Partitioning of nutrients to the mammary gland:

Regulation of nutrient uptake and effect of stage of lactation

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Quality Goat Milk for Cheese Production
Meeting 31.05.-1.06.2010
Department of Animal and Aquacultural Sciences
Norwegian University of Life Sciences
The mammary gland: 
A milk factory and parasite

In dairy cows: can produce >80 kg milk/day 
Requires bloodflow ~ 30 tons/day 
In early lactation:
  • Up to 85-90% of total body glucose consumption 
  • Up to 75% of total amino acid consumption 
Can induce negative energy balance in the cow 
Requires efficient mechanisms for adaptation of metabolism with onset of lactation

*Reason:* mammary gland not subject to same regulation as other body tissues.
Determinants of milk production

- Synthetic capacity of the mammary gland
  - Number of mammary epithelial cells (MEC)
    → proliferation / apoptosis
  - Synthetic activity of MEC
    → age / differentiation

- Utilisation of this capacity
  - Provision of nutrients in arterial blood (and removal of metabolic waste products)
    → Mammary blood flow (vascular system)
    → Nutrient concentrations (feeding, energy balance)
  - Transport from capillaries into MEC
    → diffusion
    → transport proteins in cell membrane
Issues to be addressed:

1. Uptake of nutrients and synthesis of milk components in the mammary gland:
   ➔ relation to changes in supply of nutrients to the mammary gland?

2. Sensitivity towards variation in nutrient supply:
   ➔ altered by cellular and morphological changes in the mammary gland during lactation?

_Ie. control of milk synthesis and composition at the level of the mammary gland (in the goat)_
Experimental methodologies

Key in vivo techniques
  • Arterio-venous difference (AVD)
  • Blood flow
  • Biopsy sampling
  • Intravascular infusions

To understand:
  • Regulation of mammary nutrient uptake
  • Regulation of capacity for milk synthesis
  • Efficiency of conversion – absorbed nutrients -> milk
AV difference (AVD) sampling

Exteriorized carotid artery

Milk vein loop

AVD = arterial - venous concentration difference
E = extraction = AVD / arterial concentration

Sampling + manual clamp of pudic vein
Mammary uptake<->milk: Systemic IV infusion

Goats: Keep catheters out of reach of goat !!!!
Mammary Biopsy

Frozen in Liquid Nitrogen

Homogenization, Dilution, Purification, Reverse-Transcription & Amplification

Gene Expression (Real Time RT-PCR)

Fixed in 4% PFA

Process of Fixation & Staining

Histological + Immunohistochemical Studies
Mammary morphology + cell turnover

**Histology:** Mammary morphology (H&E and PAS)

**Immunohistochemistry:** MEC turnover (TUNEL and Ki-67)

Alveoli#, MEC (+ differentiation), lumen, stroma (PAS: vessels)

MEC: #, proliferative / apoptotic + differentiation
Differentiation of epithelial cells (MEC)

Poorly differentiated

Intermediately differentiated

Fully differentiated
Gene expression

**Angiogenic**
- VEGF, VEGFR1 & -R2
- ANGPT1 & -2 and RTK
- COX1 & -2
- TBXAS

**Vascular function**
- COX1 and COX2
- TBXAS
- CA4
- PTGIS

**Cellular activity**
- PRLR
- LALBA
- LTF
- LPT & LPTR

**Pro-apoptotic / anti-proliferative:**
- BAX
- IGFBP3 & IGFBP5
- TGFB1, TGFB1R1 & R2
- LALBA
- LPT & LPTR

**Proliferative / anti-apoptotic:**
- BCL2
- CCND1
- IGF1 & IGF1R
- PRLR
Mammary nutrient uptake

- Mammary nutrient supply
- Milk synthesis
- Stage of lactation
Lactose synthesis: Glucose

GLUCOSE → \text{hexokinase} → \text{ATP} \uparrow \text{ADP} → \text{Glucose-6-phosphate} → \text{Phosphoglucomutase} → \text{Glucose-1-phosphate} → \text{UDP-glucose-4-epimerase} → \text{UDP-glucose} → \text{Pyrophosphorylase} → \text{UDP-galactose} → \text{Galactosyltransferase} → \text{LACTOSE}

