Biology of Glucose Transport in the Bovine Mammary Gland

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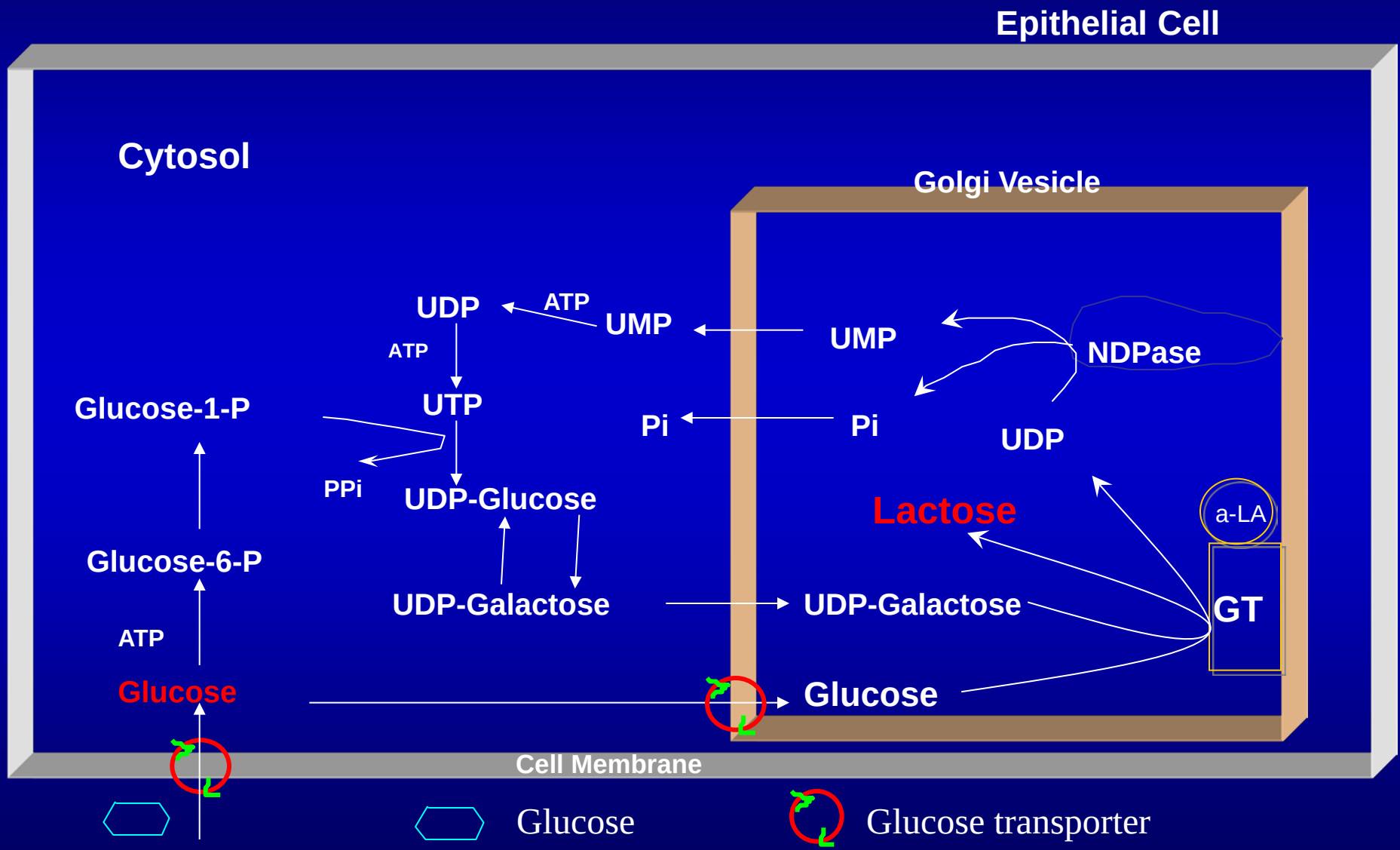
Milk Composition - Holstein

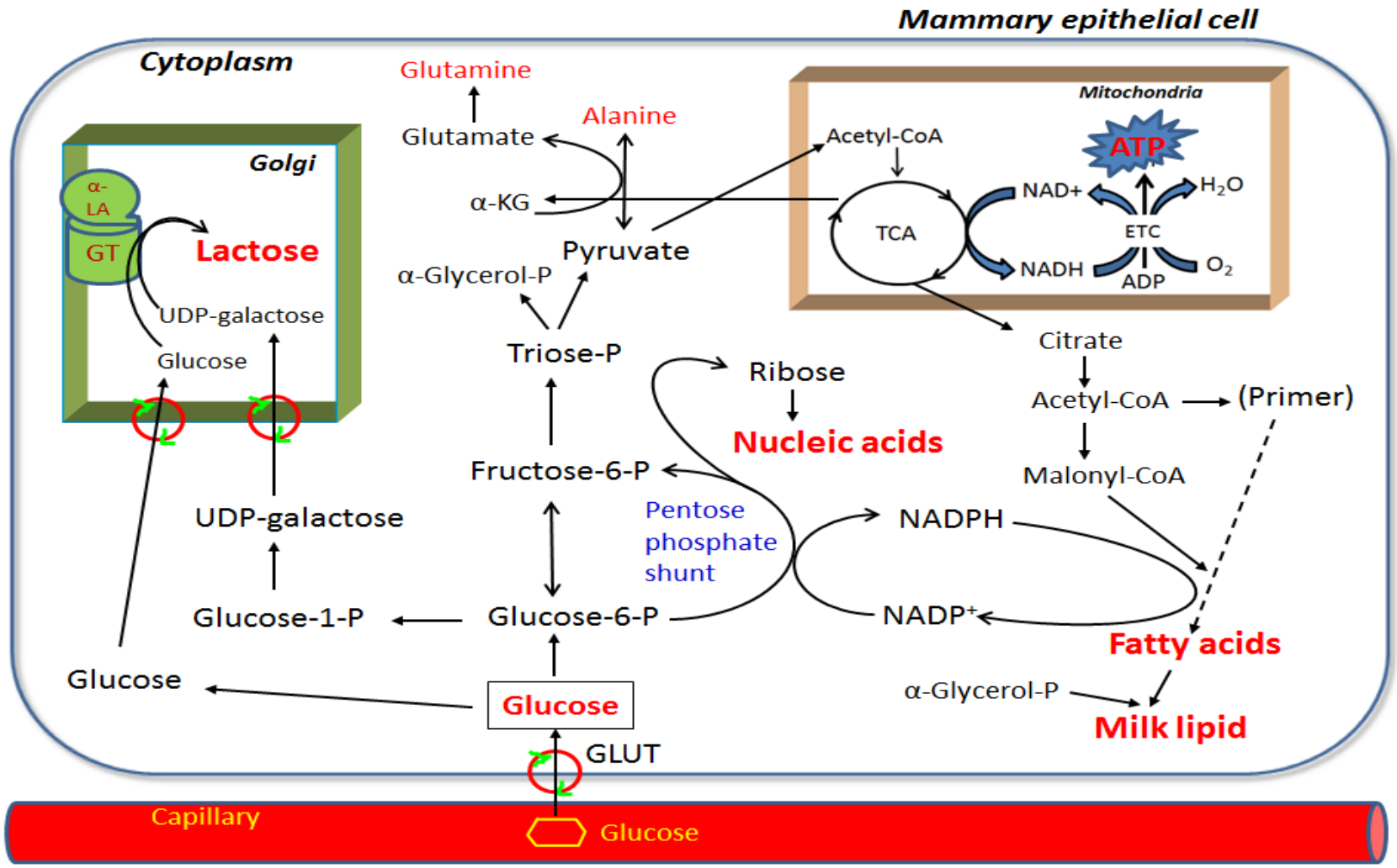
- Water 87.5%
- Total solids 12.0%
  - Fat 3.5%
  - Solids not fat 8.5%
    - Protein 3.0%
    - Lactose 4.8%
    - Ash 0.7%

Lactose Synthesis

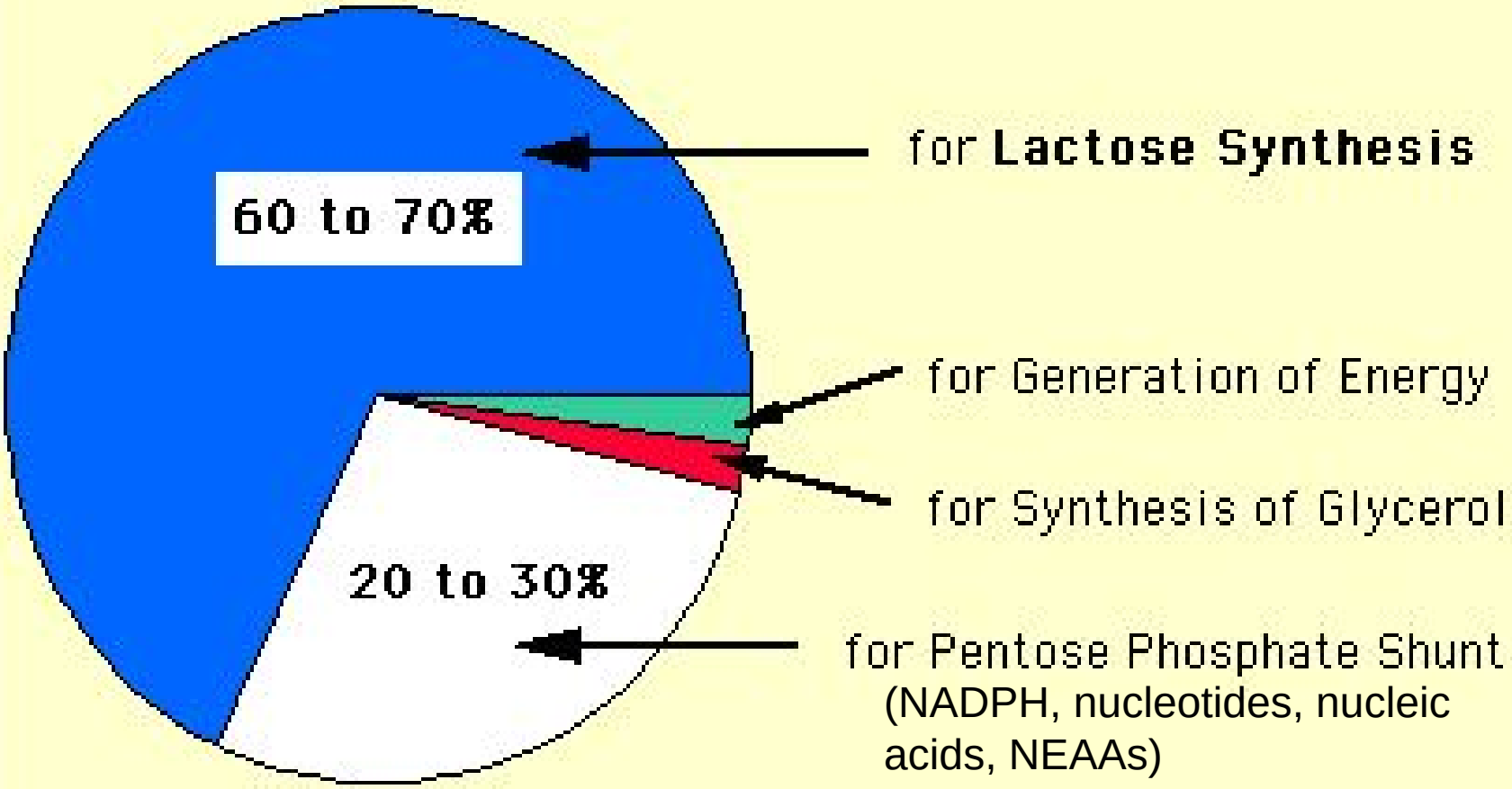


Abbreviations: GT = galactosyltransferase; a-LA = a-lactalbumin; NDPase = nucleotide diphosphatase; Pi = inorganic phosphate; PPi = inorganic diphosphate; UDP = uridine diphosphate; UDP-galactose = Uridine diphosphate galactose; UDP-glucose = uridine diphosphate glucose; UMP = uridine monophosphate; UTP = uridine triphosphate

Main Pathways of Glucose Utilization in Mammary Alveolar Epithelial Cells



Utilization of Glucose in the Bovine Mammary Gland



From: Peggy Neville

Glucose Supply and Milk Production (40 kg/per day)

- For lactose synthesis (70%):

  40 kg x 5% = **2.0** kg
- For other purposes:

  (30%) = **0.8** kg
- Total = **2.8** kg

- 72-76 g of glucose is required to produce 1 kg of milk (Kronfield, 1982)
- Mammary glucose uptake can be 60-85% of the total glucose entering the blood (Chaiyabutr et al., 1980)

Glucose Availability

=

Arterial glucose concentration

+

Mammary blood flow

+

Glucose transport

- There are no clear correlations between arterial glucose concentrations and milk yield (Kim et al., 2001; Cant et al., 2002; Rigout et al., 2002).

- Increasing mammary blood flow is also not necessarily followed by increases in milk production and lactose synthesis (Rigout et al., 2002; Lacasse & Prosser, 2003; Prosser et al., 1994).

Glucose Concentration Gradient across Mammary Epithelial Cells

- There is a steep concentration gradient of glucose across the plasma membrane of mammary epithelial cells.

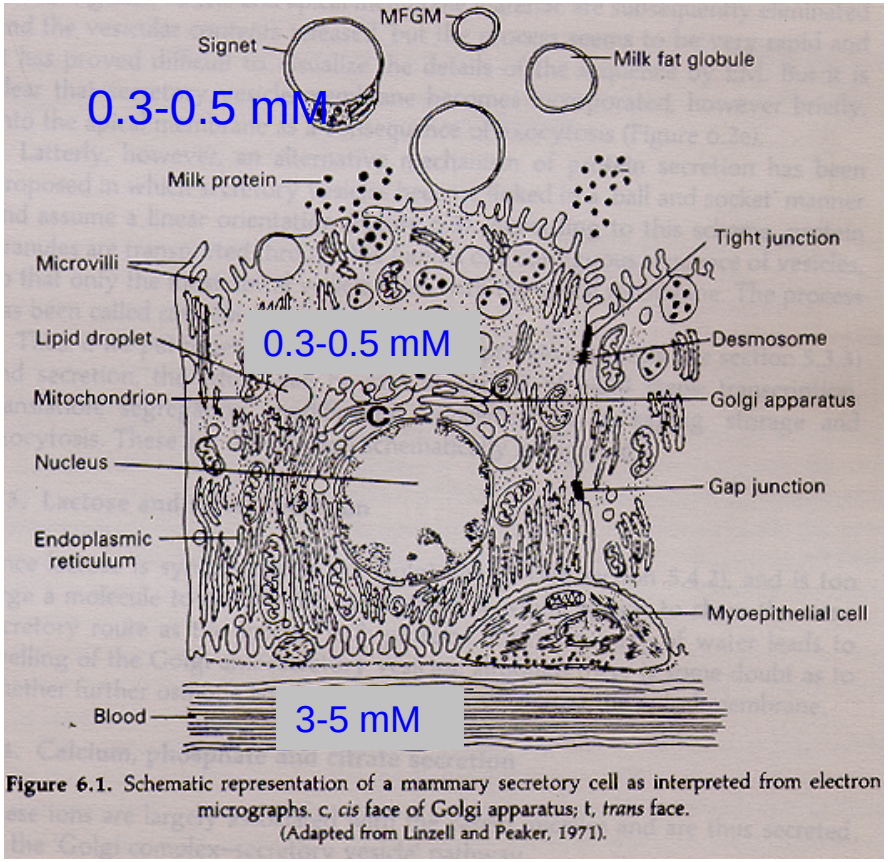


Figure 6.1. Schematic representation of a mammary secretory cell as interpreted from electron micrographs. c, *cis* face of Golgi apparatus; t, *trans* face.
(Adapted from Linzell and Peaker, 1971).

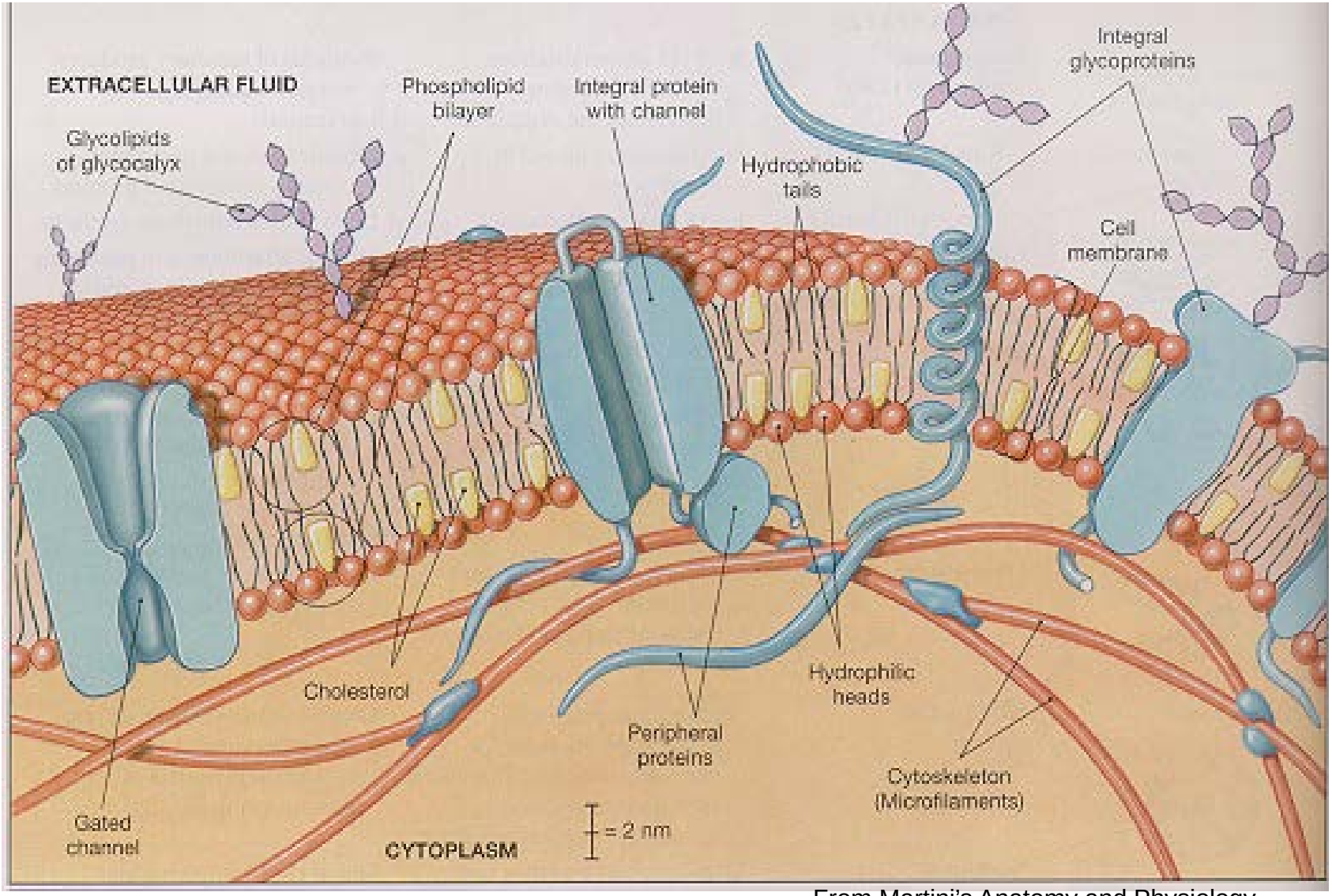
- Intracellular glucose concentration is significantly lower than K_m (3 mM) for lactose synthase (Faulkner and Peaker, 1987).

