W-1181 Project Report: Modifying Milk Fat Composition for Improved Nutritional and Market Value

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In USA, consumption and use of corn oil has progressively replaced and supplanted animal fats, so that corn and other vegetable oils now represent more than 70% of the PUFA dietary source

- The diet of our ancestors was higher in fiber, rich in fruits, vegetables, grazing livestock, and fish.
- The diet was less dense in calories, lower in total fat and saturated fat, and contained approximately equal amounts of *n*-6 and *n*-3 PUFAs
- In Western diets, the current ratio of *n*-6 to *n*-3 PUFAs is about 10 to 20:1, indicating that Western diets are deficient in *n*-3 fatty acids compared with the diet on which humans evolved

The LA/LNA ratio of US breast milk has increased from the value of **6.0–8.0** before 1970 to **14–16** since 1980.

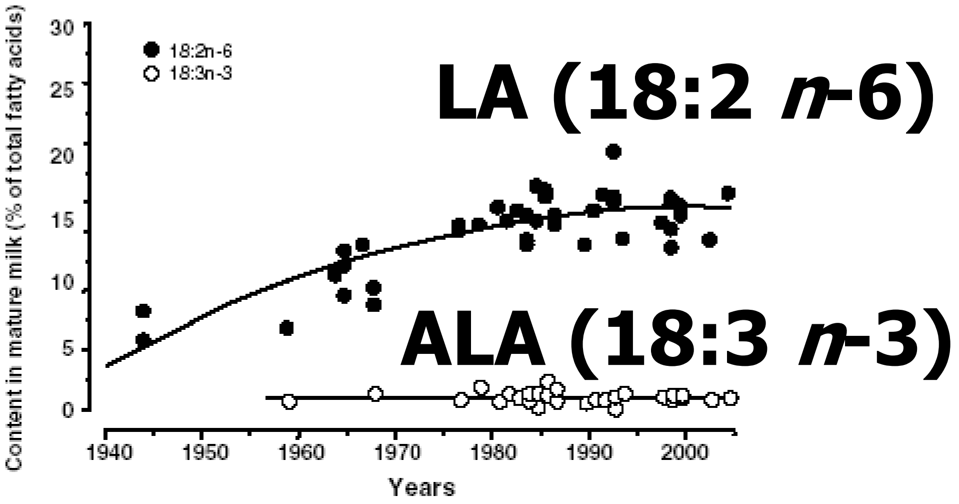


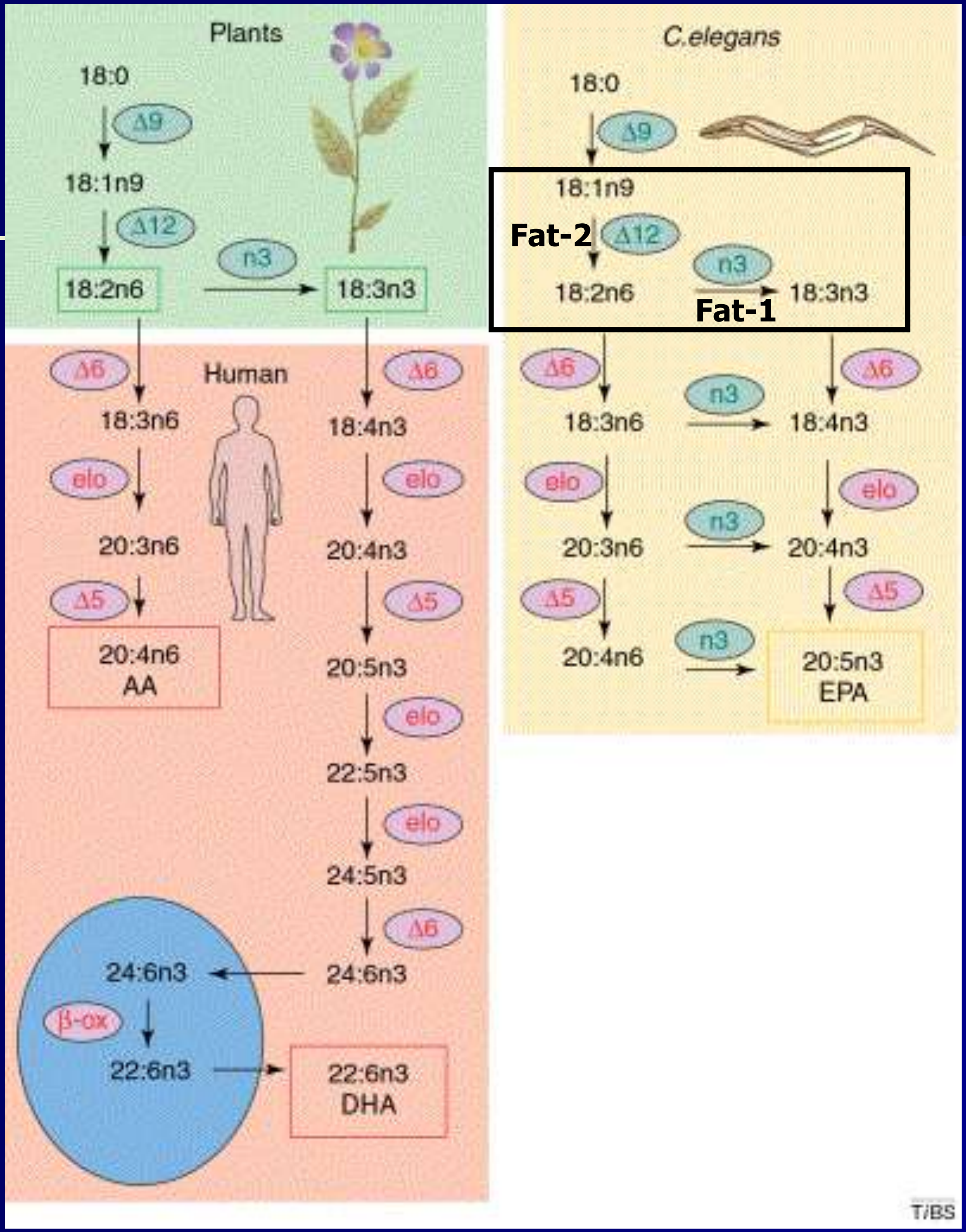
Fig. 3. Linoleic acid (LA) and α-linolenic acid (LNA) content in mature breast milk of US women from 1944 to 2005

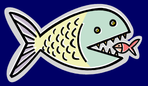
Infant "Obesity"

The percentage of US children between 6 and 11 *months* of age above the 95th percentile of the weight-for-length growth reference curve has increased in boys from 4.0% between 1976 and 1980 to 7.5% between 1988 and 1994, and in girls from 6.2% to 10.8% during the same periods of time.

Fatty acids as adipogenic hormones

- Polyunsaturated fatty acids (PUFAs) of the *n*–6 series, especially ARA (20:4n-6) are very adipogenic, and promote adipogenesis (differentiation of adipocytes)
- One key question to be addressed in humans is whether the balance of PUFAs has changed during pregnancy and/or the lactation period such that it favors excessive adipogenesis during the early stages of adipose tissue development, i.e. during fetal life and infancy.

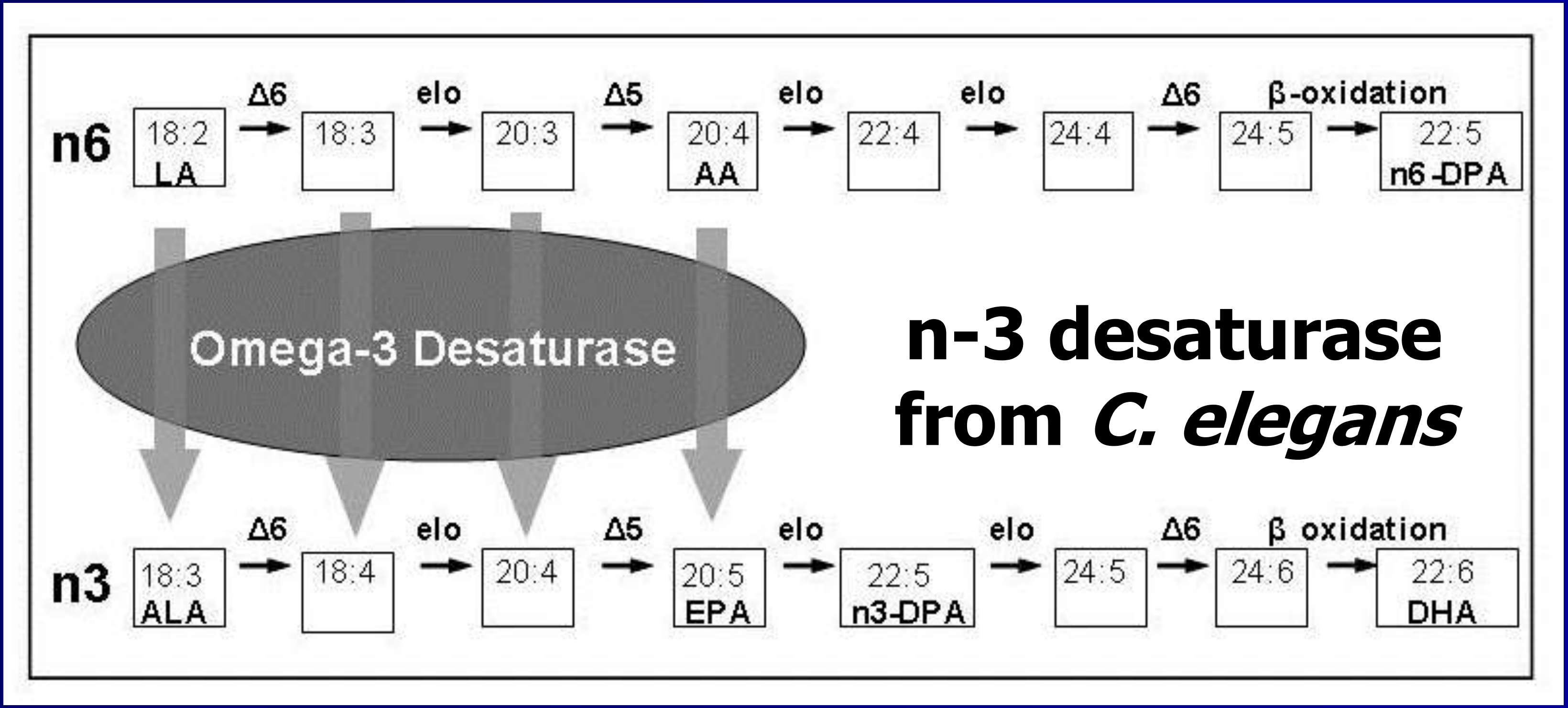




Vertebrates lack the fatty acid desaturase enzymes required for the synthesis of linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3), and are therefore dependent on dietary sources to obtain these essential PUFA.

