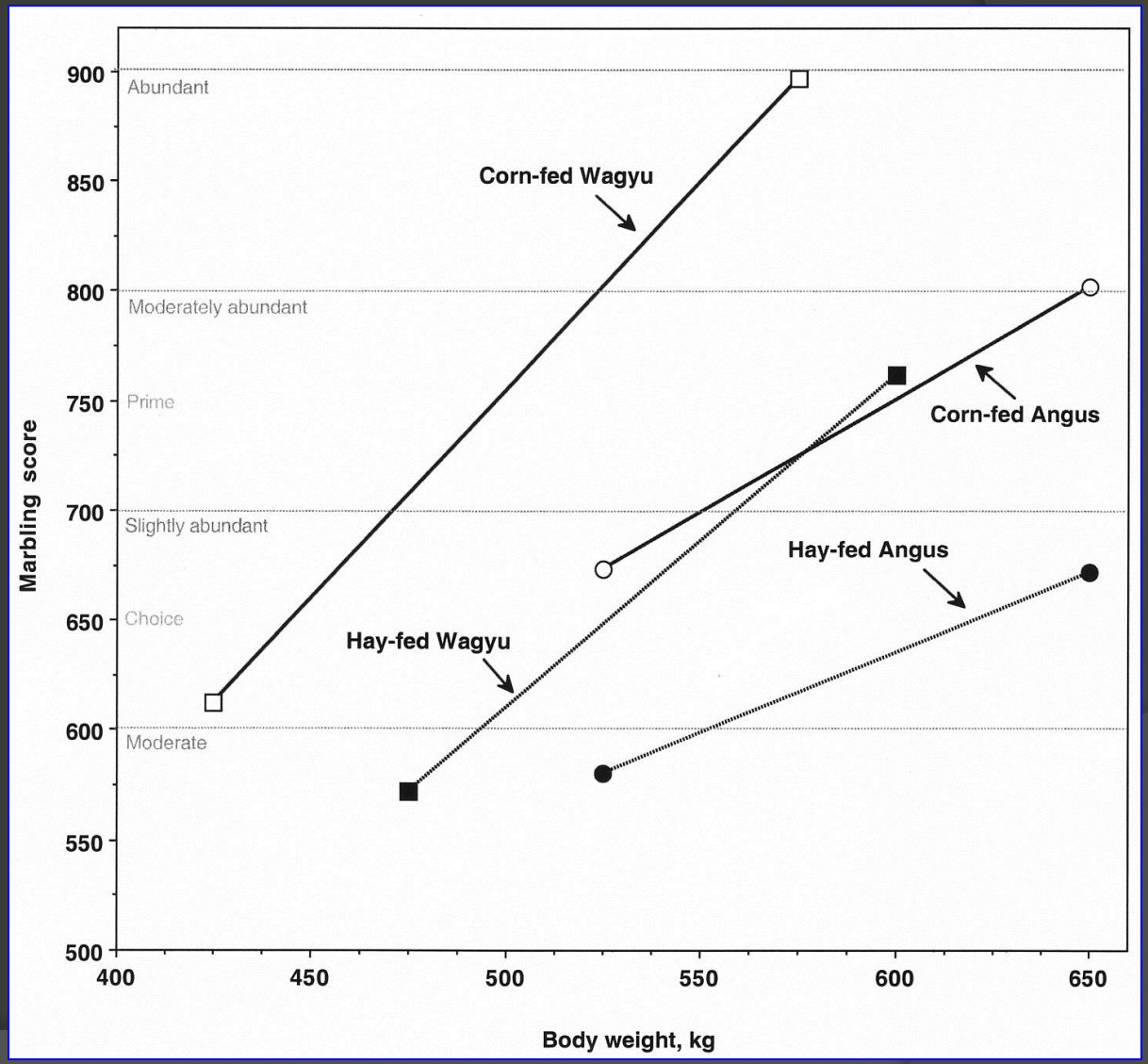
Corn-fed for 8 mo



USDA Prime

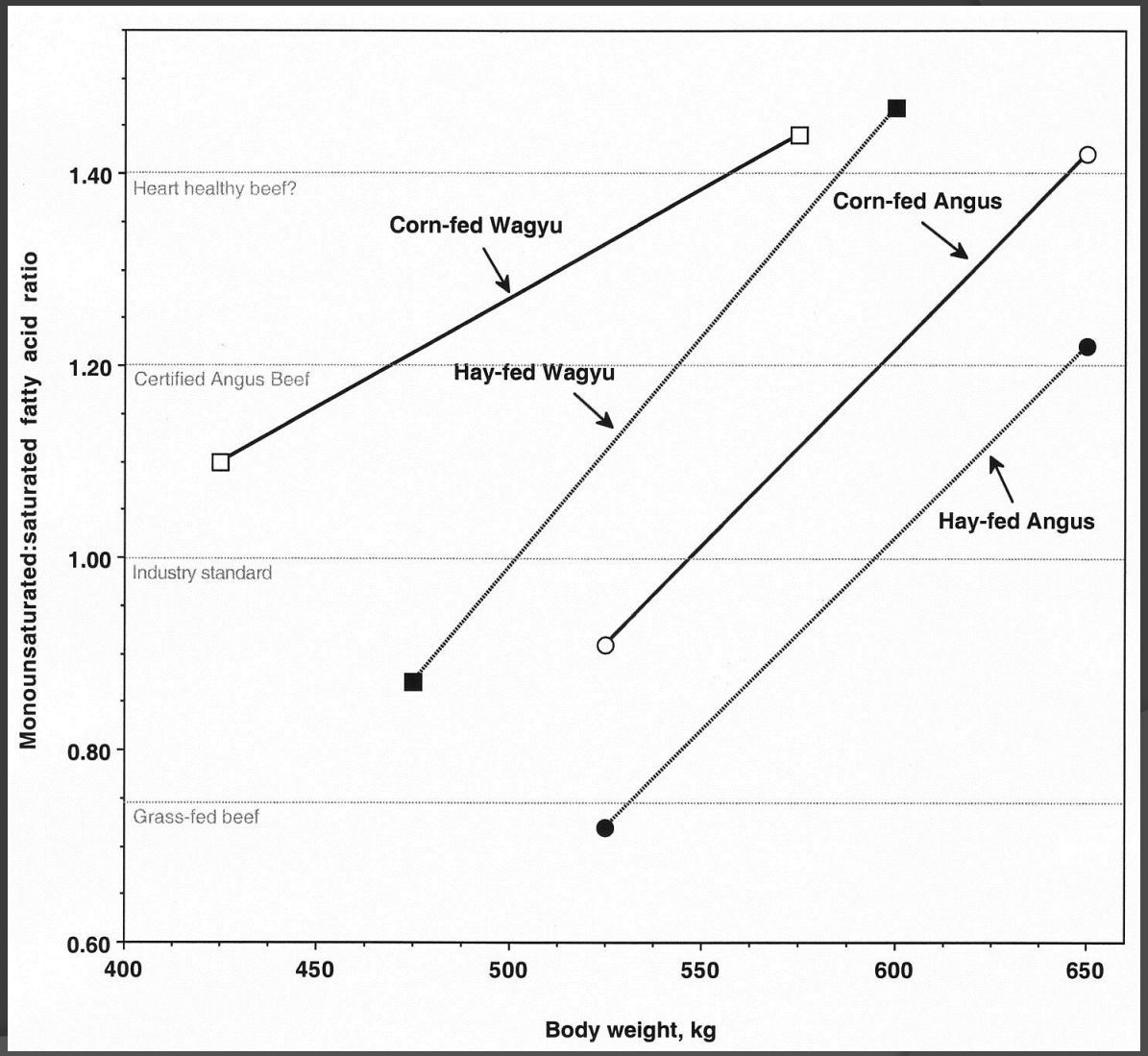
Marbling scores are independent of final body weights.

- Pasture-fed steers have lower marbling scores than corn-fed steers, even when raised to the same body weight.



Fatty acid composition also is independent of final body weight.

- Hay-fed Angus steers have lower MUFA:SFA ratios than corn-fed steers, even when raised to the same body weight.
- *Is this caused by fatty acids in pasture?*



Marbling research funded by The Beef Checkoff

- Hypotheses
  - Feeding high-concentrate diets to beef cattle stimulates marbling via the effects of endogenously produced oleic acid.
  - α-Linolenic acid (**ALA**) from pastures/grasses specifically depresses marbling development.
  - Subcutaneous adipose is less sensitive than i.m. adipose tissue to the effects of fatty acids.

Funded by The Beef Checkoff

Marbling research funded by the Beef Checkoff

- Objective
  - Demonstrate the effects of specific fatty acids on the differentiation of muscle satellite cells, i.m. and s.c. adipose tissues, and i.m. and s.c. preadipocytes

University of Idaho

BEEF

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University of Idaho

BEEF

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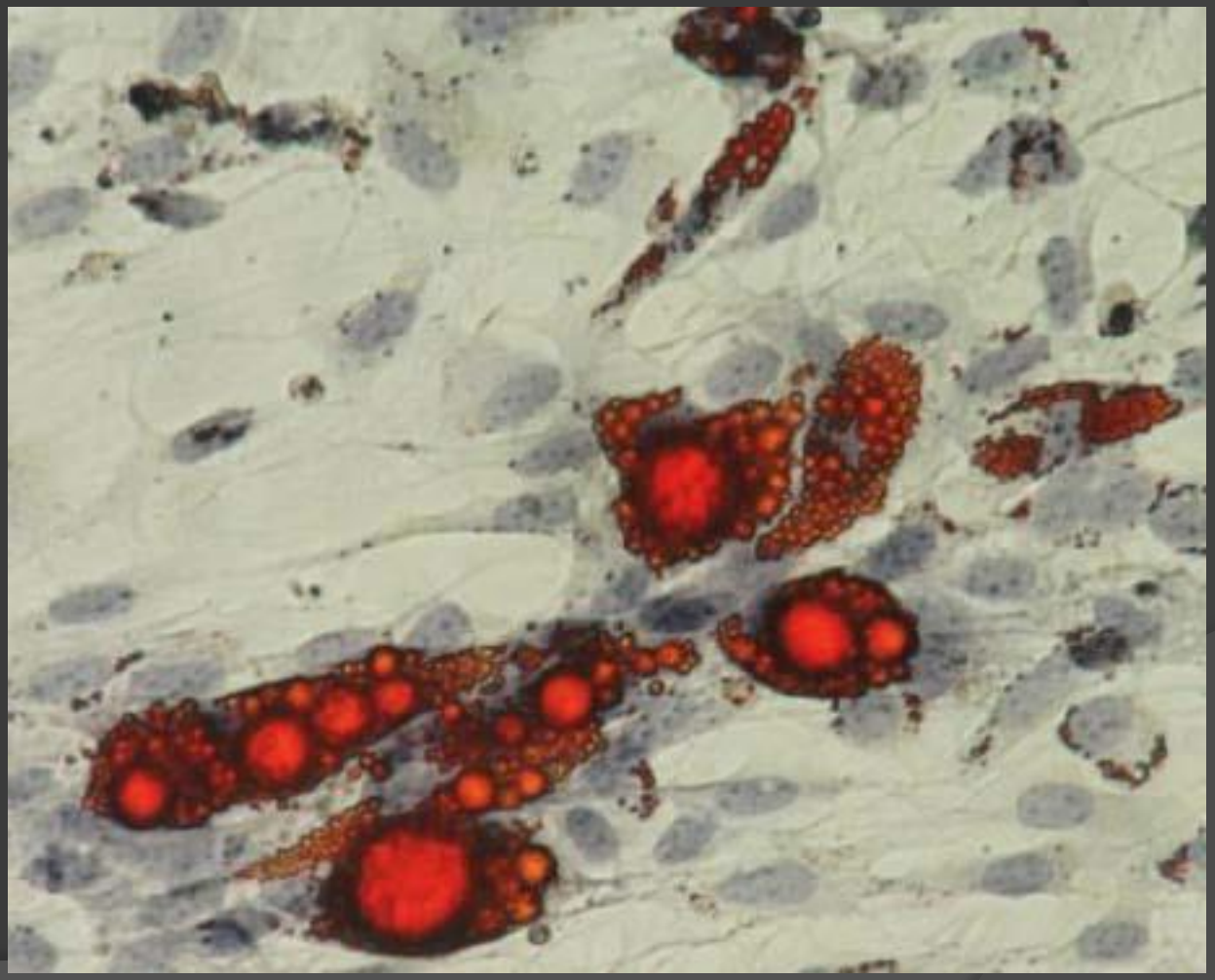
Contribution of M. Doumit

- Hypothesis: Intramuscular preadipocytes are more sensitive to media fatty acids than s.c. adipocytes.
  - Stromal-vascular cells were isolated from i.m. and s.c. adipose tissue depots and cultured.
  - Preadipocytes were treated with combinations of arachidonic, α-linolenic, linoleic, oleic, *trans*-vaccenic and stearic acid, and *trans*-10,*cis*12 CLA acid.