Take Home Message

Glucose transport in the mammary gland may be a limiting factor of milk production

- Lactose synthesis and milk yield shows a linear or positive correlation with glucose uptake in the mammary gland *(Nielsen and Jackson, 1993; Hurtaud et al., 2000; Kim et al., 2001; Nielsen et al., 2001; Cant et al., 2002; Huhtanen et al., 2002).*

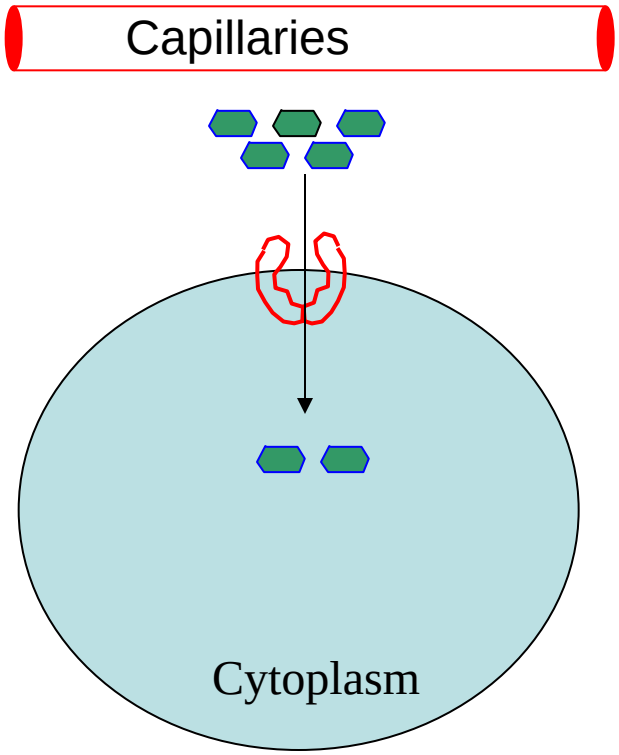
Cell Membrane: Nutrient Exchange Barrier



From Martini's Anatomy and Physiology

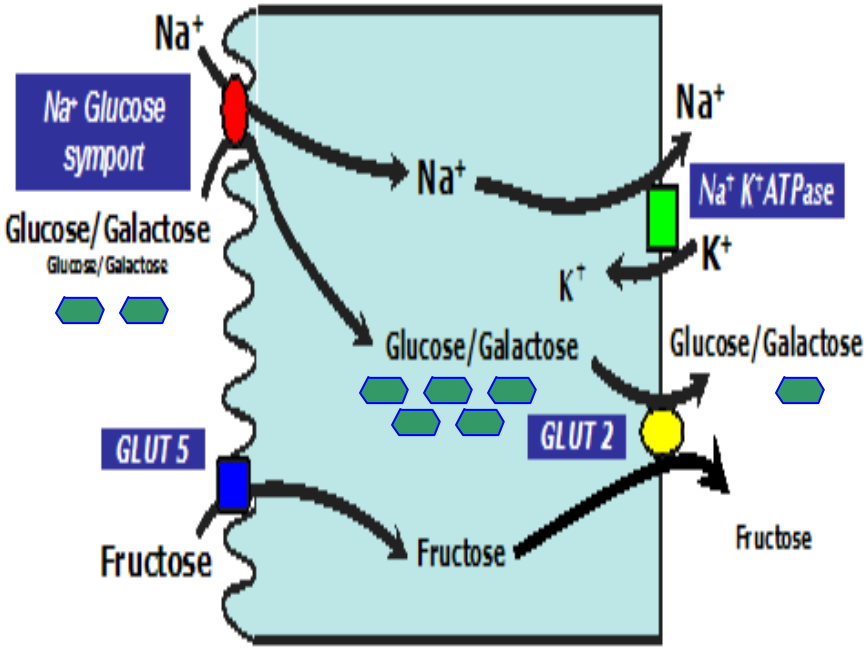
Glucose Uptake by Mammalian Cells

Facilitative diffusion



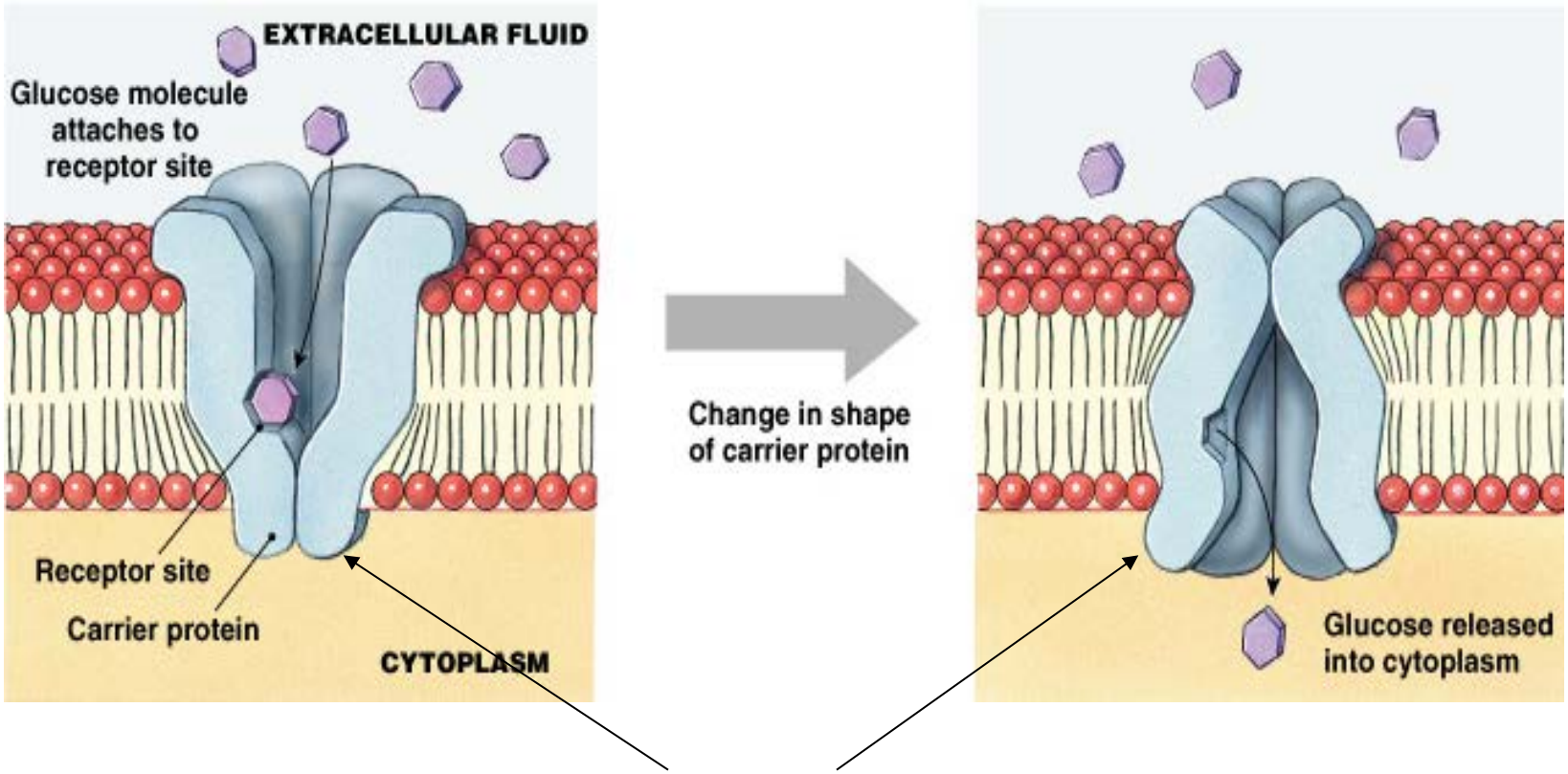
Most cells

Active transport



Small Intestinal epithelium

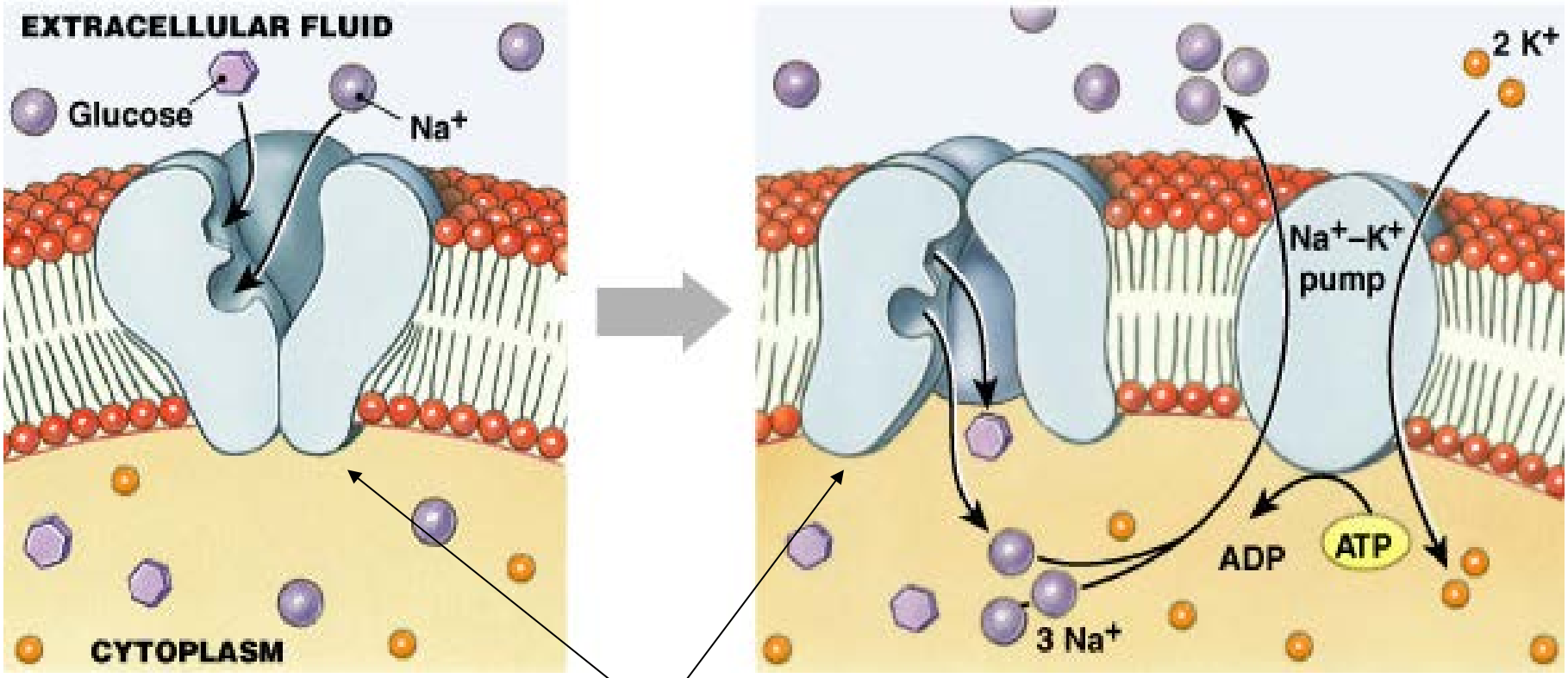
Facilitated Diffusion



Facilitative glucose transporter (GLUT)

From: Martini's Anatomy and Physiology

Secondary Active Transport

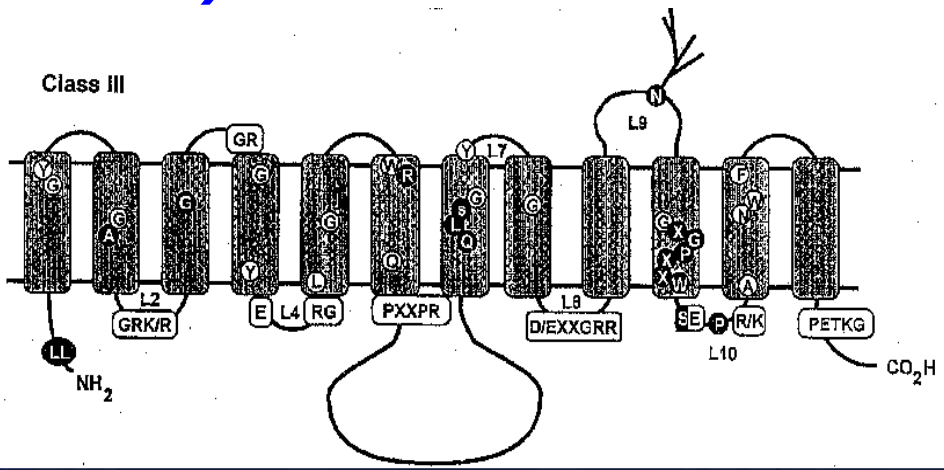


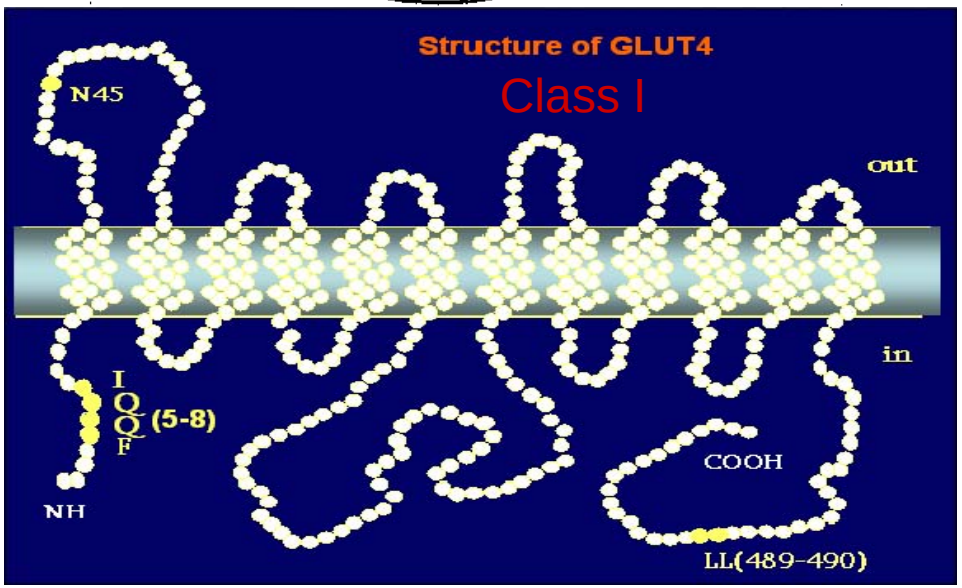
Na^+/glucose cotransporter (SGLT)

From: Martini's Anatomy and Physiology

Facilitative Glucose Transporters (GLUTs)

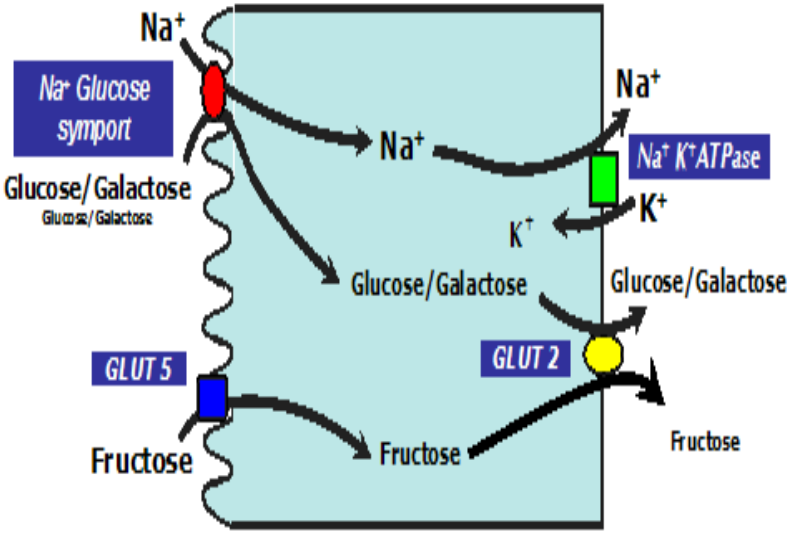
- 14 members (GLUT1-12, 14 & HMIT)
- 12 transmembrane domains
- Sugar transporter signatures
- Glycosylation sites
- Tissue-specific distribution
- Different transport kinetics
- Different regulation properties