The nematode *Caenorhabditis elegans* is able to synthesize ALA from oleic (18:1n-9) by virtue of endogenous enzymes (acyl-lipid fatty acid desaturases) called “fat-1” and “fat-2”.

Develop a transgenic mouse model expressing omega-3 desaturase

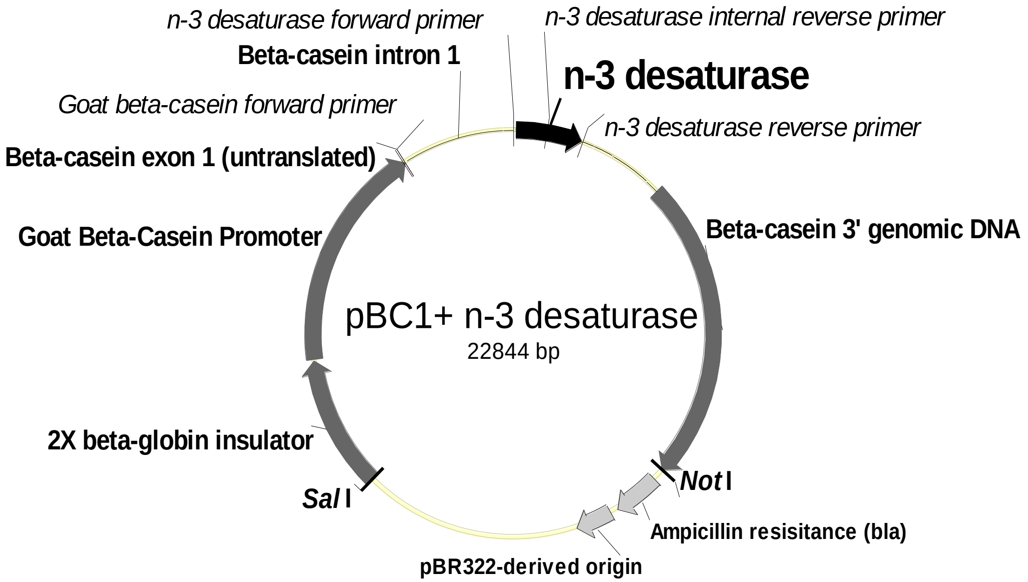


n-3 desaturase from *C. elegans*

B. T. Kao, K. A. Lewis, E. J. DePeters, and A. L. Van Eenennaam. 2006. Endogenous Production and Elevated Levels of Long-Chain n-3 Fatty Acids in the Milk of Transgenic Mice. Journal of Dairy Science. 89:3195-3201.

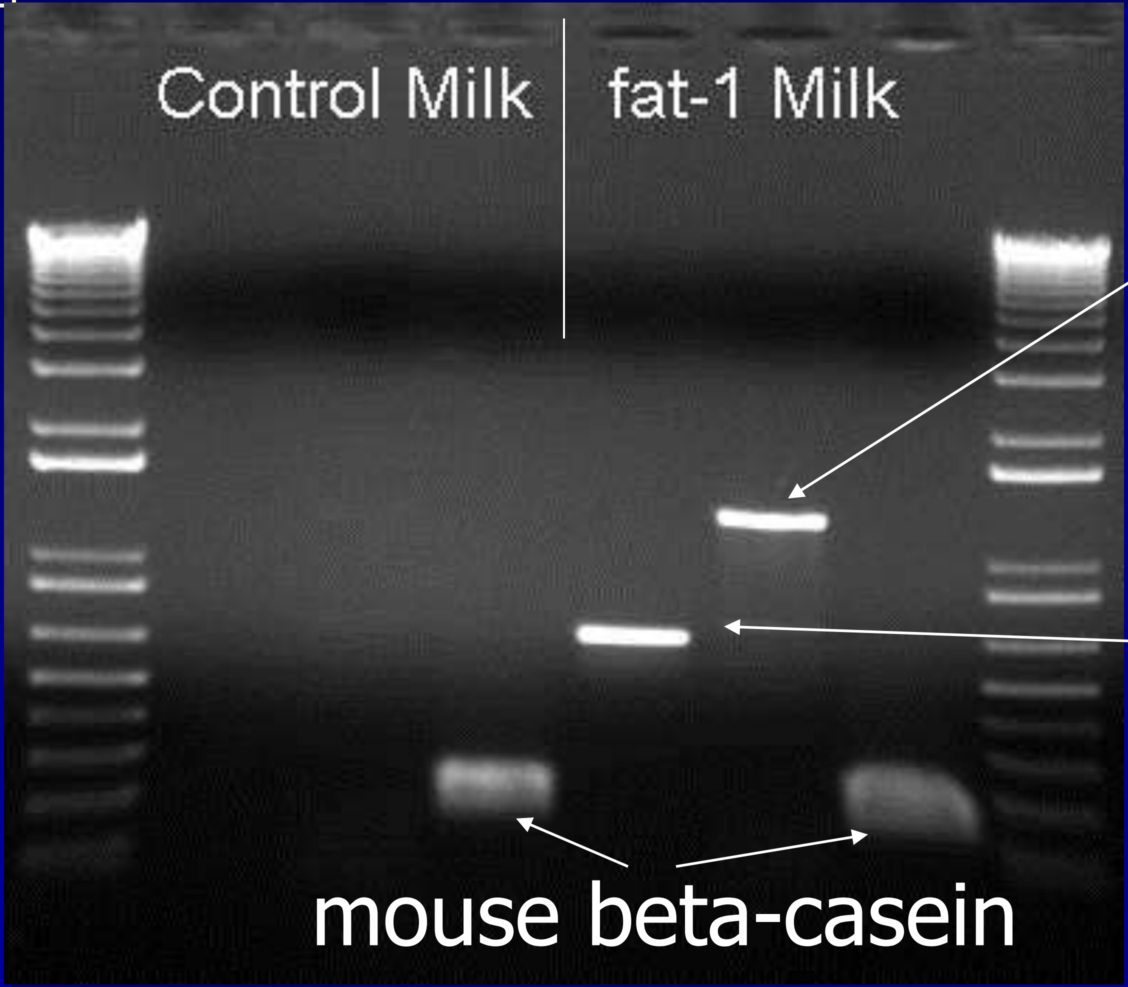
Development of the omega-3 milk mouse model

Beth Kao (MS)



Transgenic mice were generated by pronuclear microinjection of the *SalI/NotI* fragment containing the *C. elegans* omega-3 fatty acid desaturase (Fat-1) under the control of the goat beta-casein promoter of the pBC1 mammary expression vector.