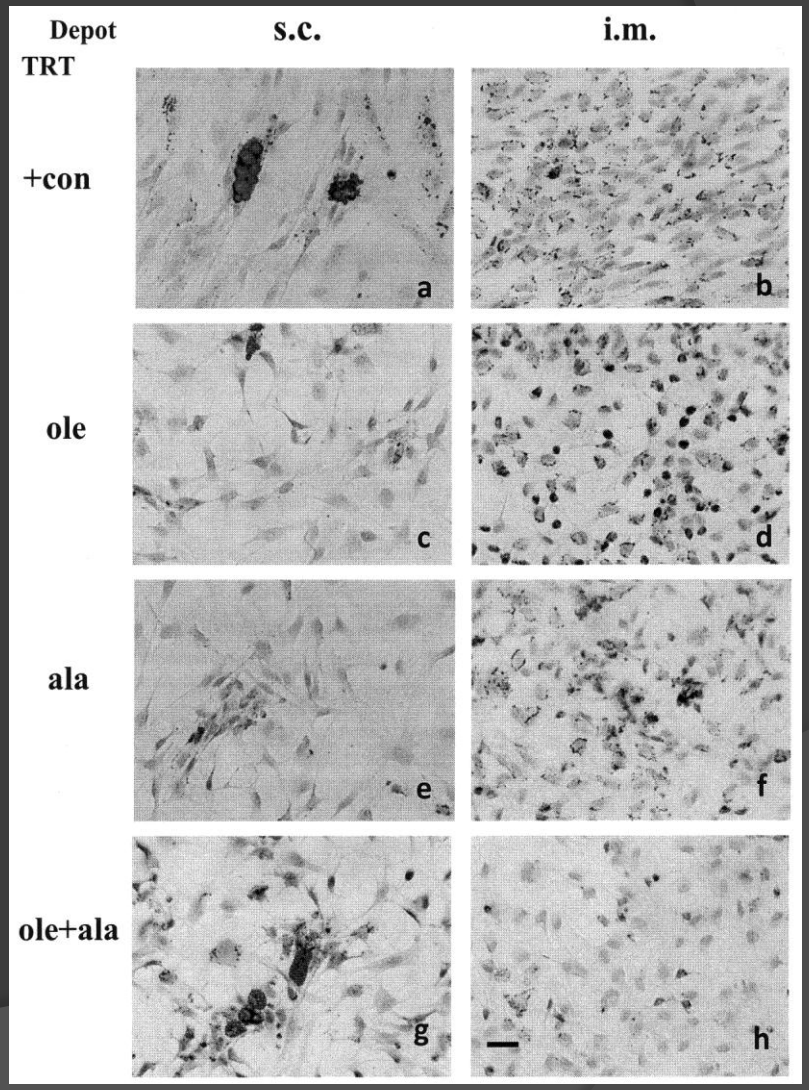
Differentiation of bovine intramuscular stromal vascular cells

- Intramuscular (marbling) preadipocytes proliferate in culture.
- Addition of specific media promotes lipid filling.



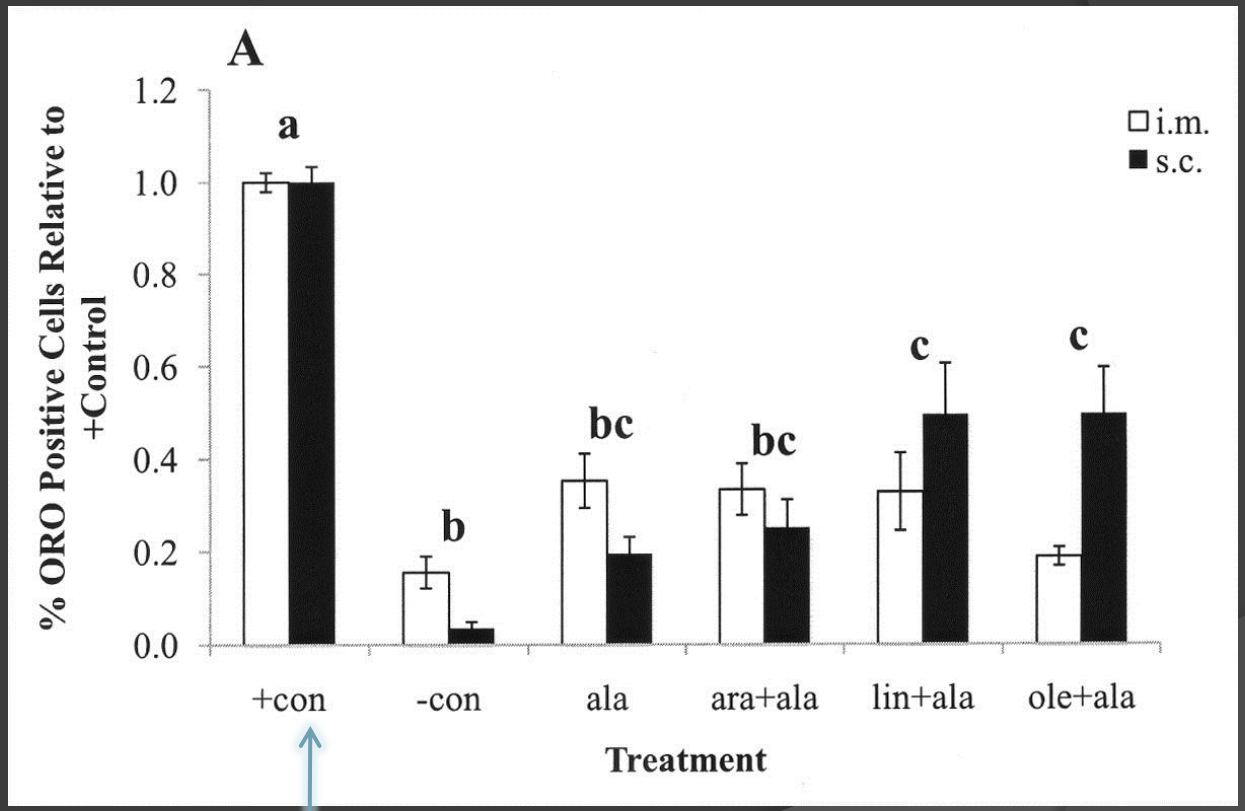
Certain fatty acids promote lipid filling of s.c. and i.m. adipocytes.

- Oleic acid promotes lipid filling in i.m. preadipocytes.
- The combination of oleic acid and ALA strongly increases lipid filling of s.c. preadipocytes.



α-Linolenic acid, arachidonic, linoleic and oleic acid combined effects

- *Linoleic acid plus ALA* and *oleic acid plus ALA* increased lipid filling in s.c. adipose tissue.
- ALA alone increased lipid filling in i.m. adipose tissue.
- *Conclusion: s.c. and i.m. preadipocytes respond differently to media fatty acids.*

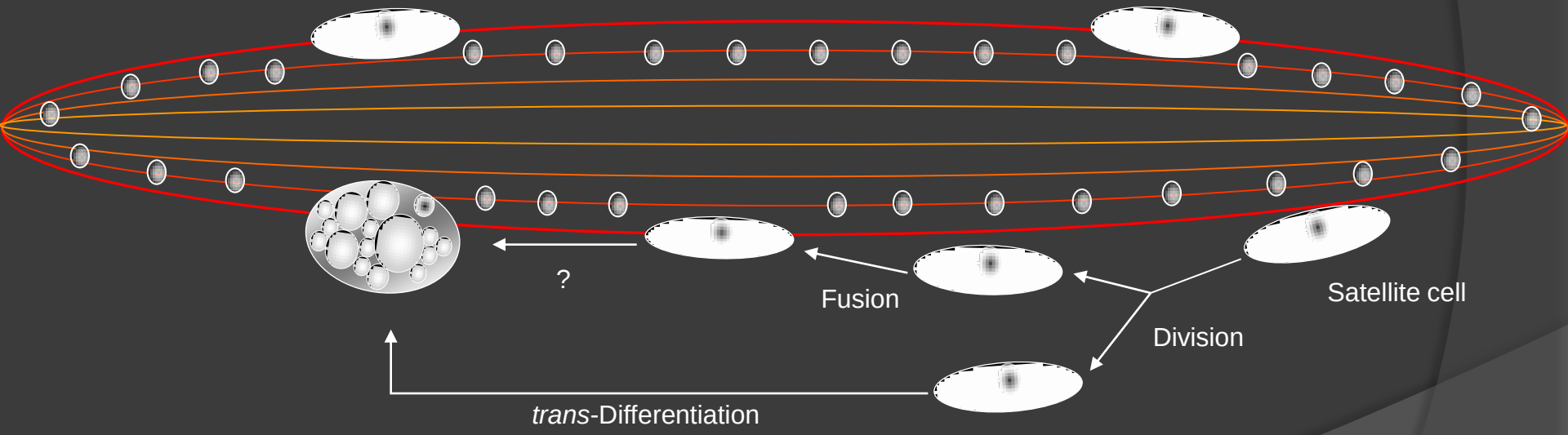


Lipid control

Contributions of Texas Tech University

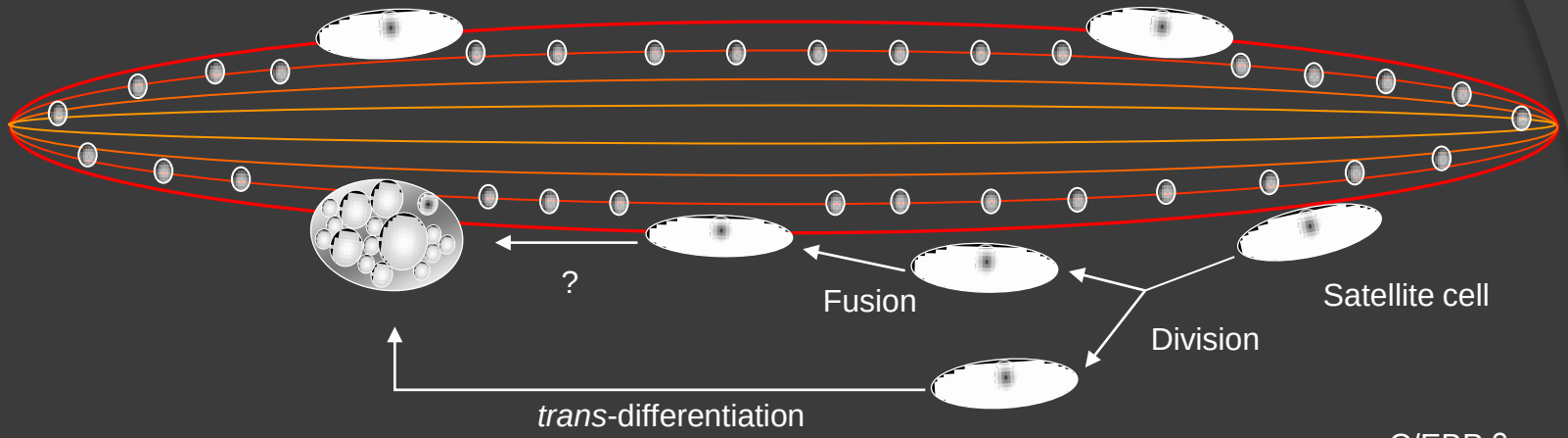
- Document:
  - *trans*-Differentiation of muscle satellite cells to preadipocytes
  - Effects of specific fatty acids on GPR receptors

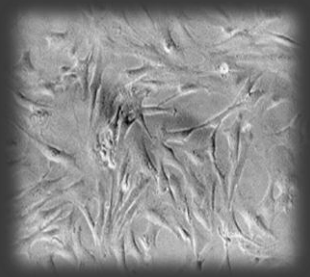
Satellite cell development and differentiation



