Discovering the invigorating osmolyte

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Abstract

Next to GENENCARE® OSMS BA (Betaine), DuPont Industrial Biosciences has now added GENENCARE® OSMS MI (INCI name: Inositol also known as myo-inositol) to the family of all-natural osmolytes. Like betaine, myo-Inositol, is highly purified and suitable for many skin care applications. It is extracted from non-GM sugar beet, 100% bio-based and Cosmos and Natrue certified.

This article focuses on myo-inositol and describes the many versatile functions of this ingredient in particular how it is involved in the regulation of three main elements in skin: energy, oxygen and water.

This is reflected and supported by an improved skin elasticity of the face in a large clinical test where an O/W cream with 3% myo-inositol was applied for 5 weeks and compared to a placebo cream and to the start of treatment.

Nature has designed our skin to adjust to external stress factors thanks to osmoprotectants. As an active osmolyte, myo-inositol is involved in the regulation of three main elements in skin – energy, oxygen and water; myo-inositol helps fight back the negative effects of stress and ageing by stimulating skin cells' metabolism and by improving skin oxygenation and elasticity.

Energy: Myo-inositol as stimulator of cell growth and cell metabolism

In skin, myo-inositol is a required nutrient for keratinocyte growth, mainly via increased production of PDGF-BB, a growth factor of dermal stem cells.[1] Also, in yeast, myo-inositol is a well-known growth stimulator.[2]

In an *in vitro* test, it was demonstrated that myo-inositol is essential for keratinocyte growth: At an optimal concentration of 55 pM, myo-inositol approximately tripled keratinocyte yield compared to paired cultures in basal medium containing 0.3 pM myo-inositol.[3]

Also, in a cell culture of dermal fibroblasts, addition of 0.1% myo-inositol, improved cell metabolism by increasing ATP (+62%) and protein biosynthesis (+84%) (Fig 1).[4]

In another *in vitro* test, we have recently found that myo-inositol is also able to enhance filaggrin expression. Filaggrin (filament aggregating protein) is an important structural protein in the epidermis and also a major precursor protein of Natural Moisturising Factors (NMF) in the stratum corneum. It is essential for the regulation of epidermal homeostasis and skin barrier function. Normal Human Epidermal Keratinocyte (NHEK's) were incubated for 72 hours with 3% myo-inositol and filaggrin expression was compared to control without myo-inositol by *in situ* immunolabelling using an appropriate fluorescent probe for filaggrin. The results of the immunolabelling test clearly shows an enhanced filaggrin expression. The images were also analysed and quantified by an INCell Analyzer® and showed that myo-inositol stimulated filaggrin expression by 65% (Fig 2).

All together, these data clearly demonstrate that myo-inositol is able to stimulate dermal stem cells, fibroblasts as well as keratinocytes and acts as an important energy source for the skin.