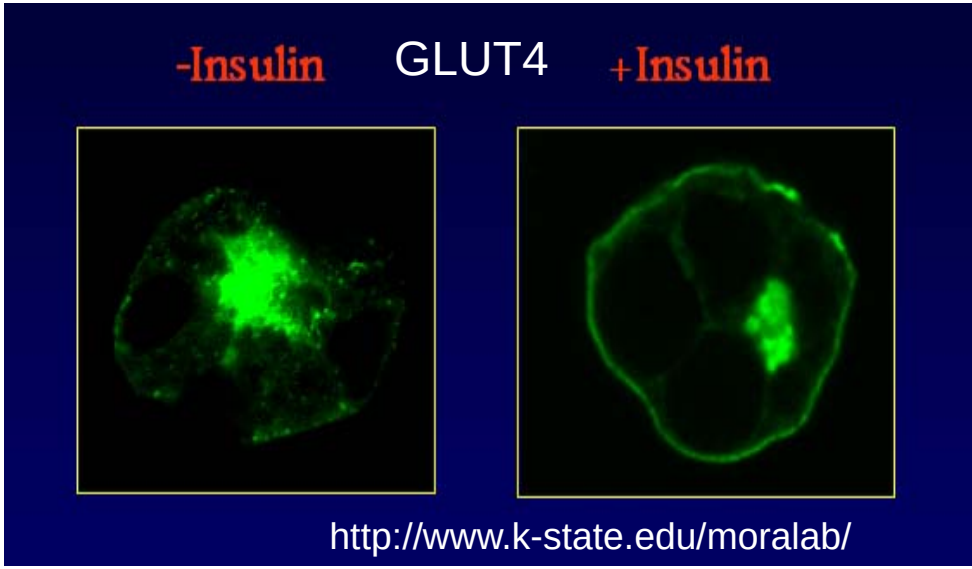




Highlights of GLUTs

- GLUT1:
  - ubiquitous expression
- GLUT2:
  - liver-type, high capacity
- GLUT3:
  - brain-type, high affinity
- GLUT4:
  - muscle and fat-type, insulin sensitive
- GLUT5:
  - fructose transporter
- GLUT8:
  - most abundant in testis, estrogen sensitive
- GLUT12:
  - localize in apical membrane of rat mammary epithelial cells





Glucose Uptake in the Mammary Gland Is Mediated by the Facilitated Transport Process

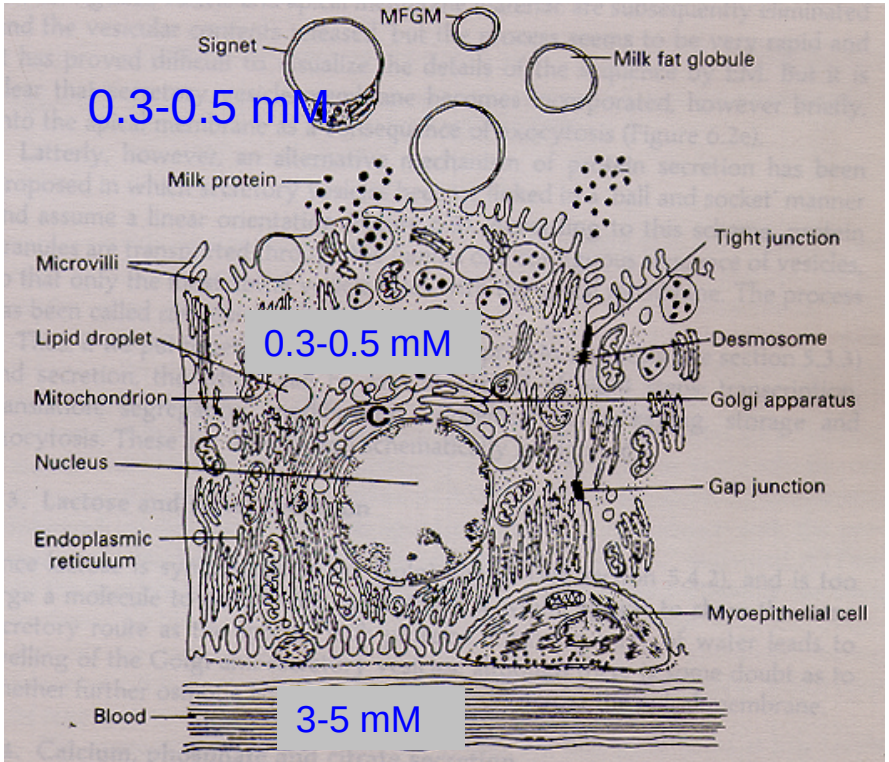
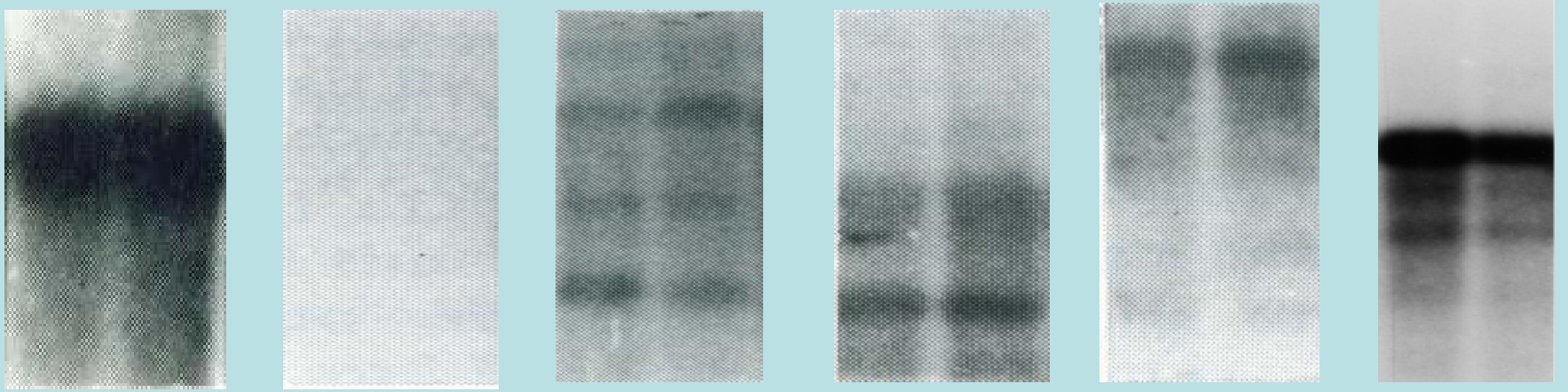


Figure 6.1. Schematic representation of a mammary secretory cell as interpreted from electron micrographs. c, *cis* face of Golgi apparatus; t, *trans* face.
(Adapted from Linzell and Peaker, 1971).

- Glucose transport into mammary epithelial cells is specific, saturable, Na^+-independent, and can be inhibited by cytochalasin-B or phloretin in guinea pig, rat, mouse, and cow.

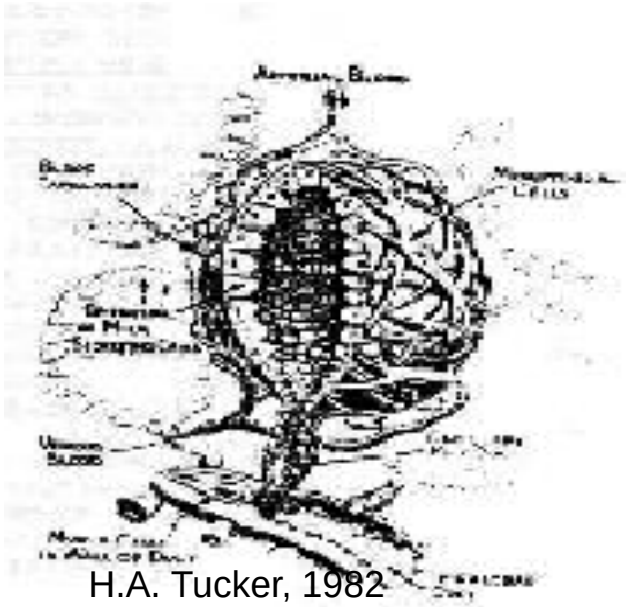
1. Expression of GLUT in Bovine Mammary Gland

- **GLUT1,** GLUT8, GLUT12
- SGLT1&2; GLUT3, 4, and 5

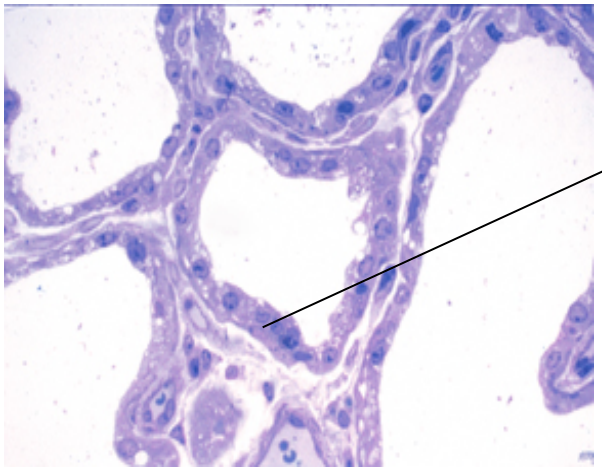


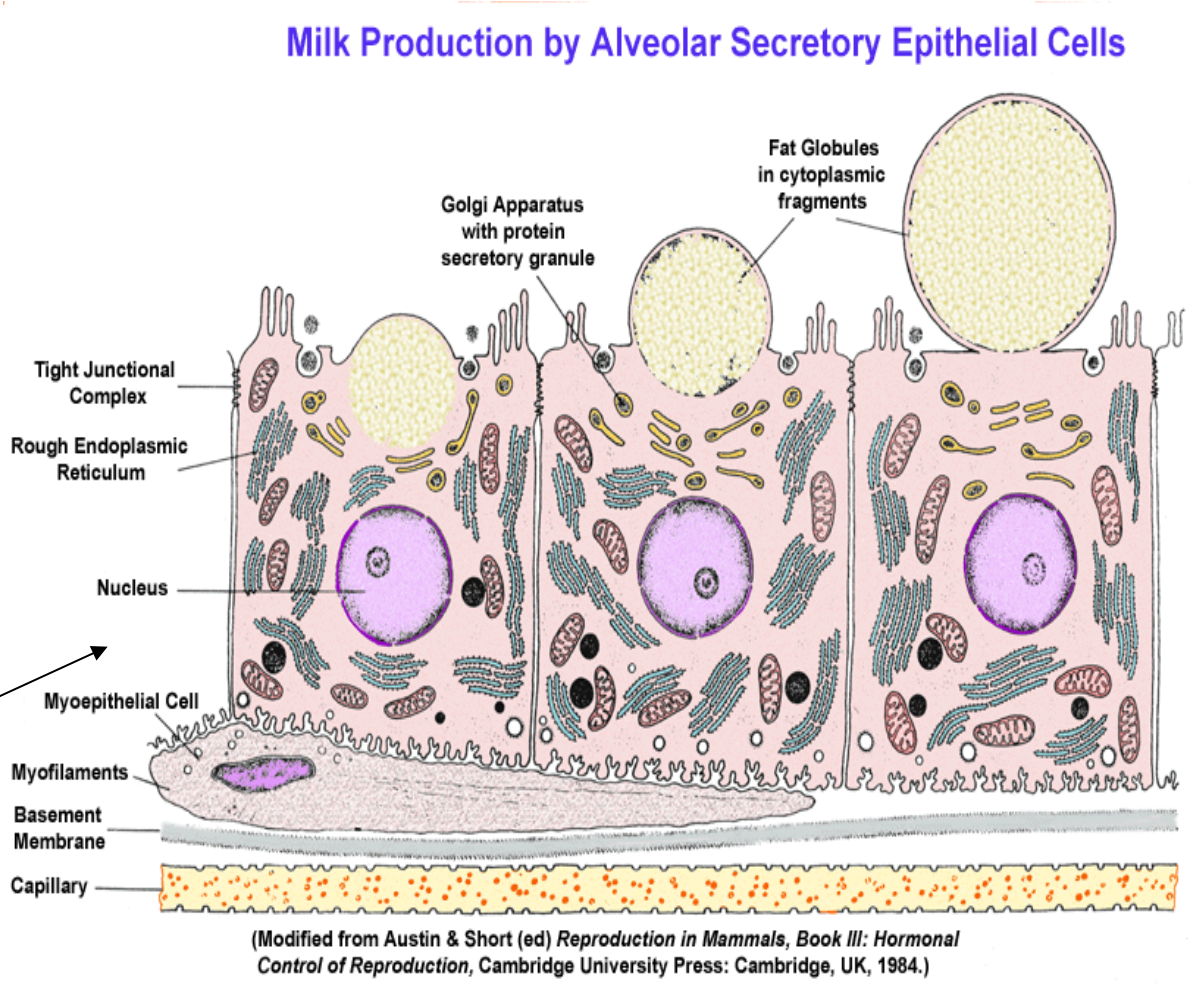
Zhao et al., 1993. Int. J. Biochem.; Zhao et al., 1999, J. Anim. Sci.

2. Cellular and Subcellular Localization of Glucose Transporters in Bovine Mammary Gland



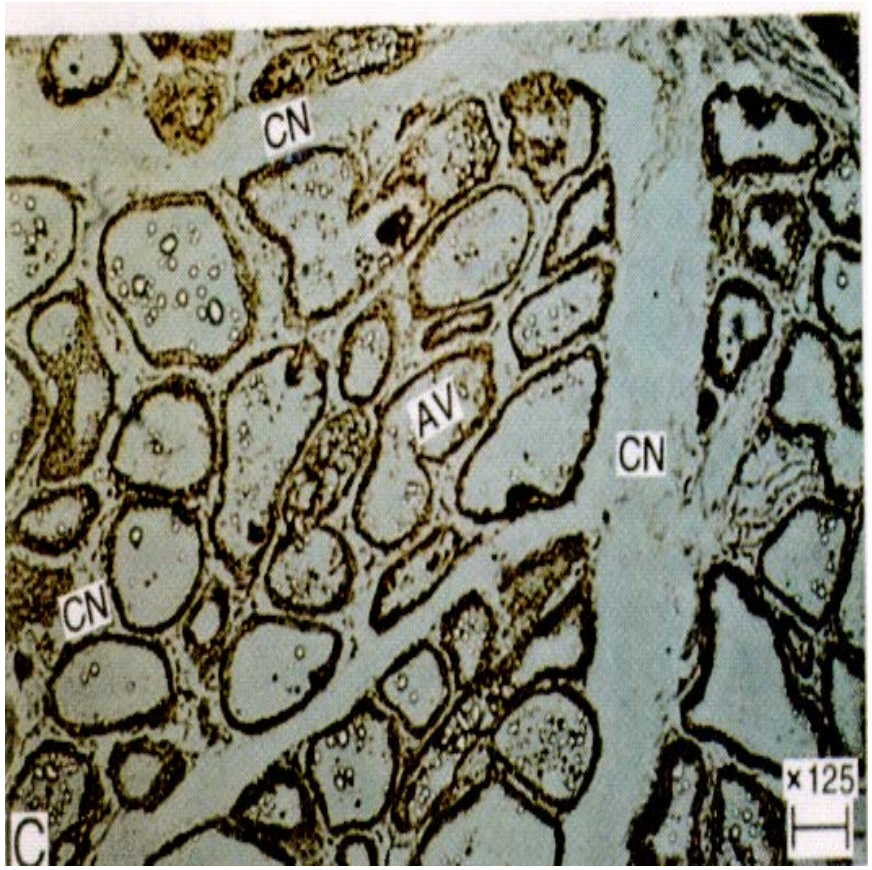
H.A. Tucker, 1982

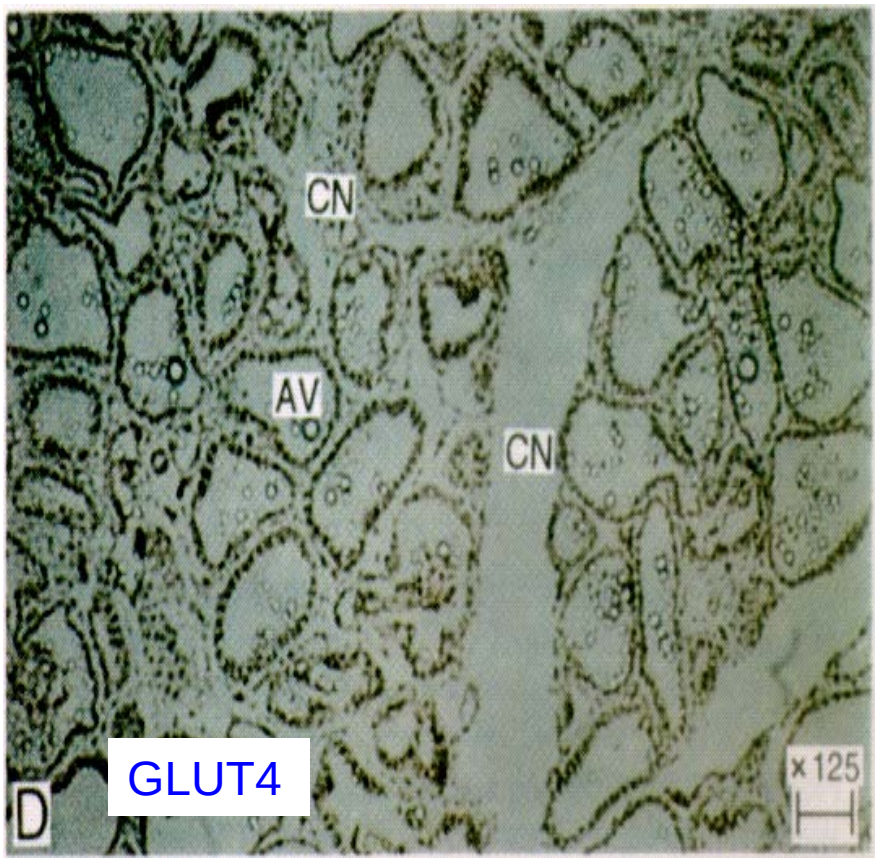




(Modified from Austin & Short (ed) *Reproduction in Mammals, Book III: Hormonal Control of Reproduction,* Cambridge University Press: Cambridge, UK, 1984.)

