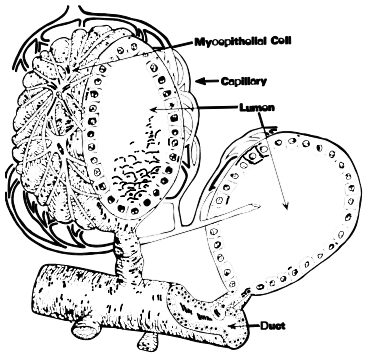


Alveolus – functional milk synthesizing unit

Former World Record Holder

Muranda Oscar Lucinda

67,914 pounds in 365 days
3.6% fat, 3.1% protein
averaged 186 pounds/day
November, 1997

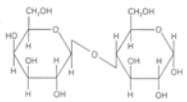
New WORLD RECORD
Hartje-Meyer 9792
76,064 pounds in 365 days
3.2% fat, 2.9% protein
peak at 276 pounds/day
averaged 208 pounds/day

Milk Synthesis Primer

- Protein (3.0%)
  - From amino acids
- Fat (3.5%)
  - Fatty acids from acetate and butyrate
  - Long chain fatty acids from diet/body stores
- Lactose (4.7%)
  - From glucose (world record holder produced 9.8 pounds of lactose per day)

Milk Lactose Synthesis

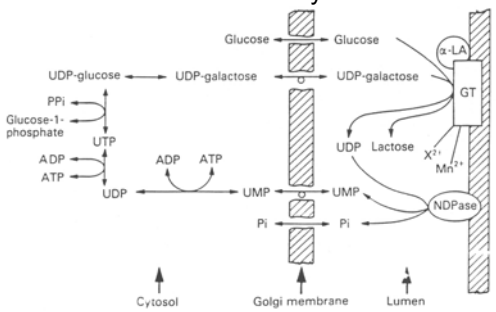
- Lactose (disaccharide of glucose and galactose) is ultimately formed entirely from glucose
  - Over 60% of whole body glucose use in the lactating dairy cow is for lactose synthesis



Milk Lactose Synthesis

- Mammary specific genes turn on when milk synthesis is active
  - α-lactalbumin is key regulatory component of lactose synthase enzyme
    - Synthesis turned on by prolactin
    - Inhibited by progesterone

Lactose Biosynthesis



α-LA = α-lactalbumin
GT = galactosyltransferase
NDPase = nucleoside diphosphatase

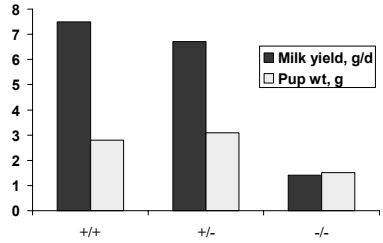
α-lactalbumin Deficiency in Mice

Variable	+/+	+/-	-/-
Lactose %	6.2	4.3	0.01
Fat %	28.2	29.6	45.3
Protein %	8.8	9.6	16.5
Mammary wt g/pup	0.34	0.40	0.35

+ is wild-type, contains α-lactalbumin gene
- is α-lactalbumin gene knockout

Stacey et al. (1995) PNAS 92:2835

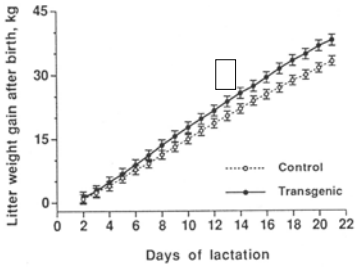
α-lactalbumin Deficiency in Mice



+ is wild-type, contains α-lactalbumin gene
- is α-lactalbumin gene knockout

Stacey et al. (1995) PNAS 92:2835

α-lactalbumin Deficiency in Pigs



Noble et al. (2002) J Anim Sci 80:1090

Low Lactose Milk in Mice

- Produce low lactose milk without problem of milk removal
- Intestinal lactase secreted into milk
- Action in alveoli and ducts
- 50-85% reduction in lactose concentration 4 to 8 hours after last milk removal

Jost et al. (1999) Nature Biotechnol 17:160

Milk Lactose Concentration

- Lactose is the major osmoregulator of milk volume
  - if you produce more lactose, more water comes into the lumen of the alveoli keeping lactose concentration fairly constant

Milk Lactose Concentration

- Least variable component
- High lactose concentration
  - have never heard of this situation
  - will not occur unless removing water after harvest
- Low lactose concentration
  - involution
  - recovery from milk stasis (illness)
  - added water

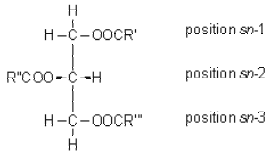
Lipids in Bovine Milk

Class	% of total lipid
Triglyceride	95.80
1,2-diglyceride	2.25
Phospholipids	1.11
Cholesterol	0.46
Free fatty acids	0.28
Monoglyceride	0.08

Adapted from Jensen and Newburg, 1995

Milk Triglyceride

- Main form is triacylglyceride
  - 3 fatty acids esterified to a glycerol molecule