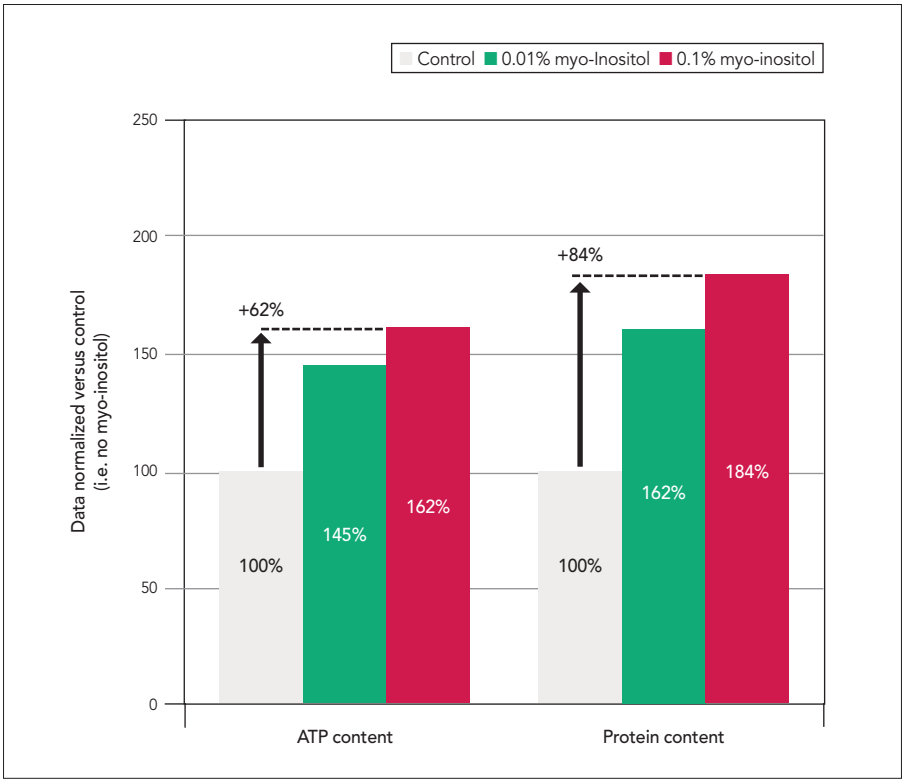

Figure 1: Improved cell metabolism by 0.1% myo-inositol.

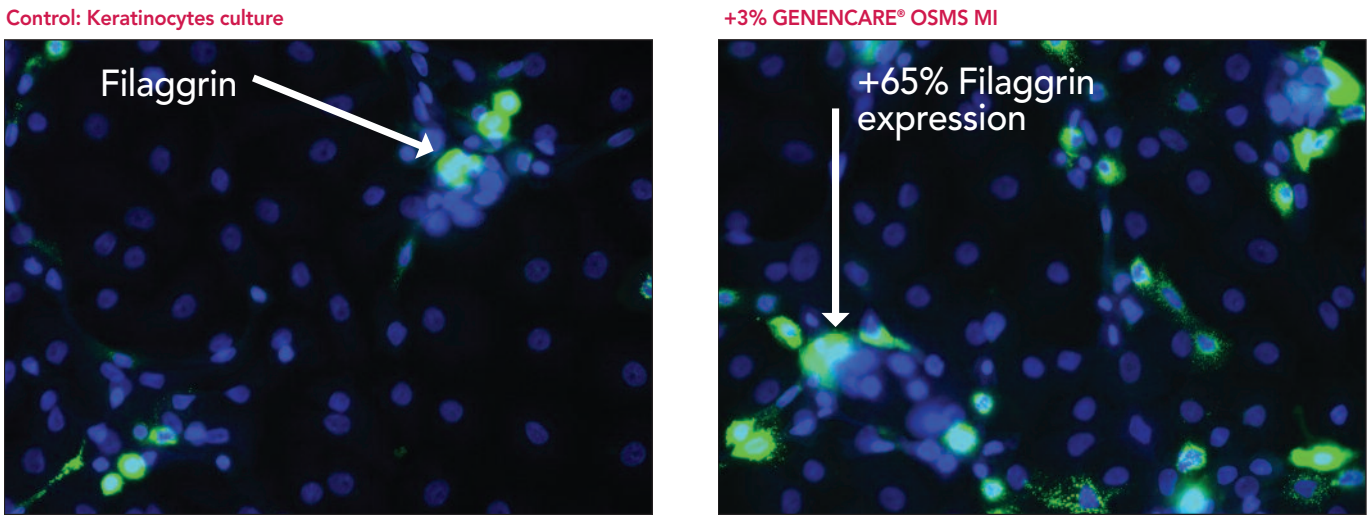

Figure 2: Improved filaggrin expression by 3% myo-inositol.

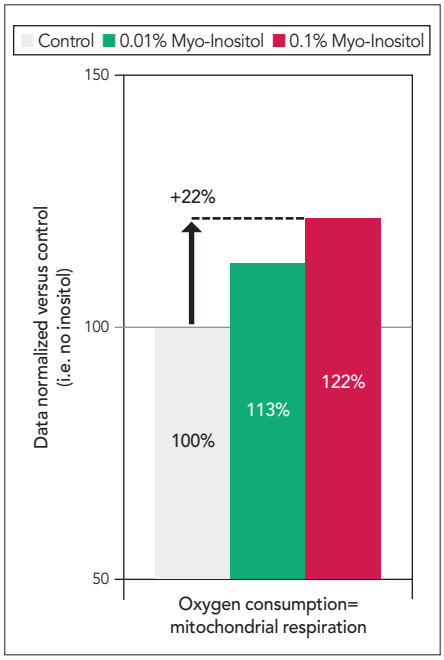

Figure 3: Improved oxygen consumption of dermal fibroblasts by 0.1% myo-inositol.

