

# Conjugated Linoleic Acid Isomers and Mammary Lipid Metabolism

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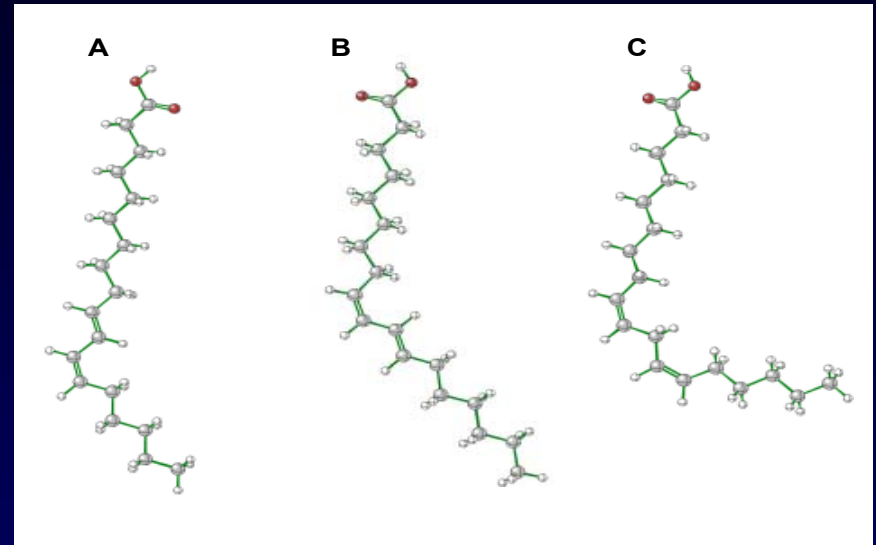
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# Conjugated Linoleic Acids (CLA)

- Many isomers (n=24) found in ruminant food products
  - $C_{18:2}$  *cis-9, trans-11*
  - $C_{18:2}$  *trans-7, cis-9*
  - $C_{18:2}$  *trans-10, cis-12*
  - $C_{18:2}$  *cis-8, trans-10*
- CLA has been shown to:
  - Anti-carcinogenic
  - Anti-atherogenic
  - Anti-diabetic
  - Enhanced immune system
  - Reduces severity of cachexia
  - Alleviates symptoms of lupus
  - Improved bone mineralization
  - **Alters lipid metabolism**



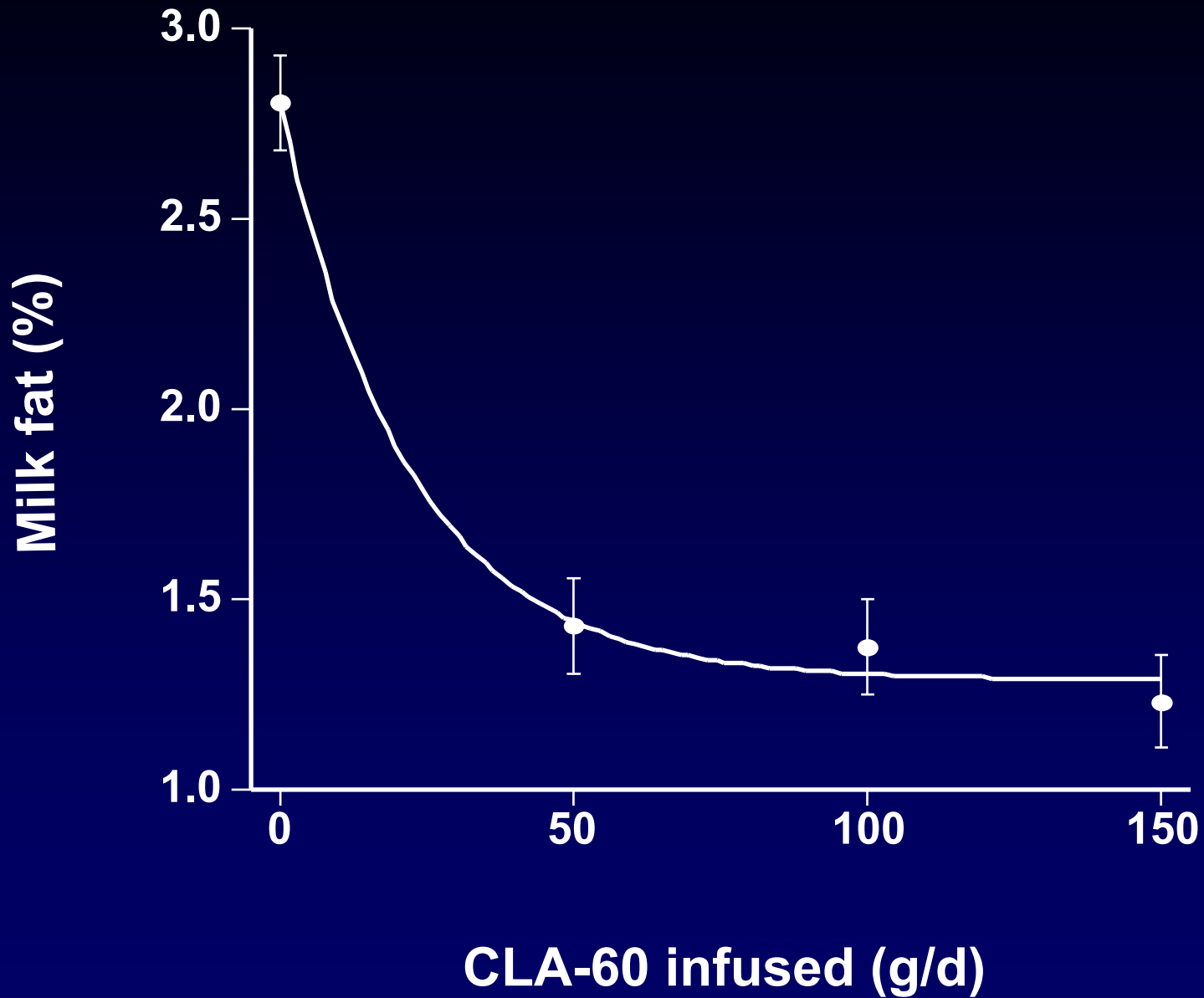
*t*10, *c*12 CLA

*c*9, *t*11 CLA

*c*9, *c*12  $C_{18:2}$

# Milk Fat CLA

- Early work focused on
  - Increasing CLA content
  - Identifying sources of variation
  - Determining composition of CLA isomers
- In attempt to enhance milk fat CLA it was serendipitously discovered that exogenous CLA causes severe milk fat depression  
(Chouinard et al., 1998)



# Abomasal Infusion of CLA

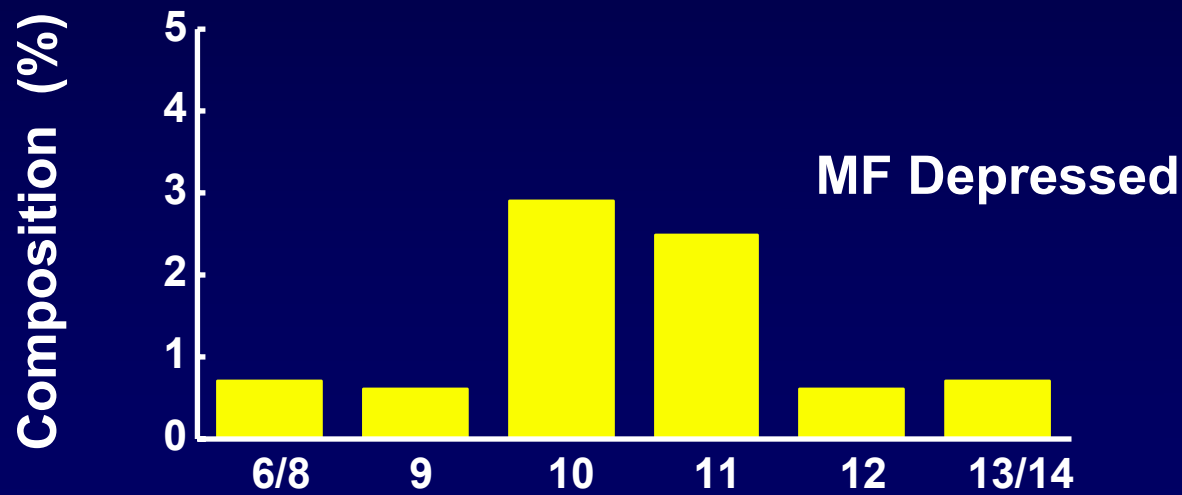
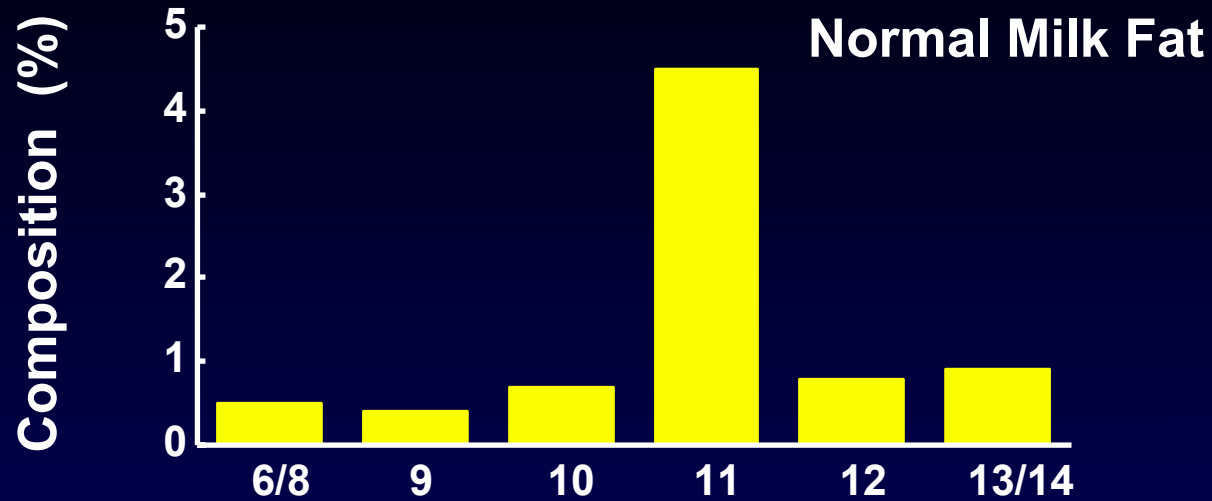
Dose		Milk yield	Milk fat		Milk protein	
g/d	days	kg/d	%	g/d	%	g/d
0 <sup>1</sup>	1	16.0	3.31 <sup>a</sup>	540 <sup>a</sup>	3.15 <sup>a</sup>	790
100		15.0	2.66 <sup>b</sup>	400 <sup>b</sup>	2.99 <sup>b</sup>	760
0 <sup>2</sup>	5	21.5	2.81 <sup>a</sup>	599 <sup>a</sup>	3.31	696
31		20.4	1.43 <sup>b</sup>	290 <sup>b</sup>	3.37	675
60		20.9	1.38 <sup>b</sup>	295 <sup>b</sup>	3.53	717
92		18.3	1.23 <sup>b</sup>	222 <sup>b</sup>	3.46	627
0 <sup>3</sup>	3	26.9	3.34 <sup>a</sup>	883 <sup>a</sup>	3.14	831
15		27.5	2.40 <sup>b</sup>	655 <sup>b</sup>	3.03	826
18		29.4	2.36 <sup>b</sup>	691 <sup>b</sup>	3.04	882
29		26.8	2.43 <sup>b</sup>	633 <sup>b</sup>	3.15	829