Good transgene expression – full length fat 1 expression

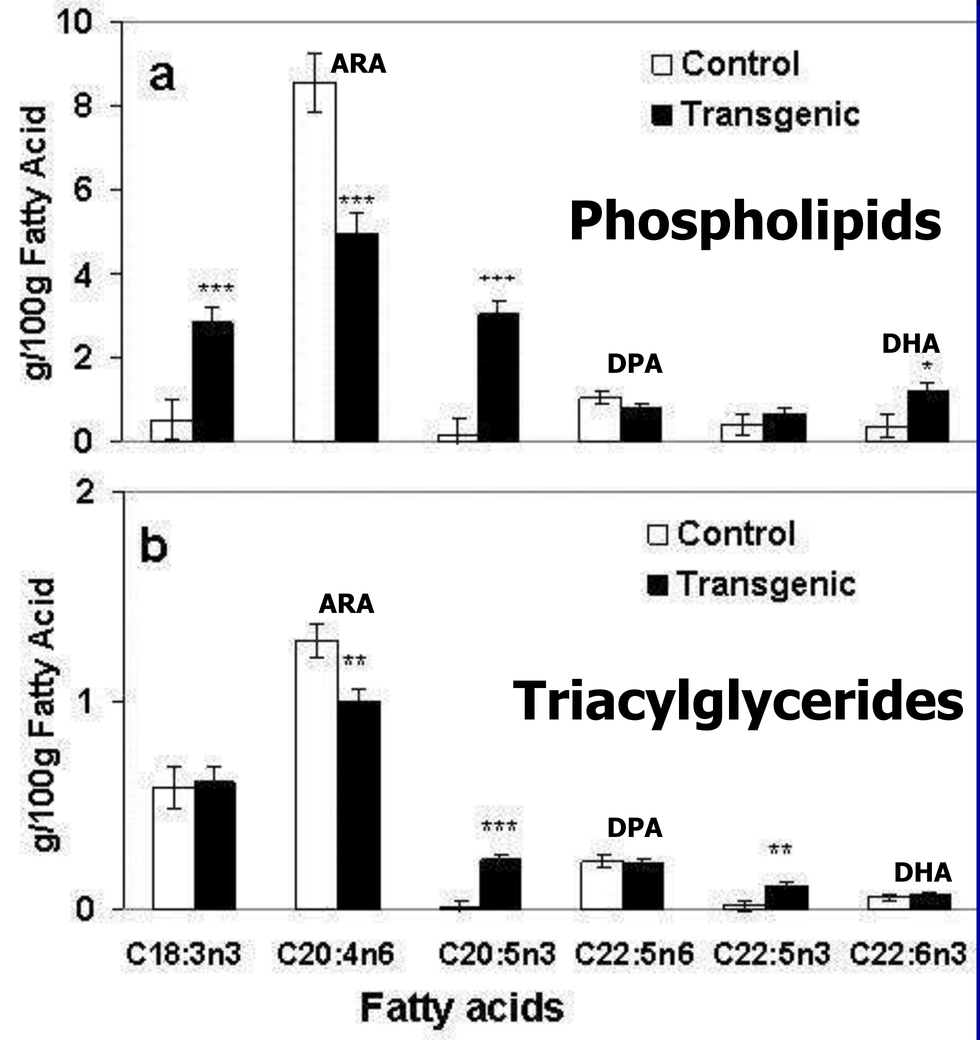


fat-1 CDS

Goat beta casein 5′ UTR / fat-1 intron spanning primers

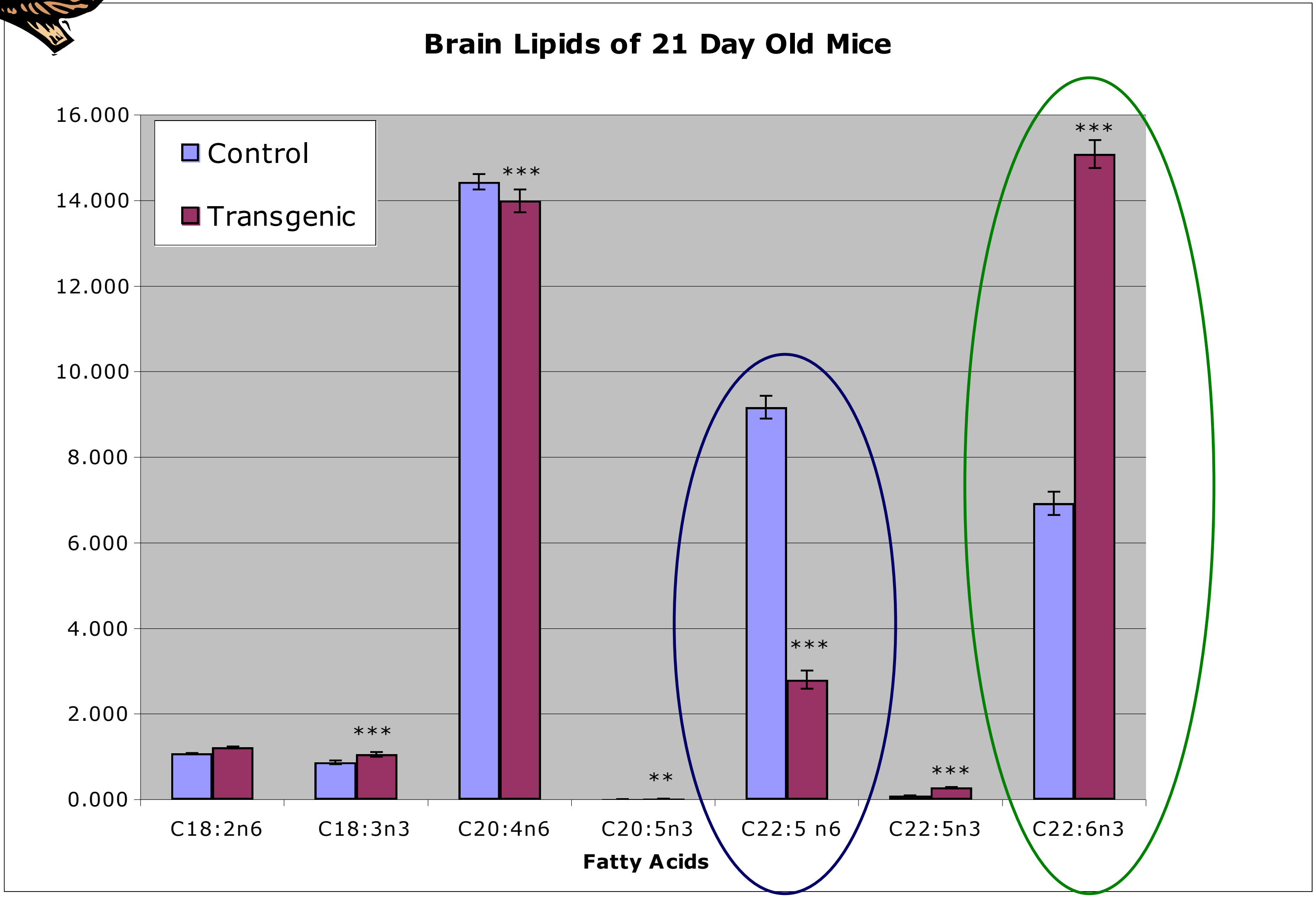
Milking day at the mouse house

Endogenous Production and Elevated Levels of Long-Chain n-3 Fatty Acids in the Milk of Transgenic Mice



- Transgenic females and nontransgenic full sib control females were placed on a high safflower oil (n-6) diet from breeding to 21 days post-parturition.
- There were 33 pups reared in each treatment group.

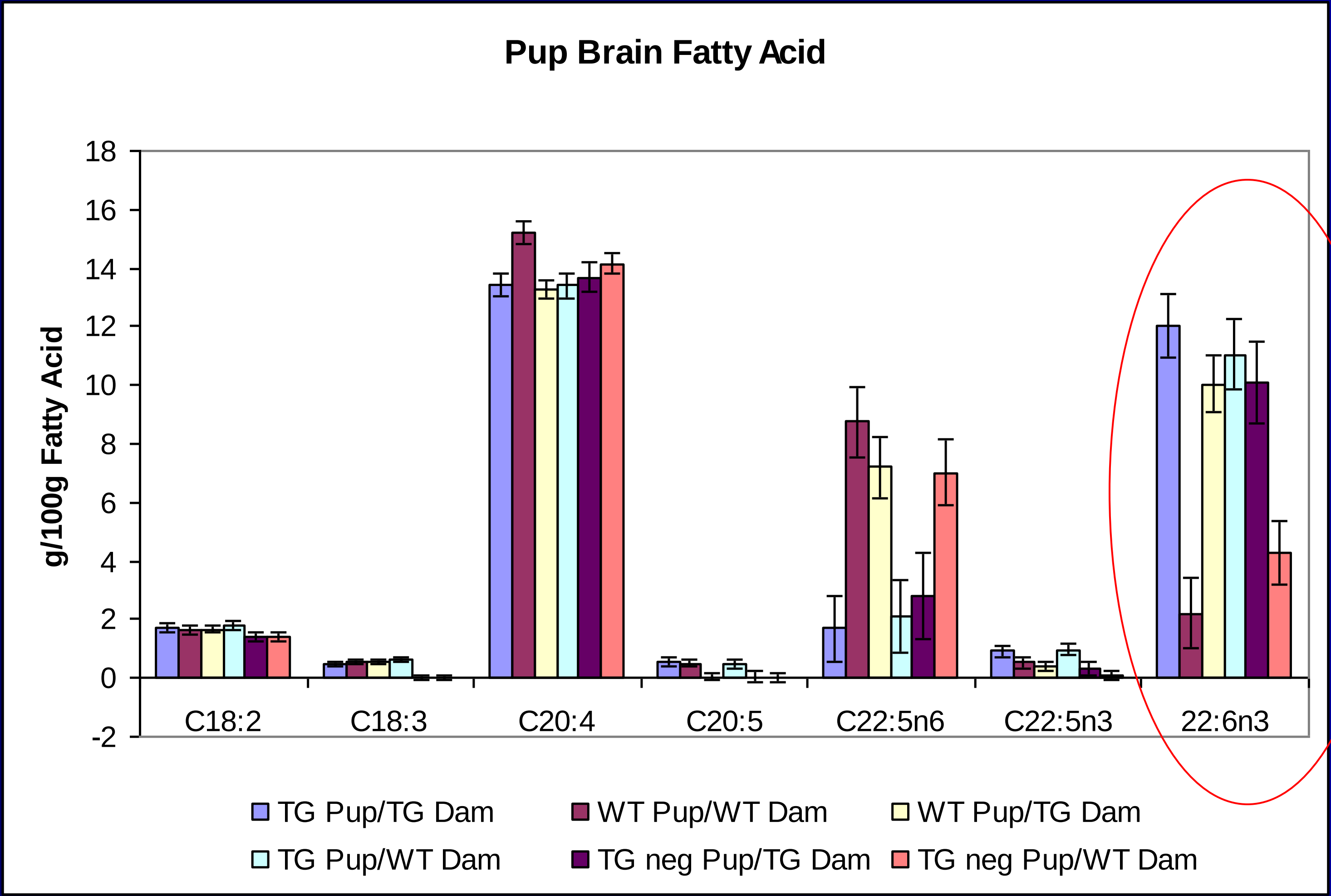
Brain lipids of 21 day pups nursed on control or transgenic dams