Location : Between basal lamina and sarcolemma

Bovine satellite cell development and *trans*-differentiation

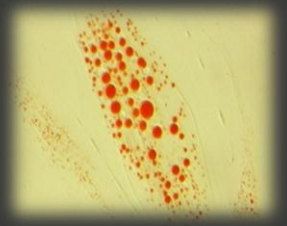


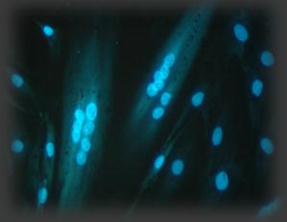


Satellite cells

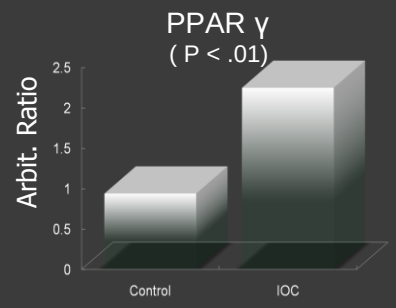
Fatty acids,
TZDs

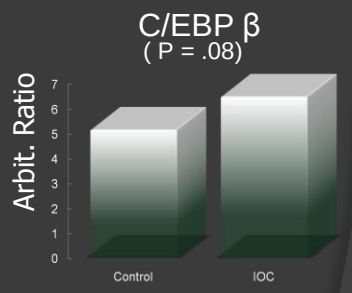
Serum,
Growth factors,
Anabolic steroids

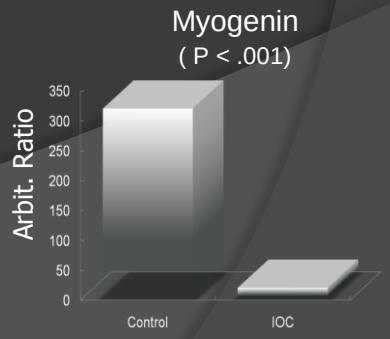




Differentiation







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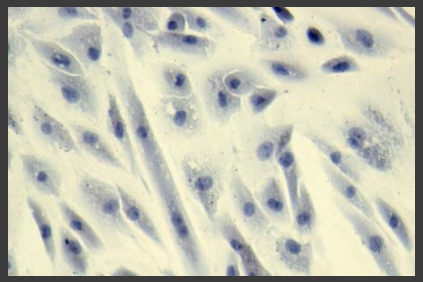
Chung and Johnson, 2009 J. Anim. Sci.

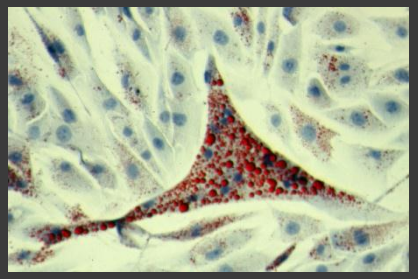
Cell Culture

Bovine Muscle Derived Cell

Differentiation

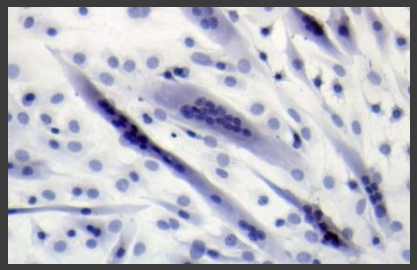


trans-differentiation

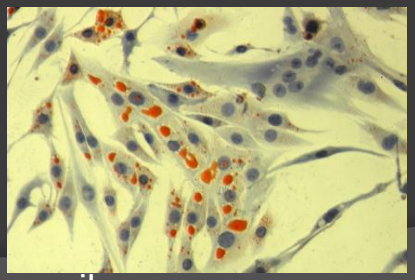


Mouse Myoblast (C2C12)

Differentiation

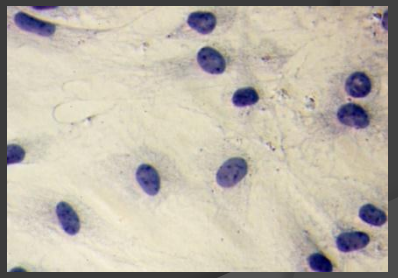


trans-Differentiation

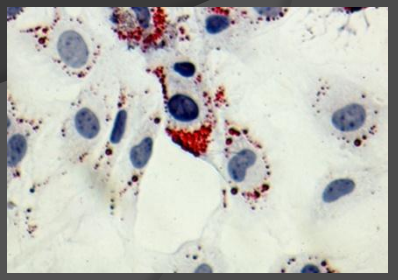


Mouse Mesenchymal Cell (10T 1/2)

Differentiation



trans-Differentiation



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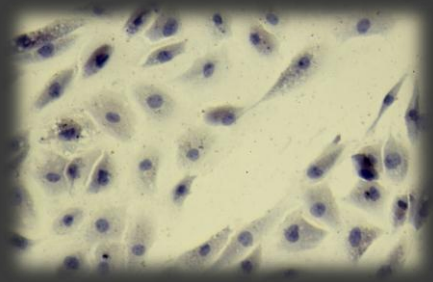
Bovine Satellite Cell (BSC) *trans*-differentiation

Adipogenic gene expression

Myogenic gene expression



Muscle-derived cells

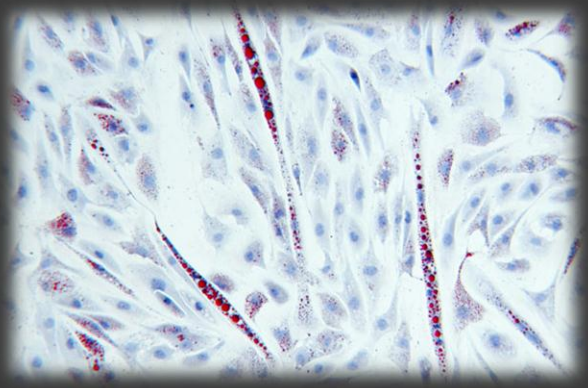
C/EBPβ
ADD/SREBP1
PPARγ, C/EBPα
SCD, ACC, FAS

MUFA,PUFA
TZD, Insulin

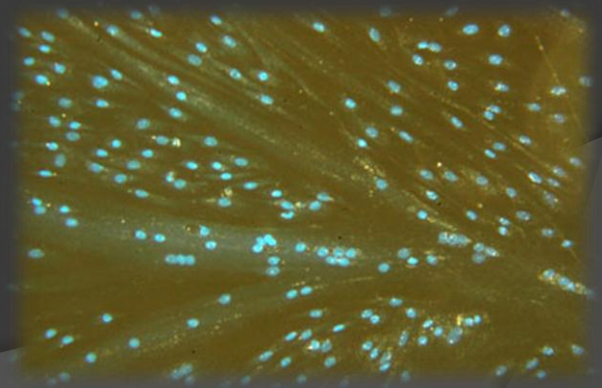
Horse Serum
PUFA

Myogenin
MyoD
Myf5
MHC I, IIA, IIX

Regulatory factors



trans-Differentiation

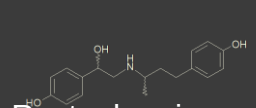


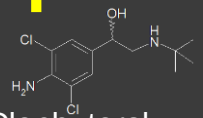
Differentiation

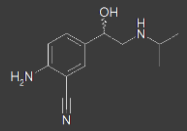
G protein-coupled receptors (GPRs)

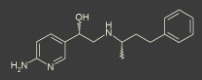
- GPR40, 41, 43, and 120 are activated by free fatty acids.
- These receptors appear to be important for normal adipocyte differentiation.

Role of β-AA and GPRs in Muscle and IMAT


Rectophamine


Clenbuterol


Cimaterol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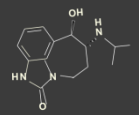
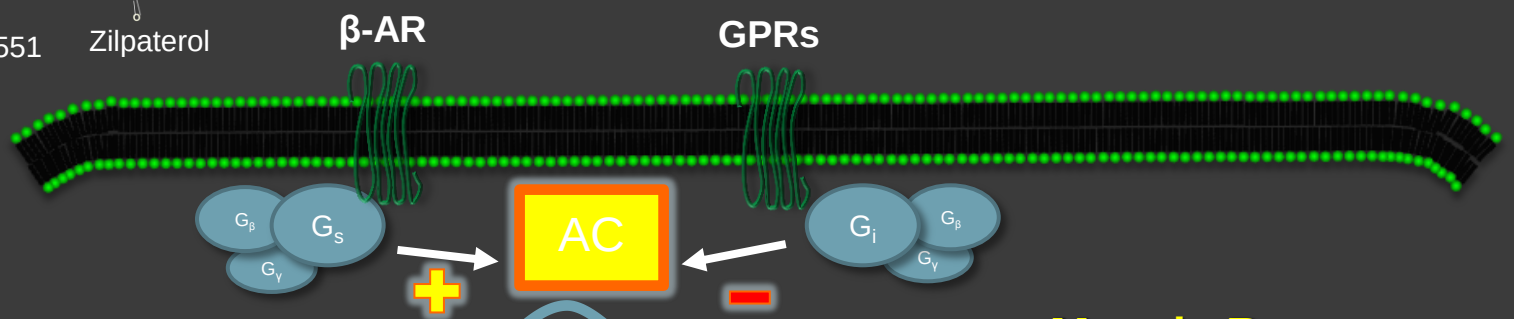

Zilpaterol

LCFA

SCFA



Muscle Response

- ↑ Protein Synthesis ↓
- ↓ Protein Degradation ↑

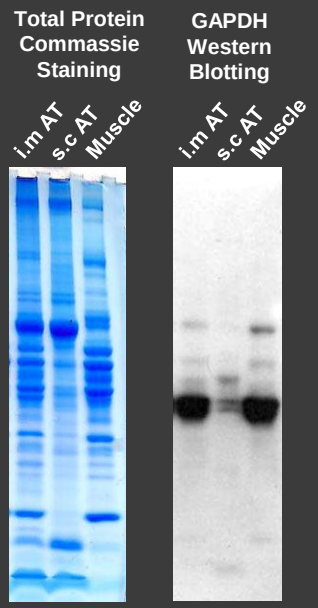
Adipose Tissue Response

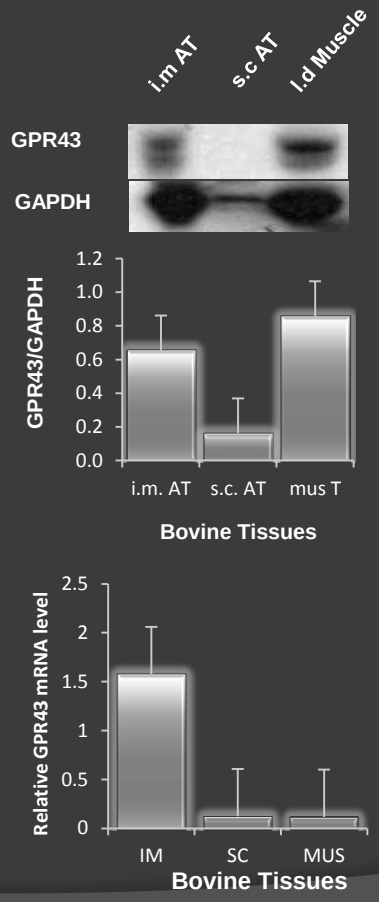
- ↓ Lipogenesis ↑
- ↑ Lipolysis ↓

GPR activity in bovine IM and SC adipocytes

GPR43 in IMAT, SCAT and MUSCLE

1. Western Blot





2. Real Time PCR

- **GPR43 membrane protein is highly distributed in IMAT and muscle tissues but not in SCAT**
- **Relative GPR43 mRNA level was greater in IMAT than SCAT or MUSCLE**