\text{Energy consumption: 3 ATP}

Lactose synthetase (rate limiting)
Mammary nutrient uptake: Glucose

Arterial glucose supply: Impacts glucose uptake in early lactation only

Mammary nutrient uptake: Glucose

Systemic bolus injection of propionate (acute)

Glucose

*Venous

Arterial

AVD

Insulin

Mammary nutrient uptake: Glucose

- Efficiency of uptake follows lactation curve
- Not related to arterial glucose or insulin
- I.e. carrier operating close to V_max (GLUT1)
- Pull effect

Protein synthesis: Amino acids

Energy consumption: 4 ATP per peptide bond

**Figure 4.32** Schematic representation of ribosomes attached to mRNA showing the growing polypeptides and a proposed mechanism for cotranslational crossing of the RER membrane (from Mercier and Gaye, 1983).
Mammary nutrient uptake: Amino acids

Jugular concentration $\sim$ Arterial concentration
Jugular-milk vein difference $\sim$ AVD

Mammary nutrient uptake: Lysine and Methionine

Lysine – early lactation
\[ y = 0.17x + 56 \]
\[ R^2 = 0.29, P=0.15 \]

Lysine – late lactation
\[ y = 0.78x - 96 \]
\[ R^2 = 0.59, P=0.03 \]

Methionine – early lactation
\[ y = 0.51x - 7 \]
\[ R^2 = 0.39, P=0.02 \]

Methionine – late lactation
\[ y = 1.29x - 66 \]
\[ R^2 = 0.89, P<0.01 \]

Uptake with supplementation:
- Linear related to arterial supply
- Sensitivity lowest in early lactation

## Mammary nutrient uptake: Amino acids

<table>
<thead>
<tr>
<th></th>
<th>Stage of lactation</th>
<th>SEM</th>
<th>P&lt;sub&gt;stage&lt;/sub&gt;</th>
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<tbody>
<tr>
<td></td>
<td>35 DIM</td>
<td>285 DIM</td>
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<tr>
<td>Milk yield (g/day)</td>
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<td>94</td>
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<tr>
<td>MPF (L/h)</td>
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<td>28</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>Arterial (mM)</td>
<td>3.40</td>
<td>2.75</td>
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<td>AVD (mM)</td>
<td>1.55</td>
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<td>0.10</td>
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<tr>
<td>Extraction (%)</td>
<td>46</td>
<td>13</td>
<td>4</td>
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<td>EAA</td>
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<tr>
<td>Arterial (mM)</td>
<td>1.61</td>
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<td>AVD (mM)</td>
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<td>Extraction (%)</td>
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<td>4</td>
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<tr>
<td>NEAA</td>
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<tr>
<td>Arterial (mM)</td>
<td>1.59</td>
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<td>AVD (mM)</td>
<td>0.30</td>
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<td>0.05</td>
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<tr>
<td>Extraction (%)</td>
<td>18</td>
<td>10</td>
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Intravascular infusions of EAA, glucose and acetate to dairy goats in early and late lactation fed an energy and protein (absorbed amino acids) restricted diet

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
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<th>P-value</th>
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<tr>
<td></td>
<td>EAA</td>
<td>ACE</td>
<td>GLU</td>
<td>SAL</td>
<td>SEM</td>
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<tr>
<td>EL</td>
<td>4821*</td>
<td>4765*</td>
<td>547</td>
<td>547</td>
<td>0.06</td>
<td></td>
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<tr>
<td>LL</td>
<td>2888</td>
<td>273*</td>
<td>106*</td>
<td>106*</td>
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<tr>
<td></td>
<td>5188</td>
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<td>9*</td>
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<td>3547*</td>
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<td>Protein (%)</td>
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<tr>
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<tr>
<td>LL</td>
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<td></td>
<td>0.14</td>
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<tr>
<td>Protein yield (g/last 48h)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EL</td>
<td>166*</td>
<td>163*</td>
<td>157*</td>
<td>139*</td>
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<tr>
<td>LL</td>
<td>114*</td>
<td>100*</td>
<td>98*</td>
<td>106*</td>
<td>14</td>
<td>0.09</td>
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<tr>
<td>Fat (%)</td>
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<td></td>
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<tr>
<td>EL</td>
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<td>Fat yield (g/last 48h)</td>
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<tr>
<td>EL</td>
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<td>272</td>
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<tr>
<td>LL</td>
<td>160*</td>
<td>130*</td>
<td>108*</td>
<td>130*</td>
<td>31</td>
<td>0.04</td>
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</tbody>
</table>