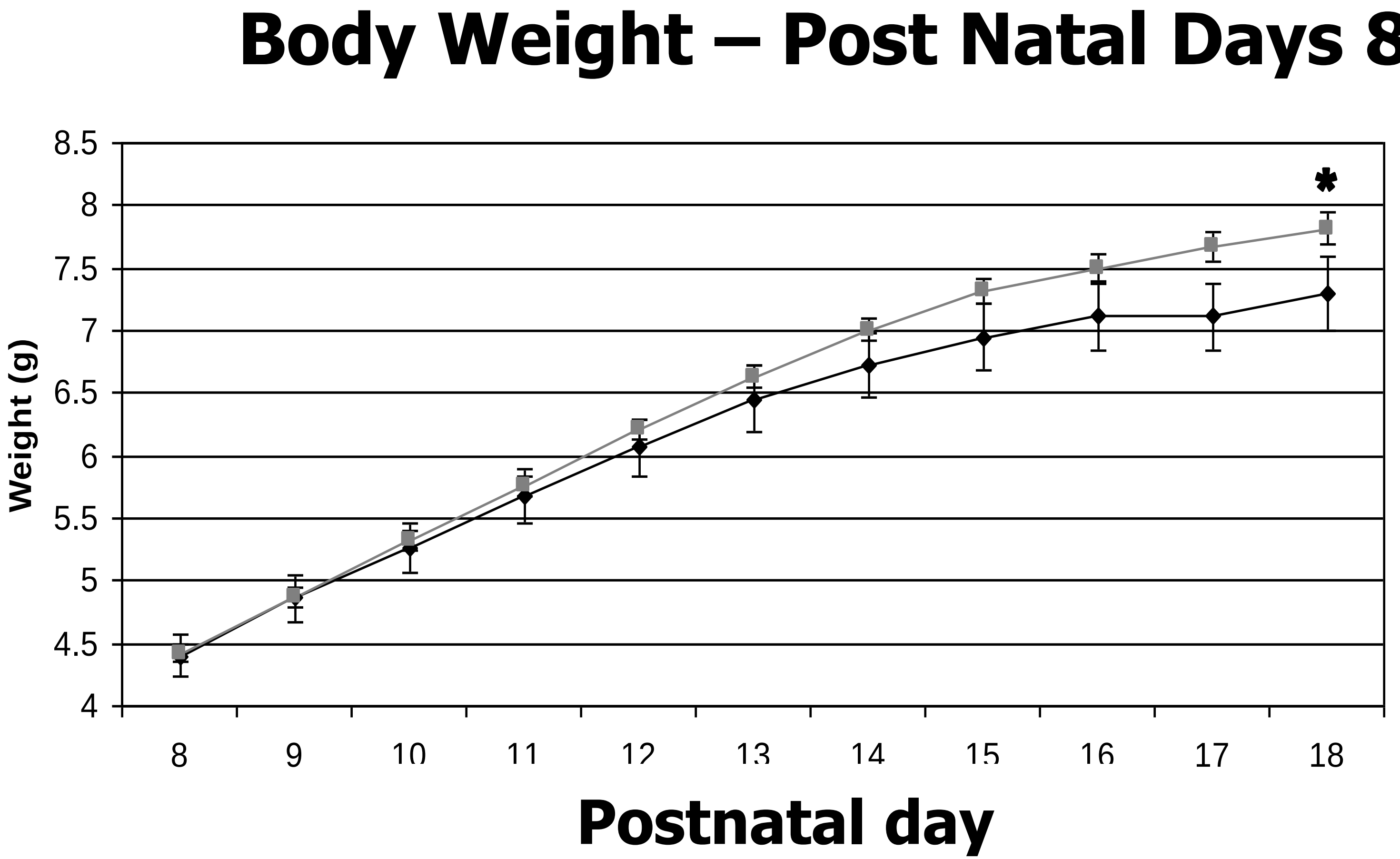
B.T. Kao, E. J. DePeters, and A. L. Van Eenennaam. 2006. Mice Raised on Milk Transgenically-Enriched with n-3 PUFA have Increased Brain Docosahexaenoic Acid. Lipids. 41(6):543-9.

Brain lipids of constitutive promoter transgenic and control pups on control or transgenic dams – cross foster

Kristin Deines (MS)

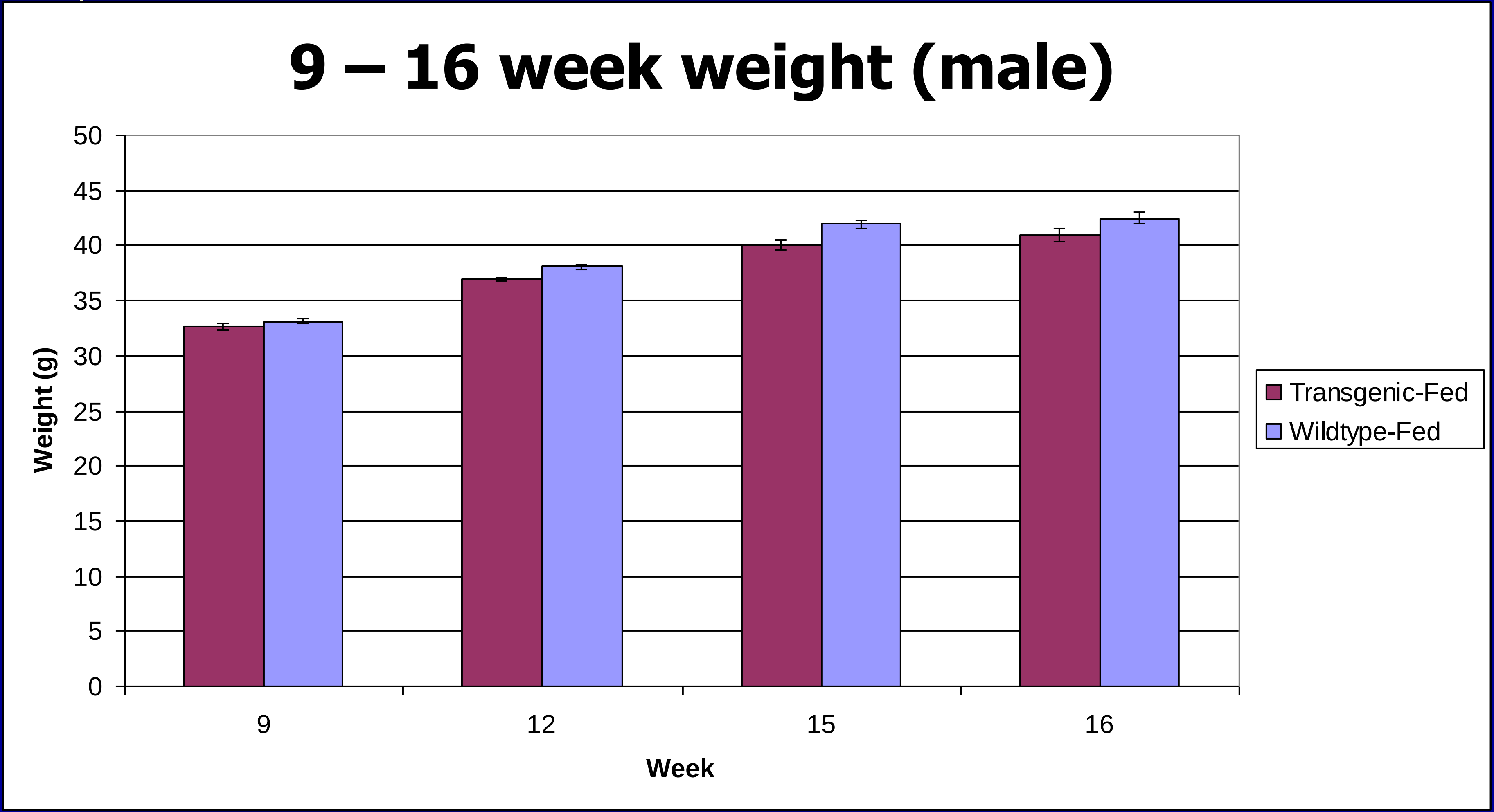


By the end of weaning the pups raised on transgenic dams were heavier than pups raised on omega-3 milk.

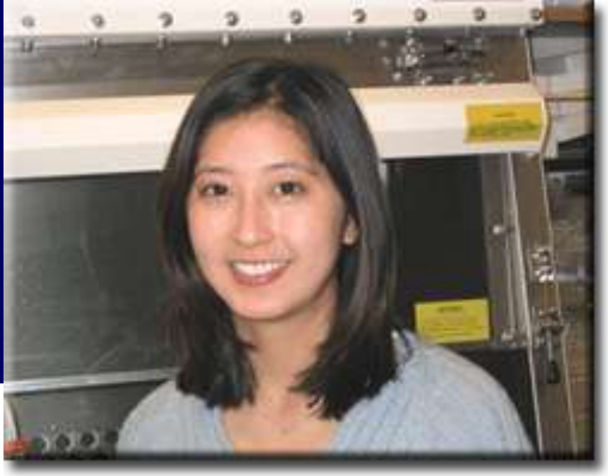


K. D. Bongiovanni, E. J. DePeters, and A. L. Van Eenennaam. 2007. Neonatal growth rate and development of mice raised on milk transgenically enriched with omega-3 fatty acids. . Pediatric Research 62(4):412-6.