<sup>1</sup>Loor and Herbein 1998; <sup>2</sup>Chouinard et al., 1999; <sup>3</sup>Chouinard et al., 1999

# Which CLA Isomer is Responsible?

- CLA supplements contained many isomers
- Predominantly *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA
- Diet-induced milk fat depression (MFD) associated with specific *trans* C<sub>18:1</sub> isomer

# C<sub>18:1</sub> *trans* Fatty Acids



**Double Bond Position**

# Hypothesis

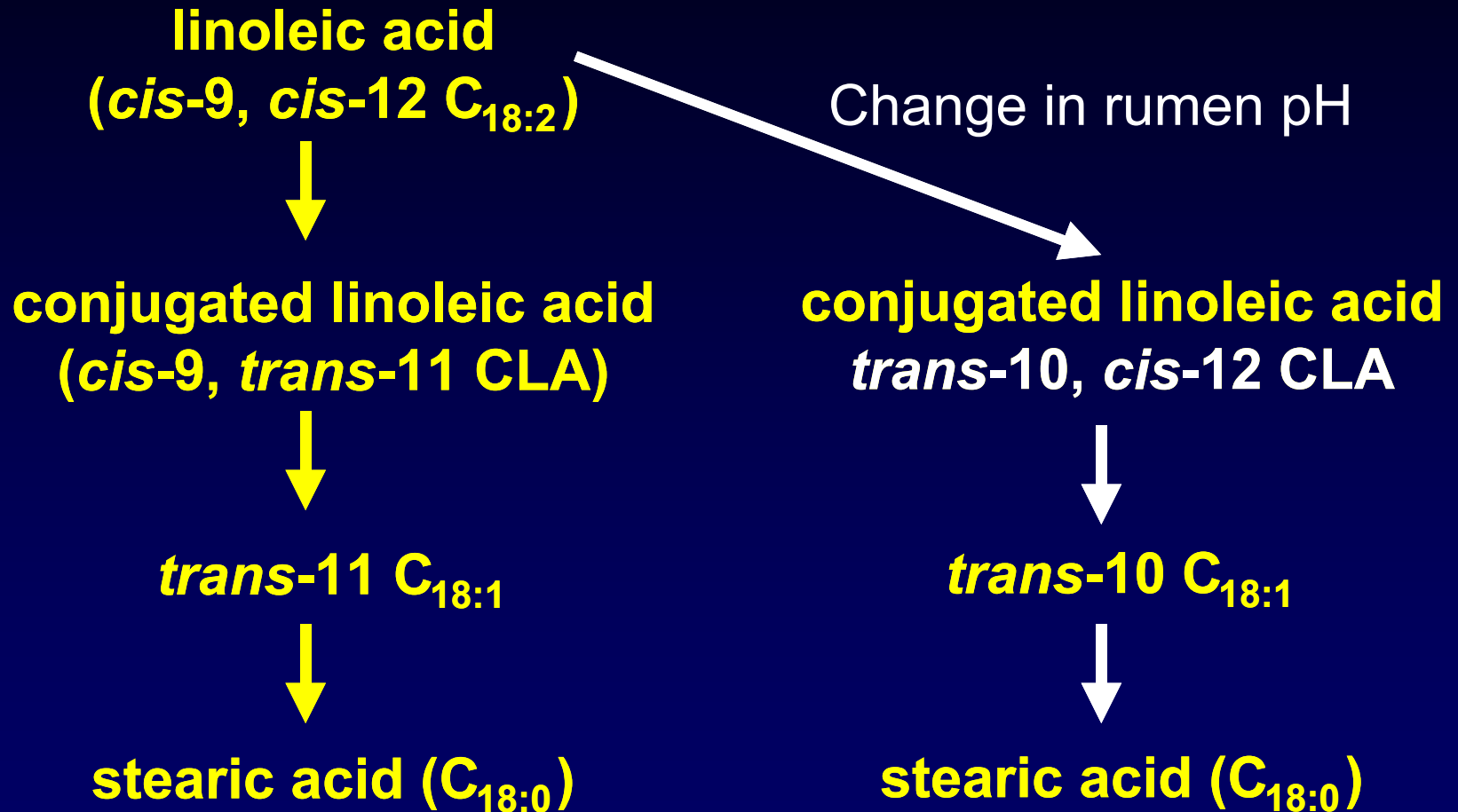
- Rumen derived CLA or related metabolites containing a *trans*-10 double bond inhibit milk fat synthesis