Major Fatty Acids of Bovine Milk

- Saturated fatty acids (65%)
  - No double bonds
  - Related to some health concerns
- Monounsaturated fatty acids (25%)
  - One double bond
  - Thought to be generally healthy
- Polyunsaturated fatty acids (10%)
  - Greater than one double bond

Saturated Fatty Acids (wt%)

4:0	Butyric acid	4.5
6:0	Hexanoic acid	2.3
8:0	Octanoic acid	1.3
10:0	Decanoic acid	2.7
12:0	Lauric acid	3.0
14:0	Myristic acid	10.6
16:0	Palmitic acid	28.2
18:0	Stearic acid	12.6

Monounsaturated Fatty Acids (wt%)

16:1	Palmitoleic acid	1.6
18:1	Oleic acid	21.4

Polyunsaturated Fatty Acids (wt%)

18:2	Linoleic acid	2.9
18:3	Linolenic acid	0.3

Milk Triglyceride Synthesis

- Glycerol backbone can be made from glucose or by using circulating glycerol
- The fatty acids are synthesized in the mammary gland ($\leq$ 16 carbons; *de novo*) and used from the diet or body reserves ($\geq$ 16 carbons; *preformed*)

Milk Fatty Acid Synthesis

- De novo synthesis (within the mammary gland) utilizes acetate as the main carbon source (β-hydroxybutyrate can also serve as the initial 4 carbons)
- Fatty acids are built 2 carbons at a time
  - Limit is 16 carbons
- Key regulatory enzymes are
  - Acetyl CoA carboxylase
  - Fatty acid synthase

Milk Fatty Acid Synthesis

Acetyl CoA carboxylase

$$\text{Acetyl-CoA} + HCO_3^- + \text{ATP} \longrightarrow \text{malonyl-CoA} + \text{ADP} + \text{Pi}$$

Milk Fatty Acid Synthesis

Fatty acid synthase

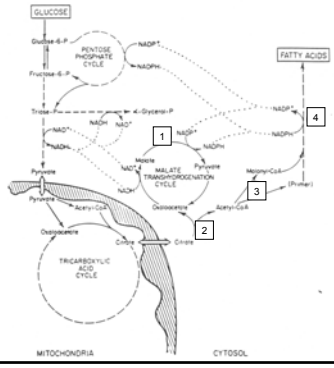
$$\text{Acetyl-CoA} + 7\ \text{malonyl-CoA} + 14\ (\text{NADPH} + H^+) \longrightarrow$$

$$\text{Palmitic acid (16 carbons)} + 7\ CO_2 + 14\ \text{NADP} + 8\ \text{CoA} + 6\ H_2O$$

Sources of Acetyl-CoA

- Acetate and β-hydroxybutyrate are primers
  - acetyl-CoA and butyryl-CoA synthase
- Addition of malonyl-CoA all from acetate
- Glucose does not contribute to carbons of fatty acids in ruminants
  - lack citrate lyase

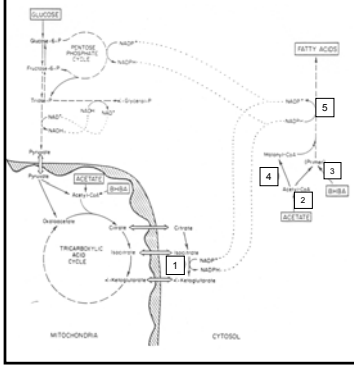
Milk Fat Synthesis in Nonruminants



1. NADP malate dehydrogenase
2. Citrate lyase
3. Acetyl-CoA carboxylase
4. Fatty acid synthase

Bauman & Davis, 1974

Milk Fat Synthesis in Ruminants



1. NADP isocitrate dehydrogenase
2. Acetyl-CoA synthase
3. Butyryl-CoA synthase
4. Acetyl-CoA carboxylase
5. Fatty acid synthase

Bauman & Davis, 1974

Preformed Fatty Acids: Triglycerides

Lipoprotein lipase (LPL)

Triglyceride ⟶ 3 free fatty acids + glycerol

- Action is on the luminal side of the vascular endothelium
- Acts on lipoproteins
  - hydrolysis of TG in VLDL and chylomicrons
- Fatty acids bound by fatty acid binding proteins (FABP) within secretory tissue
- Activated to fatty acyl-CoA

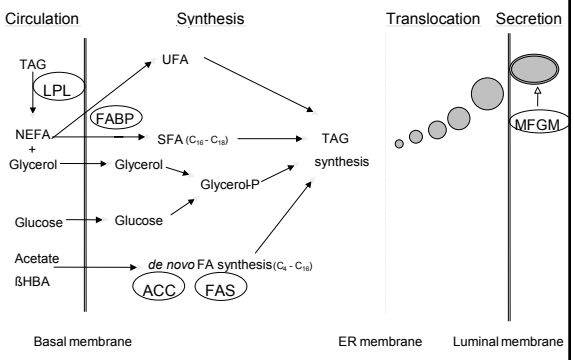
Preformed Fatty Acids: NEFA

- Released from adipose by hormone-sensitive lipase during periods of energy shortage
- Travel in blood via albumin
- Taken up by mammary gland when concentrations are >300 μM
  - only significant during first month of lactation
- Activated to fatty acyl-CoA