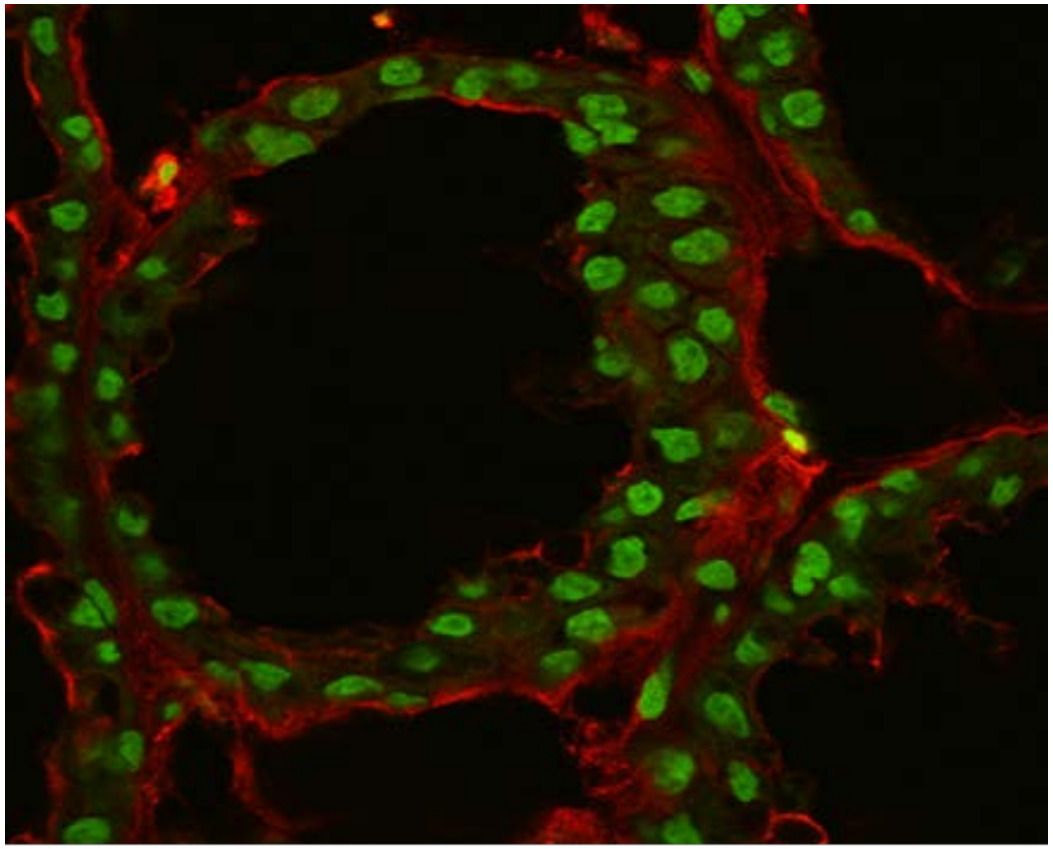
Cellular localization of GLUT1 in lactating bovine mammary gland





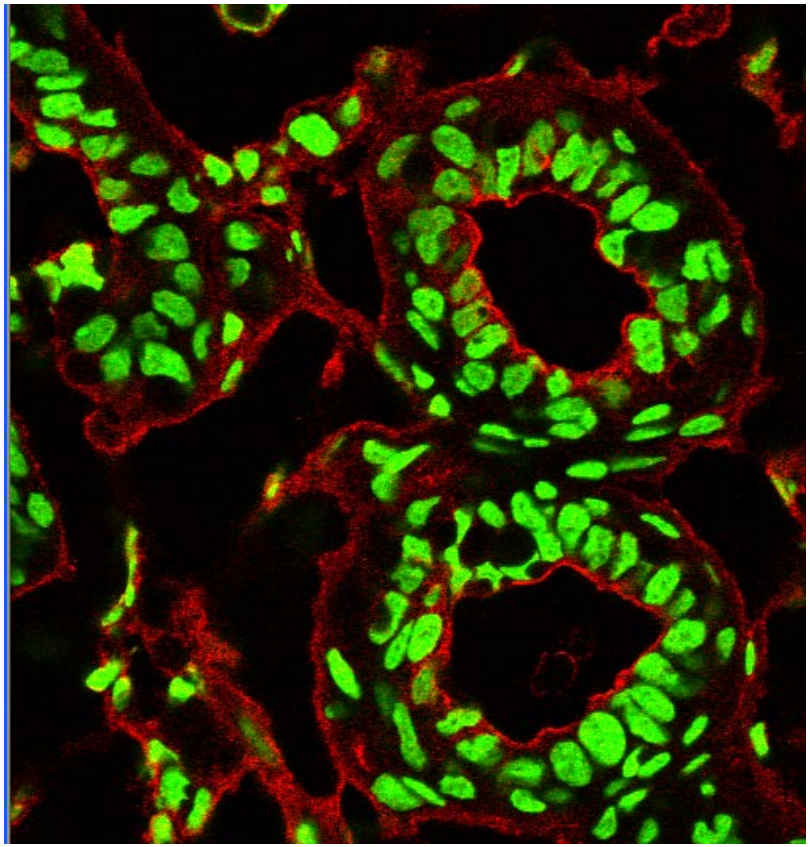
Zhao et al., 1996

Cellular localization of GLUT1 in lactating bovine mammary gland

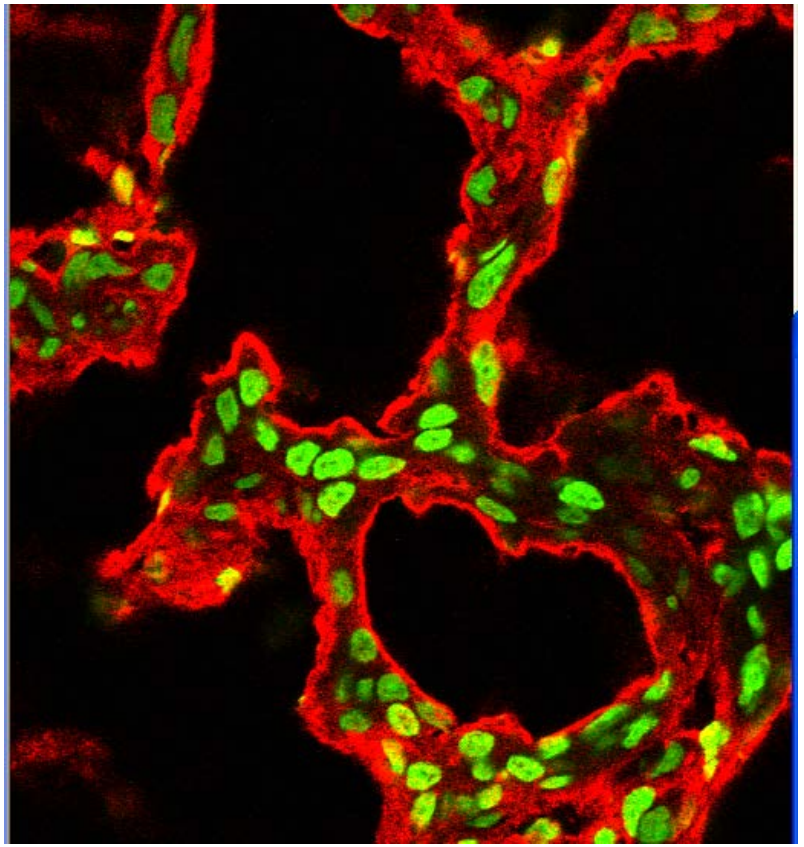


Finucane et al., 2008

GLUT8 is localized in both apical and basolateral membrane of mammary epithelial cells



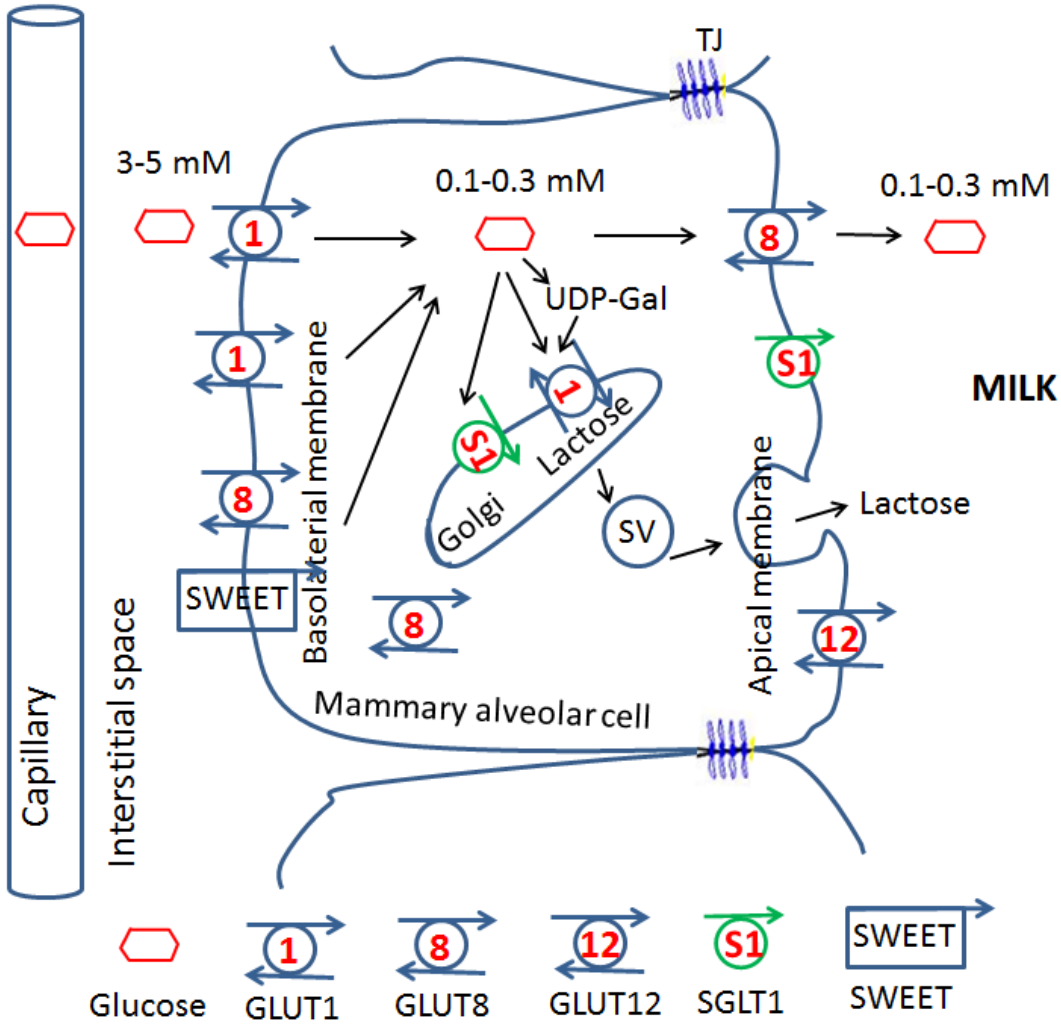
1 wk before parturition



1 wk after parturition

Finucane et al., unpublished

Model: Glucose Transporters in the Bovine Mammary Epithelial Cells



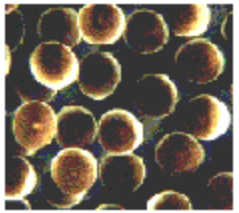
3. Kinetics of Bovine Glucose Transporters

- Blood glucose concentrations:
  - Humans: 4-8 mM; Rodents: 9-11 mM
  - Cows: 3-5 mM

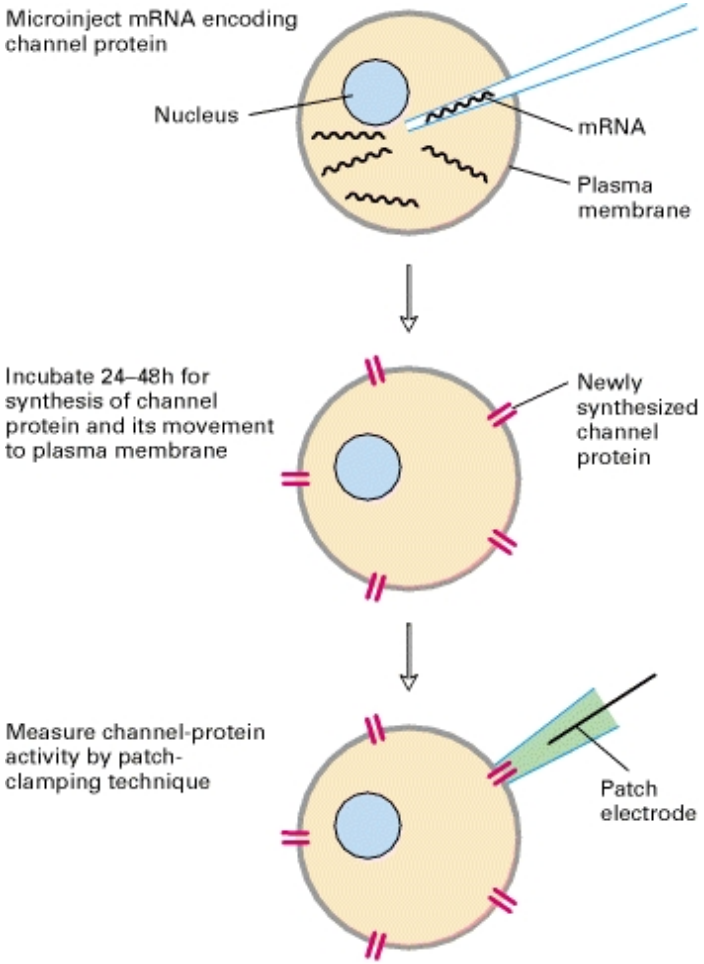
- ***Hypothesis: bovine glucose transporters have a higher affinity to glucose***

Kinetic analysis of glucose transporters

Xenopus laevis

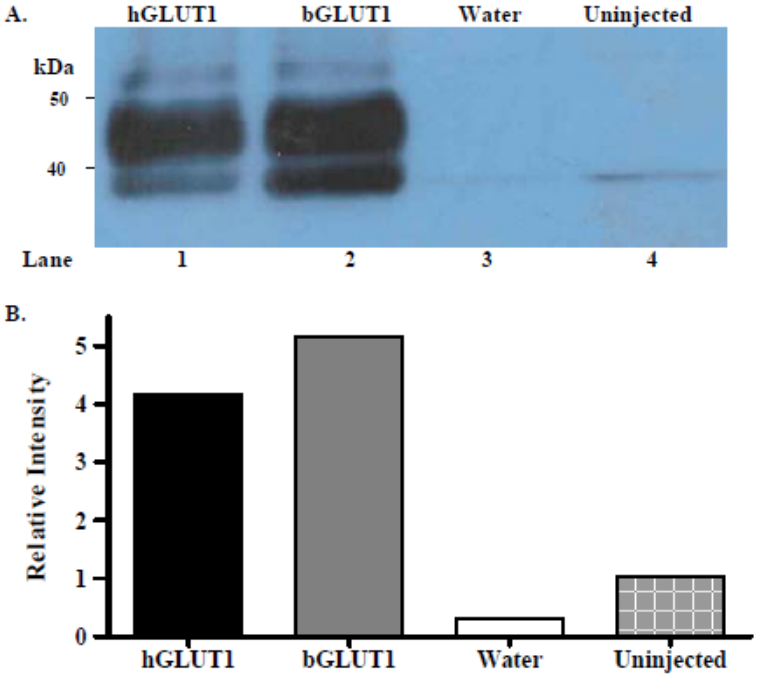


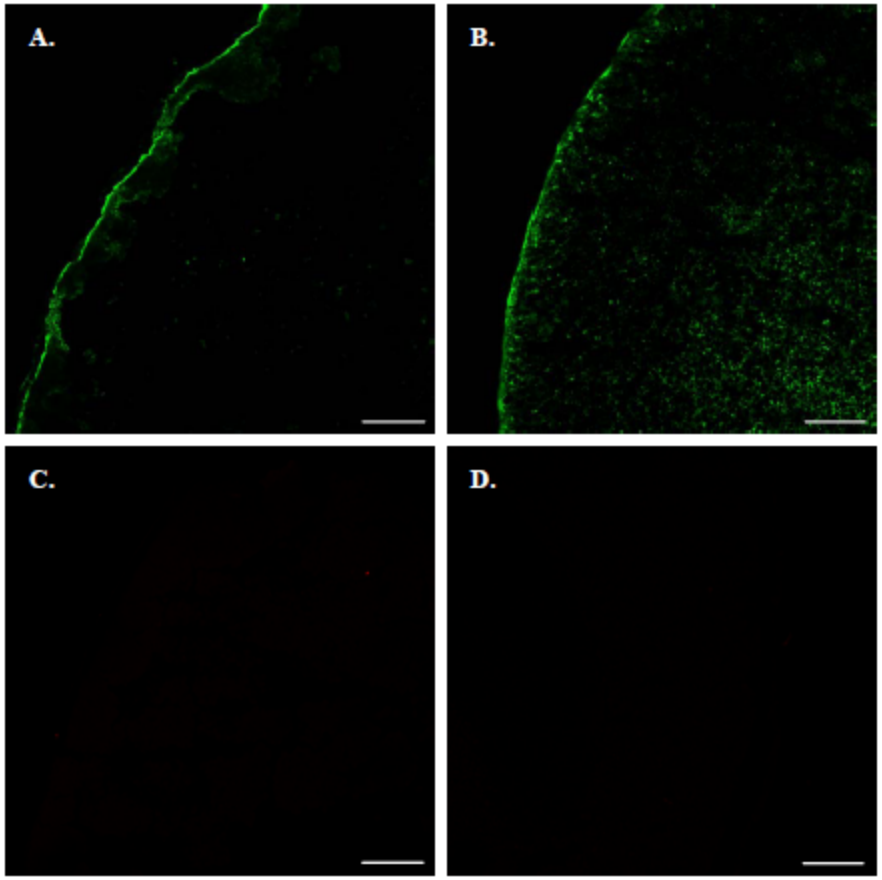
oocytes



http://www.physiologie.uni-tuebingen.de

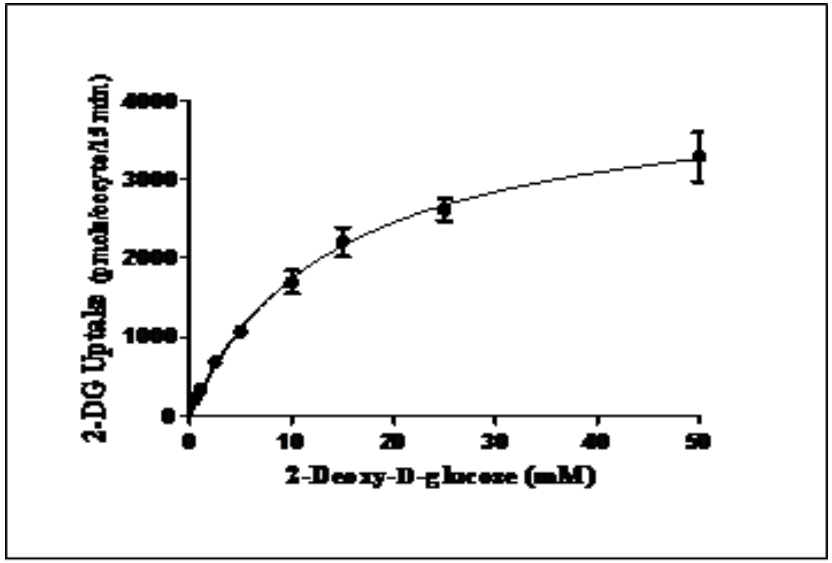
Kinetic analysis of bGLUT1 in *Xenopus* oocytes