# Rumen Biohydrogenation

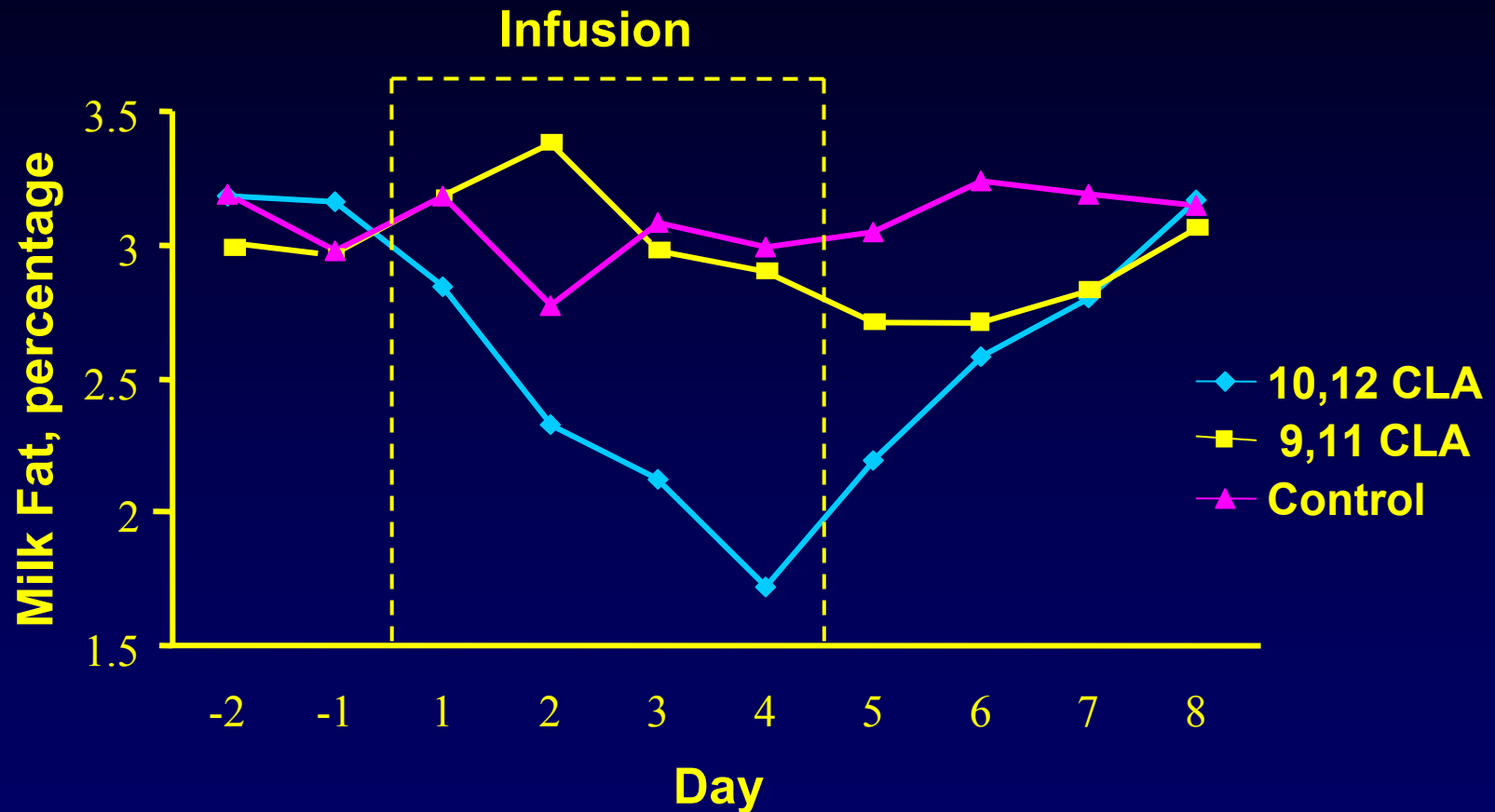
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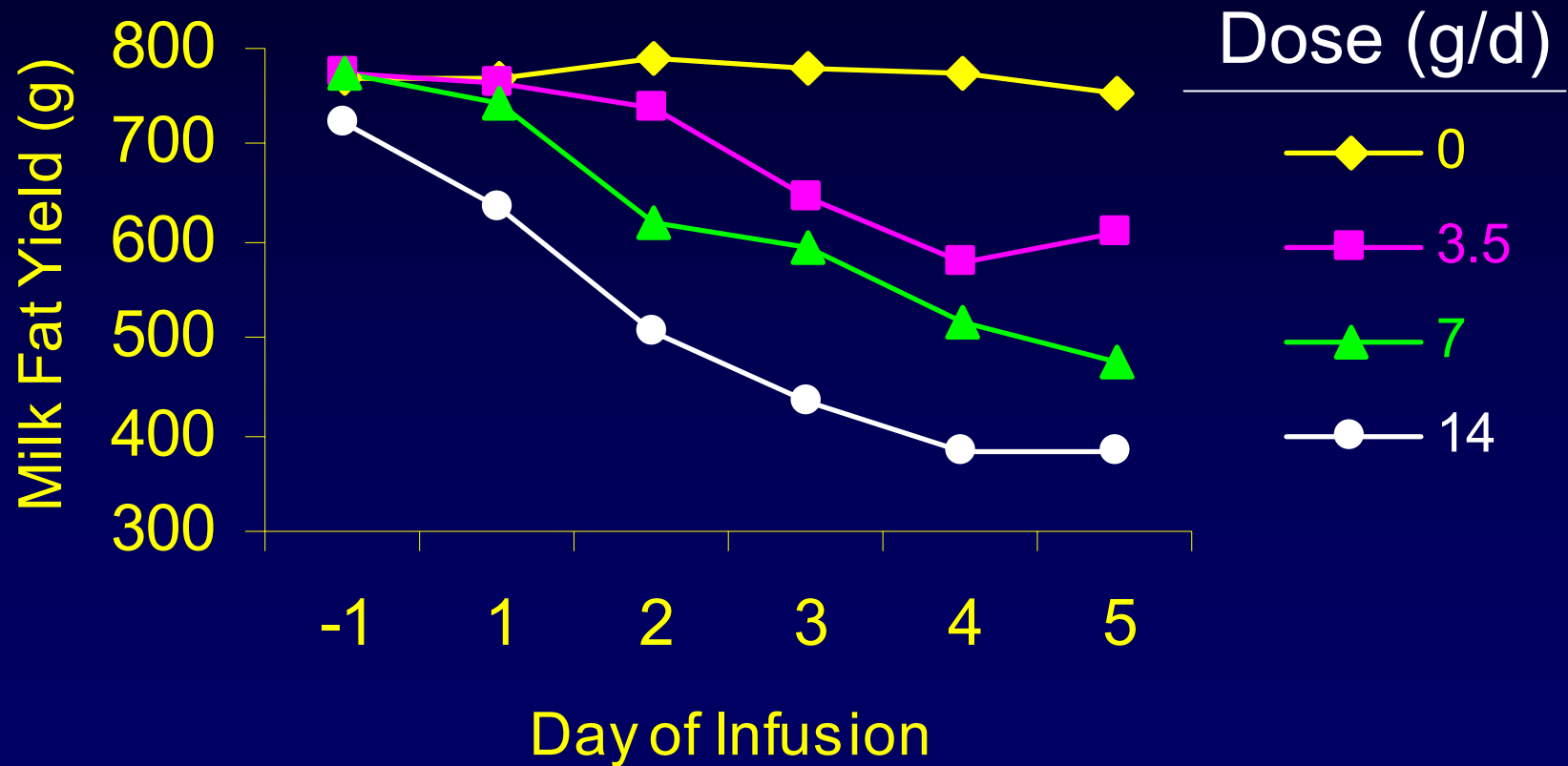


Griinari and Bauman, 1999

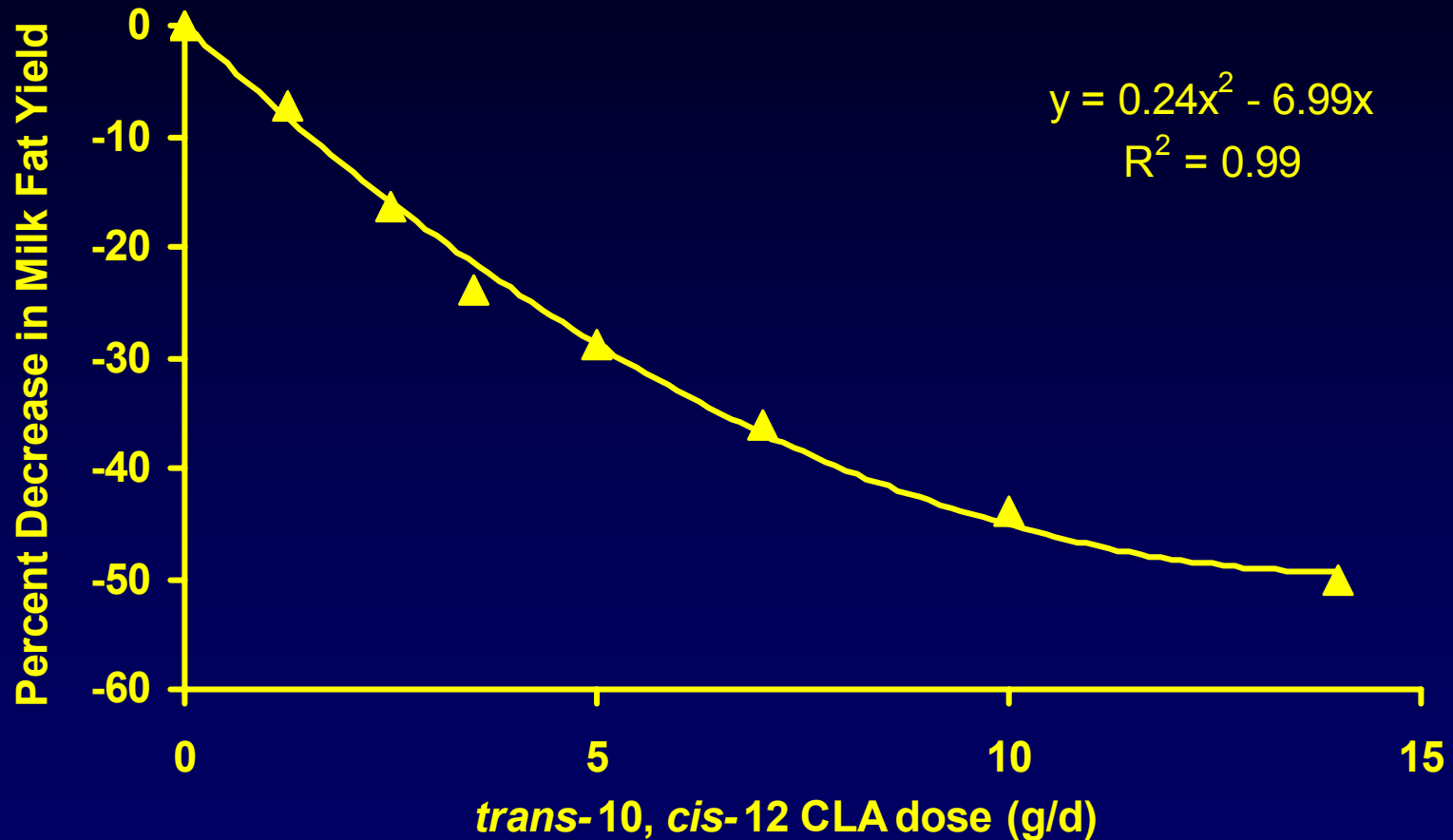
# Effects of CLA Isomers on Milk Fat %



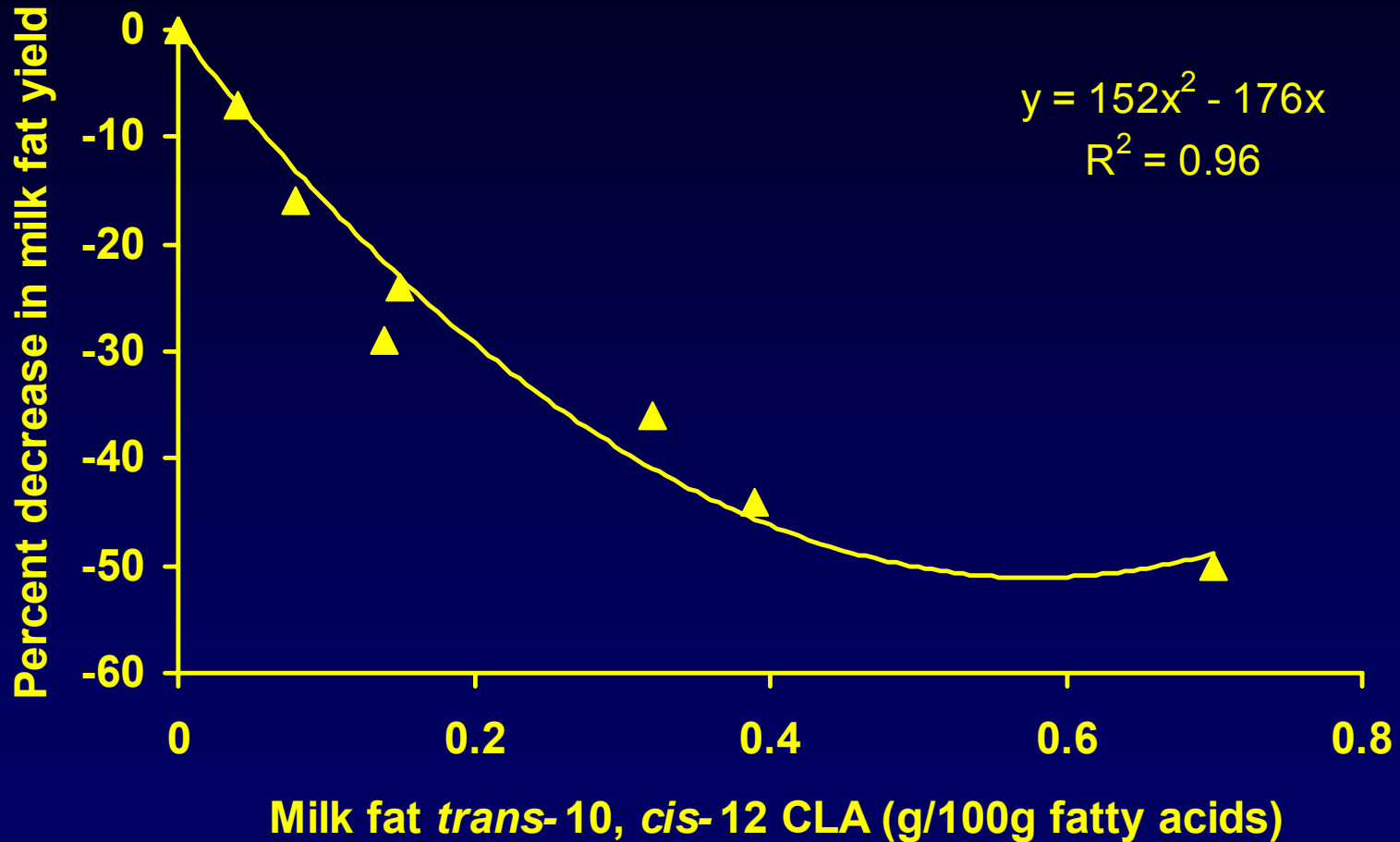
# Milk Fat Response to *trans*-10, *cis*-12 CLA



