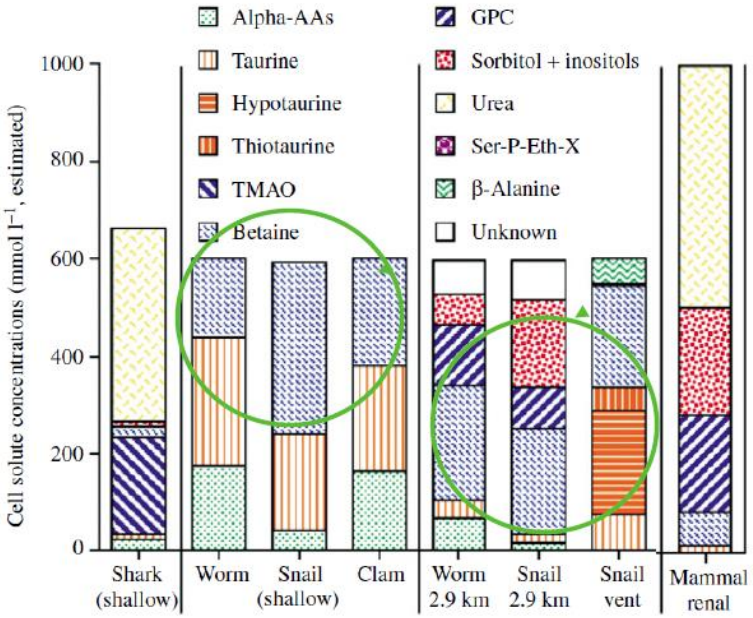
SYNERGIE SKIN
CLEAN SCIENCE

CLINICAL DATA – BETAINE

Betaine is found everywhere

The saltier and drier the environment, the higher levels of betaine are found in living cells.

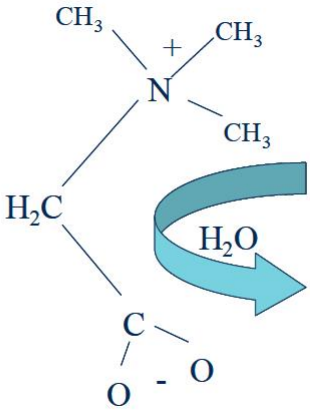
Betaine is found in every kingdom of life. Especially seawater invertebrates have high levels of betaine. In the kidney, betaine counteracts the perturbant effect of urea.



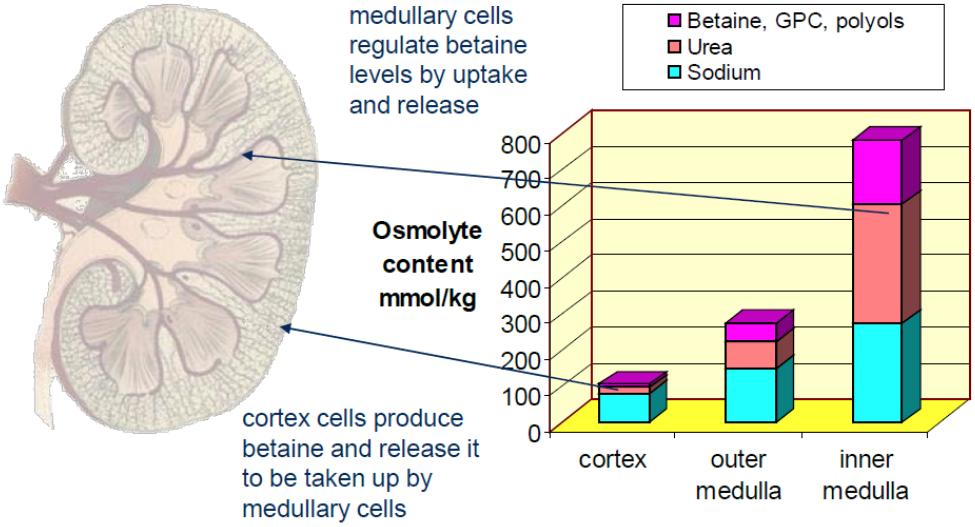
Source: Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses – Paul H Yancey. *Biology department, Whitman College, Walla Walla, WA 99362, USA.* Email: yancey@whitman.edu