Desaturation of Fatty Acids

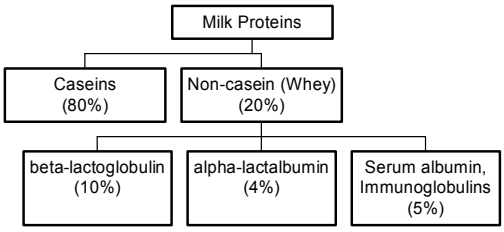
- Bovine mammary gland actively inserts a double bond
  - stearic acid (18:0) to oleic acid (18:1 n-9)
  - palmitic acid (16:0) to palmitoleic acid (16:1 n-7)
  - myristic acid (14:0) to myristoleic acid (14:1 n-5)
  - vaccenic acid (18:1 t11) to conjugated linoleic acid (18:2 c9t11)
- Thought to be important for fluidity of milk fat

Milk Fat Synthesis



Milk Protein Synthesis

- Just like any other protein in the body
  - Under the direction of gene transcription (DNA to mRNA) and translation (mRNA to protein)
- Mammary specific genes turn on when milk synthesis is active
  - α-lactalbumin, caseins
- Need amino acids and energy (5 ATP per peptide bond)

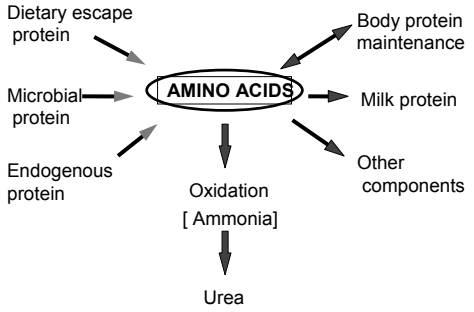


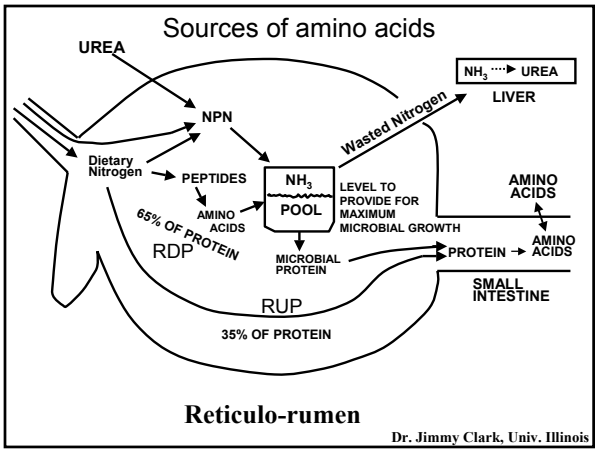
Composition of Milk Proteins

- Caseins (80% of milk proteins)
  - phosphoproteins precipitated at pH 4.6 or by rennin
    - α_{s1} 38% of total casein
    - α_{s2} 10% of total casein
    - β 36% of total casein
    - κ 13% of total casein
    - γ 3% of total casein

Composition of Milk Proteins

- Whey proteins (20% of milk proteins)
  - proteins not precipitated at pH 4.6 or by rennin
    - β-lactoglobulin
    - α-lactalbumin
    - Immunoglobulins (enriched in colostrum)
    - Others
      - Serum albumin, hormones, enzymes





Balance of C and N Across the Mammary Gland

Parameter	Amino acid C	Amino acid N
Uptake	147	46
Output	155	42
Ratio	95	110

Clark et al. (1978) Fed Proc 37:1233

Balance of C and N Across the Mammary Gland

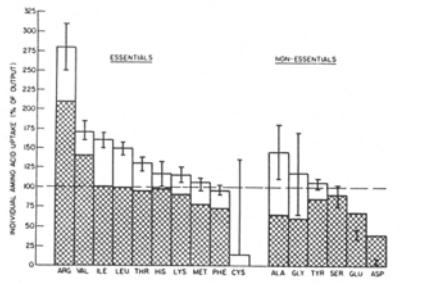
	Amino acid C		Amino acid N	
Parameter	Essential	Non	Essential	Non
Uptake	103	44	33	13
Output	87	68	25	17
Ratio	118	65	131	78

Clark et al. (1978) Fed Proc 37:1233

Amino Acid Metabolism

- Branched chain amino acids (leucine, isoleucine and valine) and arginine taken up in excess of their output in milk
  - Converted to non-essential amino acids in mammary gland

Amino Acid Metabolism



Clark et al. (1978) Fed Proc 37:1233

Amino Acid Uptake by Mammary Gland

- Variety of specific amino acid transporters
- Regulation is poorly understood