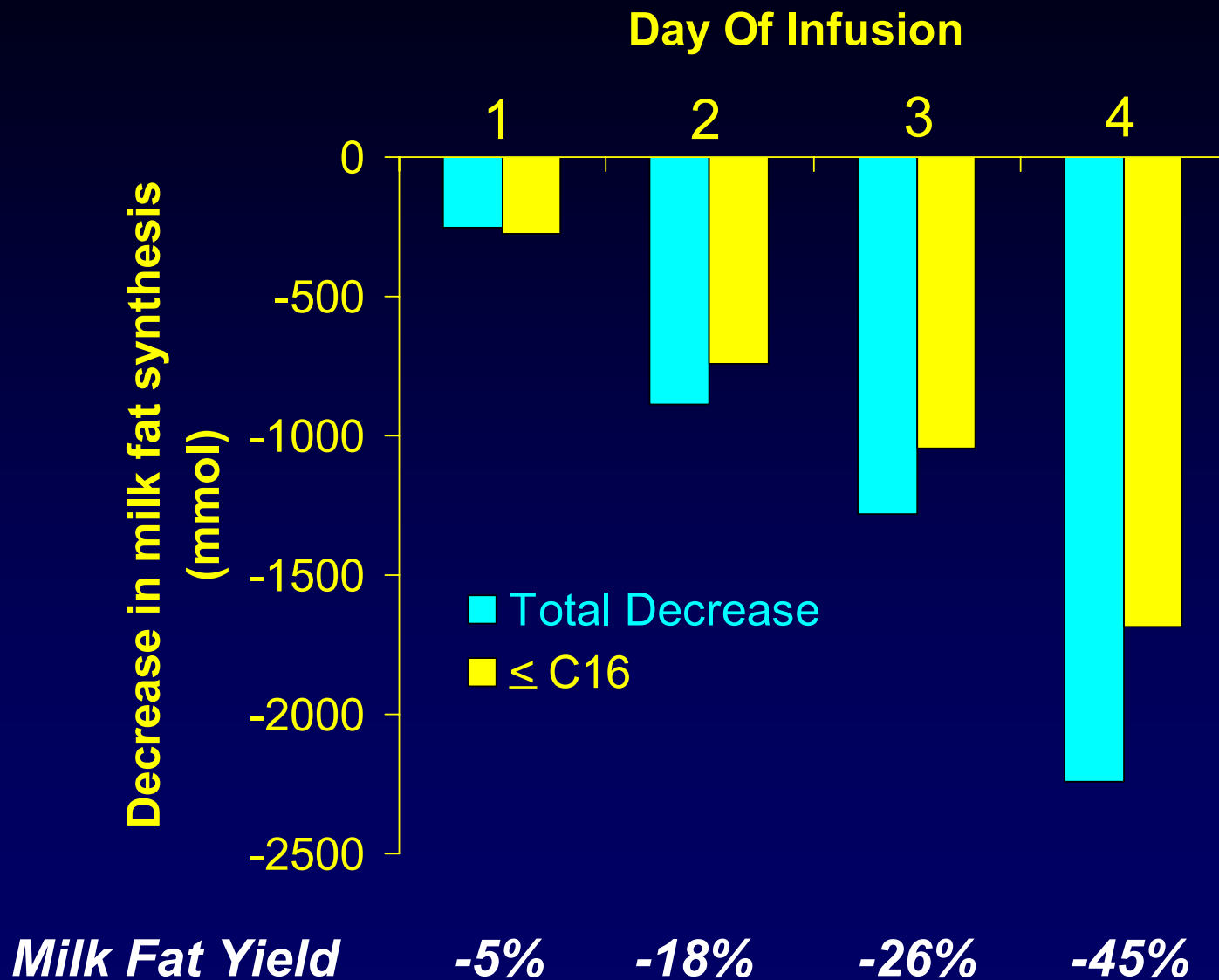
# *trans*-10, *cis*-12 CLA Dose Response



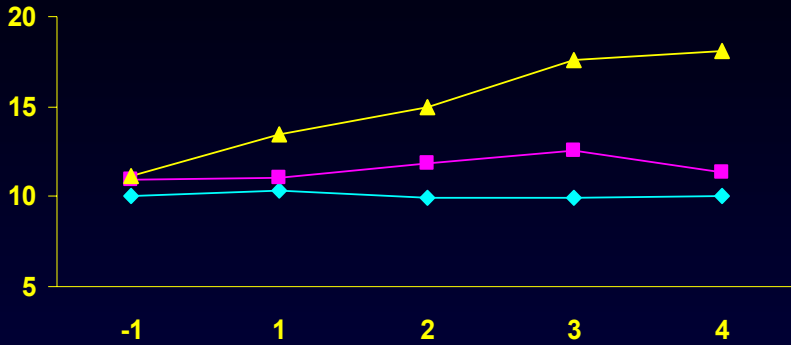
# *trans*-10, *cis*-12 CLA in Milk Fat



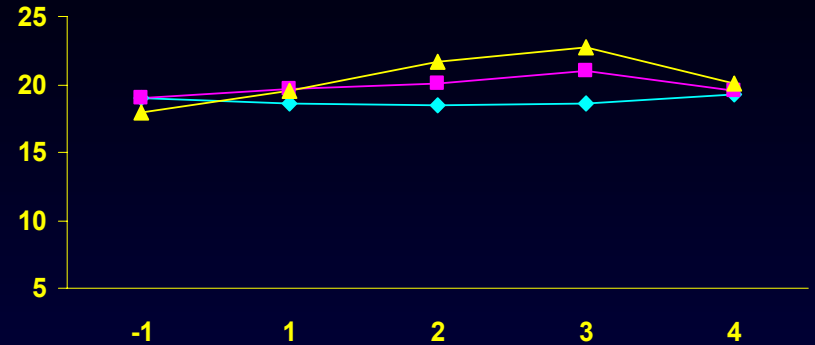
# Effect of *trans*-10, *cis*-12 CLA (10 g/d) on Fatty Acid Composition