Safayi S (2009). PhD thesis. Faculty of Life Sciences, University of Copenhagen, Denmark
Milk yield and its fat and protein contents in early and late lactating dairy goats fed a restricted diet supplemented by intravascular infusion of nutrients.

<table>
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<tr>
<th>Treatment</th>
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<th>ACE (g/kg)</th>
<th>GLU (g/kg)</th>
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</thead>
<tbody>
<tr>
<td>EL</td>
<td>5761</td>
<td>5393</td>
<td>4765</td>
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<tr>
<td>LL</td>
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<td>2772</td>
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<tr>
<td>Protein (%)</td>
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<tr>
<td>EL</td>
<td>3.48</td>
<td>3.46</td>
<td>3.38</td>
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<tr>
<td>LL</td>
<td>3.98</td>
<td>3.76</td>
<td>3.52</td>
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<td>Protein yield (g/last 48h)</td>
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<tr>
<td>EL</td>
<td>166</td>
<td>163</td>
<td>172</td>
</tr>
<tr>
<td>LL</td>
<td>114</td>
<td>98</td>
<td>100</td>
</tr>
</tbody>
</table>

Confirmed.: Minor differences in dietary provision of protein can be compensated by provision of energy (ATP) yielding substrates to sustain milk (protein) synthesis in EL but not in LL!

Abundance of AA transporters in MEC membrane?

Sensitivity towards variations in AA supply: EL < LL

Higher levels of AA in blood circulation required in LL to drive mammary uptake?

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Milk yield and its fat and protein contents in early and late lactating dairy goats fed a restricted diet supplemented by intravascular infusion of nutrients

<table>
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<th>GLU (g/day)</th>
<th>SAL (g/day)</th>
<th>SEM (g/day)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>EL</td>
<td>4821&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4765&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4237&lt;sup&gt;b&lt;/sup&gt;</td>
<td>547</td>
<td>0.06</td>
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<tr>
<td>LL</td>
<td>2888</td>
<td>2732</td>
<td>2787</td>
<td>2861</td>
<td>606</td>
<td>0.71</td>
</tr>
</tbody>
</table>

ACE (ATP) ↔ GLU (NADPH) → necessity of providing ATP

Extra-mammary tissue consumption + ↑ lactose synthesis → dilution effect

Safayi S (2009). PhD thesis. Faculty of Life Sciences, University of Copenhagen, Denmark
Milk fat synthesis

**Intracellular**

- Glucose
- Glycerol-P
- Acetyl-CoA
- FA-CoA
- Acetate (β-OH-butyrate)
- Acetate (3-OH-butyrate)
- NEFA
- Lipoproteins

**Blood**

- Glucose
- Acetate (3-OH-butyrate)
- NEFA
- FA
- Lipoproteins

- **FA** = Long chain fatty acid
- **FA-CoA** = Fatty acid – coenzyme A ester (activated form)
- **Rate limiting:** Acetyl-CoA carboxylase
- **FA-synthetase**
- **Lipoprotein lipase**

**Not ruminants**

**Ruminants**
De novo fatty acid synthesis

ATP citrate lyase
Very low activity in ruminants
Mammary nutrient uptake: Acetate and BOHB

- Uptake very closely linked to supply
- I.e. determined by concentration gradients

⇒ Same pattern in early and late lactation

Madsen et al. (2005). Small ruminant Res. 56. 151-164
Arterial NEFA and AVD during lactation