Betaine attracts water

- Betaine is an organic osmolyte that attracts water but does not immobilise it.
- Betaine protects cellular macromolecules from external disturbances
- To maintain cell water balance cells can accumulate and release betaine

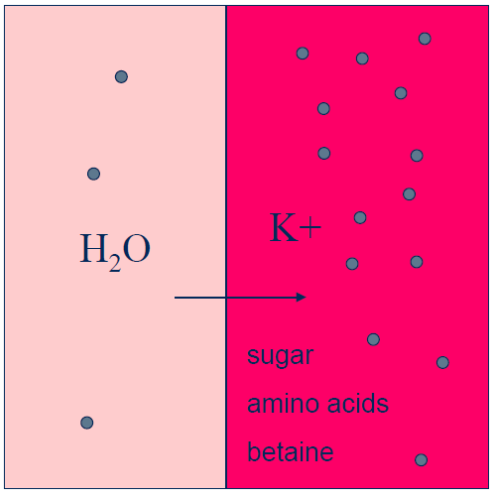


Betaine counteracts the harmful effects of urea and salt in kidney cells



Diffusion across the membrane

Water follows osmotically active compounds such as potassium and organic osmolytes.



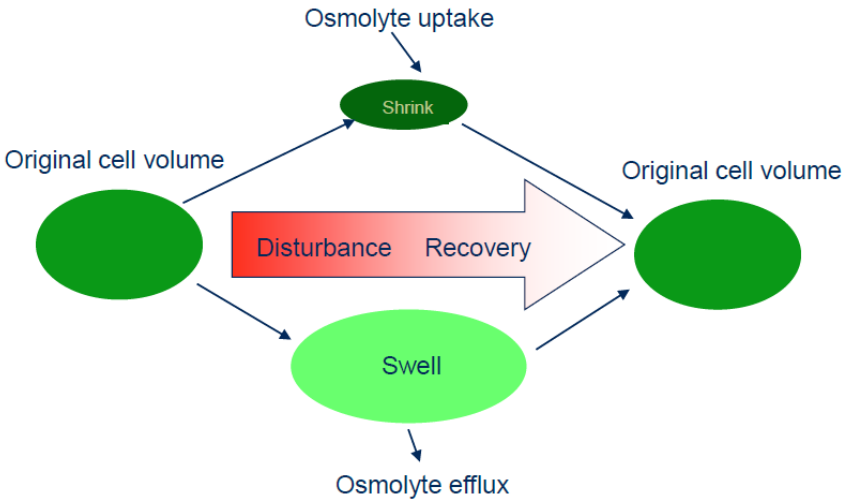
Nature has selected osmolytes that do not…

- Interfere cellular processes
- Upset electrostatic balance of the cell
- Perturb cellular structures

Osmolytes are compounds that preserve intracellular solution for metabolic activities and macromolecular structure against osmotic stress.

Cell volume regulation

Cells regulate their volume and water balance by absorbing or effluxing osmolytes