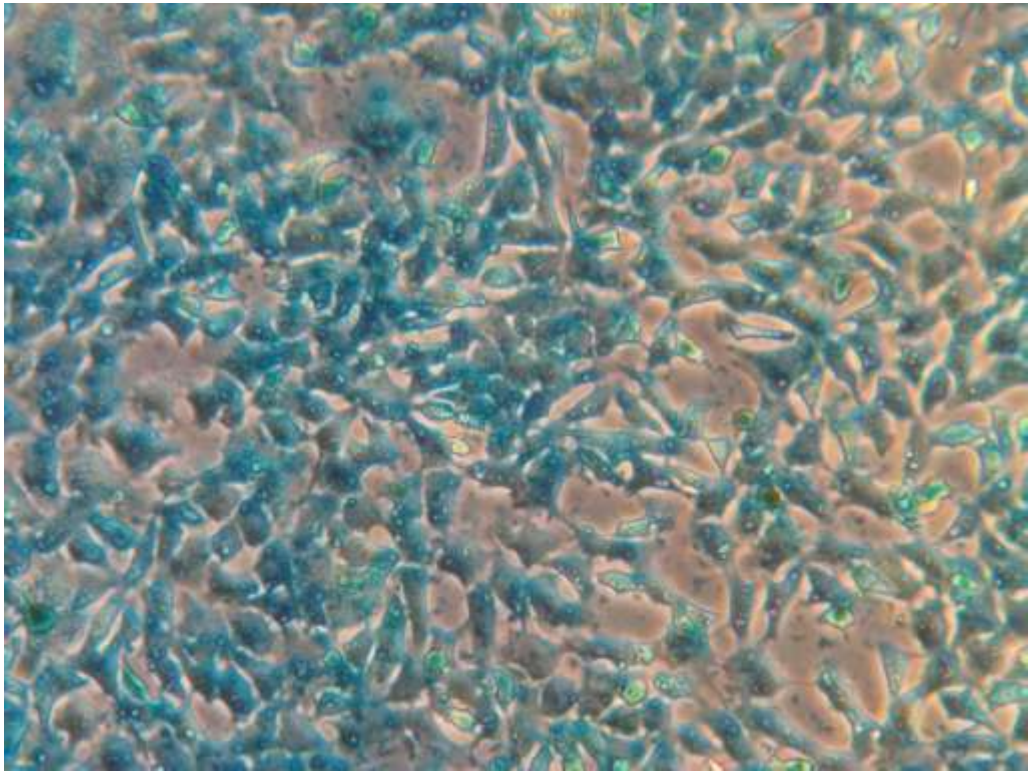
Weight of adult mice (n=21 or 26)