14:0/14:1



16:0/16:1



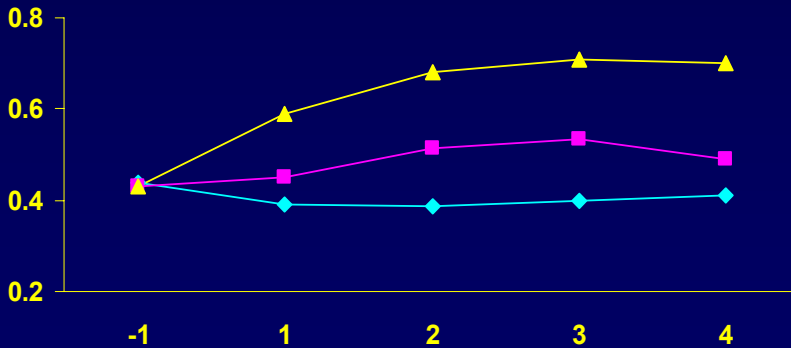
Treatment

control

cis-9, trans-11 CLA

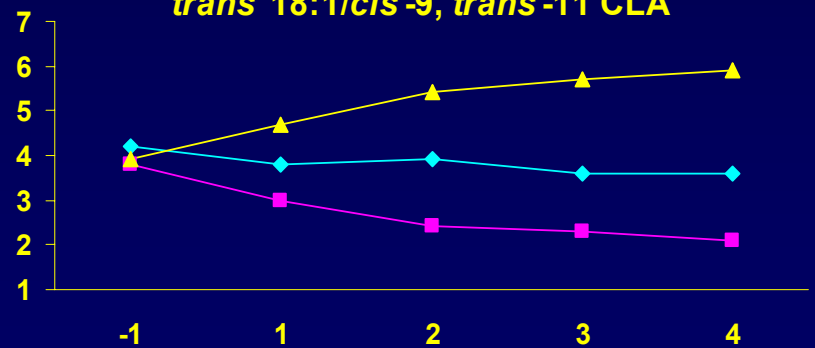
trans-10, cis-12 CLA

18:0/18:1



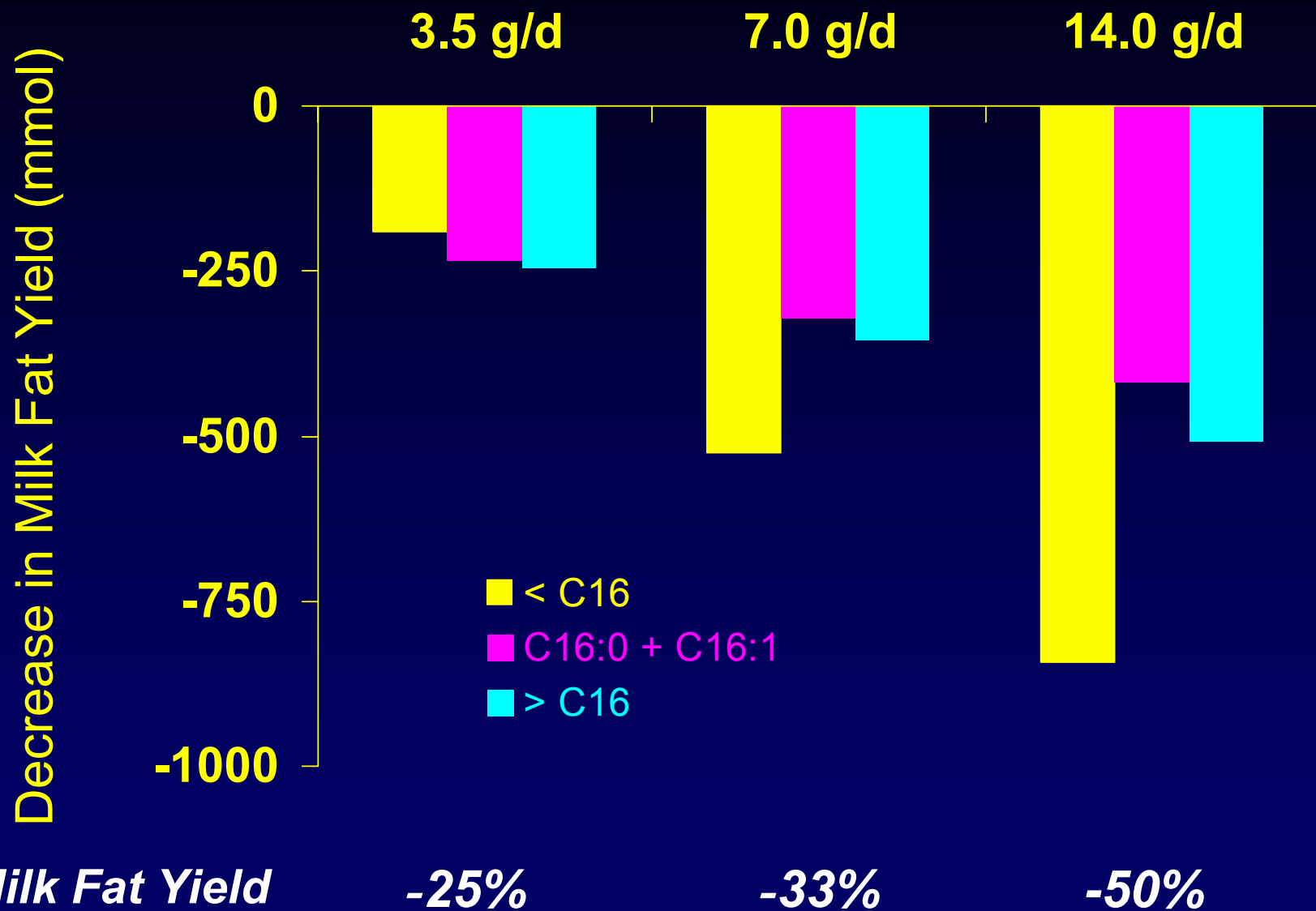
Day of Infusion

trans 18:1/cis-9, trans-11 CLA



Day of Infusion

# Effect of *trans*-10, *cis*-12 CLA on Milk Fatty Acid Composition



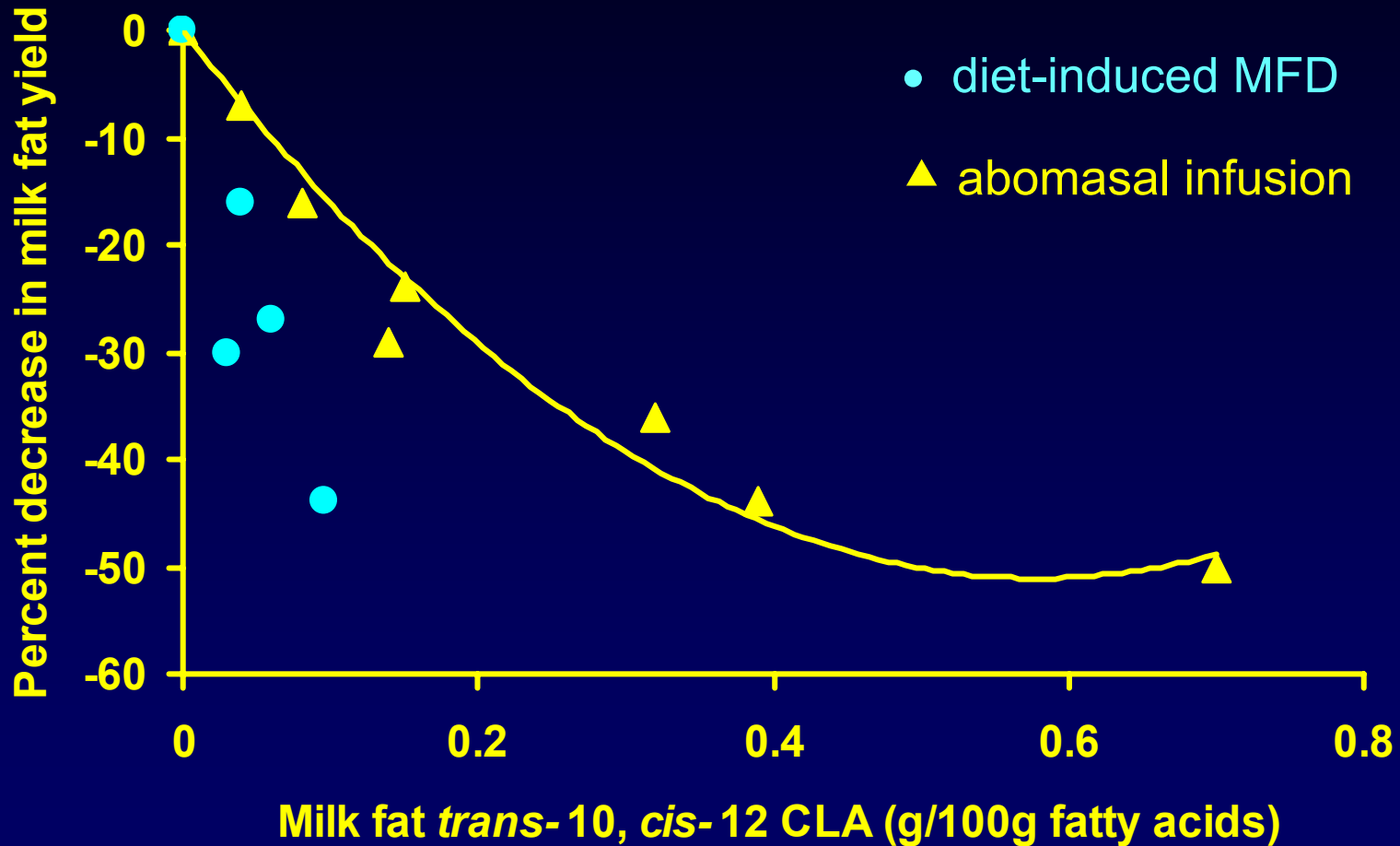


# Effect of Dose on $\Delta^9$ -desaturase

Substrate/product	<i>trans</i> -10, <i>cis</i> -12 CLA, g/d				SEM	<i>P</i>
	0	3.5	7.0	14.0		
	Ratios					
14:0/ <i>c</i> 9 14:1	12.1 <sup>a</sup>	12.7 <sup>a</sup>	14.8 <sup>a</sup>	20.2 <sup>b</sup>	1.4	<0.01
16:0/ <i>c</i> 9 16:1	18.5	20.0	19.2	24.0	1.4	0.06
18:0/ <i>c</i> 9 18:1	0.4 <sup>a</sup>	0.5 <sup>a</sup>	0.6 <sup>b</sup>	0.8 <sup>c</sup>	<0.1	<0.01
<i>t</i> 11 18:1/ <i>c</i> 9, <i>t</i> 11 CLA	2.2 <sup>a</sup>	2.6 <sup>a</sup>	3.2 <sup>b</sup>	4.1 <sup>c</sup>	0.1	<0.01