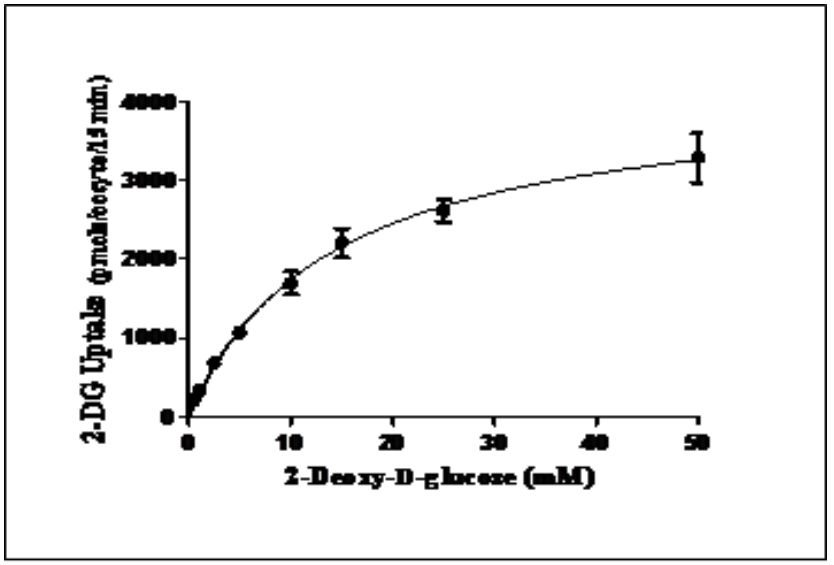
Bentley et al., JDS 2012

Kinetic analysis of bGLUT1 and hGLUT1 in *Xenopus* oocytes



bGLUT1

K_m = 9.8 œ3.0 mM

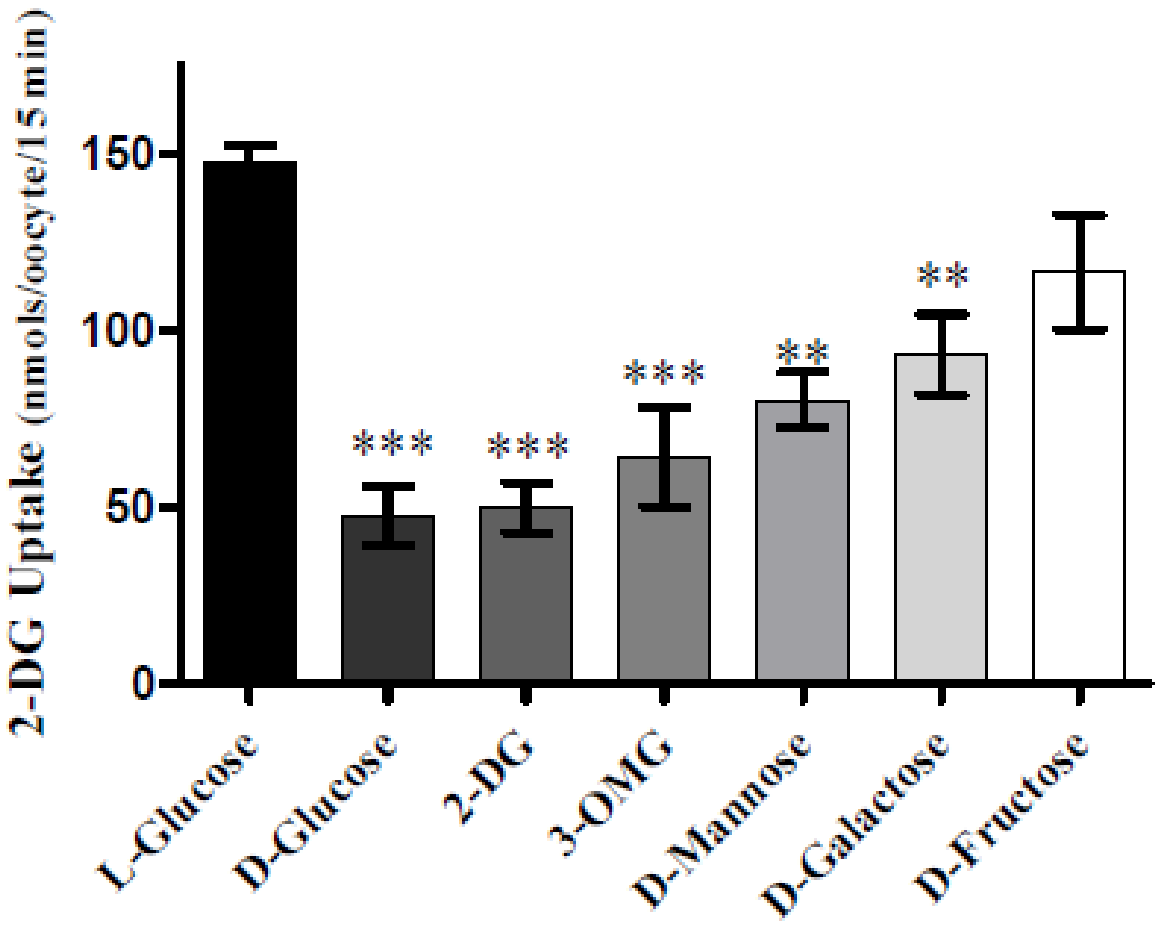


hGLUT1

K_m = 11.7 œ3.7 mM

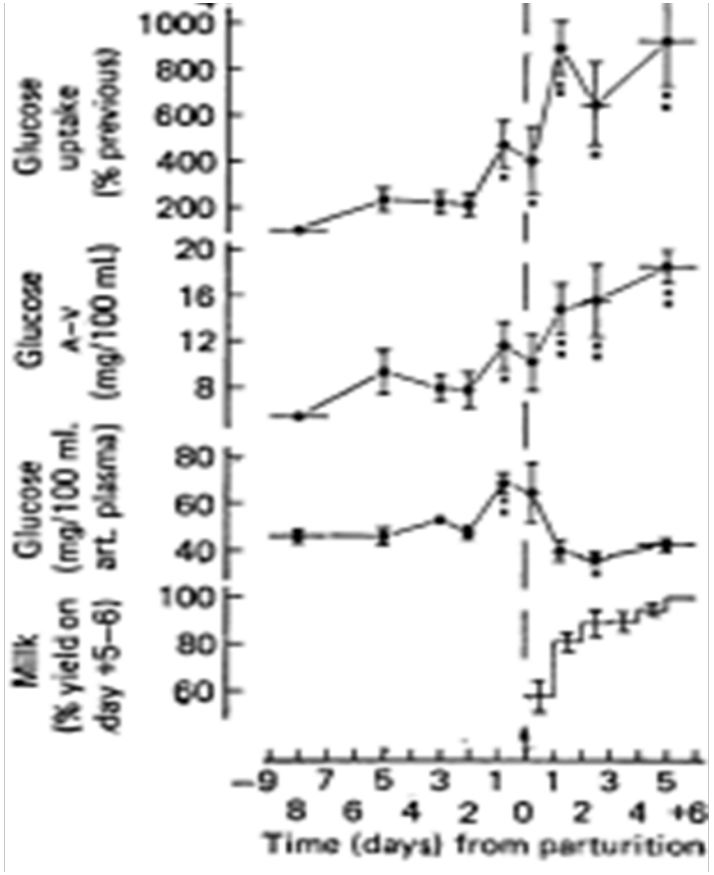
Bentley et al., JDS 2012

bGLUT1 substrate specificity



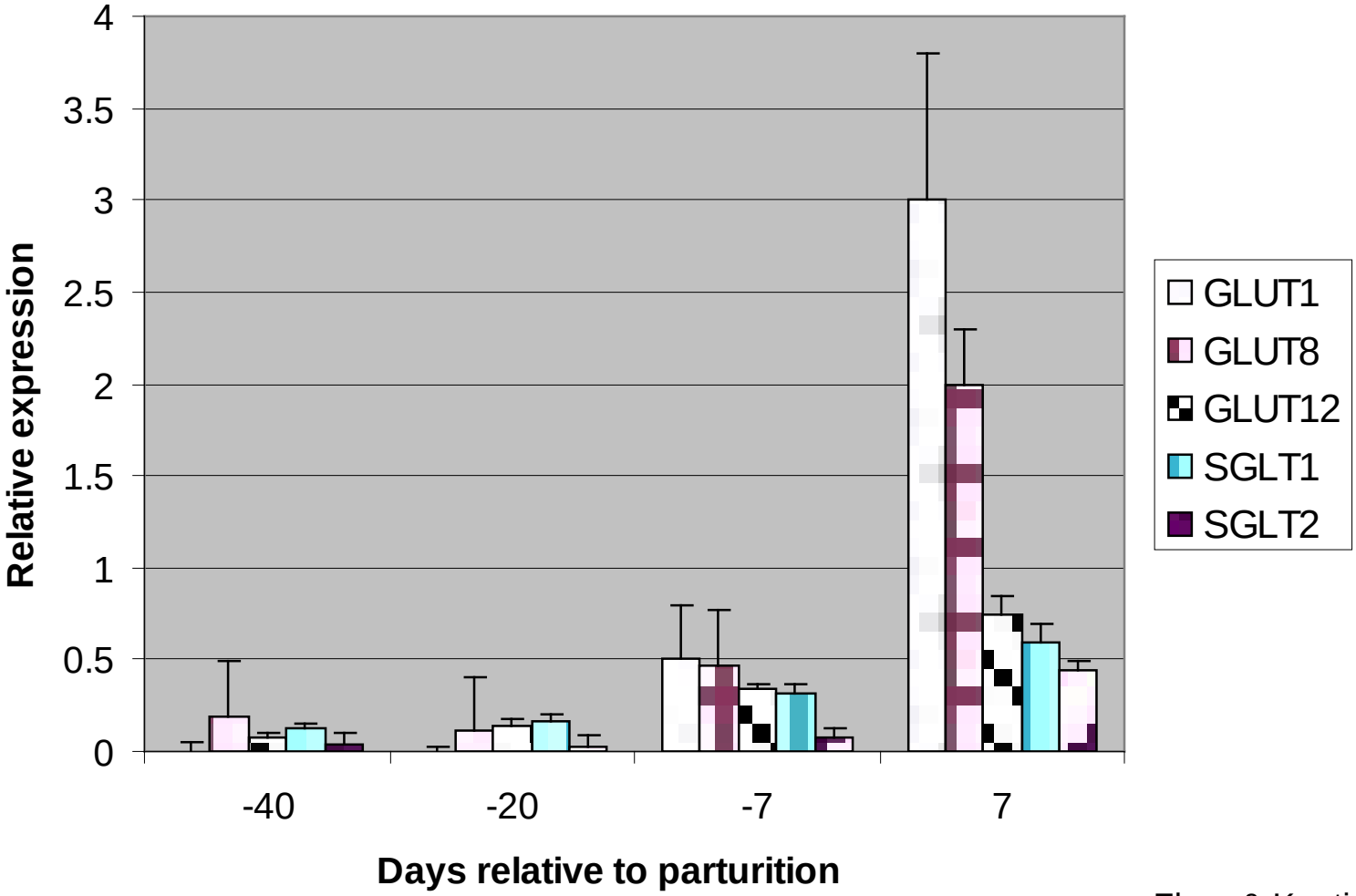
Bentley et al., JDS 2012

4. Developmental Regulation of Glucose Transporters in Bovine Mammary Gland



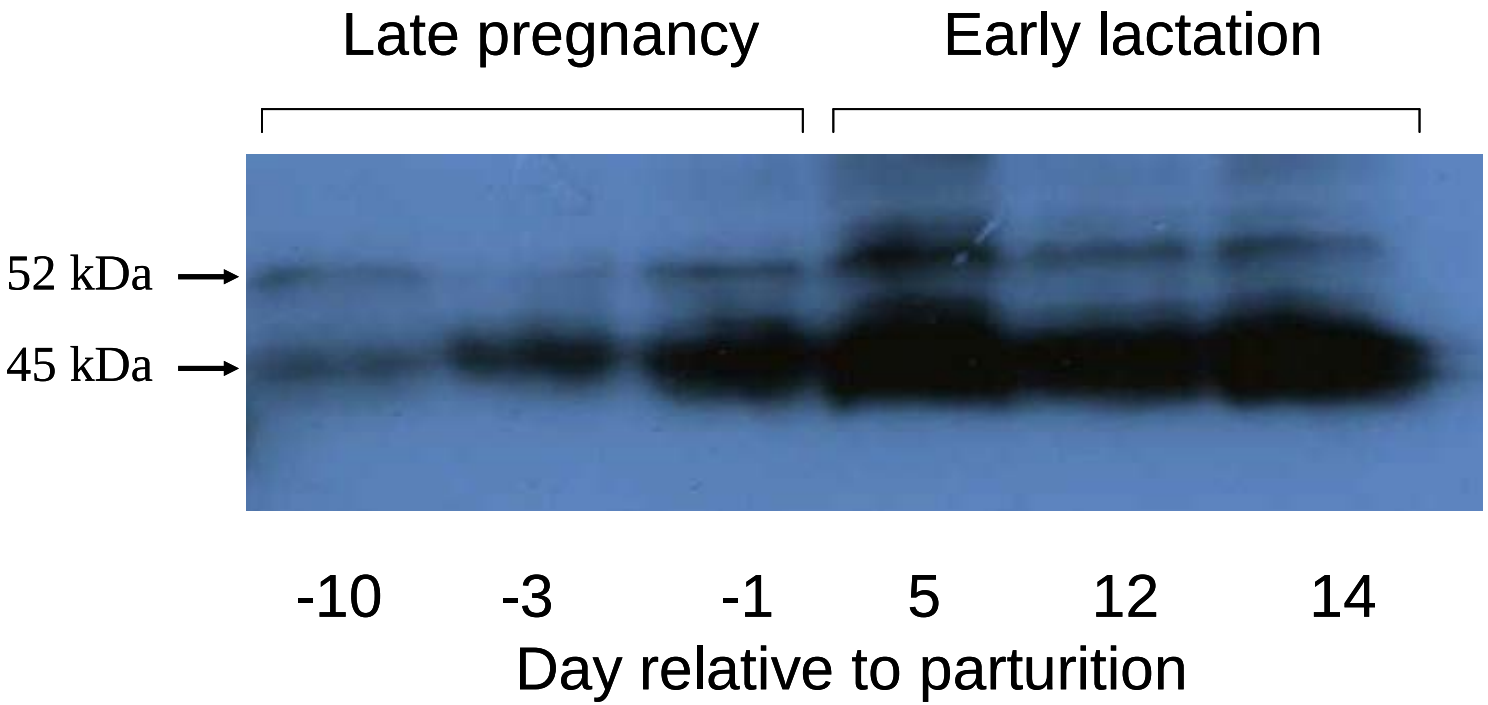
Davis et al., 1979

Glucose transporter mRNA expression in bovine mammary gland around parturition



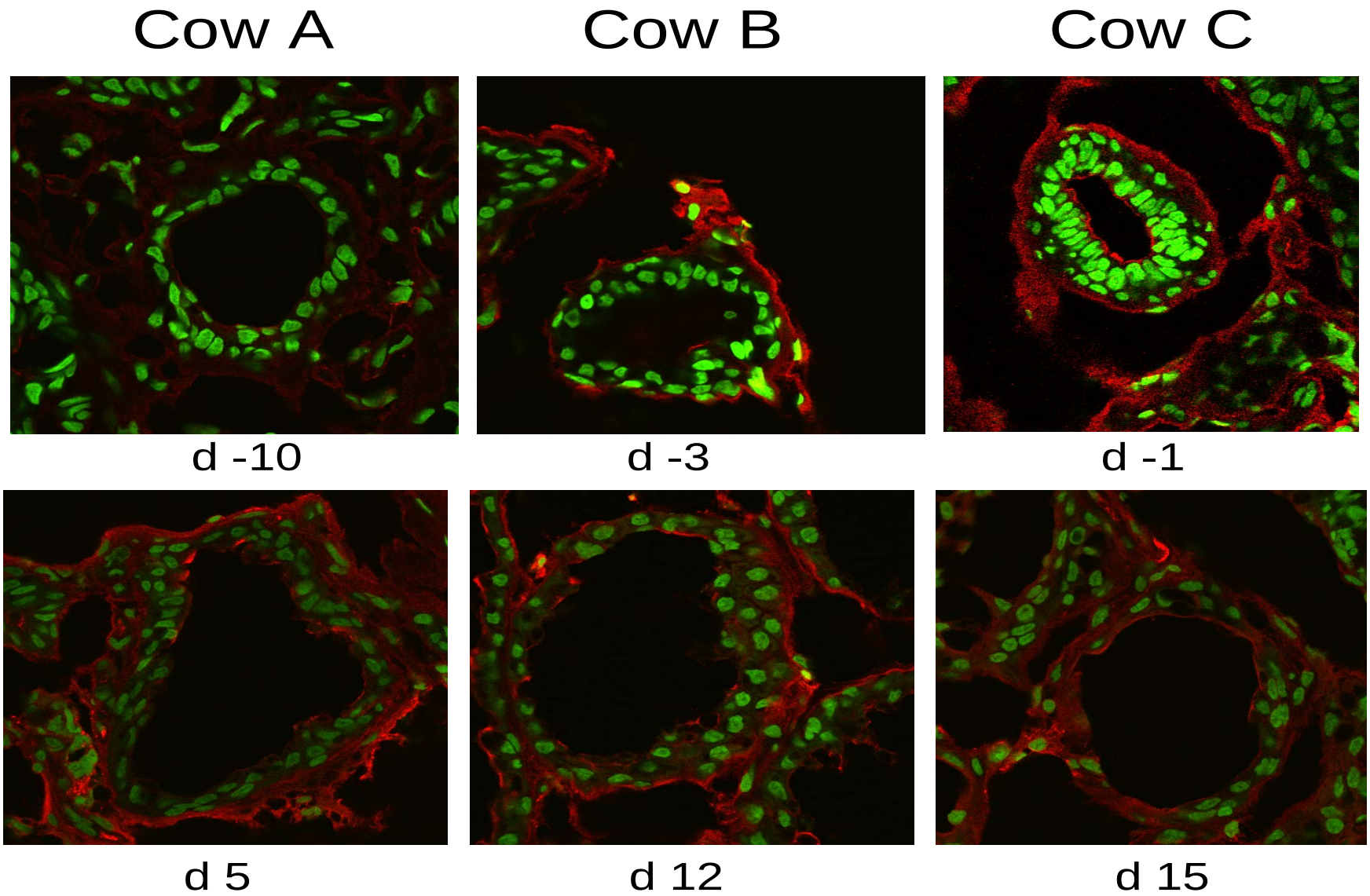
Zhao & Keating, 2007

Glucose transporter protein expression in bovine mammary gland around parturition



Finucane et al., 2008

GLUT1 expression around parturition



Finucane et al., 2008

5. Regulation of Glucose Transporter Gene Expression in Bovine Mammary Gland

Hypothesis:

Expression increase of GLUTs in the mammary gland during the transition period is mediated by the lactogenic hormones

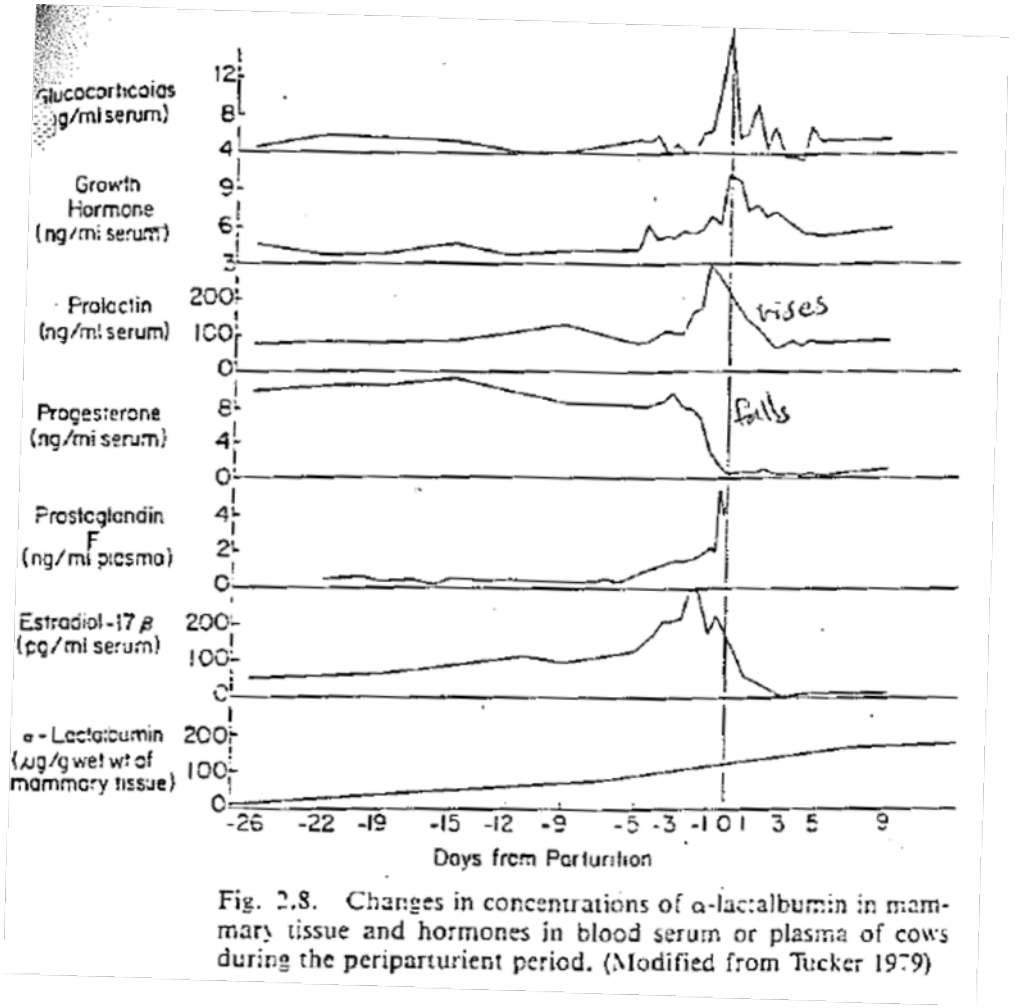