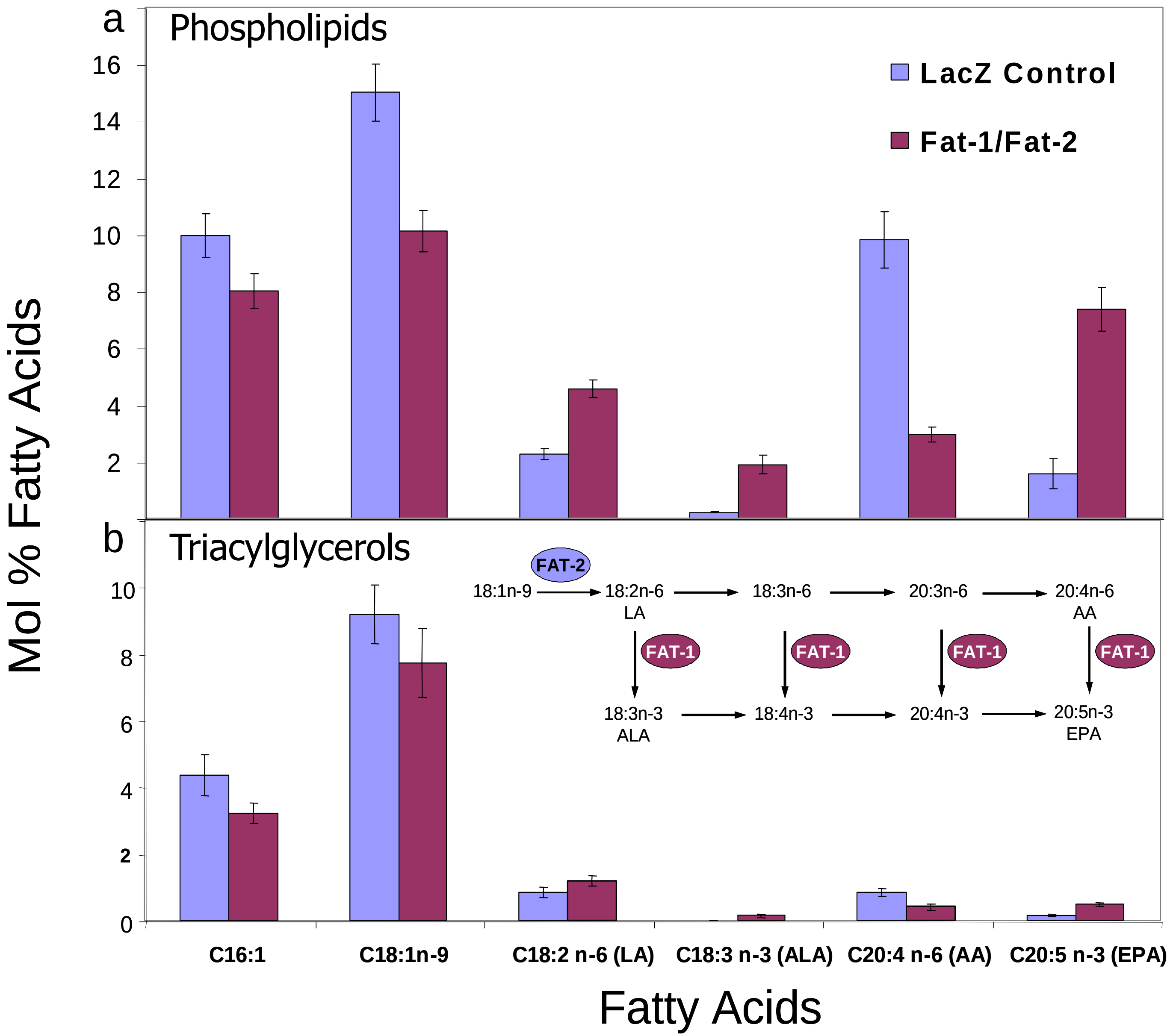
HC11 cell transfection using adenovirus



Kerri Morimoto

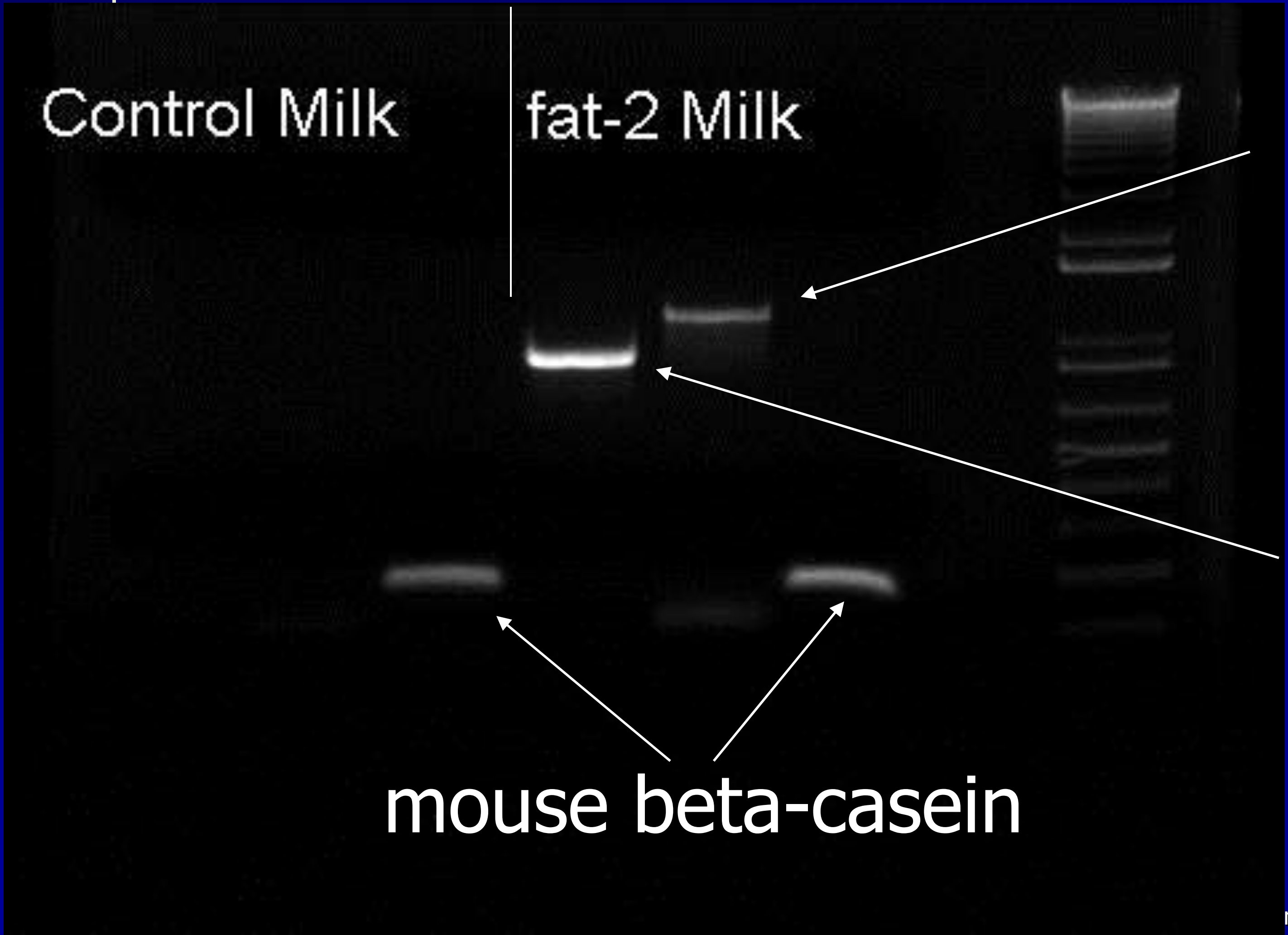


Endogenous Production of Omega-3 and Omega-6 Fatty Acids in Mammalian Cells



K. C. Morimoto, A. L. Van Eenennaam, E. J. De Peters, J. F. Medrano. 2005. J Dairy Sci.

Good transgene expression – full length fat 2 expression



fat-2 CDS

Goat beta casein 5′ UTR / fat-2 intron spanning primers

mouse beta-casein

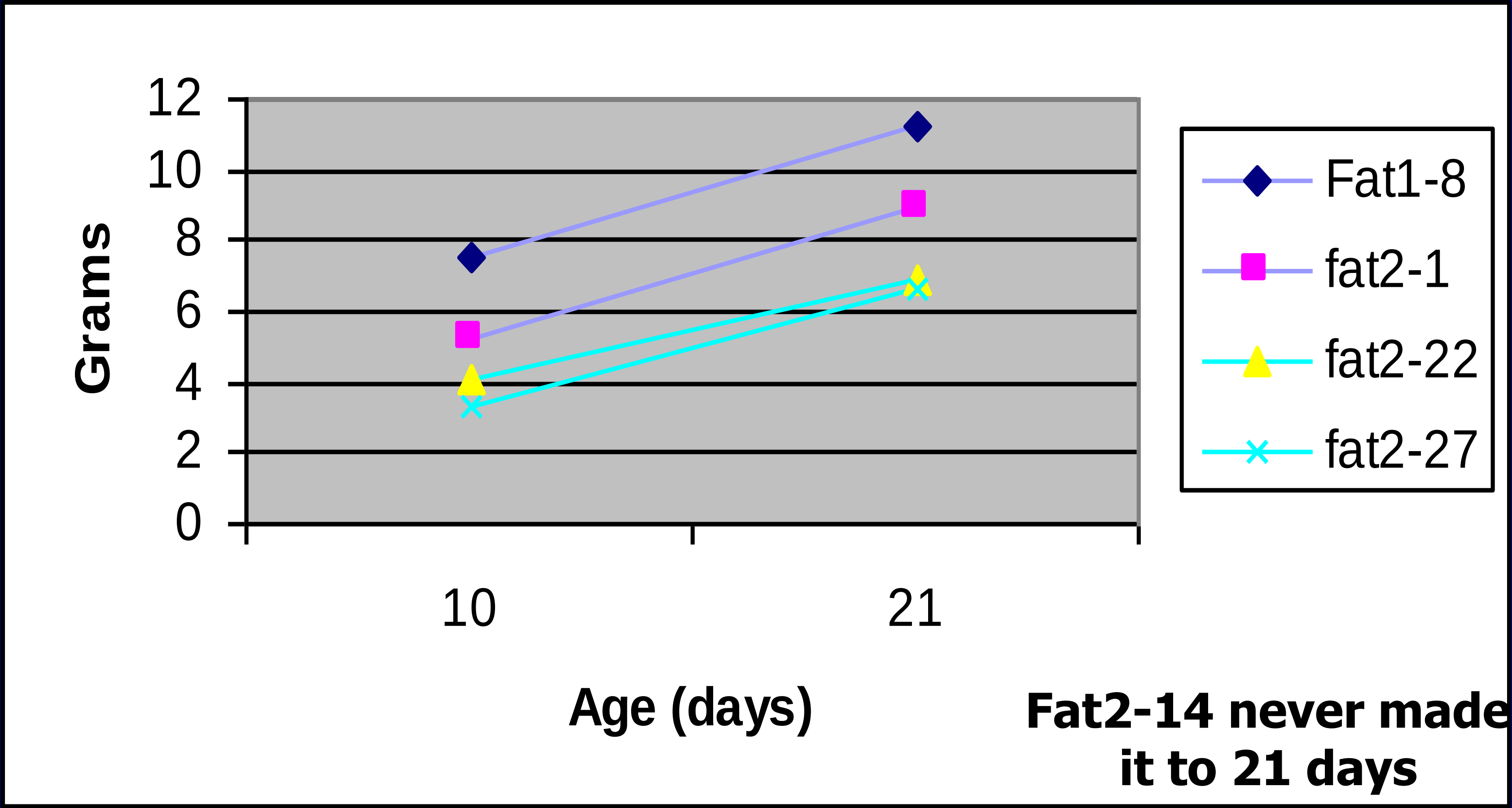
Abnormal growth in FAT-2 mice – apparent shut down of lactation

10-day old pups

Fat2 pup raised on control dam

Control raised on Fat-2 dam

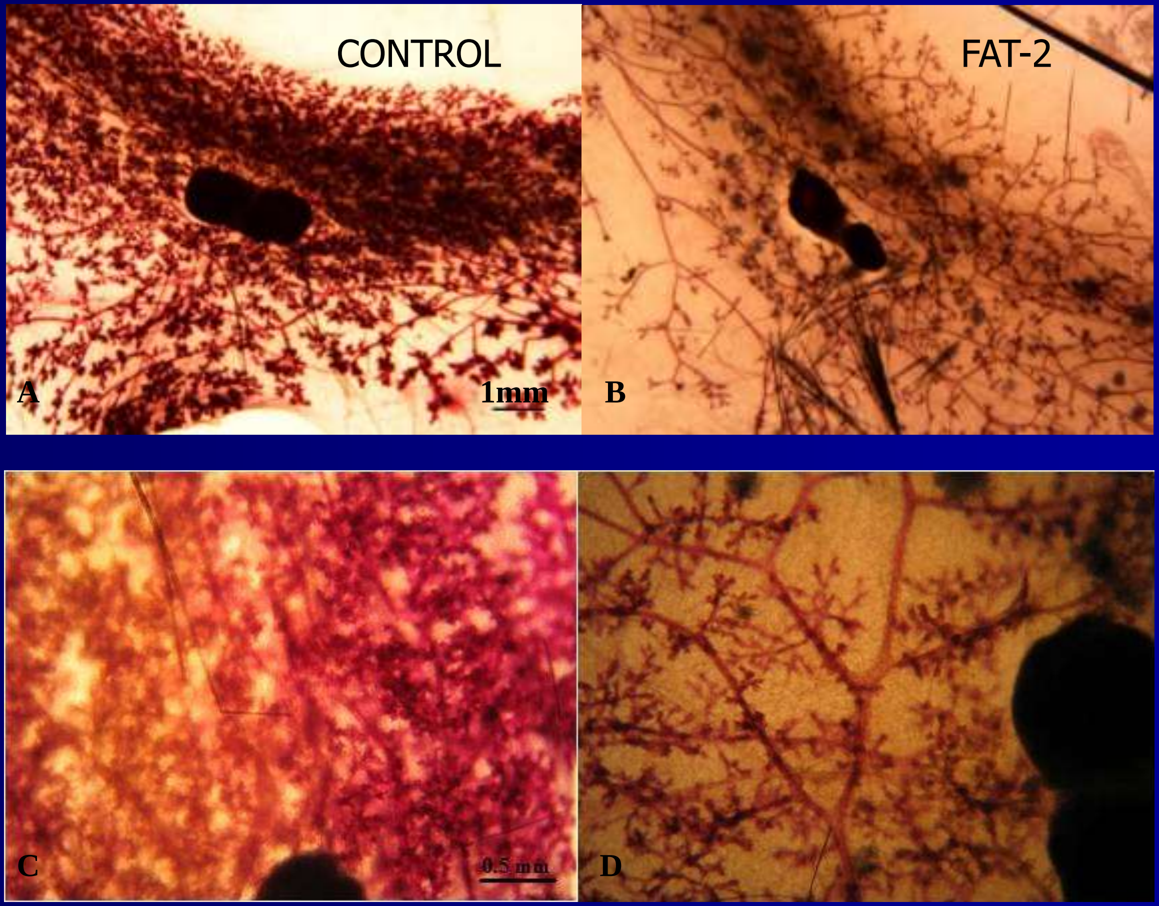
Four lines FAT4 - never grow as well as FAT1 or C57 controls



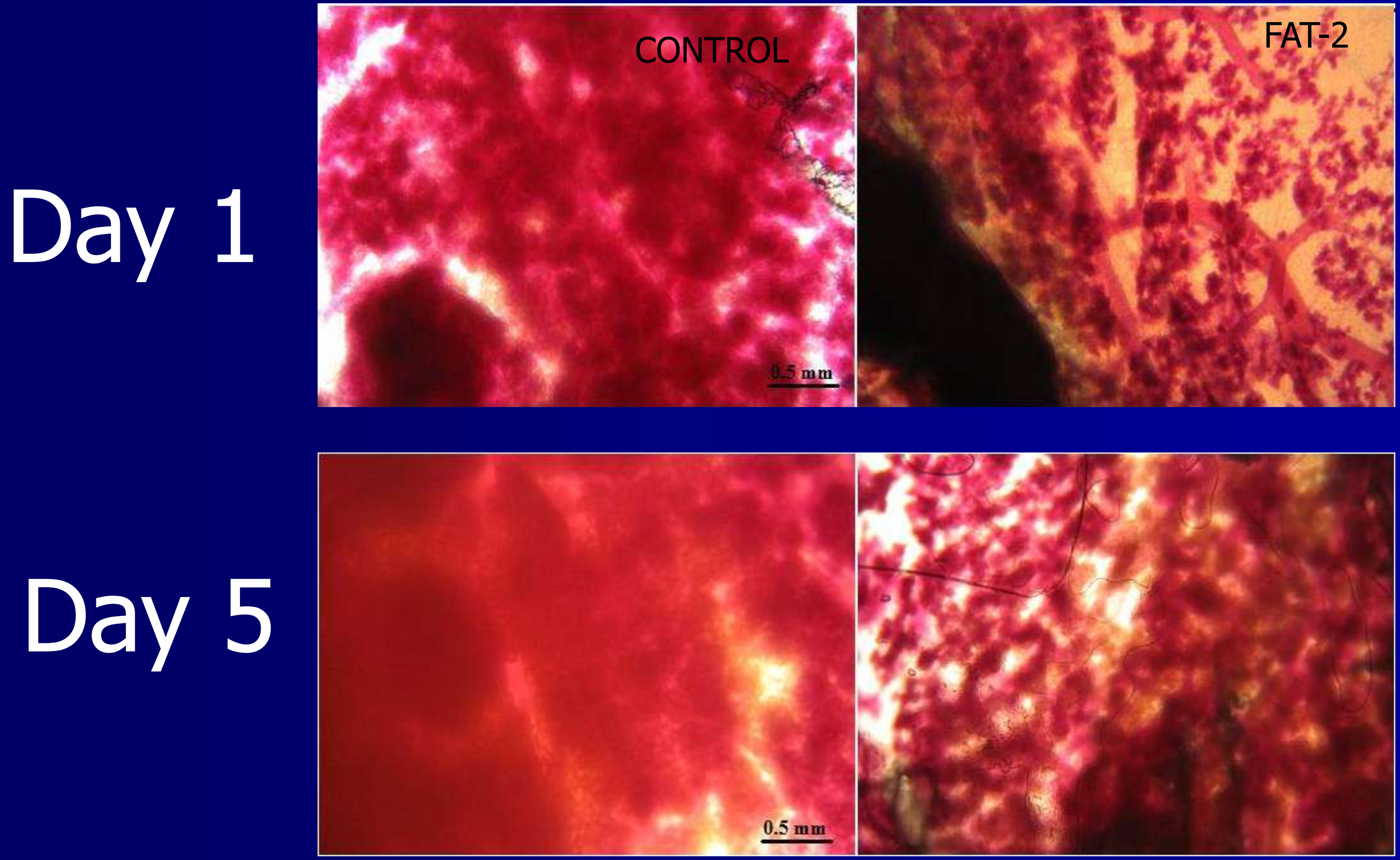
Fat2-14 never made it to 21 days

Mammary gland from a 19 day pregnant non-transgenic (control) and FAT-2 transgenic