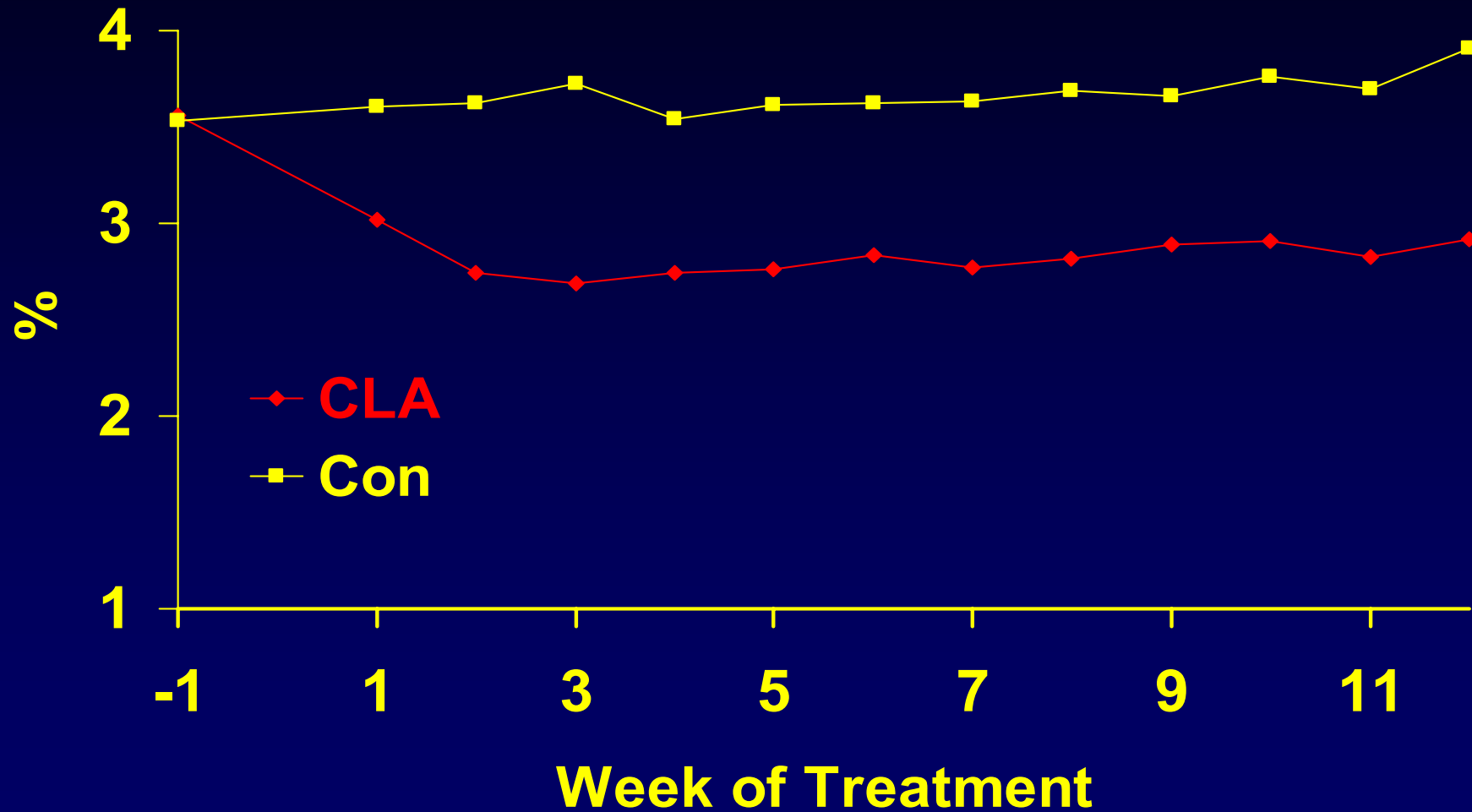
Means within row with different superscripts differ ( $P < 0.05$ )

# *trans*-10, *cis*-12 CLA in Milk Fat

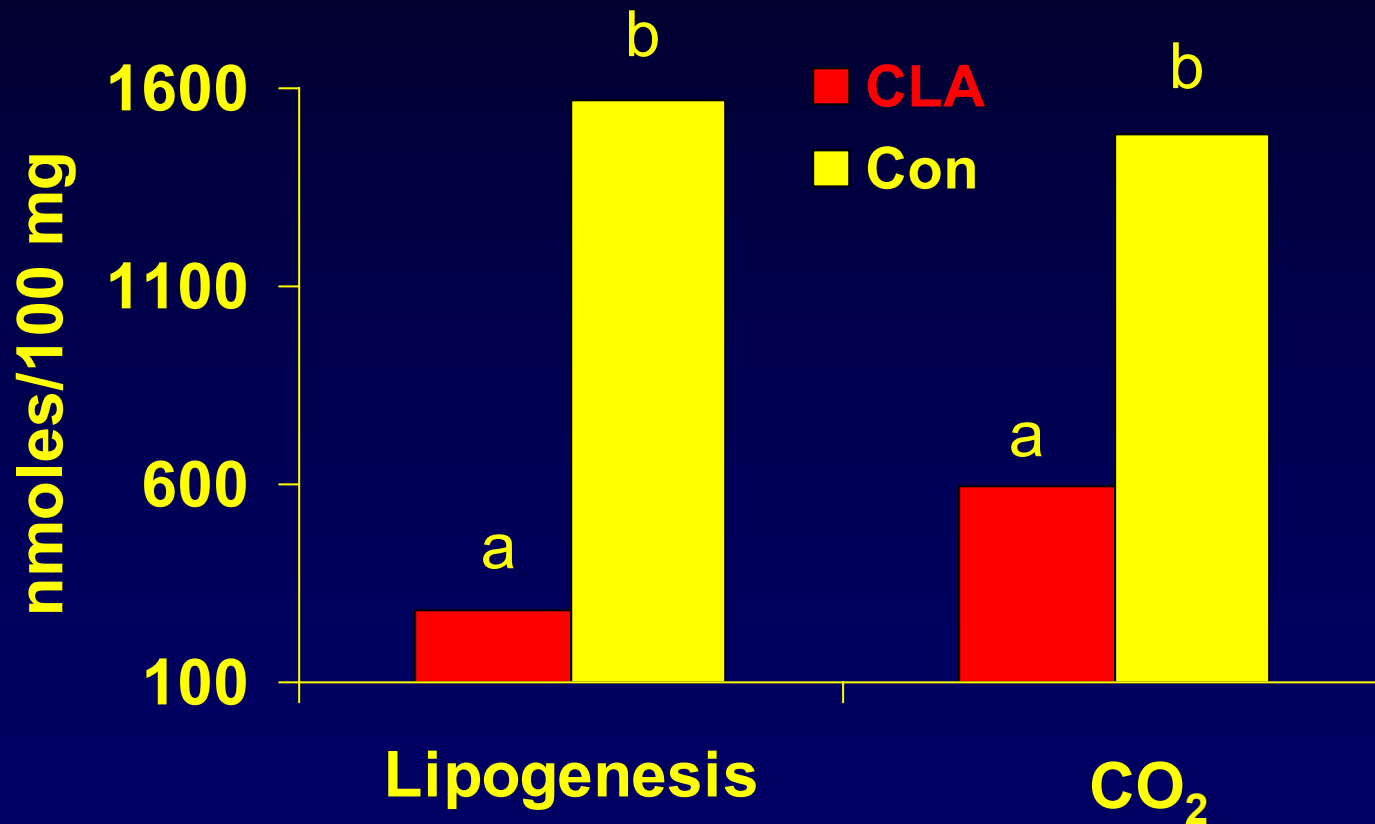


Circles are from Whitlock et al. (2002), Piperova et al. (2000) and Peterson et al., (2002)

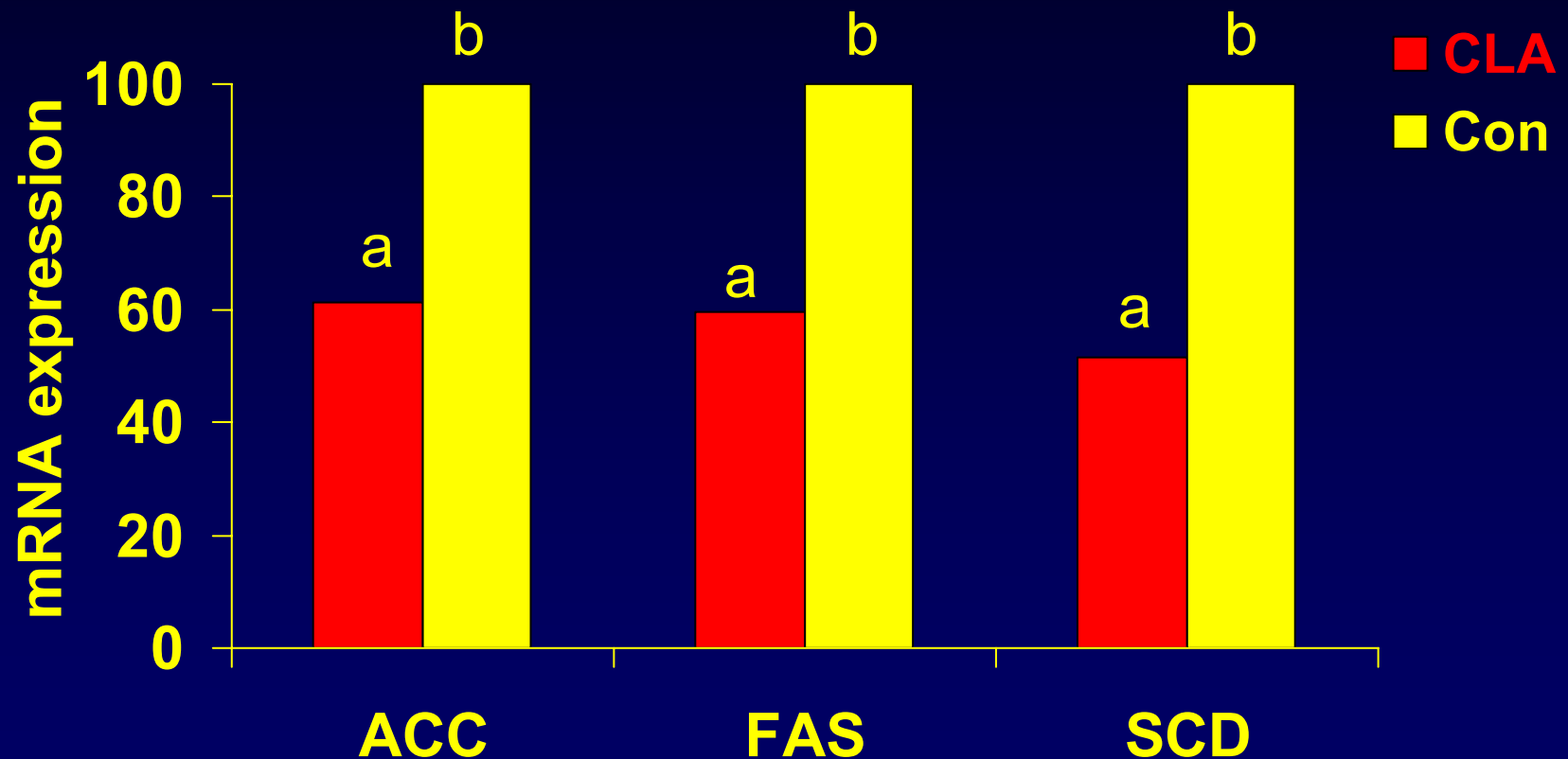
# Effects of CLA on Milk Fat Content During Late Lactation