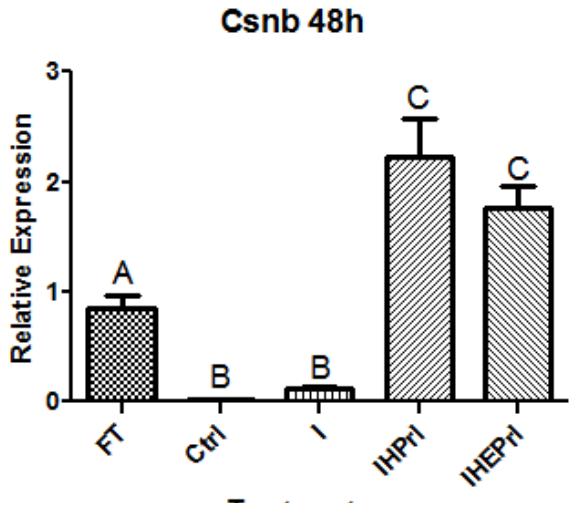
Fig. 2.8. Changes in concentrations of α-lactalbumin in mammary tissue and hormones in blood serum or plasma of cows during the periparturient period. (Modified from Tucker 1979)

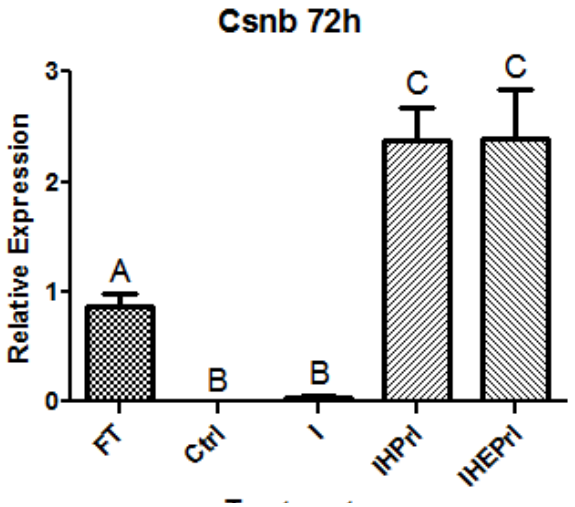
Bovine mammary explants

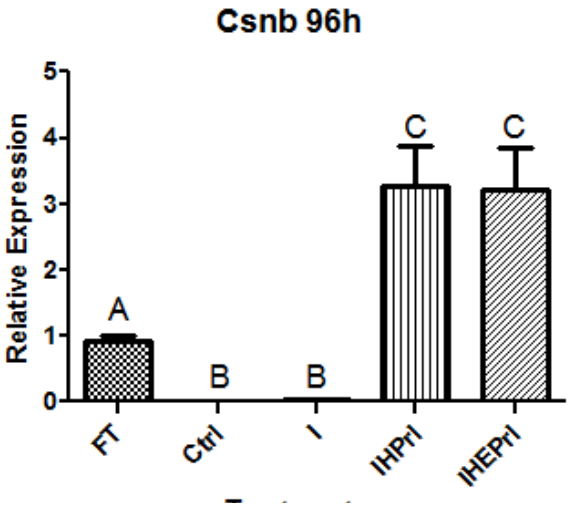
- Hormone treatments:
  - Ctrl: Basal medium (BM): medium 199
  - I: BM + Insulin (5 µg/ml)
  - IHPrl: BM + I + Prolactin (5 µg/ml) + Hydrocortisone (1 µg/ml)
  - IHEPrl: BM + I + Prl + H + Estrogen (500 ng/ml)
- Explants harvest: 48h, 72h and 96h

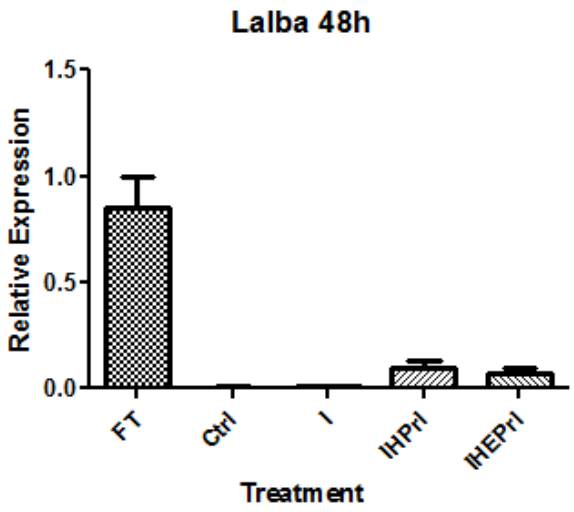
Shao et al. 2012

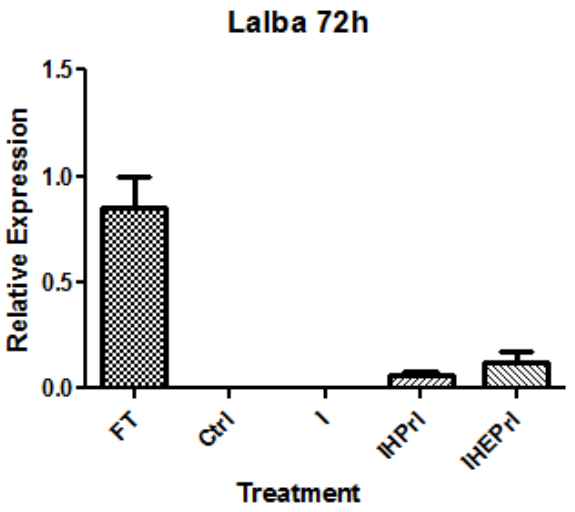
^{2} -Casein and ±-lactalbumin expression in explants treated with lactogenic hormones

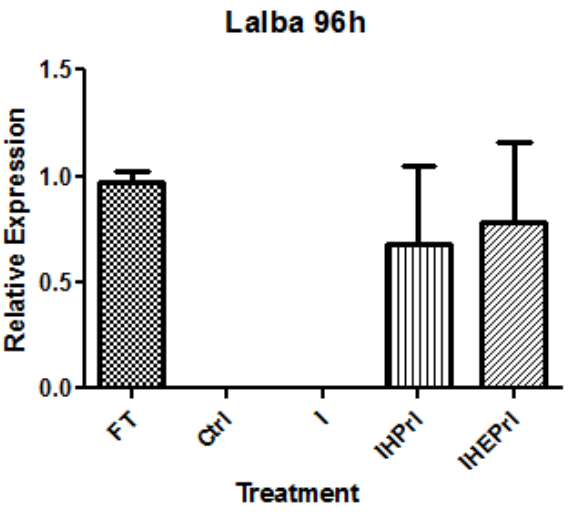




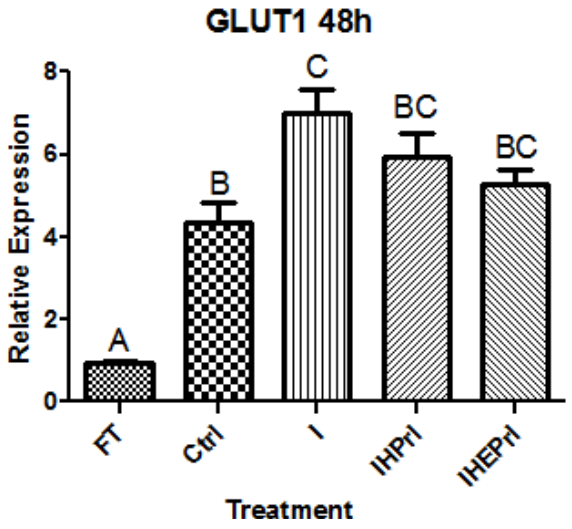


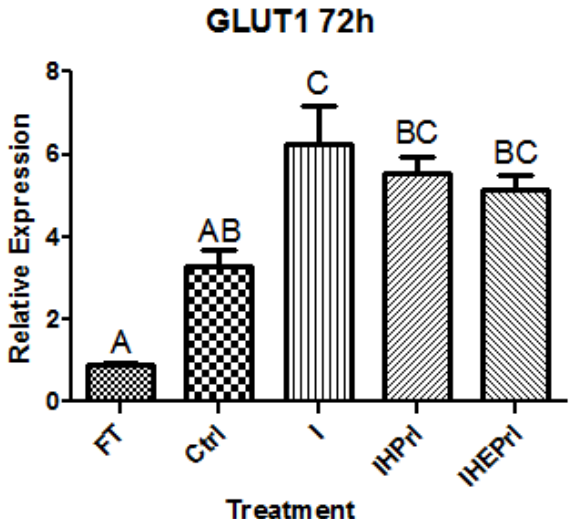


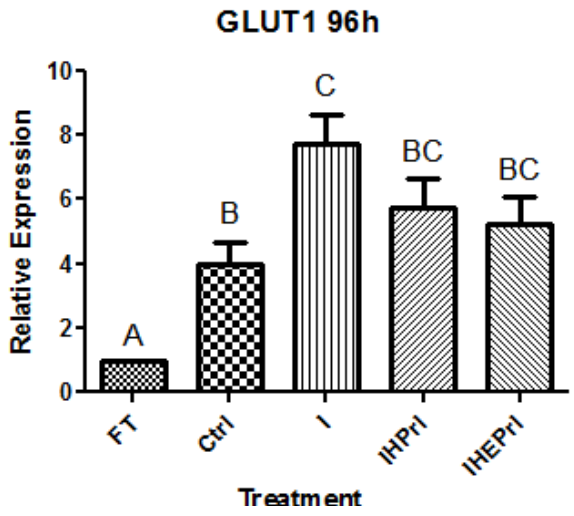


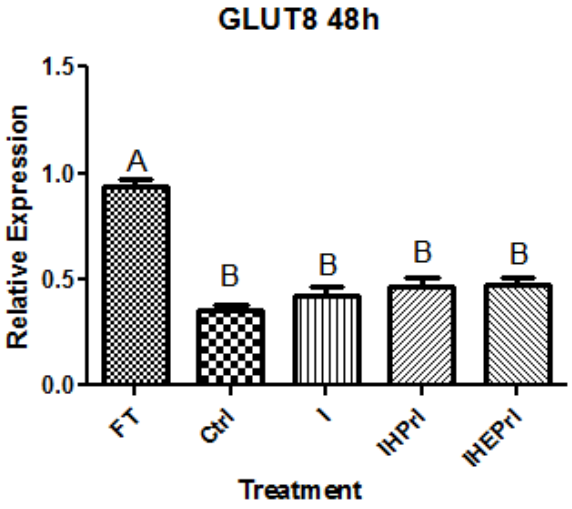


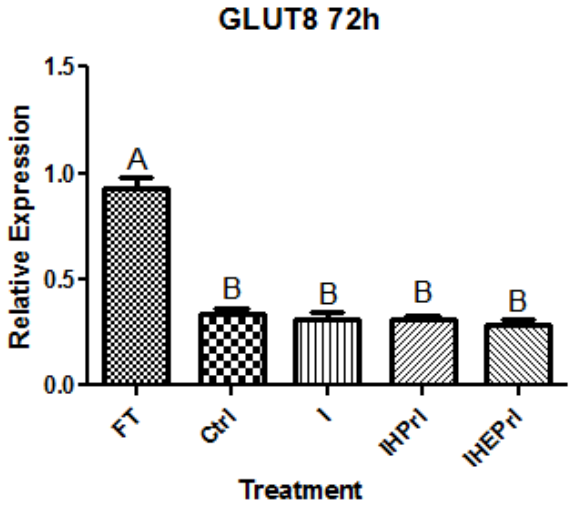
GLUT1 and GLUT8 mRNA expression in explants treated with lactogenic hormones