Oxygen: Myo-inositol contributes to increase skin respiration

For healthy skin, proper cell respiration is essential. It has been demonstrated that addition of 0.1% myo-inositol to a cell culture of dermal fibroblasts improved oxygen consumption by 22% (Fig 3).[4]

Also, in a clinical test, it has been shown that myo-inositol contributes to an increase of oxygen consumption *in vivo*. After a 1-week application of a cream containing 0.4% inositol, skin oxygenation increases by 10% versus control (Fig 4).

Water: Myo-inositol helps to protect keratinocytes against hyper-osmotic stress

Myo-inositol is a major osmolyte in the skin and plays an essential role in protecting skin cells from osmotic stress.

Osmolytes represent different chemical classes that occur naturally in all living organisms. In nature, three different classes of osmolytes exist: (1) amino acids (e.g. proline and glycine), (2) methyl-amines (e.g. betaine and trimethylamine-N-oxide) and (3) polyols (e.g. myo-inositol and sorbitol).[6,7]

Osmolytes fulfil two major roles: (1) Controlling and regulating osmotic processes in order to preserve intracellular solution for metabolic activities and (2) Protect macromolecular structures against osmotic stress. Many organisms and organs (like skin) accumulate osmolytes in response to dry or saline conditions, and therefore osmolytes are also often called osmoprotectants.[8]

In dry skin, keratinocytes are under hyperosmotic stress. The direct consequence would be a water efflux resulting in cell shrinkage. Keratinocytes respond by increasing the production of osmolyte transporters on their cell membrane, which results in increased uptake of osmolytes.

The main osmolytes functioning in skin are betaine, myo-inositol and taurine and their transporters are betaine/γ-amino-n-butyric acid (GABA) transporter (BGT1), sodium dependent myo-inositol transporter (SMIT) and taurine transporter (TAUT), respectively. In response to hypertonicity the