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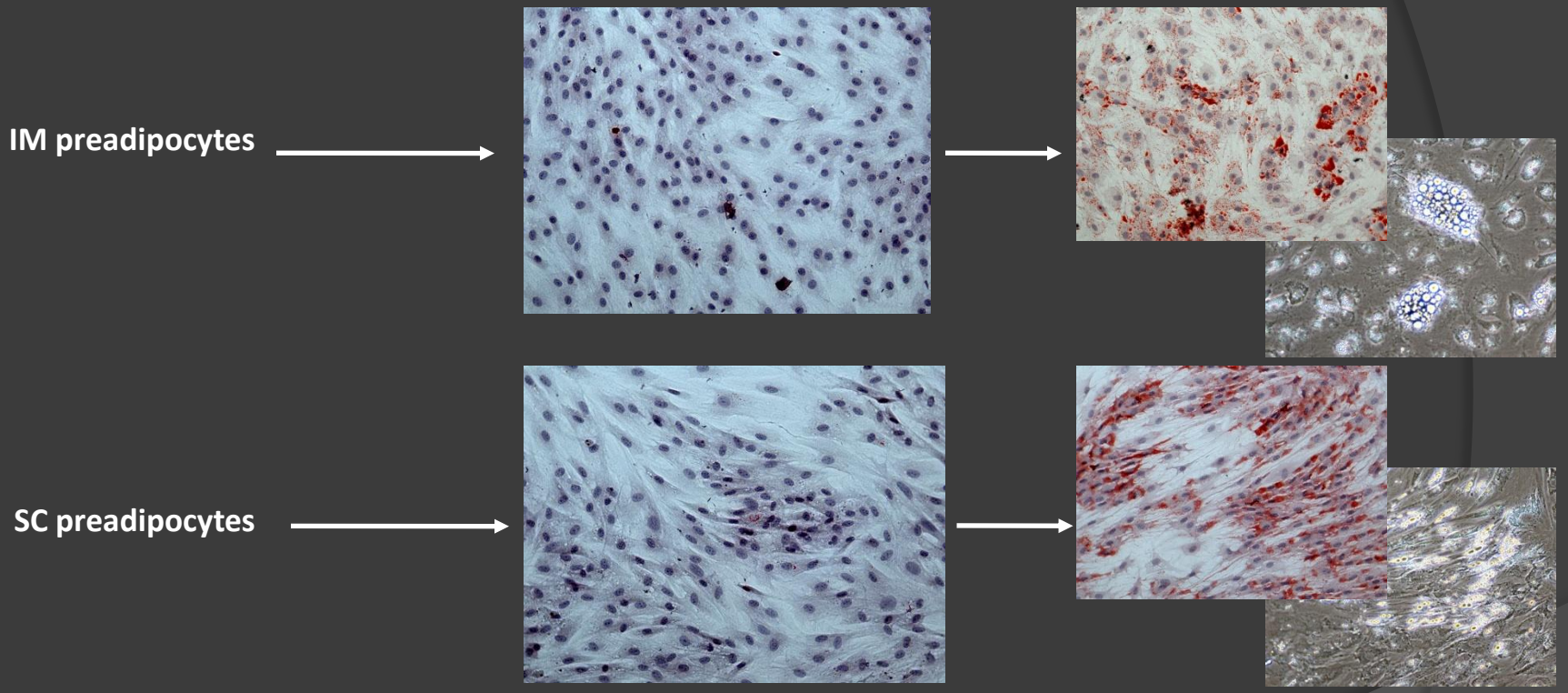
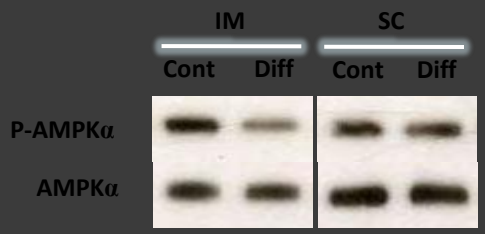
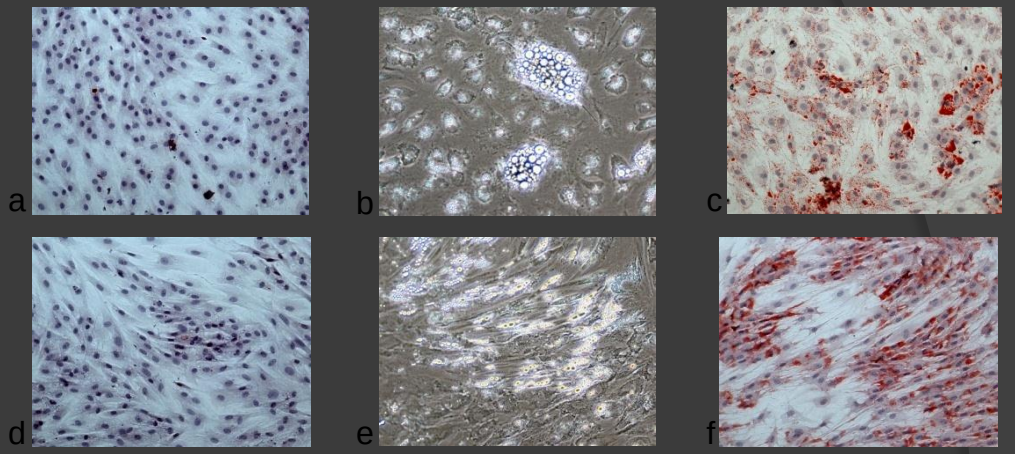
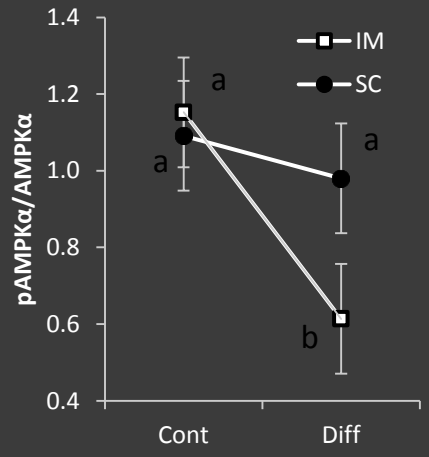
Bovine Preadipocyte Culture

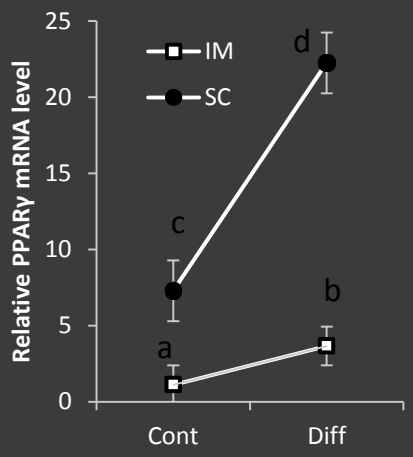


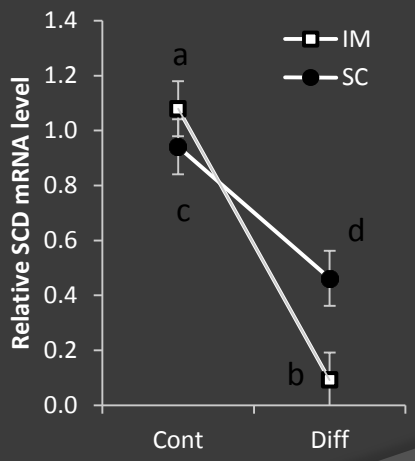
Time Frame of Cell Culture

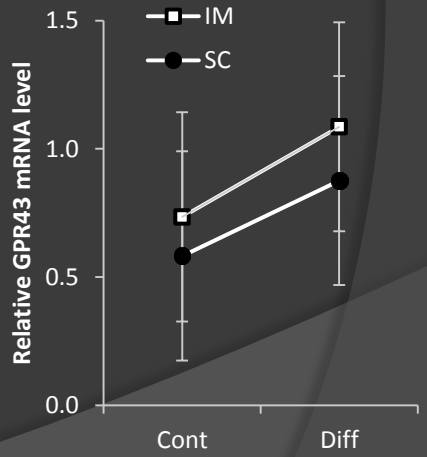












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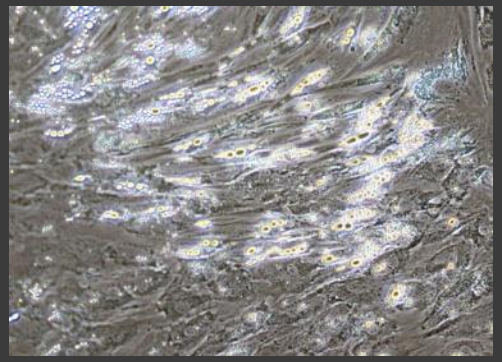

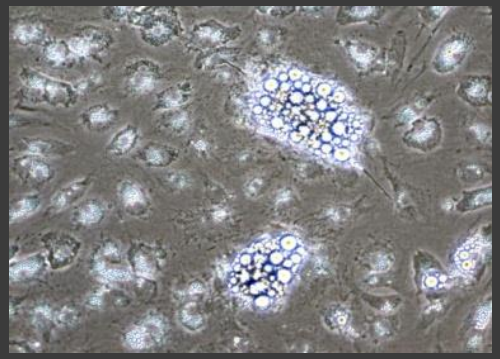

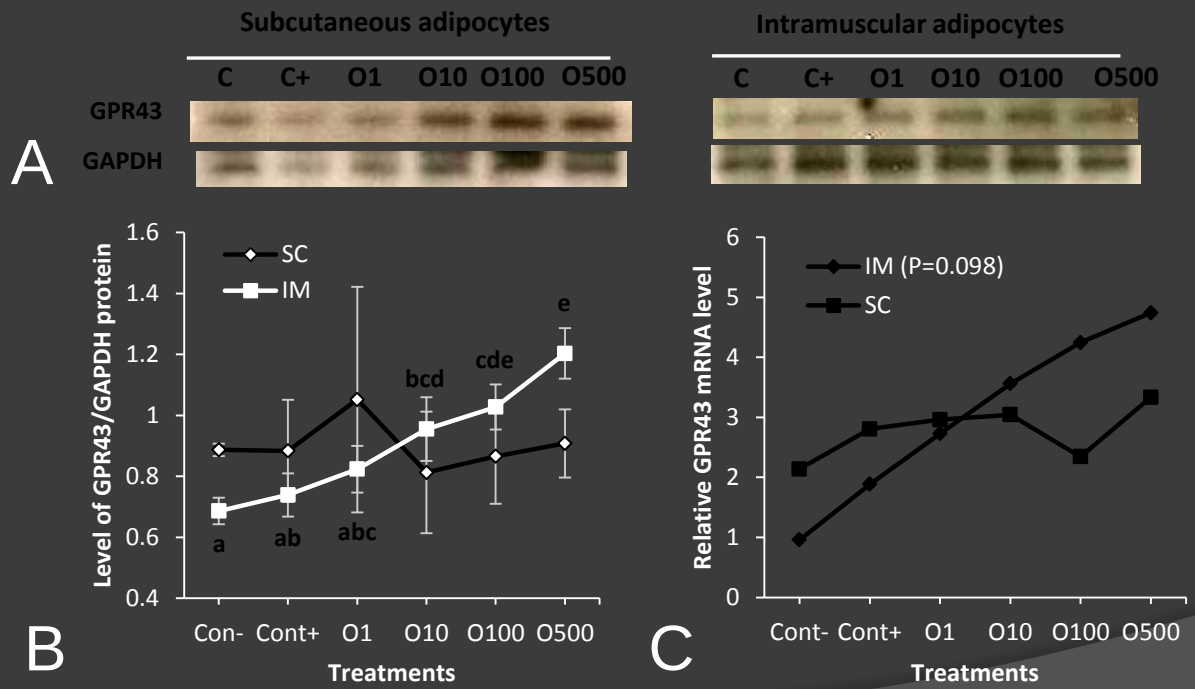

A. Oleic acid increased amount of GPR43 protein in intramuscular and subcutaneous adipocyte cultures in a dose-dependent fashion.
B. Oleic acid increased GPR43/GAPDH protein level more in intramuscular adipocytes than in subcutaneous adipocytes.
C. Oleic acid increased GPR43 mRNA in intramuscular adipocytes but not in subcutaneous adipocytes.