Mammary nutrient uptake: NEFA

0.2-0.3 mM
Mammary nutrient uptake: Triglycerid

Normal plasma concentration: ~20-30-mg/100 ml

Lipoprotein lipase
Conclusion - 1

1. Nutrient uptake in the mammary gland:
   1. is highly related to arterial supply for acetate, 3-OH-B and NEFA and amino acids
   2. but not for glucose
2. Mammary nutrient uptake is affected by mammary synthetic activity
   1. Efficiency of transmembrane transport mechanisms
      1. Glucose !!
      2. Amino acids ?
3. Increasing mammary glucose uptake increases secretion of milk and changes milk composition
   • Particularly in early lactation
   • But systemic provision of glucose alters distribution in disfavour of mammary gland
4. Mammary gland is less sensitive to deficiencies in amino acid supply in early compared to late lactation
   1. In early (but not late) lactation: amino acid deficiency can be partly compensated by provision of energy in the form of acetate (ATP)
      1. Provided enough energy (ATP ~ acetate)
   2. Scope for differential protein recommendations?
Mammary synthetic capacity

- Pregnancy - lactation cycle
  - MEC <-> microvascular system

- Two examples:
  - Parity
  - Omission of dry period (continuous lactation)
Mammary capillary : alveolar ratio

Early lactation:
- Large alveoli
- Many small capillaries
- High CA activity (staining)
- Efficient nutrient extraction

Late lactation:
- Small alveoli (~75%)
- Few (↓61%) and large capillaries (3x)
- Lower CA activity (less black staining)
- Less efficient nutrient extraction

Mammary remodelling: Parity

Multiparous (MP)

Primiparous (PP)

Mammary glands of PP vs. MP goats:
- less developed at parturition
- more differentiated throughout lactation

Safayi et al 2010 J Dairy Sci 93 1478-1490
Angiogenesis

Safayi et al 2010 J Dairy Sci 93 1478-1490

Cellular activity
Vascular function

Proliferation

Cellular activity (LPT, LPTR)

Safayi et al 2010 J Dairy Sci 93 1478-1490
Mammary remodelling and parity

Postpartum changes in PP compared to MP animals:
1. Mammary growth and lactogenesis continues further into lactation → MEC as well as mammary vasculature
2. MEC survival appears to be higher during lactation
   - higher rate of cell proliferation (IGF1)
   - lower rate of apoptosis (BCL2)
   - higher rate of differentiation (LPT, LPTR)
   → explains lactation persistency
3. More vascular development during lactation
   → gives better utilization of the synthetic capacity of the MEC?

*Not only MEC but also vascular function is involved in the lactation performance and persistency → deserves to receive more attention*
Continuous lactation in goats

- MEC undergoing proliferation occurred predominantly in the intermediately differentiated rather than in the poorly and fully differentiated MEC (69%, 23% and 9%, respectively).

Safayi et al 2010 J Dairy Sci 93 203-217
Continuous lactation

Lactogenesis after continuous lactation:
- Less cell proliferation -> older (more fully differentiated) cells?
- No differences in milk yield in goats
  - Fully differentiated MEC -> older and higher metabolically active?
- Apoptotic cells only in the intermediately or fully differentiated MEC (not poorly)

None of the studied genomic factors could account for these treatment differences!
Efficiency of mammary nutrient extraction was not affected!
Conclusions - 2

1. Mammary microvascular system undergoes as marked changes during lactation as the MEC’s
   1. Contributes to explain poorer nutrient extraction as lactation advances
2. Primiparous vs multiparous
   1. morphological and cell turnover differences during lactation are related to differences in range of gene expressions
   2. MEC as well as vascular level
3. Continuous lactation vs normal lactation (dry period)
   1. Cellular changes indicate smaller and older cell population at parturition (agrees with cow studies)
   2. Not related to gene expression for any measured genes
   3. But milk yield is not negatively affected in goats (in contrast to cows)

- Omission of the dry period in late gestation interferes with mammary remodelling through regulatory factors that are not identical to the factors responsible for differential mammary remodelling in PP versus MP goats
Puzzling questions?

1. Why is MEC renewal inhibited just because of continuous milk removal from a mammary gland throughout gestation?
   1. When MEC can respond to lactogenic stimuli?
   2. When MEC’s (even fully differentiated) can undergo proliferation?

2. Why is continuous lactation associated with:
   1. a marked depression in milk yield in dairy cows in the subsequent lactation
   2. but apparently not in the dairy goat that (with some difficulty) manage to lactate continuously?
The end !?.....