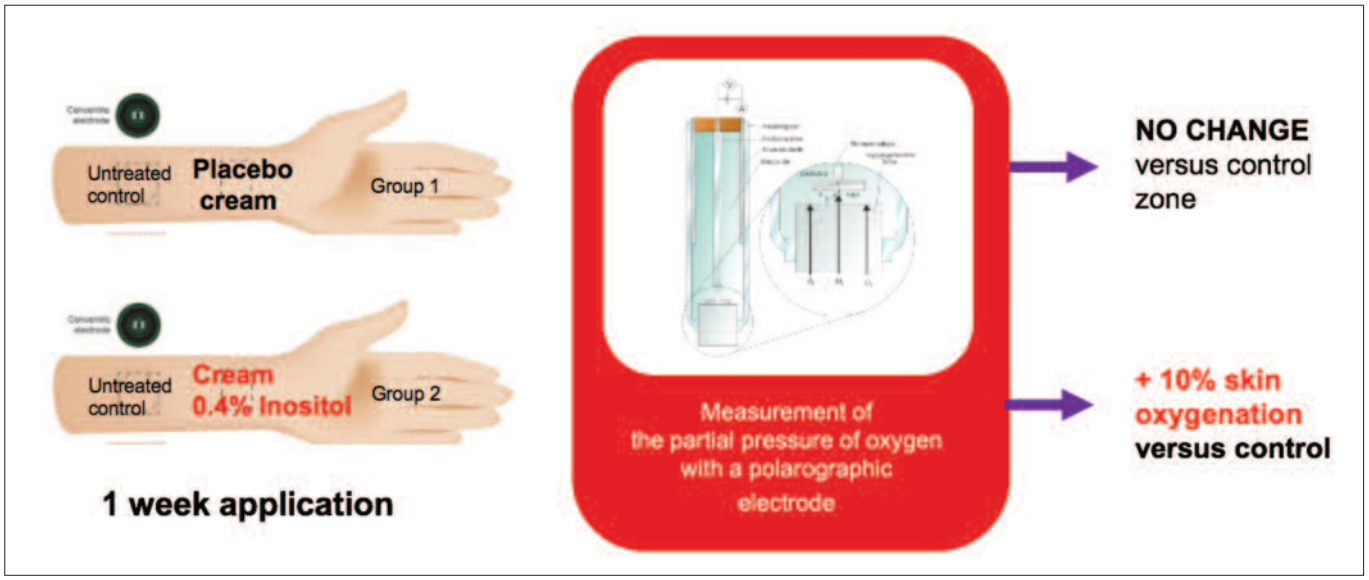

Figure 4: Improved skin oxygenation *in vivo* by 0.4% myo-inositol.

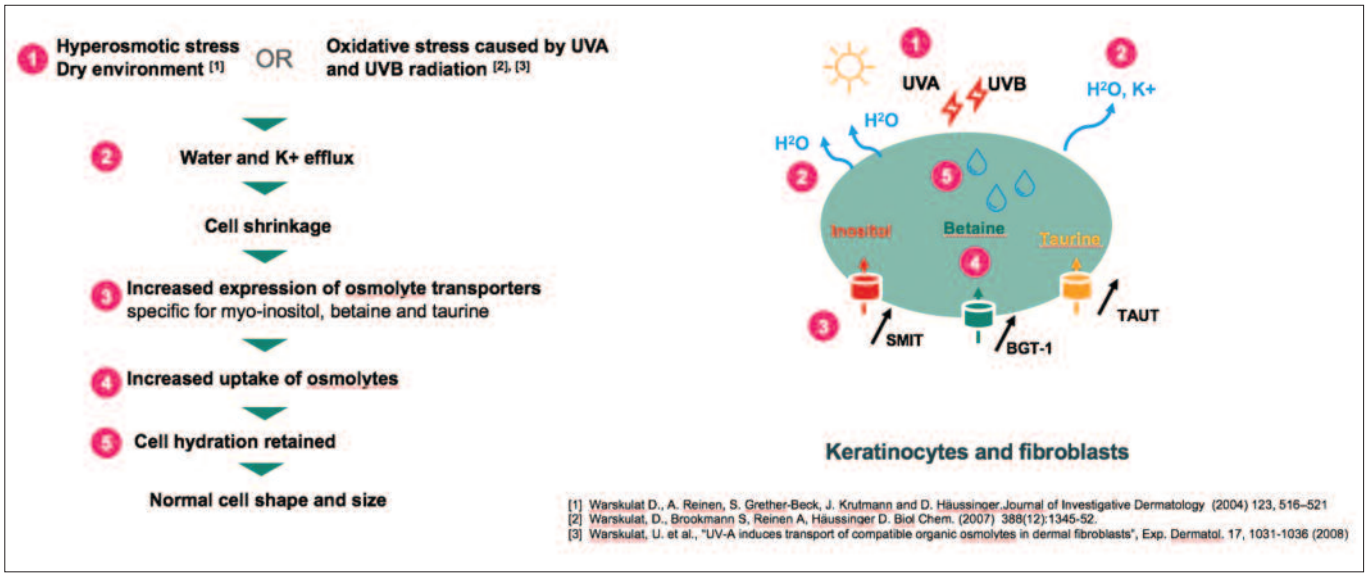

Figure 5: Control of water balance in skin by osmolytes like -myo-inositol.

transport of osmolytes betaine and myo-inositol is increased and accumulate inside the keratinocytes, followed by an osmotic influx of water that restores cell volume.[9,10]

This is an important regulatory mechanism in skin, such that, in order to maintain water balance and homeostasis of physiological processes, cells can accumulate and release osmolytes like betaine and myo-inositol in response to different external conditions, thus acting as water carriers in or out of the cell, depending on external conditions.

Keratinocytes respond by increased uptake of osmolytes like inositol. This increased uptake of osmolytes restores cell hydration and thus normalisation of dry skin to normal, well moisturised skin condition (Fig 5).

In an elegant *in vitro* experiment we have demonstrated that myo-inositol is able to manage the water balance of keratinocytes and protects them from hyperosmotic stress.