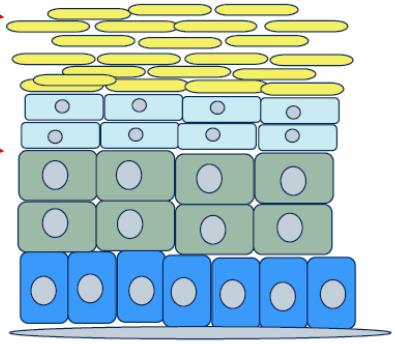


Water in skin

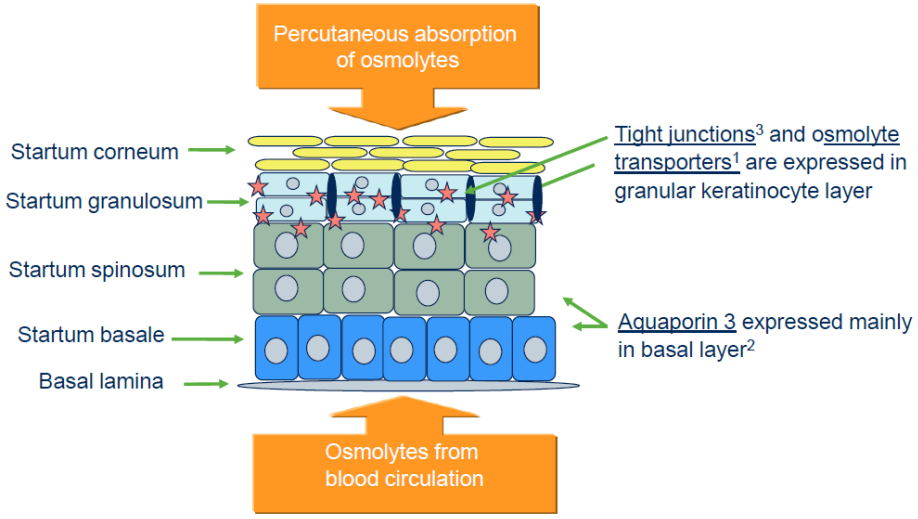
- Skin is needed to prevent water loss from the body
- Hydration of the upper part of skin is important for preserving its elasticity and structure
- Water is needed to manage the normal differentiation of the skin (enzyme reactions)

Factors contributing barrier function of the skin

- Stratum corneum – dead skin layer:
  - Extracellular lipids (ceramides, cholesterol etc.)
  - Keratin and natural moisturizing factor (NMF)
- Stratum granulosum – living skin layer:
  - Tight junctions
  - Osmolyte transporters (esim. BGT-I, SMIT, TAUT)
  - Extracellular hyaluronic acid.



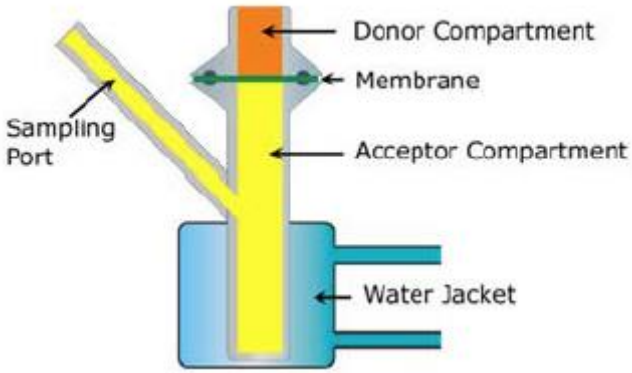
Water transport in skin



1Janeke et al 2003, 2 Boury-Jamot et al 2006, 3Kirchner et al. 2010

Betaine transport through the Stratum corneum

Tiihonen, K[1]., Suhonen, M.[2] and Tolonen, A[3].



- To evaluate the amount of topically applied betaine reaching the living keratinocytes the permeability of betaine across the stratum corneum (SC) was measured in Franz chambers. Betaine concentrations were analysed using LC/MS/MS method.
- Apparent permeability coefficient for betaine in water was 2.5 x 10^{-9} (cm/s) and in emulsion 4 x 10^{-9} (cm/s). The values are comparable to osmolytes such as glycerol and mannitol.

Betaine can protect skin cells against UV radiation

Oxidative stress caused by the UV:

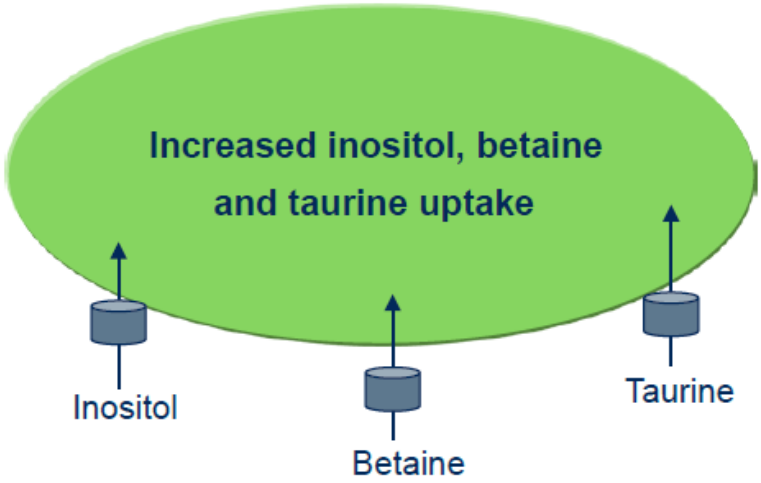
- Speed up collagen break down. Betaine can increase fibroblast growth and collagen production[1] which are important for skin structure and elasticity.