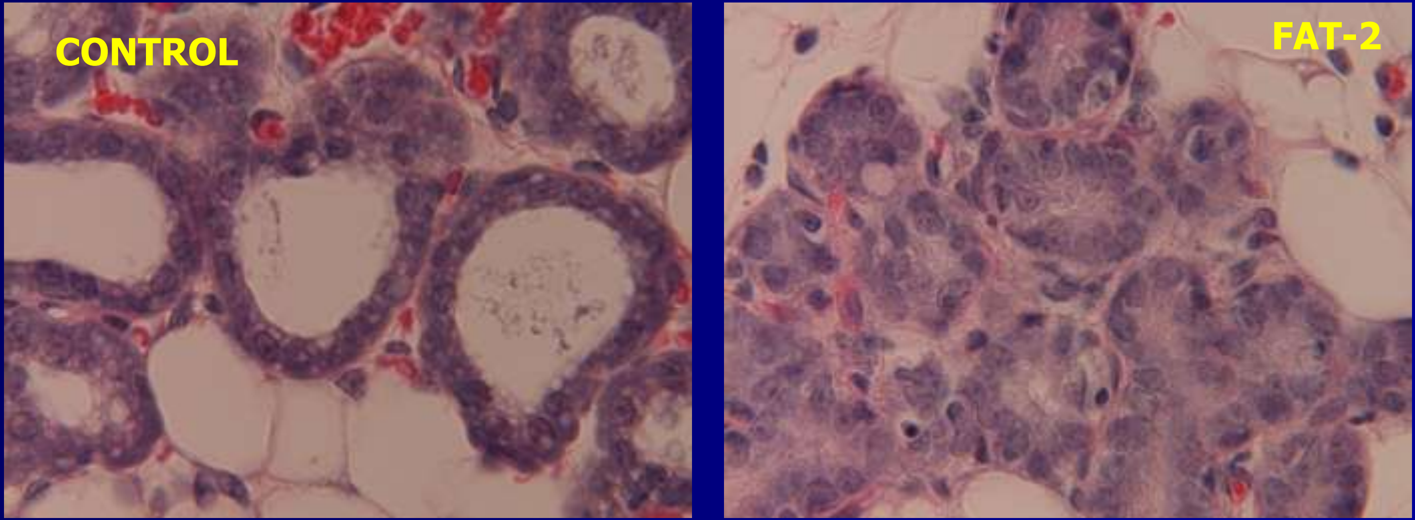
Kristin Deines (MS)



Mammary glands from day 1 and day 5 of lactation



Histological sectioning from day 1 postpartum



Histological sectioning from day 1 postpartum control (A) and *fat-2* transgenic (B) mammary glands at 400x. Hollow alveoli present in control mammary gland demonstrate the ability of the gland to become engorged with milk. Expanded alveoli are not visible in the *fat-2* transgenic mammary gland

Similarity to prolactin receptor knockout phenotype

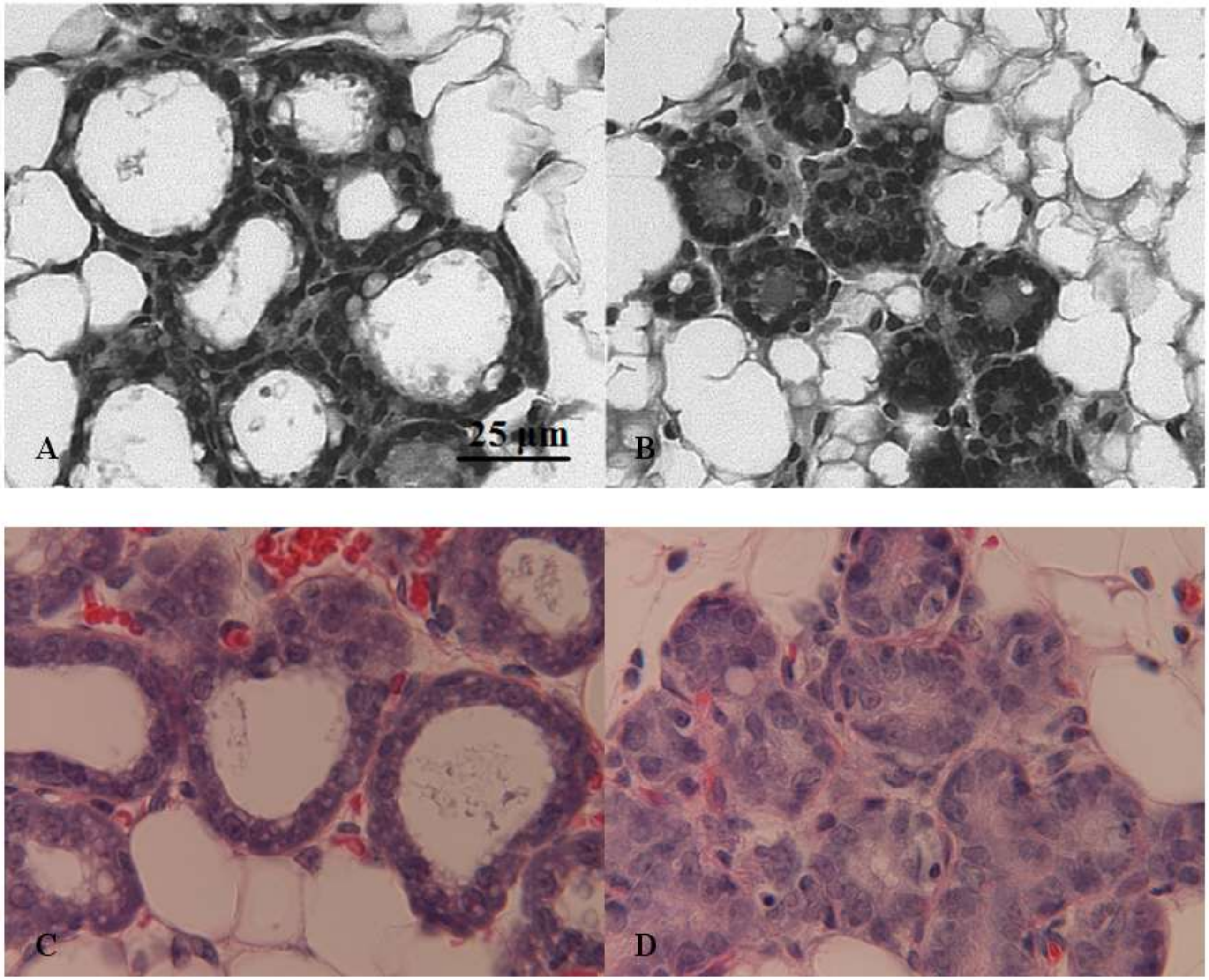


Figure 3.15 Histological sections of day 1 postpartum alveoli. Sections by Brisken et al. (1999) (A and B) and present study (C and D). Control mammary glands (A and C) compared to PRLR+/- mice (B) and *fat-2* (D) mammary glands.