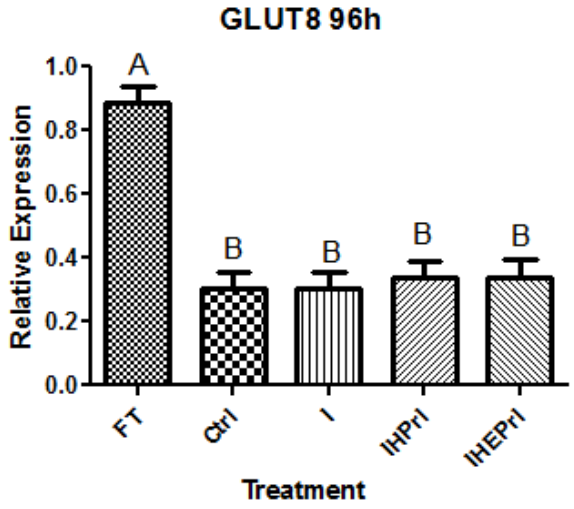












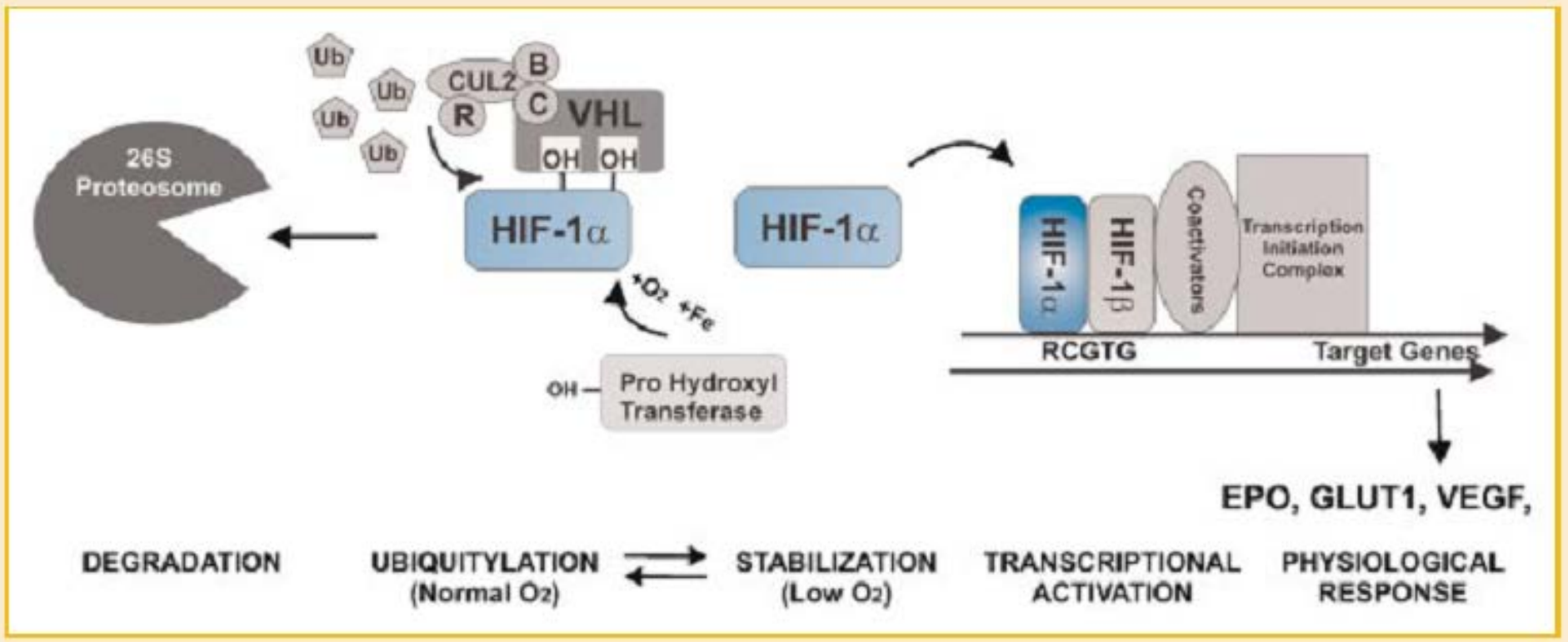
Regulation of Glucose Transporter Gene Expression in Bovine Mammary Gland

New Hypothesis: Expression increase of GLUTs in the mammary gland during the transition period is mediated by hypoxia

Hypoxia

- The reduction of normal oxygen tension
- The pO_2 ranges from 21% (the upper airway) to 1% (in the retina)
- Most tissues trigger hypoxia responses when pO_2 is below 6% (Simon and Keith, 2008)
  - Increases glucose uptake and switch to anaerobic metabolism
  - Stimulate angiogenesis and erythropoiesis

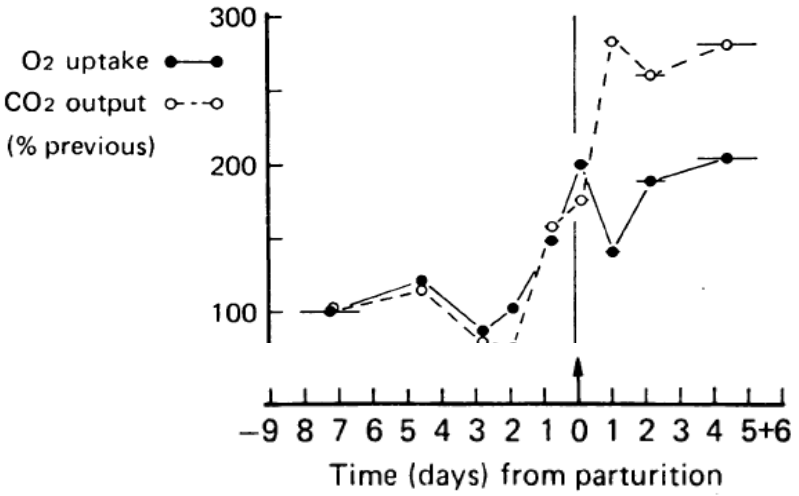
HIF-1α plays a central role in mediating hypoxia responses



Cassavaugh and Lounsbury, 2011

Mammary oxygen uptake

- The mammary O_2 uptake increases during late pregnancy and peaks in lactation



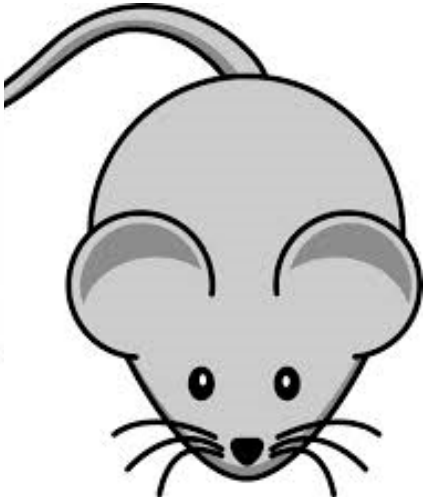
Davis et al., 1979

➡ Possible chronic local hypoxia in the mammary gland during late pregnancy and lactation

- HIF-1± deletion in the mammary gland of mice resulted in impaired mammary differentiation and failure in lactation

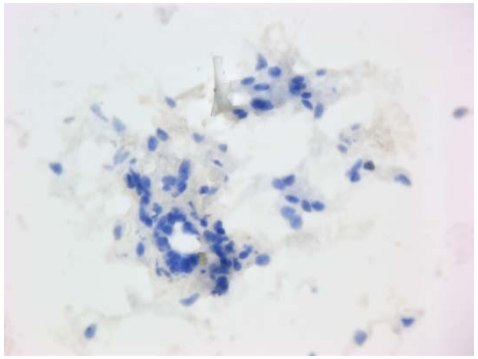
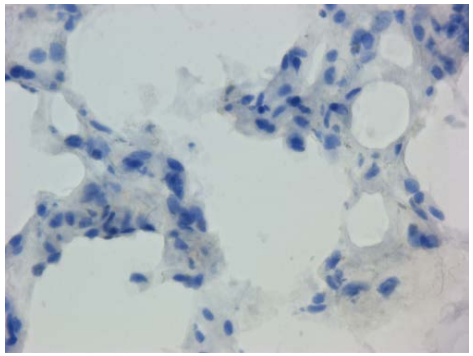
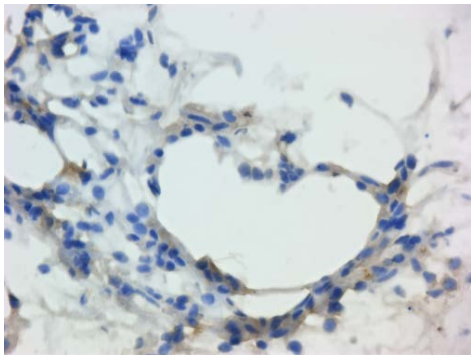
O_2 tension in the mammary gland during developmental stages

Pimonidazole HCl
(Hypoxia marker)

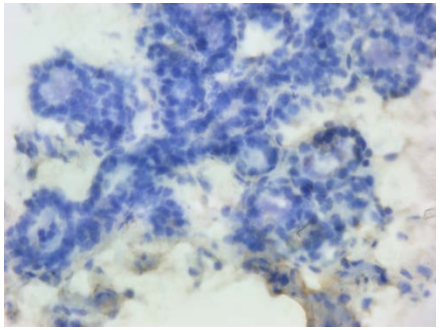
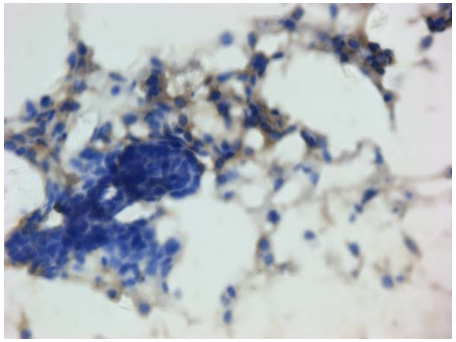
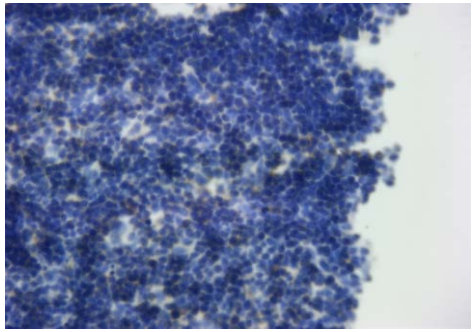


- Pimonidazole HCl is a chemical that forms adducts with thiol groups in proteins, peptides, and amino acids in hypoxic cells (http://www.hypoxyprobe.com/).
- This binding can be detected by immunohistochemical staining using specific antibodies to pimonidazole adducts.

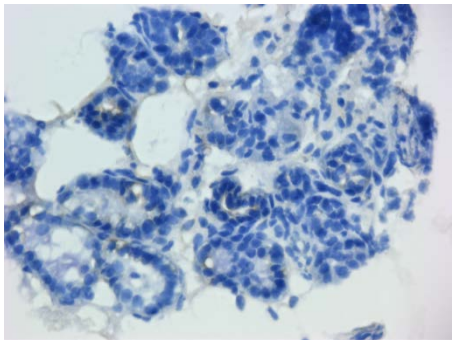
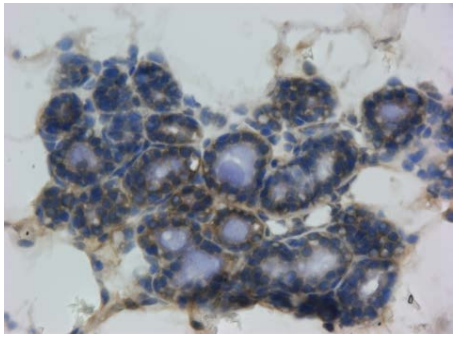
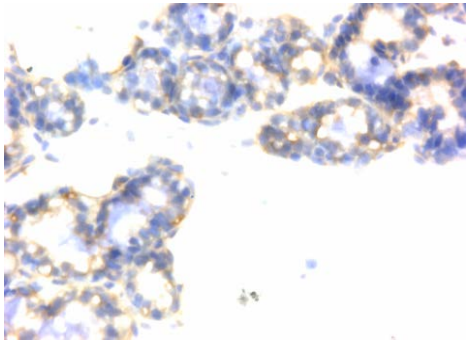
Virgin

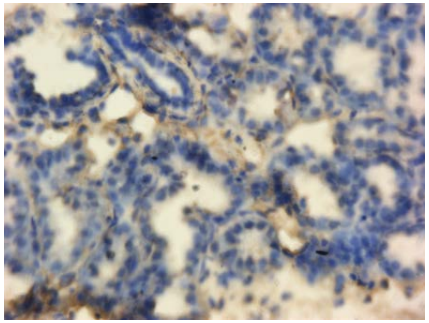
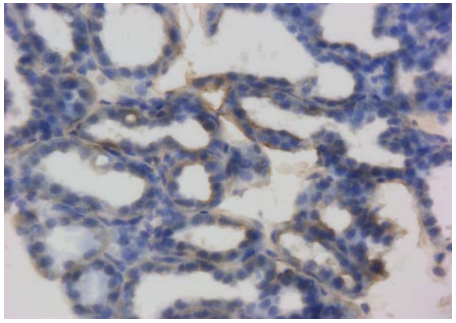
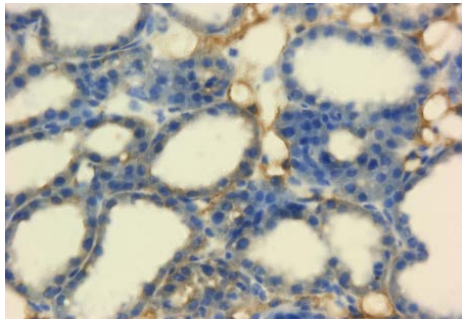
Mid-pregnancy

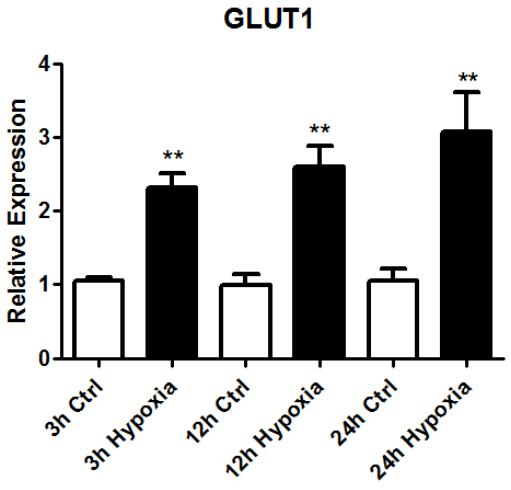
Late-pregnancy

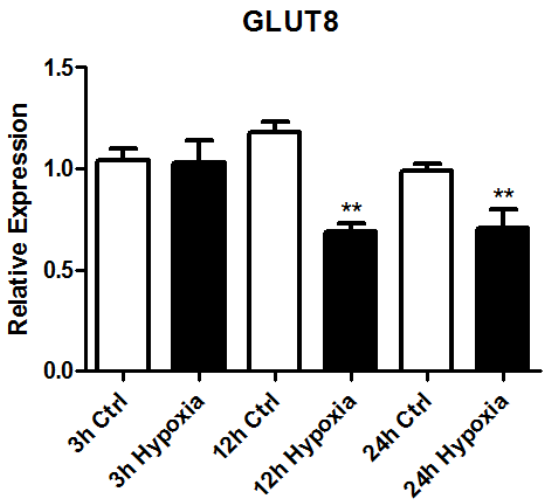




Early lactation

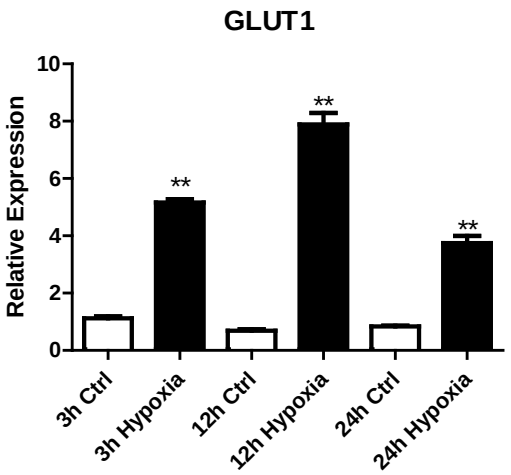




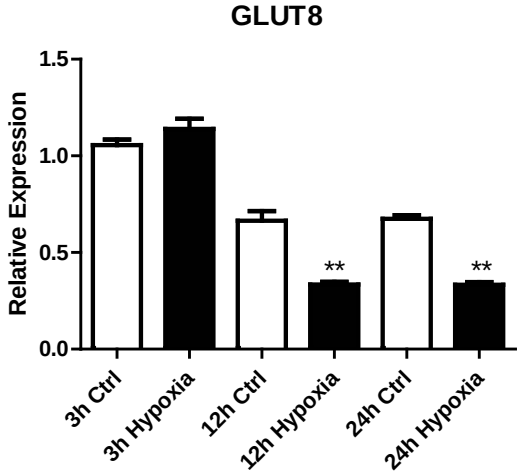
GLUT mRNA level changes in bovine mammary epithelial cells after 3, 12 and 24 h hypoxia treatment