- Dehydrates the skin cells. Keratinocytes[2,3] and fibroblasts[4] use betaine to maintain cell water balance against dehydration. Water is need to optimal keratinocyte differentiation.

Source:
1) Viennet et al. J Invest Dermatol 2002 118, 1099
2) Warskulat et al. J Invest Dermatol. 2004 Sep; 123(3):516-21.
3) Warskulat et al Biol Chem. 2007 Dec; 388(12):1345-52.
4) Warskulat et al. Experimental Dermatology 17; 1031-1036, 2008.

Osmolyte strategy of the keratinocytes [1,2]
Hyperosmotic stress:

- Watter efflux > cell shrinkage
- Increased expression of betaine, taurine and myo-inositol transporters (BGT-I, SMIT, TAUT)
- Increased uptake of osmolytes > cell hydration retained



Oxidative stress caused by UVA and UVB radiation

- Opening of K+ channels > K+ and water efflux > cell shrinkage
- Increased betaine, inositol and taurine transporters (BGT-I, SMIT, TAUT)
- Increased uptake of the osmolytes > cell hydration retained

Source:
1) Warskulat D., A. Reinen, S. Grether-Beck, J. Krutmann and D. Häussinger. Journal of Investigative Dermatology (2004) 123, 516–521
2) Warskulat, D., Brookmann S, Reinen A, Häussinger D. Biol Chem. (2007) 388(12):1345-52.

SYNERGIE SKIN

The Osmolyte Strategy of Normal Human Keratinocytes in Maintaining Cell Homeostasis

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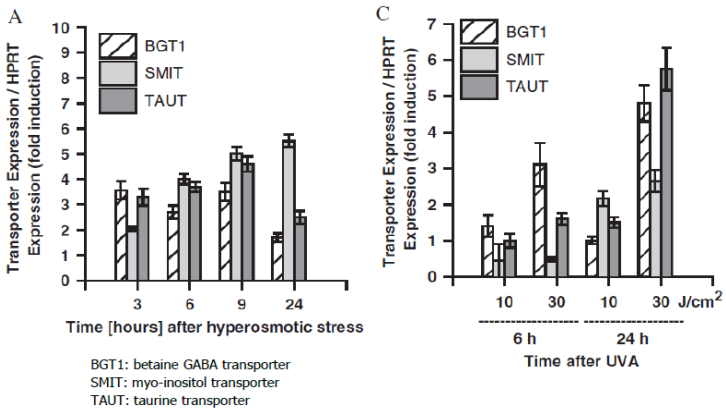

BGT1: betaine GABA transporter
SMIT: myo-inositol transporter
TAUT: taurine transporter

Induction of betaine, inositol and taurine transporters after hyperosmotic stress and UVA radiation in normal human keratinocytes. *J. Invest Dermatol* 123; 516-521, 2004.

Ultraviolet A induces transport of compatible organic osmolytes in human dermal fibroblasts