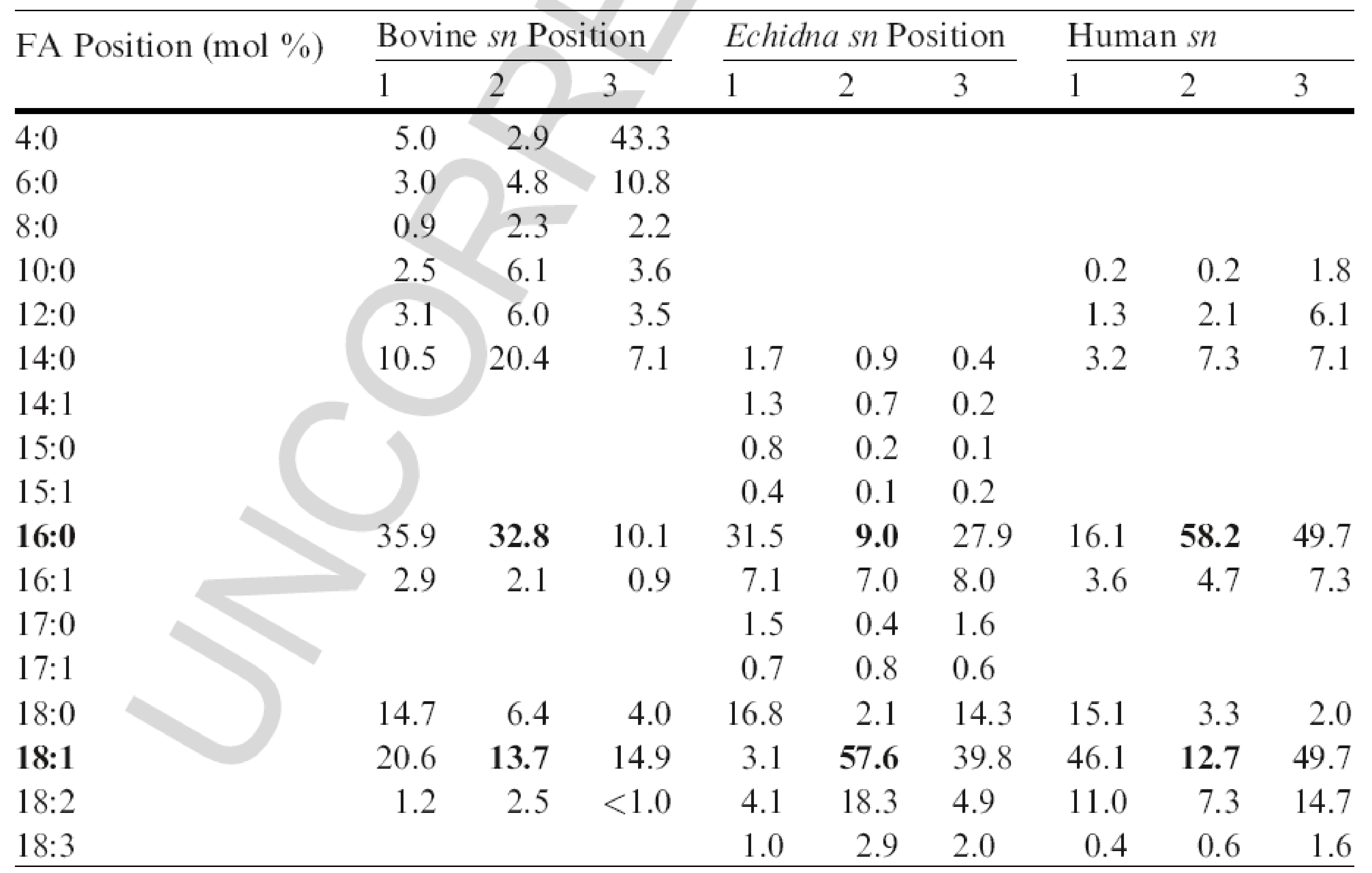
Echidna – a Monotreme (egg laying mammal)

"When the baby is between 30-40 days of age it no longer fits in the pouch and the mother is forced to walk on tippy-toes as the growing "puggle" clings to the belly hairs. At 50-60 days it gets deposited in a nursery burrow"

“For 5 months the baby lives in the nursery burrow and the mother returns only every 5-6 days for 1-2 hours ! The baby suckles hardily at the milk patch where it ingests up to 40% of its own body mass in one feeding !!! The rest of the time the bay sleeps, pees and poops like all babies”

The Echidna - Australia’s Enigma by Dr. Peggy Rismiller

Positional Distribution of Major FA in Triglycerides from Bovine, Echidna, and Human Milk



FA Position (mol %)	Bovine *sn* Position			*Echidna sn* Position			Human *sn*		
	1	2	3	1	2	3	1	2	3
4:0	5.0	2.9	43.3						
6:0	3.0	4.8	10.8						
8:0	0.9	2.3	2.2						
10:0	2.5	6.1	3.6				0.2	0.2	1.8
12:0	3.1	6.0	3.5				1.3	2.1	6.1
14:0	10.5	20.4	7.1	1.7	0.9	0.4	3.2	7.3	7.1
14:1				1.3	0.7	0.2			
15:0				0.8	0.2	0.1			
15:1				0.4	0.1	0.2			
16:0	35.9	**32.8**	10.1	31.5	**9.0**	27.9	16.1	**58.2**	49.7
16:1	2.9	2.1	0.9	7.1	7.0	8.0	3.6	4.7	7.3
17:0				1.5	0.4	1.6			
17:1				0.7	0.8	0.6			
18:0	14.7	6.4	4.0	16.8	2.1	14.3	15.1	3.3	2.0
18:1	20.6	**13.7**	14.9	3.1	**57.6**	39.8	46.1	**12.7**	49.7
18:2	1.2	2.5	<1.0	4.1	18.3	4.9	11.0	7.3	14.7
18:3				1.0	2.9	2.0	0.4	0.6	1.6

A. L. Van Eenennaam, and J. F. Medrano. 2008. Manipulation of Milk Fat Composition Through Transgenesis, pages 343-354 in "*Bioactive Components in Milk*", Edited by Z. Bösze. Springer. 483 pp.

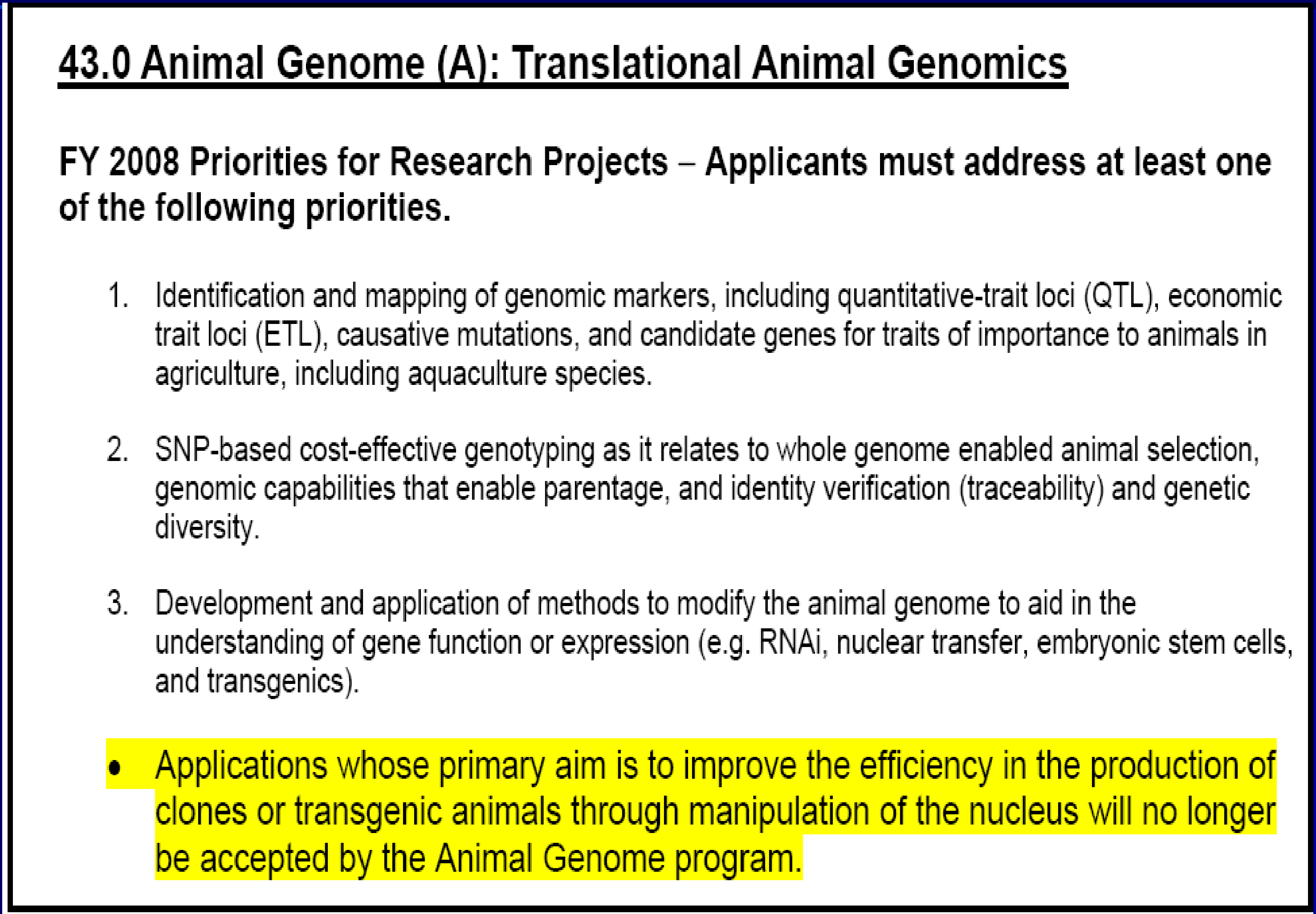
Future plans

- Fat-2 agalactic phenotype follow up
- Reproductive difficulties observed in Fat-1 mice (Bill Pohlmeier, MS)
- Continue trying to clone the echidna LPAAT gene
- Alterations in membrane composition and function with calorie restriction. NIH RO1 10/1/07 - 9/30/12 PI: Jon Ramsey
- Utilization of natural genomic variation to enhance nutritional and health values of beef. Pfizer. PI: Jim Reecy, Iowa State

Reviewer comment on USDA omega-3 mouse grant application

"Given the "pure and wholesome" public perception of milk products, it may be particularly difficult to gain wide spread public acceptance for transgenic milk products – despite their health benefits. Thus, while the technology could open new markets for the American dairy industry it could close even more."

2008 USDA NRI Animal Genome no longer funds transgenic animals



43.0 Animal Genome (A): Translational Animal Genomics

FY 2008 Priorities for Research Projects – Applicants must address at least one of the following priorities.

1. Identification and mapping of genomic markers, including quantitative-trait loci (QTL), economic trait loci (ETL), causative mutations, and candidate genes for traits of importance to animals in agriculture, including aquaculture species.

2. SNP-based cost-effective genotyping as it relates to whole genome enabled animal selection, genomic capabilities that enable parentage, and identity verification (traceability) and genetic diversity.

3. Development and application of methods to modify the animal genome to aid in the understanding of gene function or expression (e.g. RNAi, nuclear transfer, embryonic stem cells, and transgenics).

- Applications whose primary aim is to improve the efficiency in the production of clones or transgenic animals through manipulation of the nucleus will no longer be accepted by the Animal Genome program.

Questions ?

GE Chocolate ice cream ?