# Effect of *trans*-10, *cis*-12 CLA on Metabolic Flux Rates

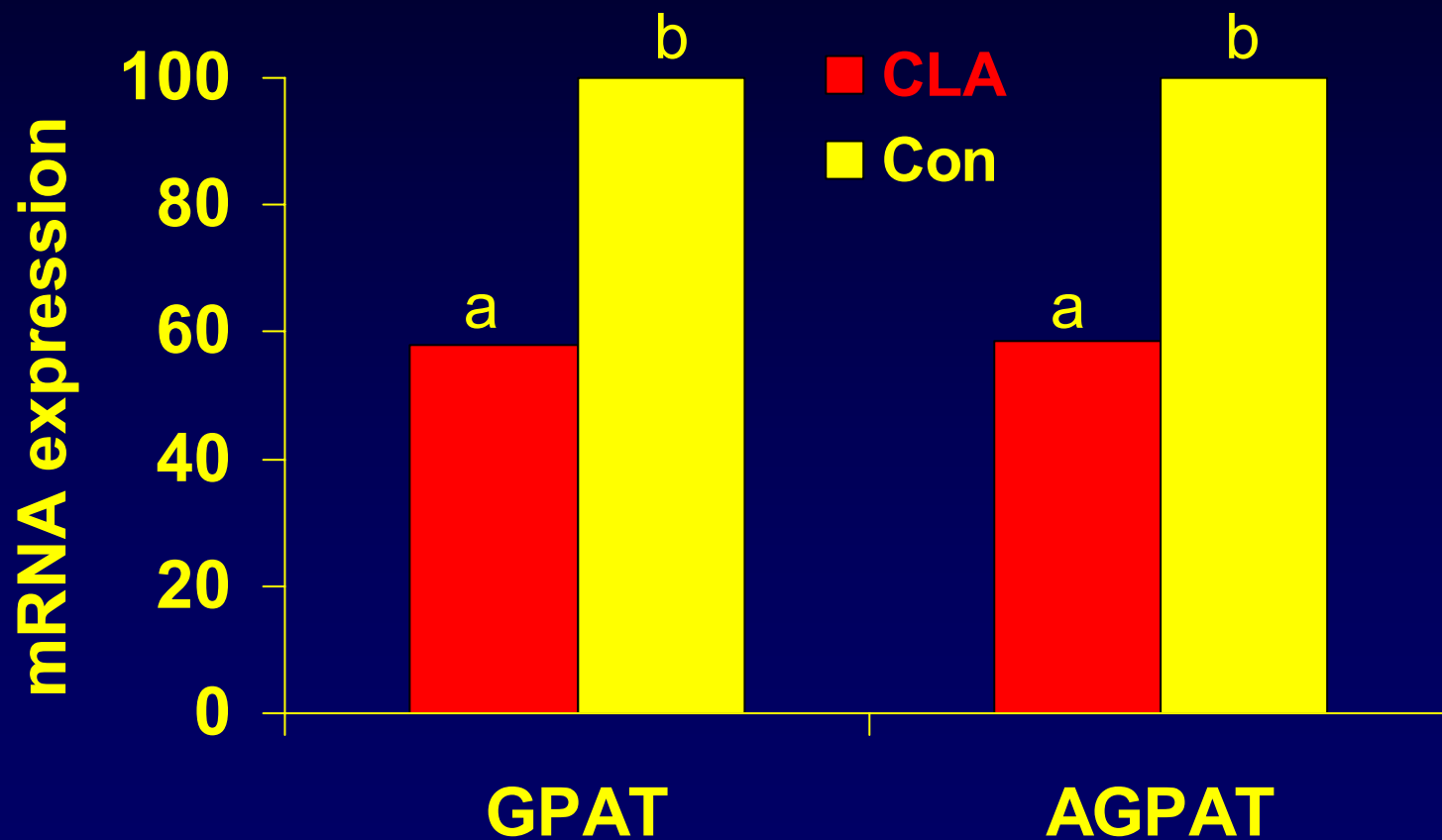


# *trans*-10, *cis*-12 CLA and Mammary *de novo* Fatty Acid Synthesis

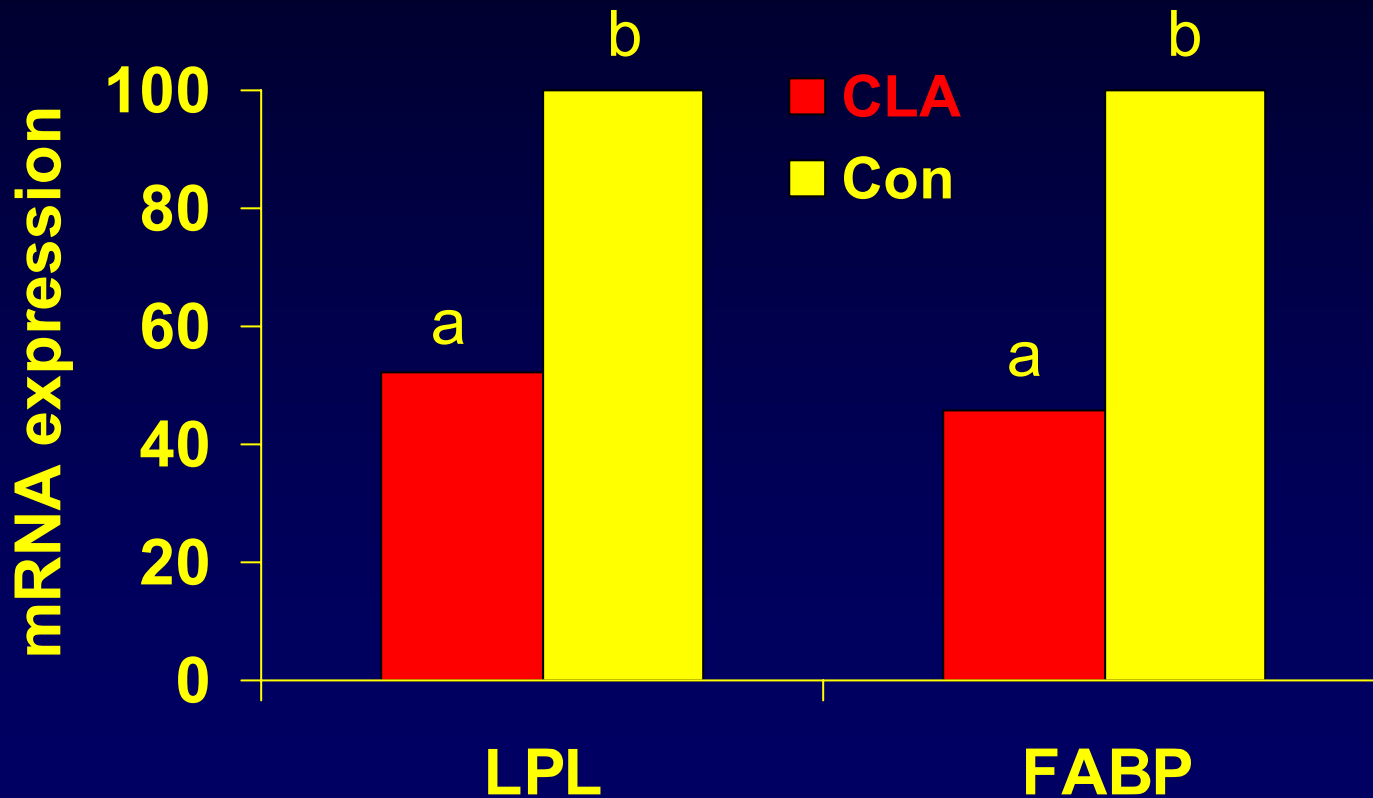


Baumgard et al., (in press)