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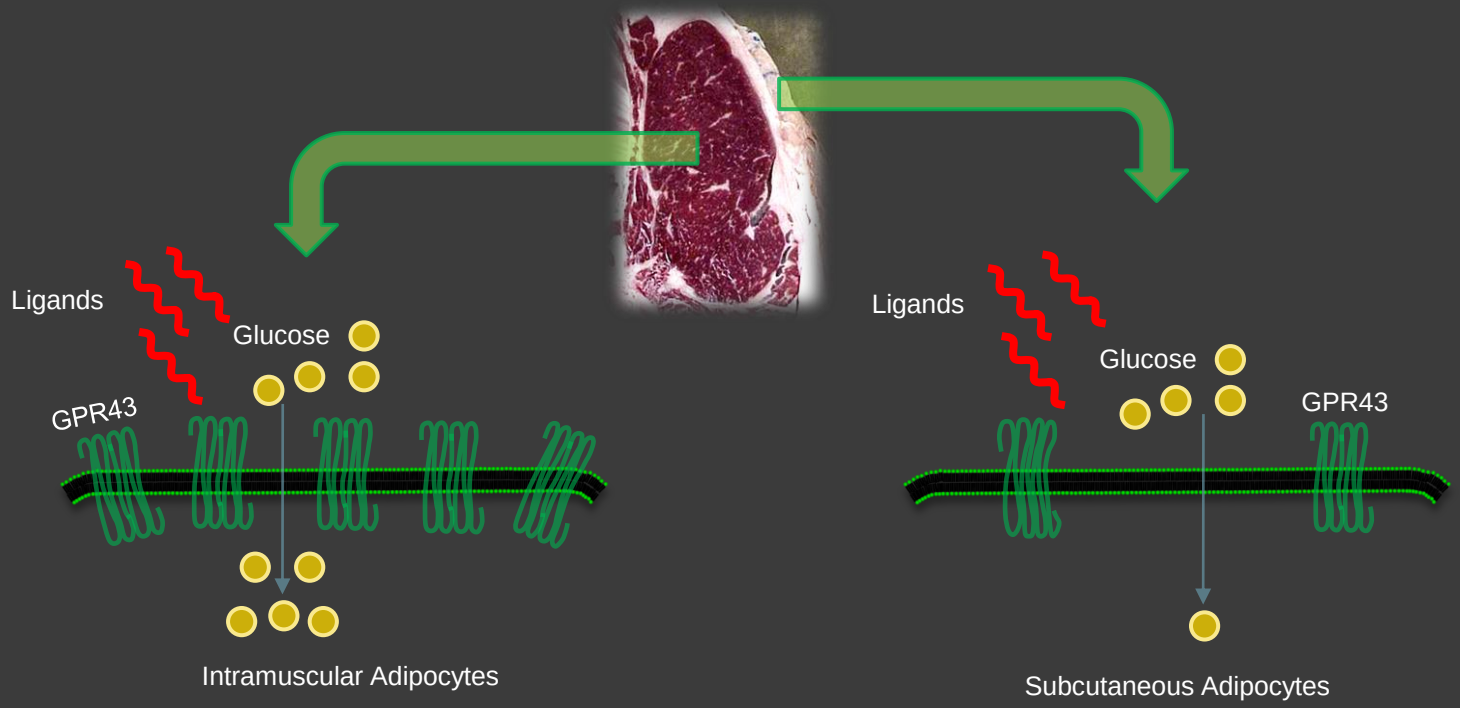
IMAT and Muscle Development



- Study on the effect of fatty acids on different adipose depots
- Development of GPR43 ligands for enhancing marbling in the beef cattle.

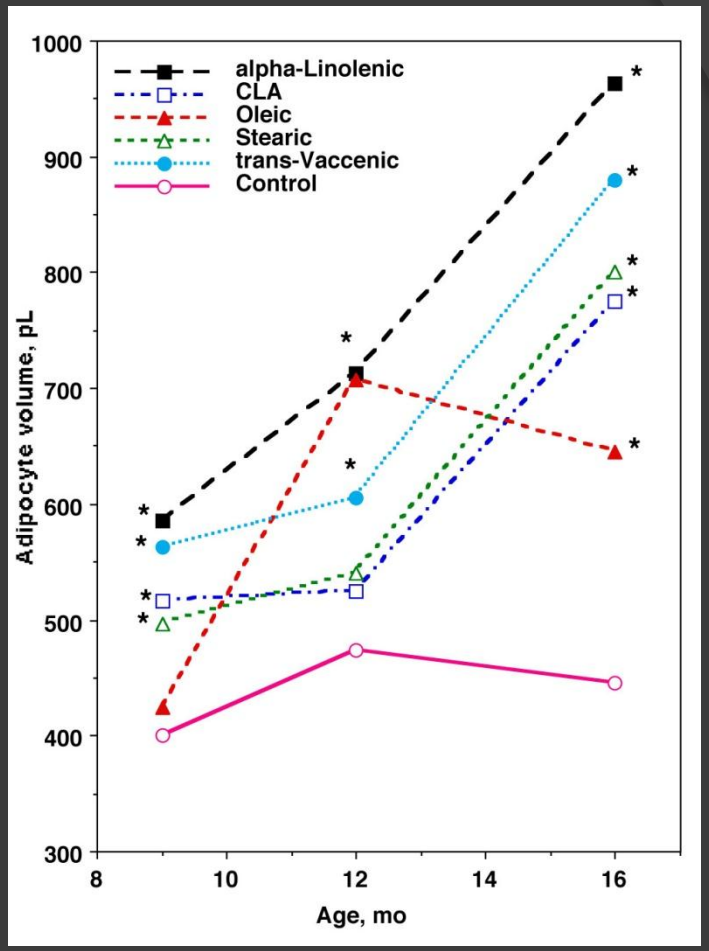
Contributions of S. B. Smith

- Hypothesis: Oleic acid increases lipid filling and gene expression in marbling adipose tissue.
- Study 1
  - Angus steers were harvested at 12, 14, and 16 months of age.
  - The 5-8th thoracic rib region was removed immediately after hide removal.
  - Samples of i.m. and s.c. adipose tissue were dissected and cultured for 48 h with 0 or 40 µM media fatty acids.

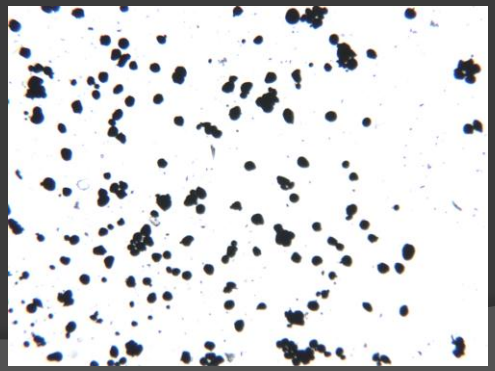
Funded by The Beef Checkoff

Intramuscular adipocyte volume

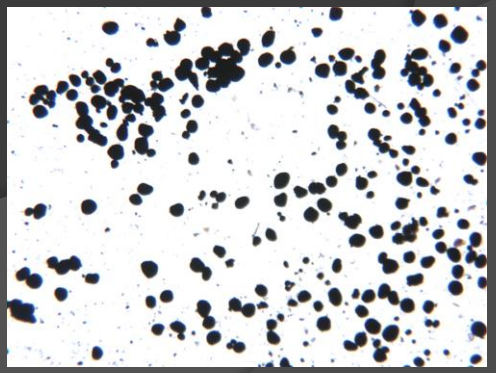
- All fatty acids increased i.m. volume by 16 mo of age.



Control

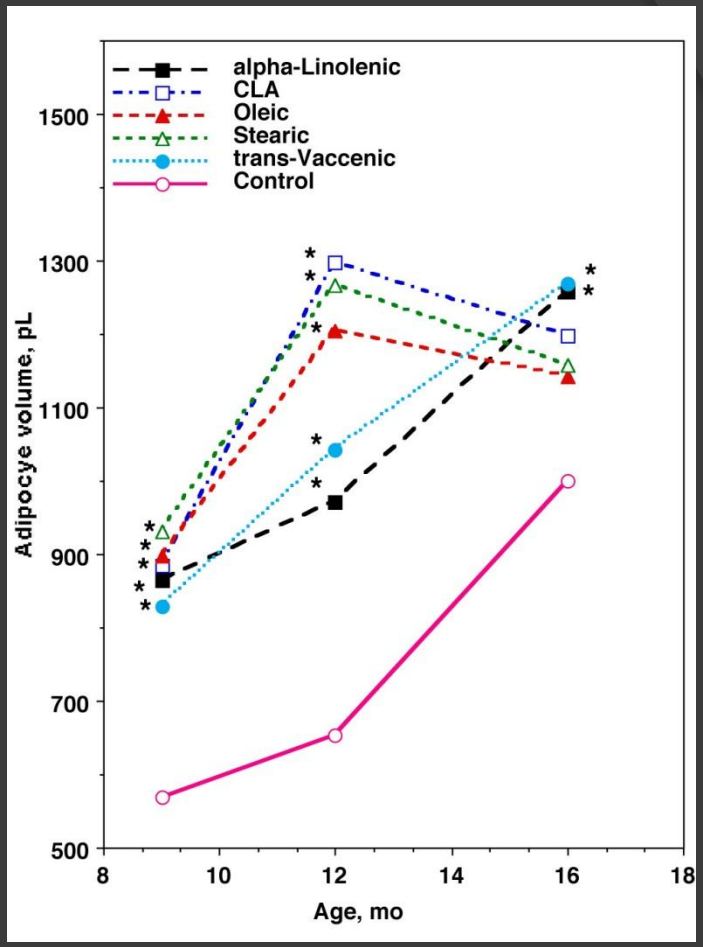


α-Linolenic

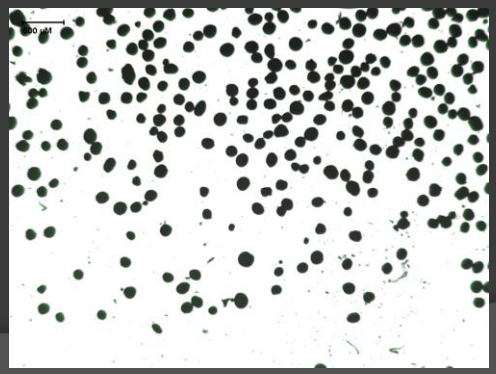


Subcutaneous adipocyte volume

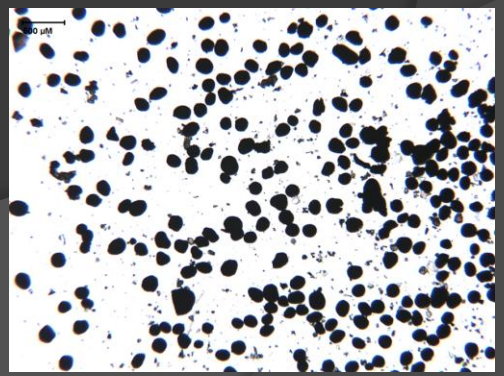
- All media fatty acids increased s.c. adipocyte volume.