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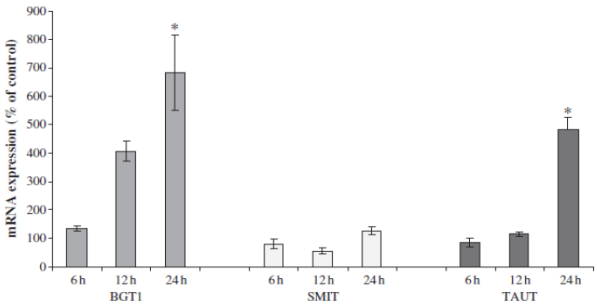
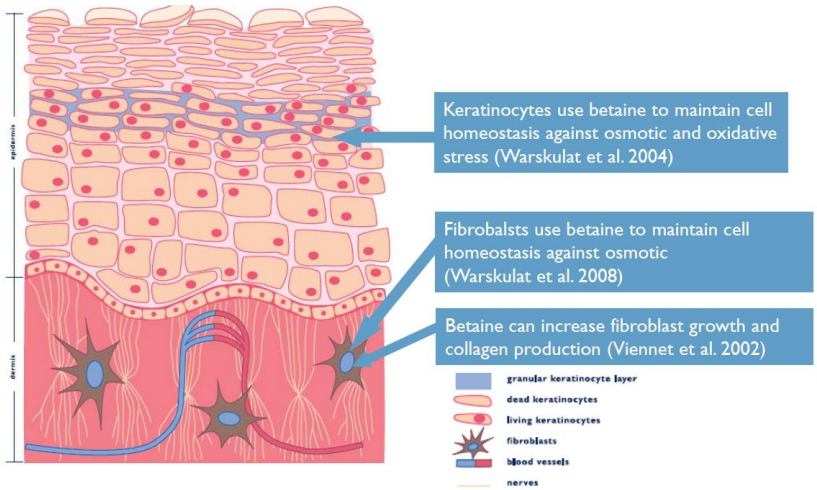


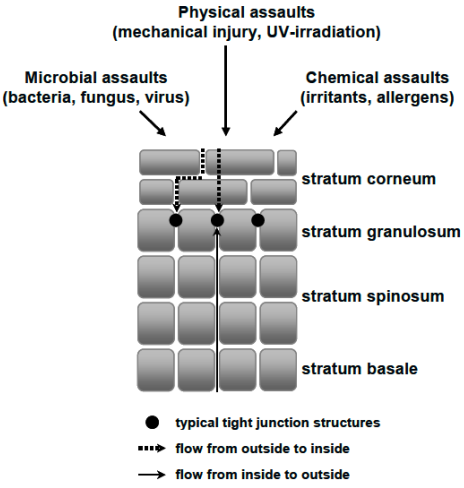
Induction of betaine, inositol and taurine transporters after UVA radiation in human dermal fibroblasts. *Experimental Dermatology* 17; 1031-1036, 2008.

Effects of betaine in skin cells



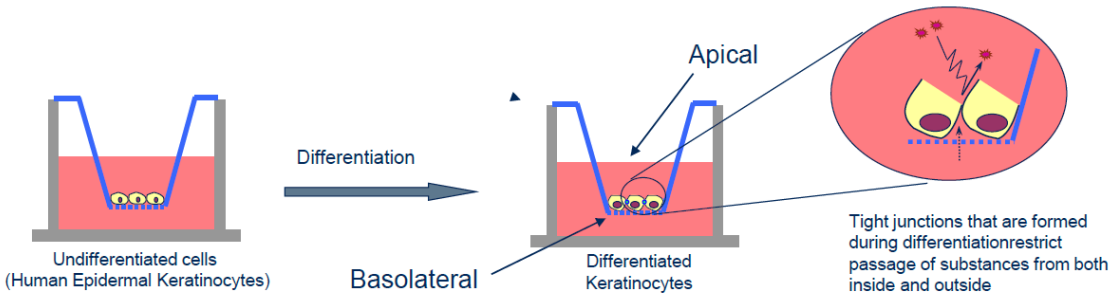
Keratinocytes and epidermal tight junction barrier

- The Stratum corneum serves as the principal barrier against the percutaneous penetration of chemicals and microbes and withstands mechanical forces.
- Epidermal tight junctions at the level of granular layer contribute to the inside-out and to the outside-in skin barrier and are important for preventing water loss
- Proper functioning of tight junctions in skin has also been implicated to be important in preventing bacterial and viral infections, allergy, and in UV-induced epidermal barrier perturbation.