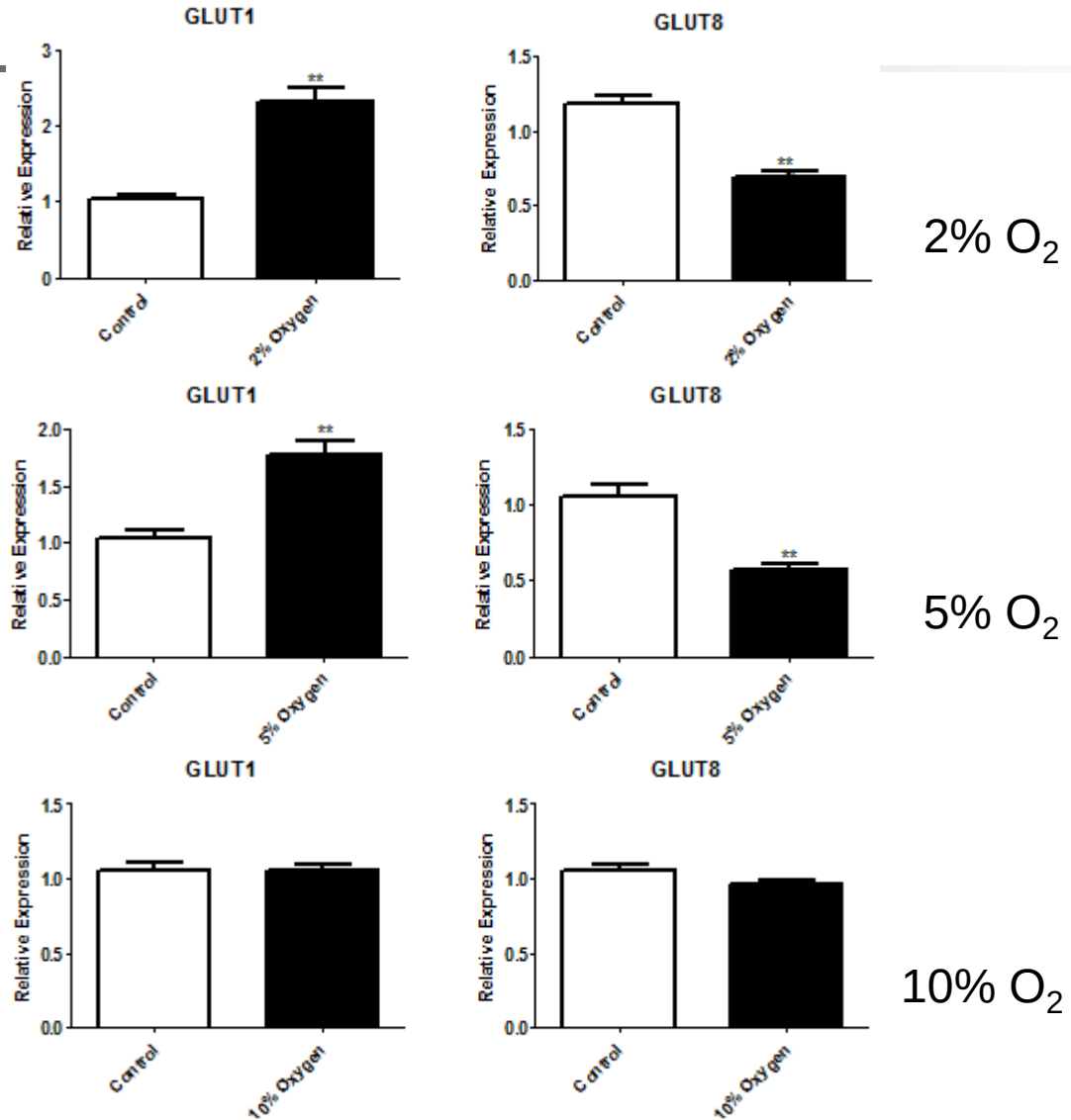
Mac-T cells



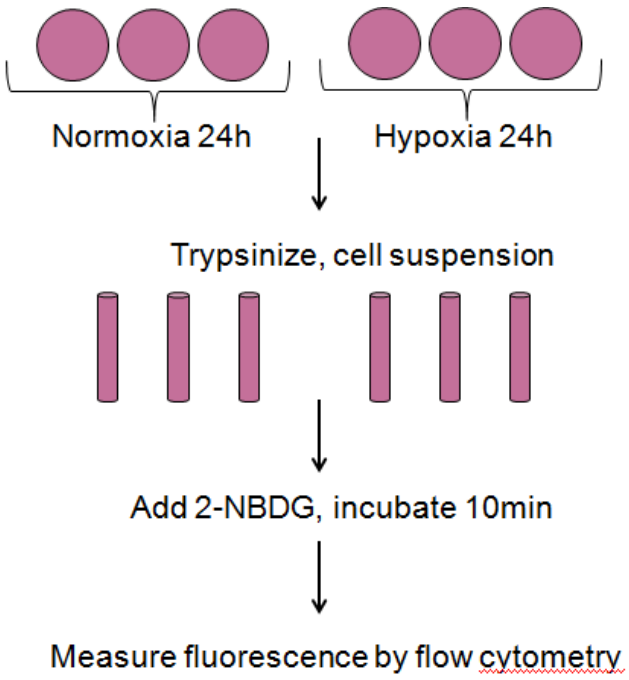


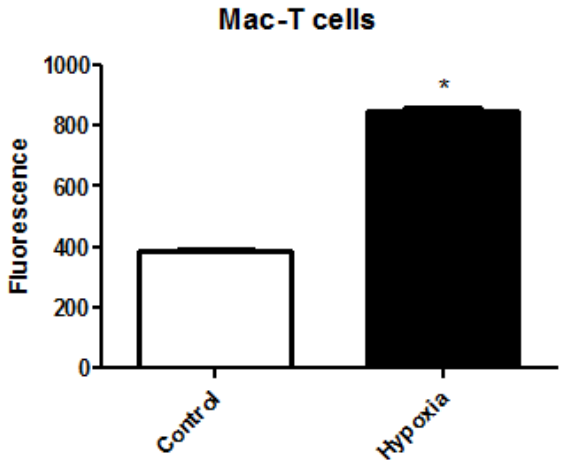
Primary cells

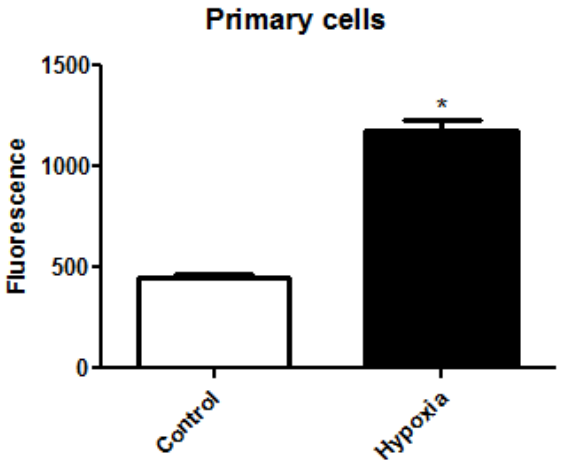
GLUT mRNA levels in Mac-T cells treated with different pO_2



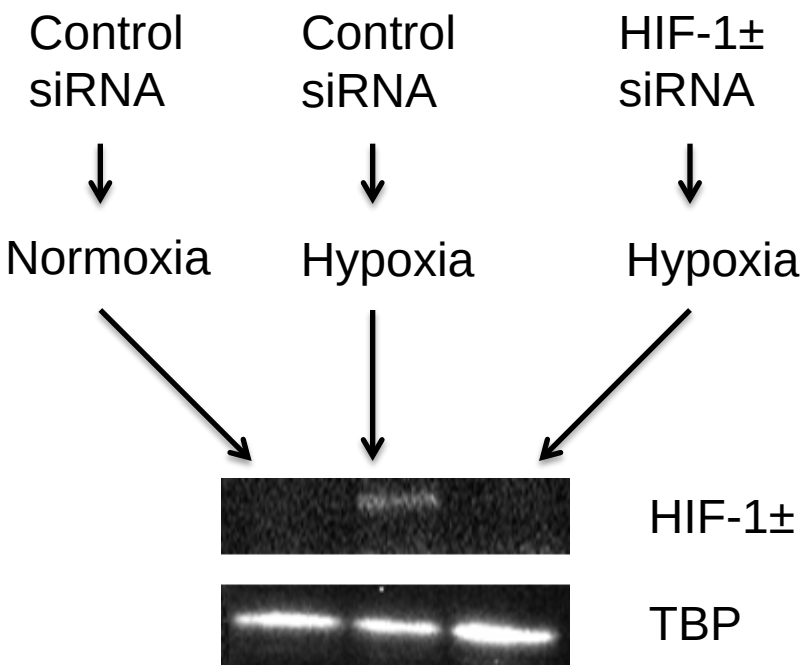
Effect of hypoxia on glucose uptake in mammary epithelial cells



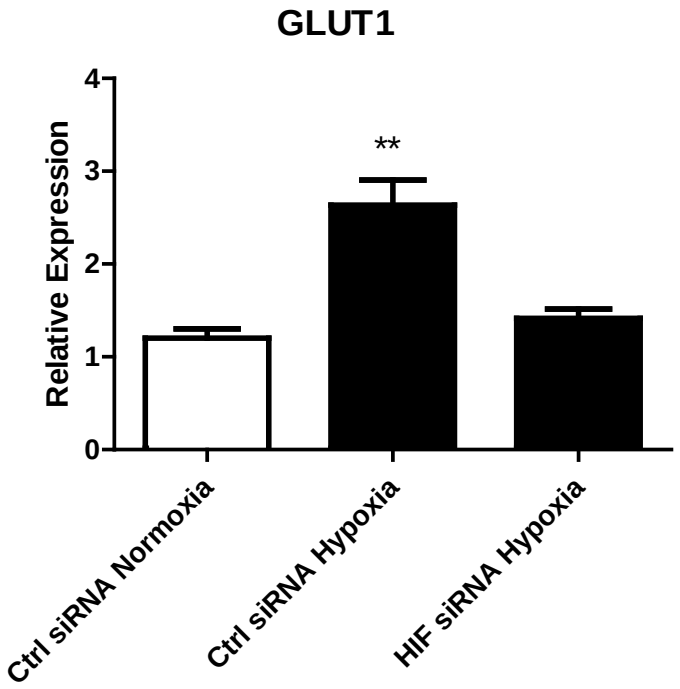




Induction of GLUT1 expression in Mac-T cells by hypoxia is HIF-1±-dependent



HIF-1± expression
Western blot



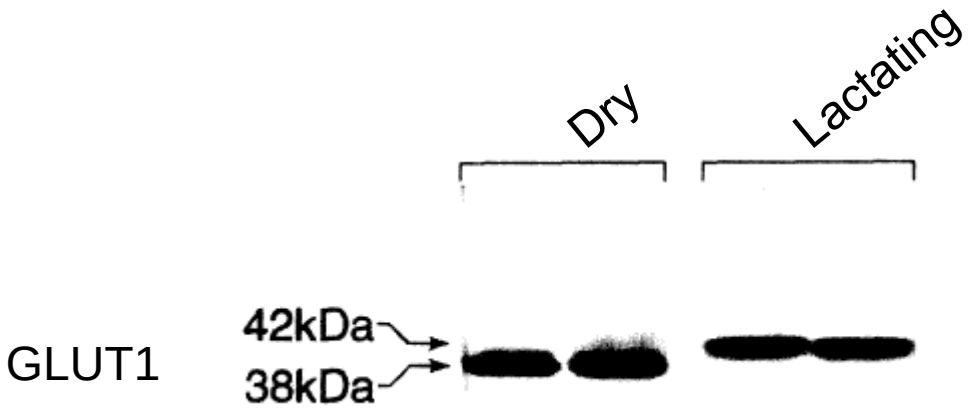
GLUT1 expression
qPCR

6. Other Possible Regulatory Mechanisms

- Translocations of GLUTs
  - The sharp decrease in the V_{max} of the transport activity and the number of GLUTs in the plasma membrane of mammary epithelial cells in fasted mice can be completely reversed by refeeding for only 2-3 h, suggesting ***a redistribution of the glucose transporters to the plasma membrane***. (Prosser, 1988, Threadgold & Kuhn, 1984)
  - In CIT_3 mouse mammary epithelial cells, lactogenic hormone complex (prolactin and hydrocortisone) stimulates the translocation of ***GLUT1 from the plasma membrane to the Golgi membrane***. (Haney, 2001, Riskin et al., 2008)

6. Other Possible Regulatory Mechanisms

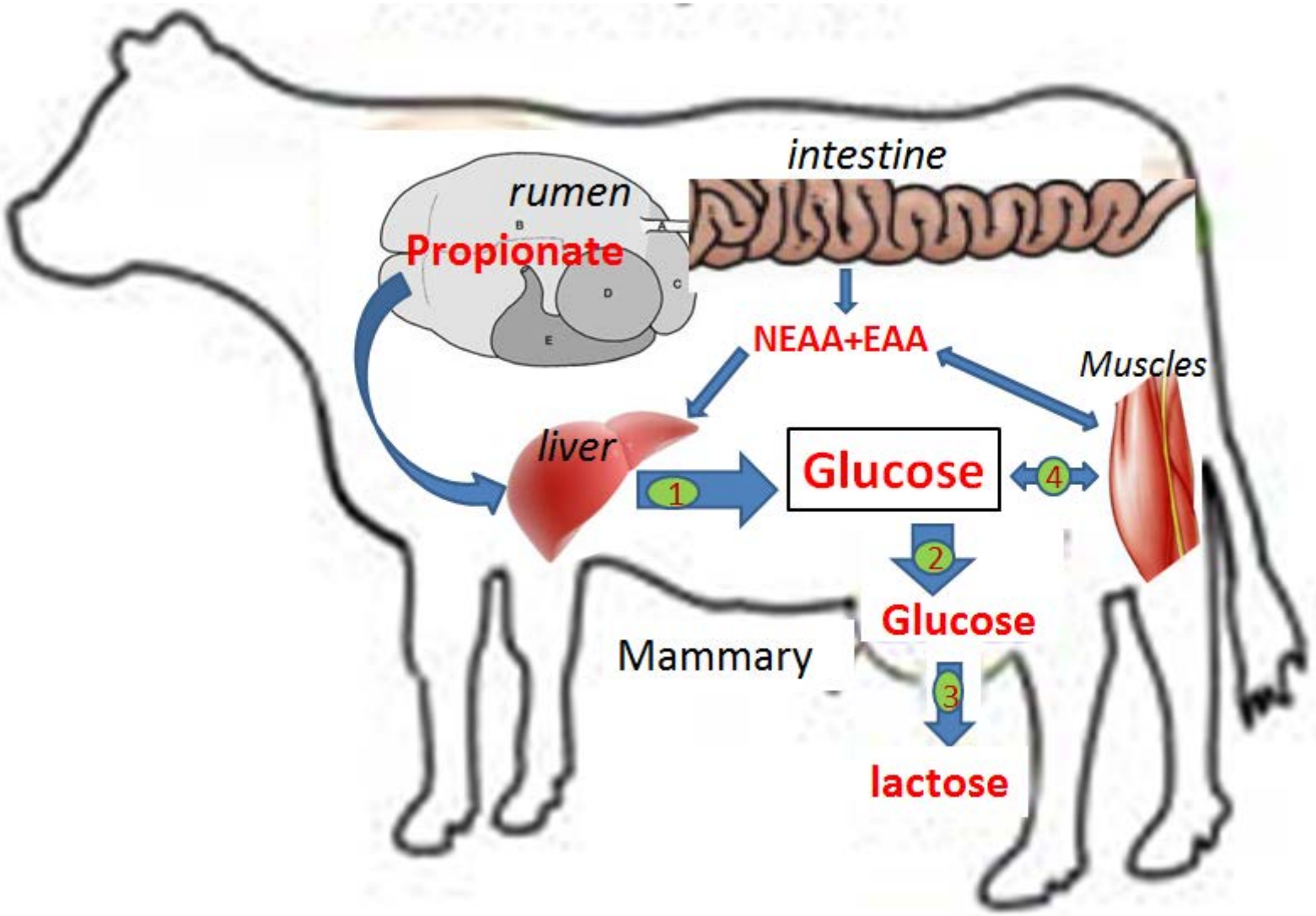
- Glycosylation of GLUTs
  - The *N*- and *O*-glycosylation of GLUT1 have an important impact on GLUT1 transport activity.
  - In CIT_3 mouse mammary epithelial cells, the lactogenic hormone complex induces GLUT1 glycosylation (Haney, 2001).
  - Different sizes of GLUT1 in lactating and non-lactating bovine mammary glands were detected: (Zhao et al., 1996)



Summary

- Lactating bovine mammary gland mainly expresses **GLUT1** and GLUT8. GLUT1 is mainly responsible for glucose uptake in mammary epithelial cells.
- Expression of glucose transporters in bovine mammary gland is developmentally regulated, with the highest expression in early lactation.
- Developmental regulation of glucose transporter expression in the bovine mammary gland may be mediated by hypoxia, but not by lactogenic hormones.

THE BIG PICTURE



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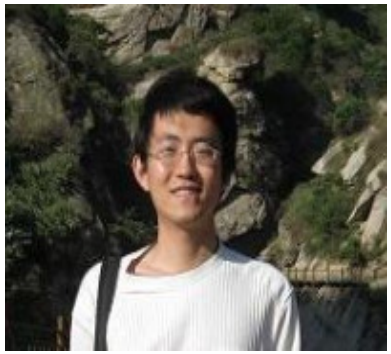

Yong

Aileen

University of Vermont


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