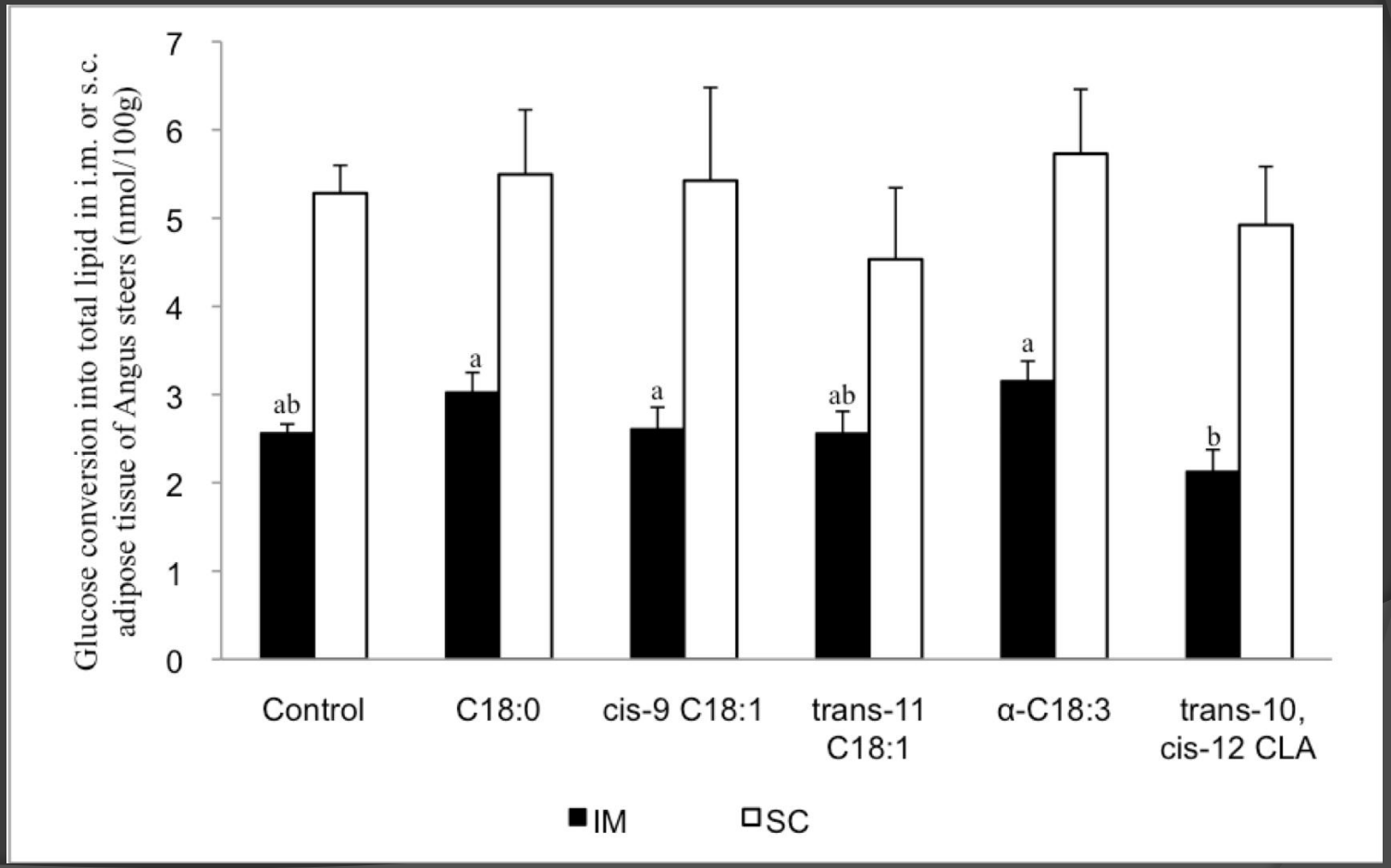
Control



α-Linolenic

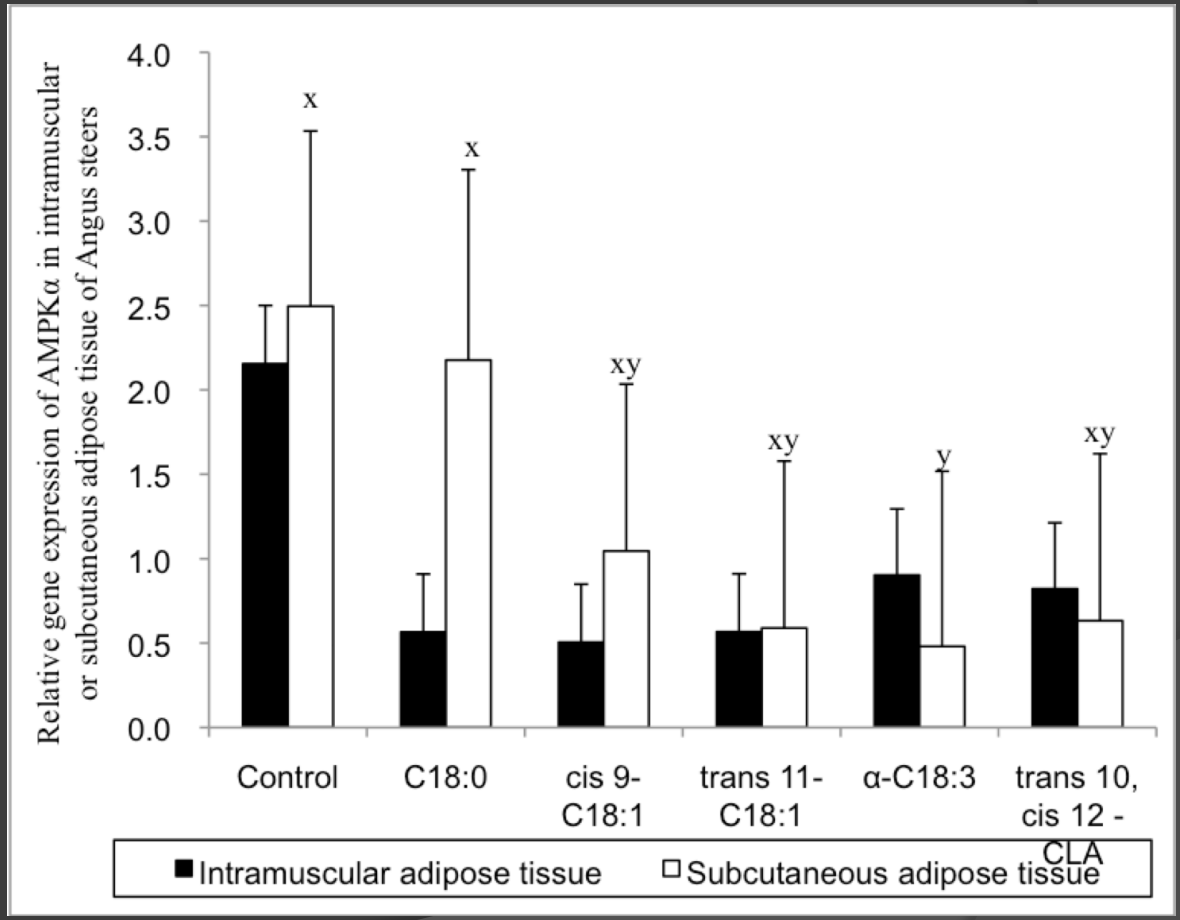


Lipogenesis from glucose in was lowest in i.m. adipose tissue with 40 μM CLA.



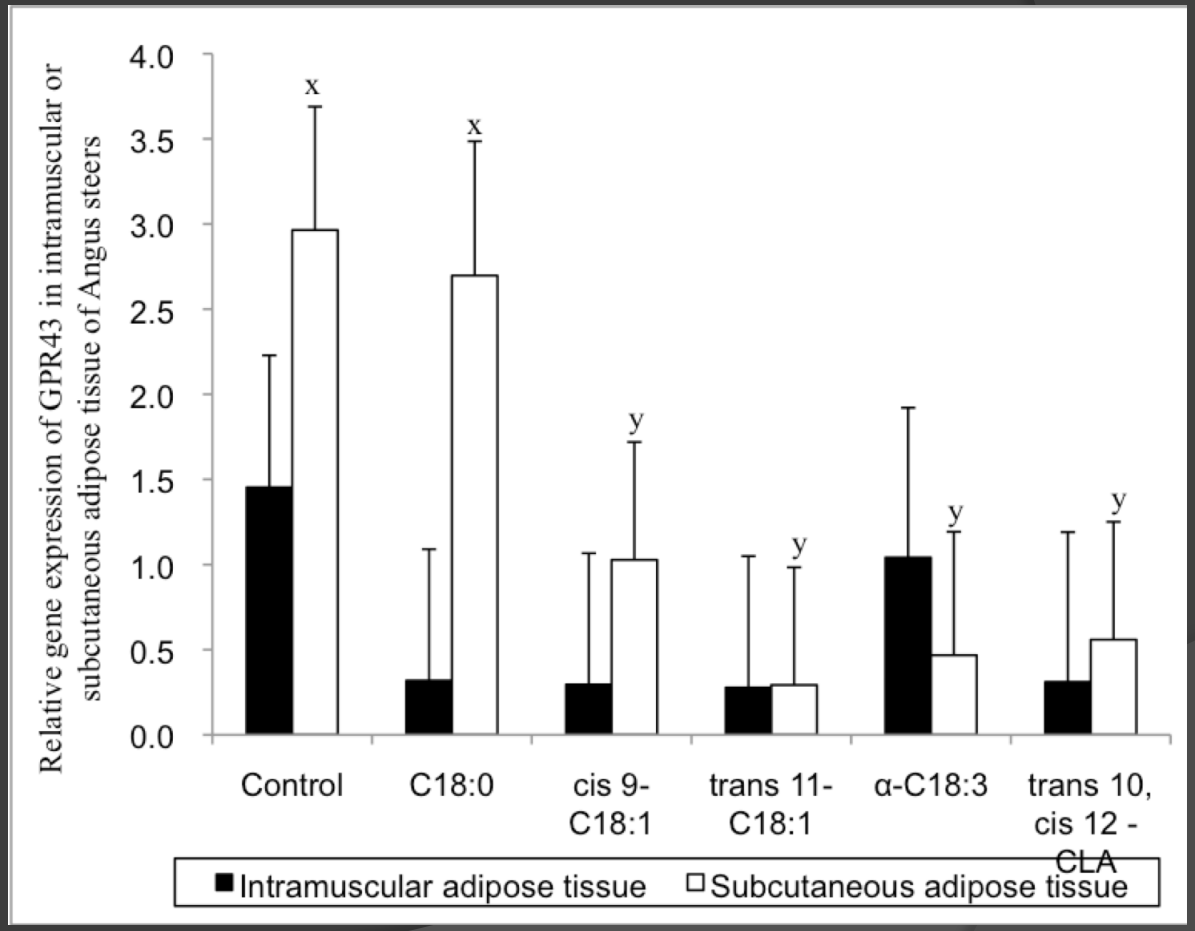
AMPK gene expression

- Stearic acid did not affect AMPK mRNA levels.
- α-Linolenic acid depressed AMPK mRNA levels in s.c. adipose tissue.