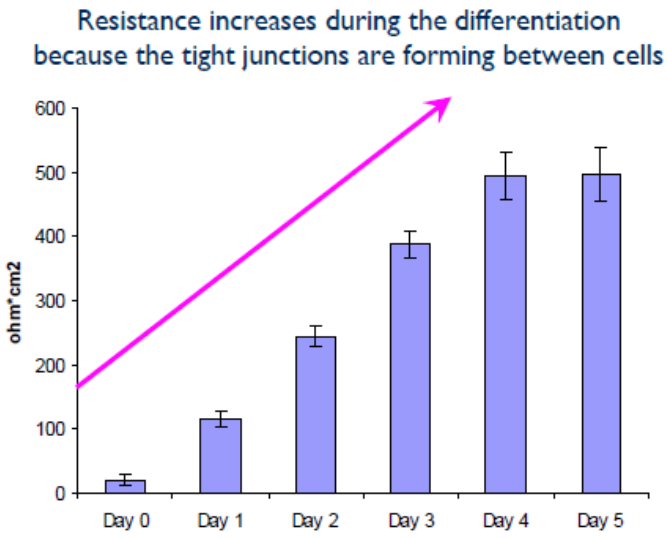


Schematic structure of the epidermis

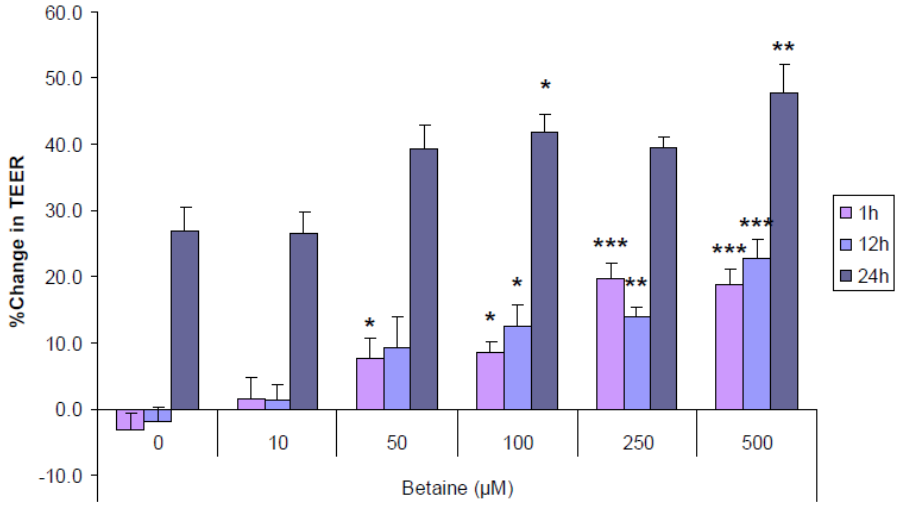
Keratinocytes as a barrier model *in vitro*



- Keratinocytes isolated from normal adult human skin are different in cell culture inserts. During the differentiation, the cells form tight junctions (=TJs) between the cells.
- The cell layer in itself is impermeable to water and water-soluble substances, but the flow of these solutes through TJs can be measured with chopstick electrodes (Transepithelial electrical resistance = TEER).
- The greater the resistance to ion flux across the TJs, the stronger the TJs between the cells and the higher the TEER values are.



Betaine Increase Tight Junction Strength



Source: Poster presentation: **Betaine increases tight junction integrity in epidermal keratinocytes;** H. Putaala, K. Tiihonen, N. Rautonen: Danisco Finland Oy, Health & Nutrition, Sokeritehtaantie20, 02460 Finland. 40th Annual Meeting of the European Society for Dermatological Research, Helsinki, Finland, September 8th to 10th 2010.

Differentiated keratinocytes were treated with betaine (0-500µM) from the apical side and the strength of tight junctions was measured using chopstick electrodes at different time points.

Betaine increases the strength of tight junctions compared to control without betaine at 24 h from application, but partially already after 1 h.

% Change in TEER = Percentage change in TEER calculated from time point 0 h, mean ± SE shown, *p<0.05, **p<0.01, ***p<0.001.