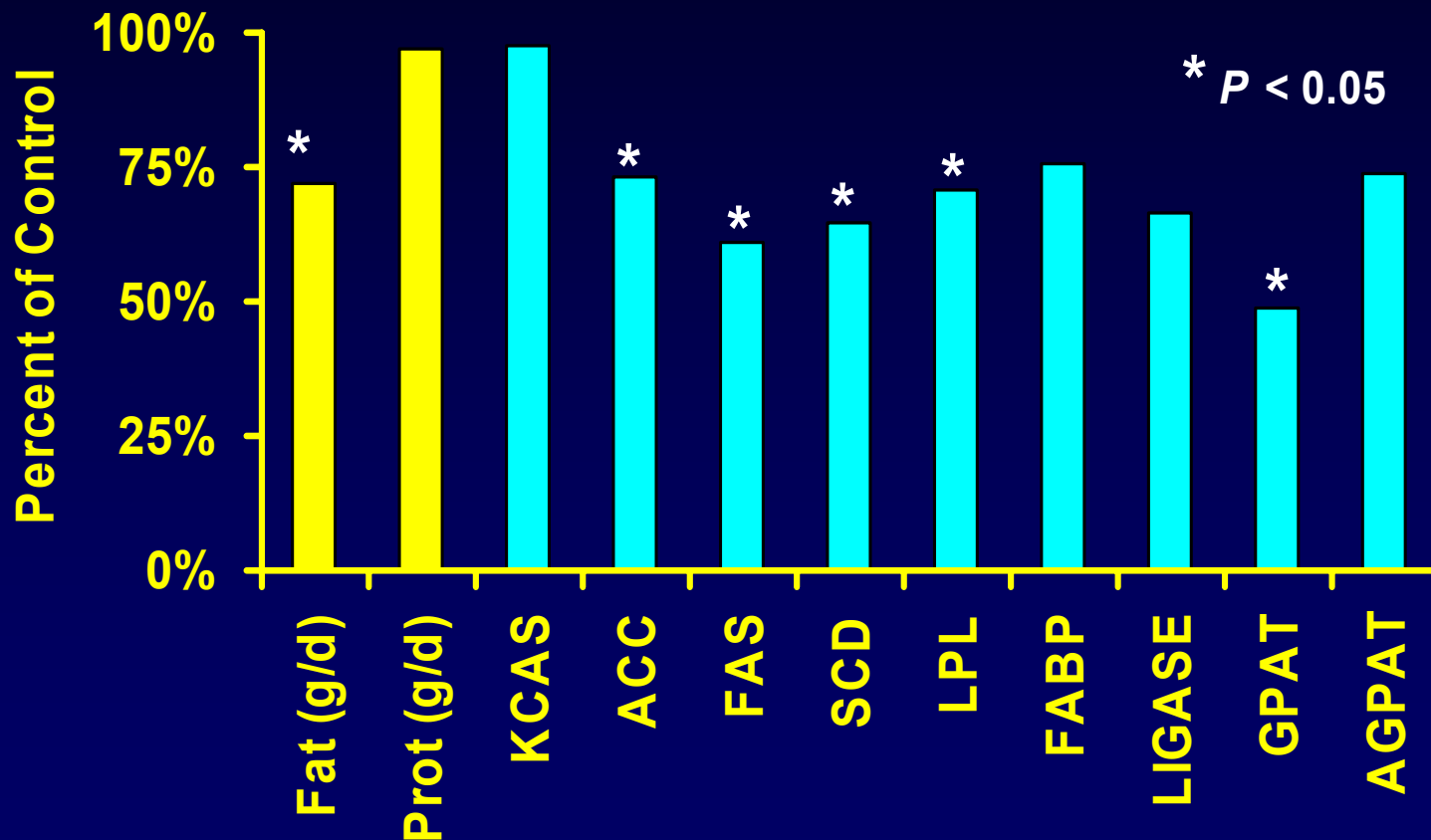
# Effect of *trans*-10, *cis*-12 CLA on Mammary Triglyceride Synthesis



# Effect of *trans*-10, *cis*-12 CLA on Mammary Fatty Acid Transport



# mRNA Abundance with Diet-Induced MFD



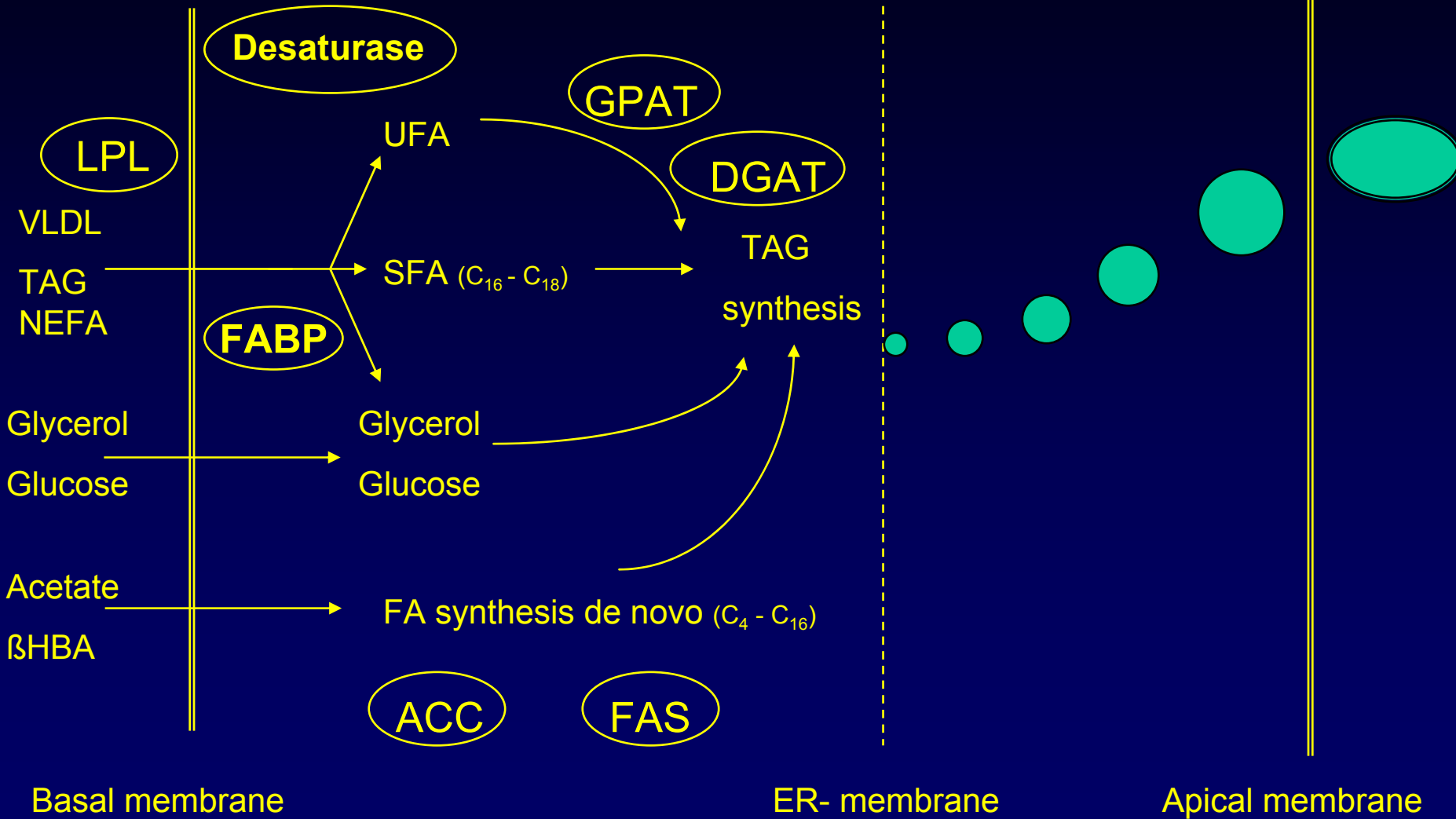


Circulation

Synthesis

Translocation

Secretion



# Common Coordination

- Central coordinator of lipid gene expression?
  - PPAR
  - SREBP
  - HNF

# Effects on Metabolic Processes

## *trans*-10, *cis*-12 CLA Inhibition of Milk Fat

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Process	Effect
<b>Lipolysis</b>	
basal	plasma non-esterified fatty acids unchanged
stimulated	response to $\beta$ -adenergic stimulation unchanged
<b>Leptin</b>	plasma concentration unchanged
<b>Glucose homeostasis</b>	
set-point	plasma glucose unchanged
basal insulin	plasma concentration unchanged
stimulated	glucose response to insulin unchanged
<b>IGF-I</b>	plasma concentration unchanged
<b>Ketogenesis</b>	plasma $\beta$ -hydroxybutyrate unchanged
<b>Feed intake</b>	unchanged
<b>Mammary epithelial cell apoptosis</b>	synthesis of protein and lactose unchanged or increased

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# CLA and Other Species

- Feeding CLA supplements decreases milk fat content in nursing women and lactating pigs
- Both species depend largely upon preformed fatty acid uptake and little on *de novo* fatty acid synthesis for milk fat production

# Effective CLA Dose Body Fat vs. Lactation

Species	CLA in diet	CLA mg/d	CLA mg/kg <sup>0.75</sup>	Duration Days	Fat % Decrease	Ref
Body Fat						
Mice	1.0 %	35	1,045	42	64	West et al
	1.2 %	42	1,267	42	55	
Pigs	0.125%	1,900	71	50	NS	Ostrowska et al
	1.000%	15,400	567	50	31	
Humans	1.9 g/d	1,950	86	94	NS	Zambell et al
Humans	6.8 g/d	6,800	234	84	4	Blankson et al
Humans	3.4 g/d	3,400	117	84	NS	Berven et al
Milk Fat						
Women	1.2 g/d	1,200	57	5	25	Masters et al
Cows	0.014%	3,500	27	5	25	Baumgard et al
Cows	0.04 %	10,000	78	5	50	Baumgard et al

NS, not significant

# CLA and Human Trials

- Compared to rodent studies, CLA dose is markedly lower (based upon % of diet and mg CLA/BW<sup>0.75</sup>) in human trials
- 20-25 g CLA/d is necessary to compare human trials to rodent experiments
- Due to high fat diets, humans rely little on *de novo* fatty acid synthesis
  - Therefore, if CLA reduces body fat by a similar mechanism as in the mammary gland, CLA may be less effective in humans

# Summary

- *trans*-10, *cis*-12 CLA markedly reduces milk fat synthesis
- *cis*-9, *trans*-11 CLA has little or no effect on mammary lipid metabolism
- Effects most aspects of lipid synthesis but especially pronounced on *de novo* fatty acid synthesis
- Solution to an old problem
  - MFD has been studied for over a century
  - Many theories have been proposed and subsequently been proved inadequate

# Summary Continued

- Little or no effect on other milk components
- CLA reduces milk fat synthesis in pigs and women
- Mammary gland lipid metabolism is much more sensitive (~ 20 x) to CLA than adipose lipid metabolism
- Other rumen derived conjugated dienes (*trans*-8, *cis*-10) and trienes (*trans*-10, *cis*-12, *cis*-15) probably reduce milk fat synthesis



# Acknowledgements and Collaborators

- **Dr. Bauman**
  - Debra Dwyer
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  - Dr. Bob Harrell
  - Dr. Tim Mackle
  - Dr. Mikko Griinari
  - Dr. Mark McGuire
  - Dr. Frank Dunshea
  - Dr. Martin Auldish

# Any Questions?