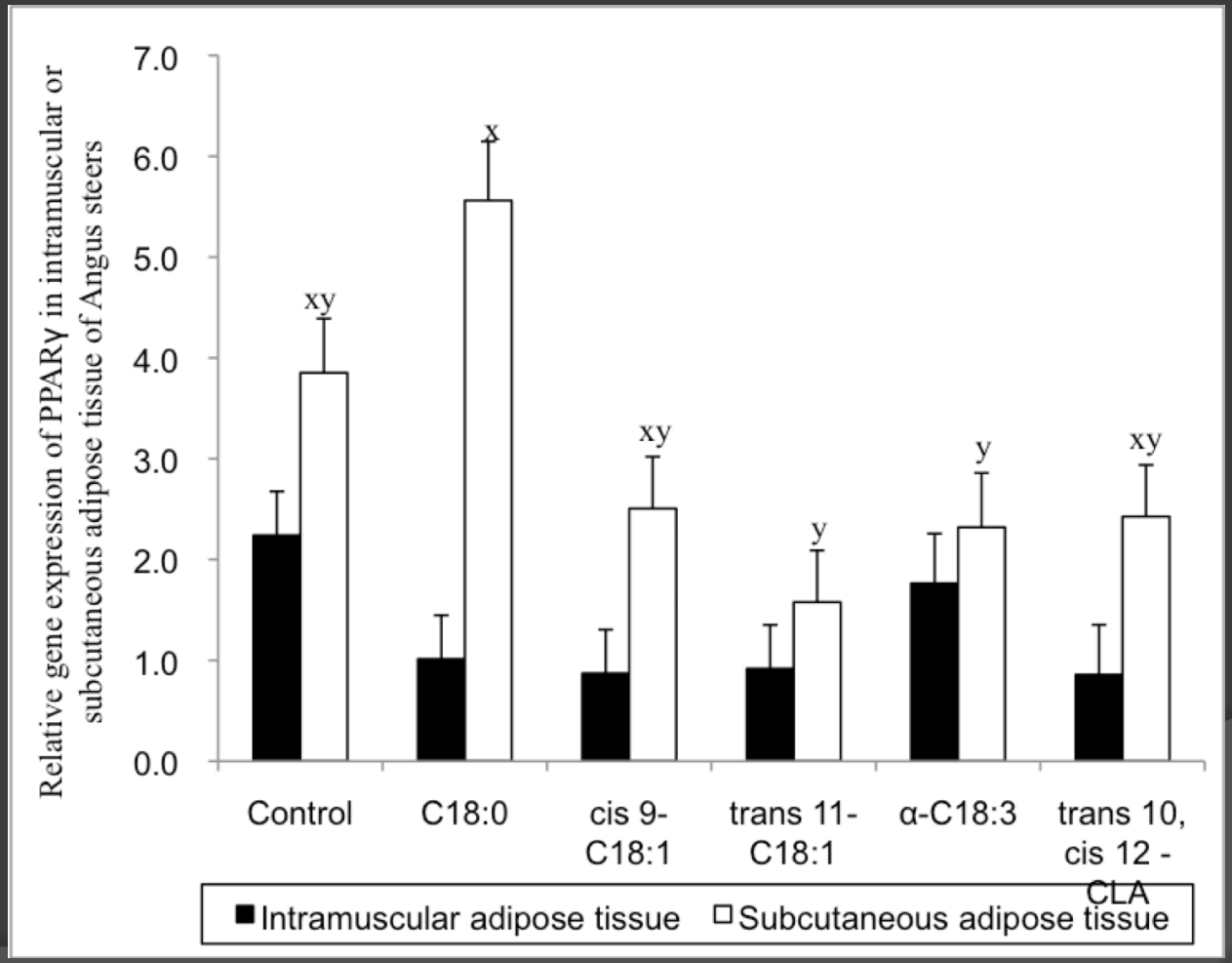


GPR43 gene expression

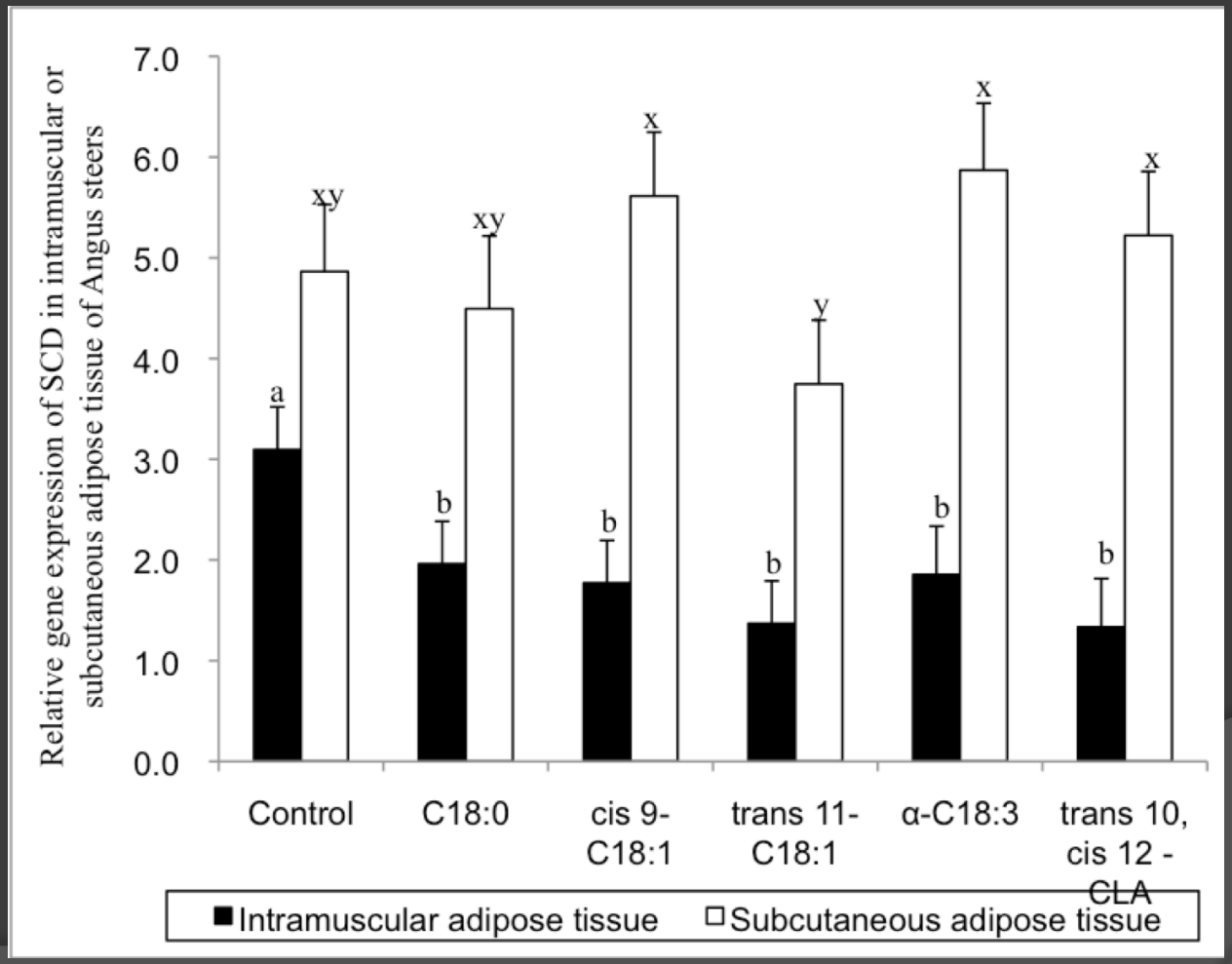
- Stearic acid did not affect GPR43 mRNA levels in s.c. adipose tissue.
- Other media fatty acids depressed GPR43 gene expression in s.c. adipose tissue.



PPARγ gene expression was highest in s.c. adipose tissues incubated with stearic acid.



All fatty acids depressed SCD gene expression in i.m. adipose tissue.



Additional contributions of S. B. Smith

- Hypothesis: Oleic acid and ALA have additive effects on AMPK and GPR43 gene expression in i.m. adipose tissue explants and preadipocytes.
- Study 2
  - Wagyu crossbred steers were harvested at 16 months of age.
  - The 5-8th thoracic rib region was removed immediately after hide removal.
  - Samples of i.m. adipose tissue and i.m. preadipocytes were dissected and cultured for 48 h with 0, 25, or 50 µM media oleic acid and/or ALA.

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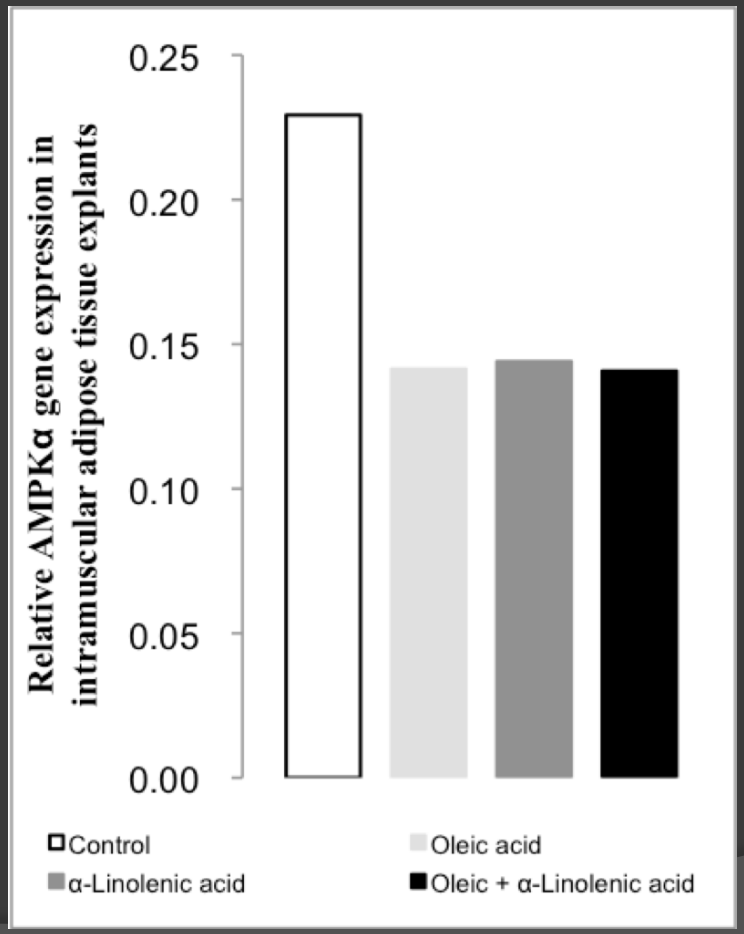
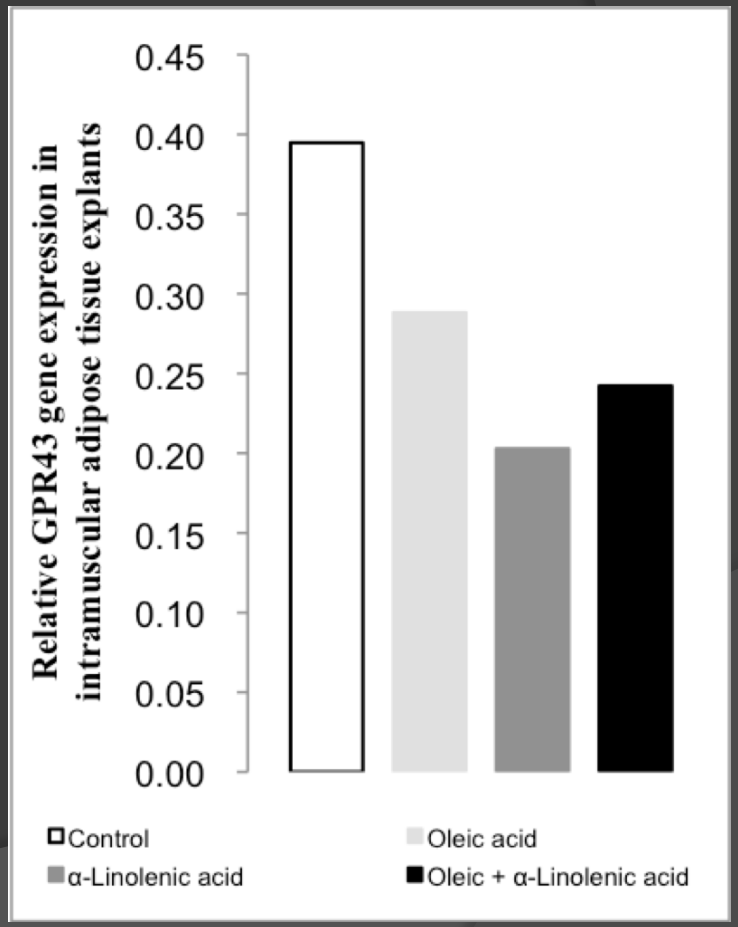
Neither oleic acid nor ALA affected AMPK or GPR43 mRNA in i.m. adipose tissue explants.

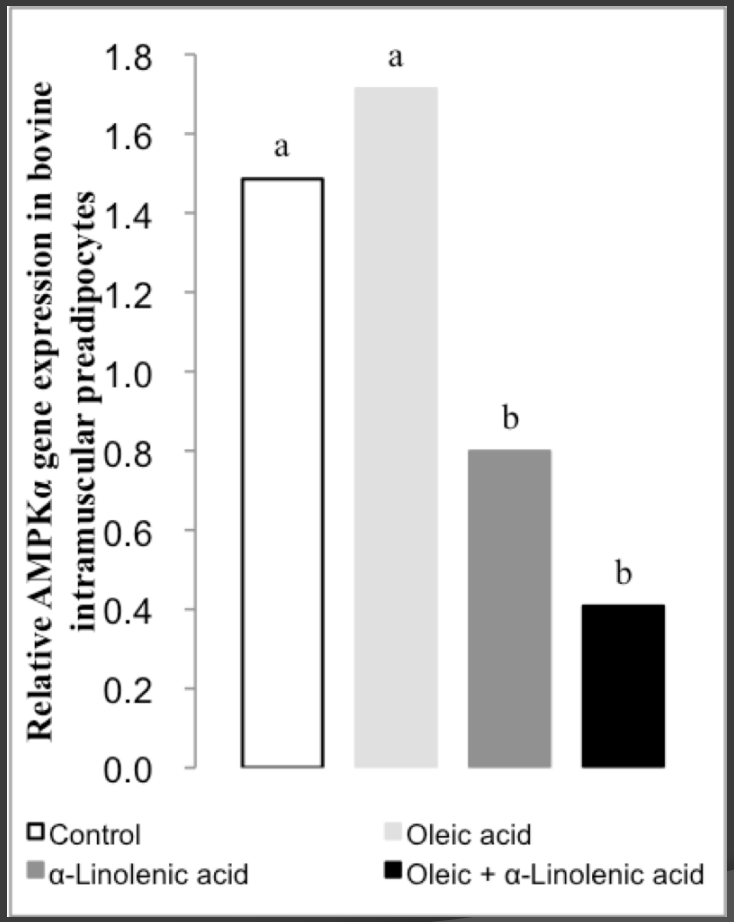
Control = 0 µM
Oleic = 50 µM
ALA = 50 µM
Oleic + ALA = 25 µM + 25 µM

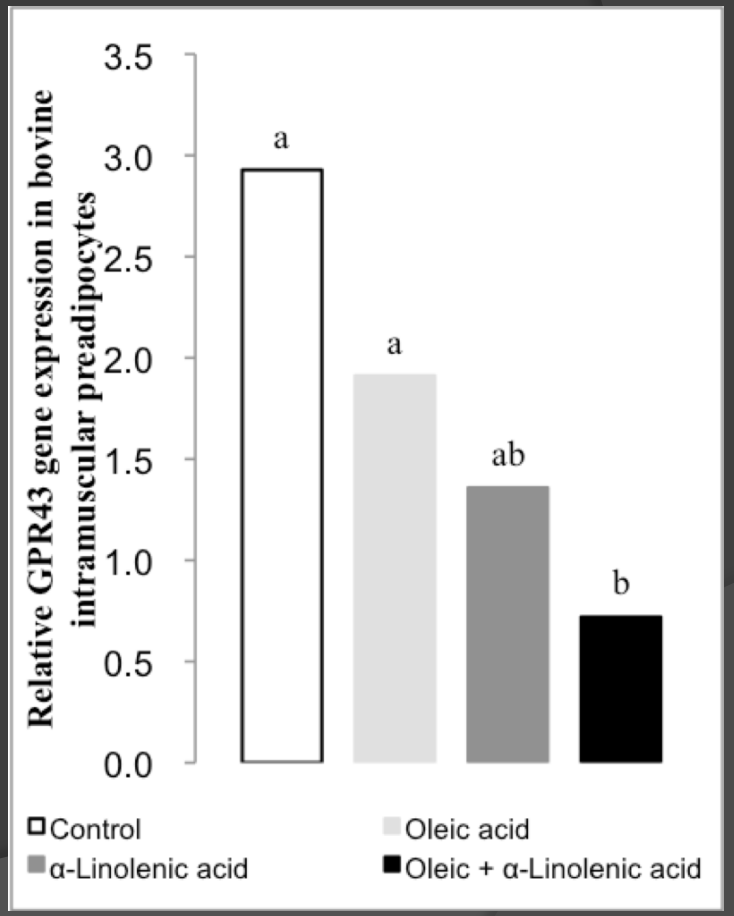




However, ALA depressed AMPK and GPR43 mRNA in i.m. preadipocytes.

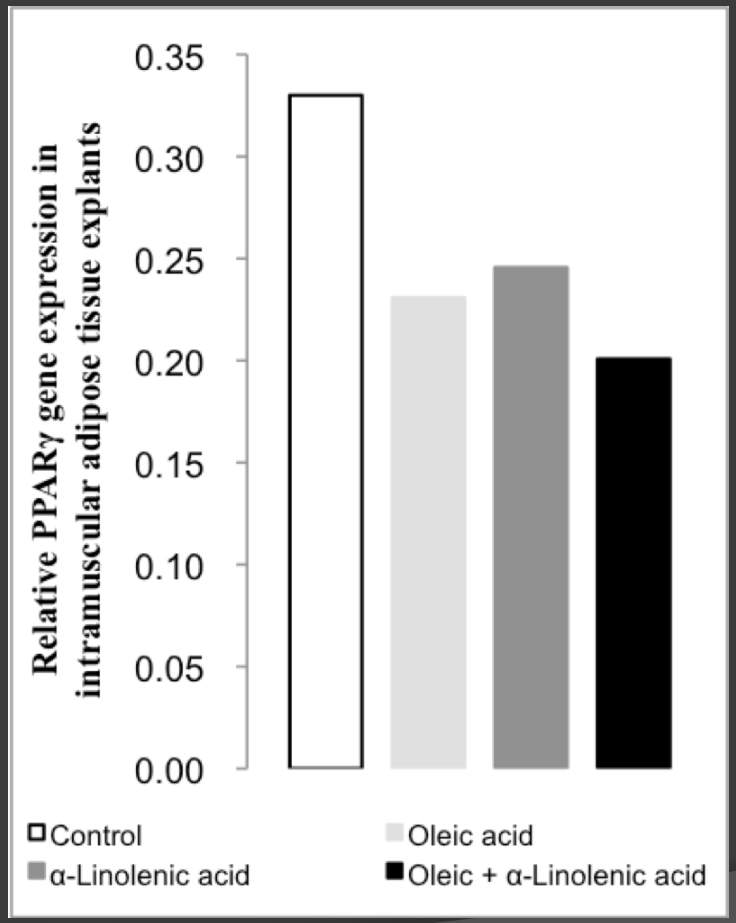
Control = 0 µM
Oleic = 50 µM
ALA = 50 µM
Oleic + ALA = 25 µM + 25 µM

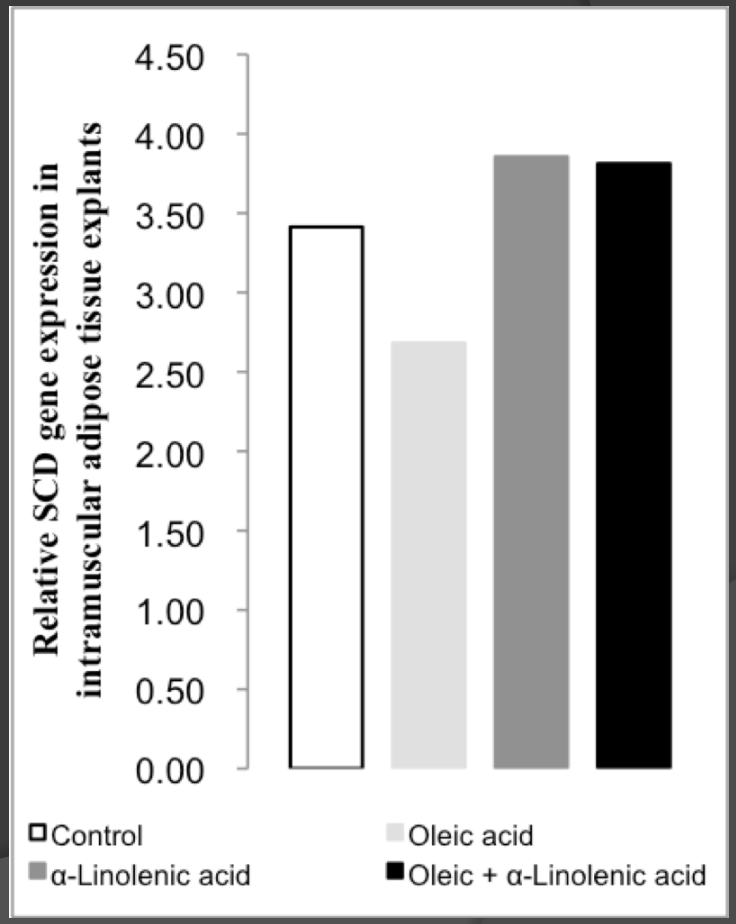




Oleic acid and ALA did not affect PPARγ or SCD mRNA in i.m. adipose tissue explants.

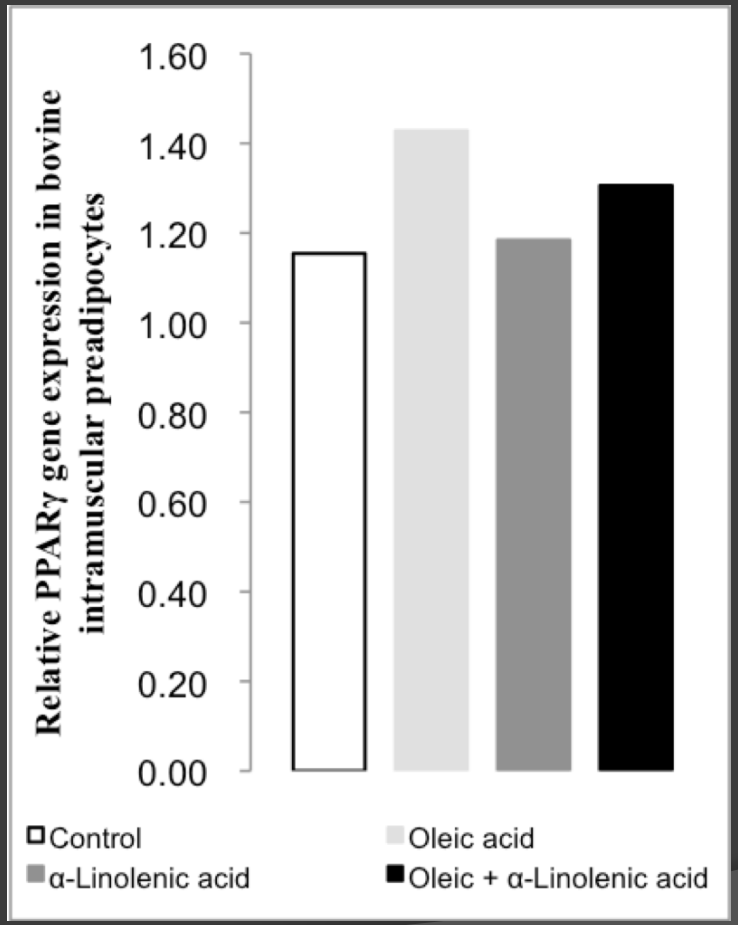
Control = 0 µM
Oleic = 50 µM
ALA = 50 µM
Oleic + ALA = 25 µM + 25 µM

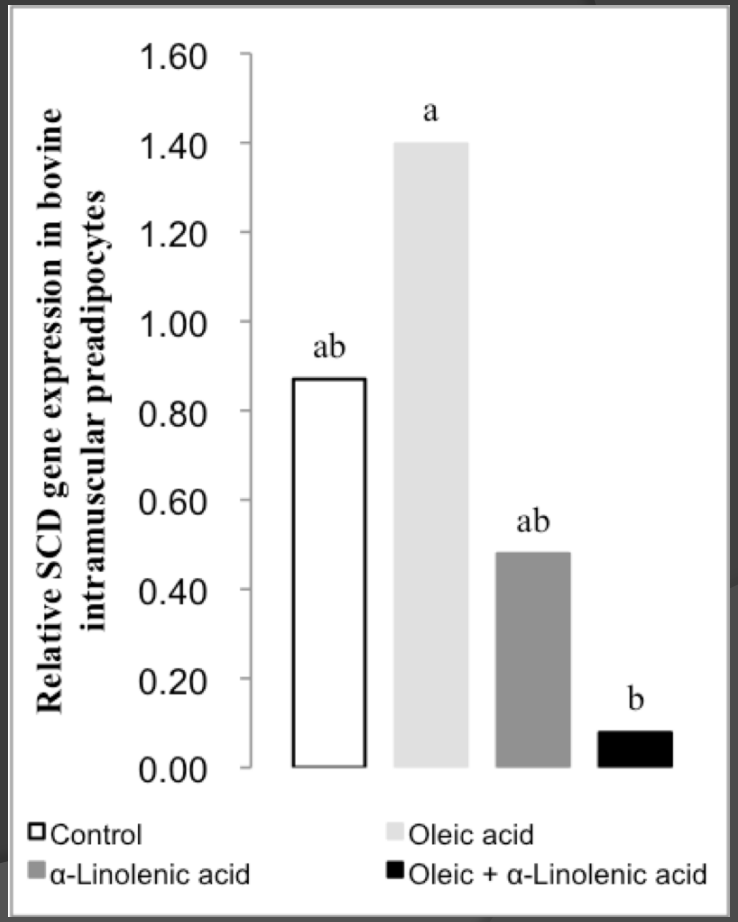




However, oleic acid plus ALA depressed SCD mRNA in i.m. preadipocytes.

Control = 0 µM
Oleic = 50 µM
ALA = 50 µM
Oleic + ALA = 25 µM + 25 µM





Conclusions of these studies

- We had predicted that oleic acid would stimulate and ALA would depress adipogenesis.
  - Oleic acid did not stimulate adipogenesis.
  - Oleic acid plus ALA often additively depressed adipogenic gene expression.
- We also predicted that oleic acid would stimulate adipogenesis and *trans*-differentiation of satellite cells.
  - This was hypothesis was correct.

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Additional conclusions

- Other studies included saturated fatty acids.
  - Stearic acid and palmitic acid often stimulated adipogenic gene expression.
- Our next study:
  - Document the effects of dietary saturated and polyunsaturated fatty acids on adipogenesis in beef cattle.

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How does this apply to marbling in beef cattle?

- α-Linolenic acid in pastures may be causative in depressing marbling development.
- Dietary or endogenous oleic acid may increase marbling by promoting *trans*-differentiation of muscle satellite cells.
- *Effects of fatty acids are much more pronounced during the earliest stages of marbling adipocyte differentiation.*

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Thank You!

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