Figure 6 shows the microscopic images of NHEK cells under normo-osmotic conditions, hyperosmotic conditions and hyperosmotic conditions in the presence of 3% myo-inositol, respectively.

These results show a strong osmoprotective effect of myo-inositol, demonstrated by maintenance of total number of cells, cell size and volume and cell viability, comparable to NHEK cells under normal iso-osmotic conditions.

Treatment of NHEK's with 150 mM NaCl for 48 hours clearly decreased the number of cells and cell size (15% and 57% of the non-treated control, respectively), and decreased the % of viable cells from 68% to 32%, indicating loss of water under these hyperosmotic conditions. Remarkably, the presence of 3% myo-inositol (30 mg/ml) resulted in a strong protection of total number of cells (72% compared to the normo-osmotic control), cell-size (98% compared to the normo-osmotic control) and cell-viability (90% of viable cells), showing a clear protecting
effect against hyperosmotic stress and a reverse to almost normo-osmotic conditions (Fig 7).

Overall effect: Improvement of skin elasticity

Based on the above positive results of myo-inositol on the controlled management of energy, oxygen and water, we decided to perform another *in vivo* in-use test to assess improvement of the overall biomechanical properties of facial skin (40 volunteers, 5-week application test, twice daily on face). An O/W cream with 3% GENENCARE® OSMS MI was applied for 5 weeks on the face and compared to a placebo cream and to start of treatment (t=0). The results (Fig 8) clearly shows that the repeated application of a 3% myo-inositol containing cream significantly increased both parameters Ur / Uf (Rate of elastic recovery to the total deformation) as well as Ur / Ue (Rate of elastic deformation), while Uf (Total deformation) is stable, which overall is representative for a more elastic skin.[11,12,13]

It is interesting to mention in this respect that ageing skin shows a continuous decrease in Ur/Uf, which may amount to more than 50% after seven decades (Figure 9; from ref 14) and thus, the demonstrated significant increase of especially the R7 (Ur / Uf) elastic recovery parameter by GENENCARE® OSMS MI

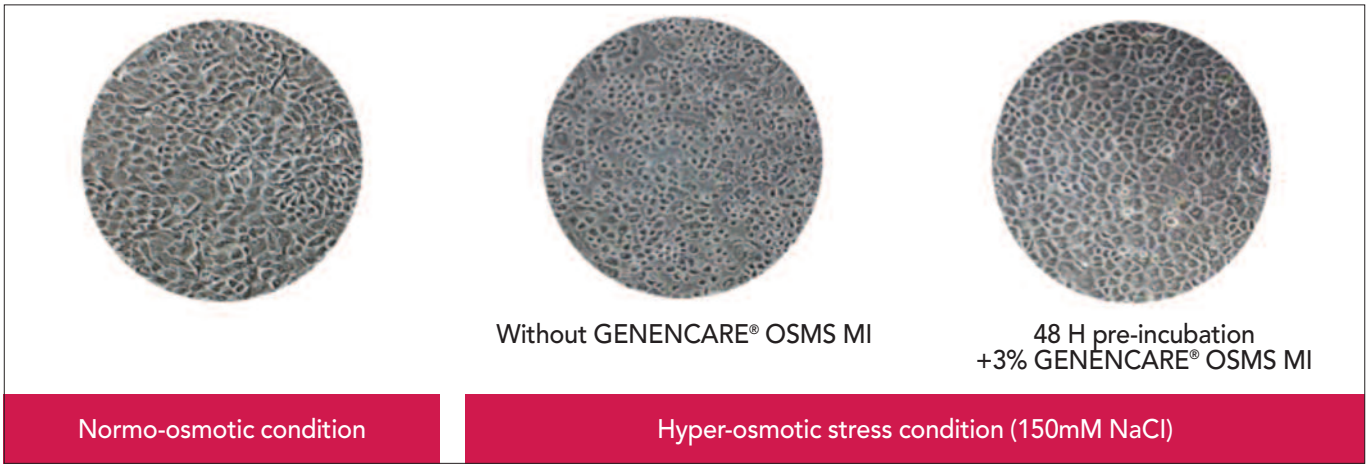

Figure 6: Cell survival and morphology assessment of keratinocytes under hyperosmotic stress. Microscopic pictures.

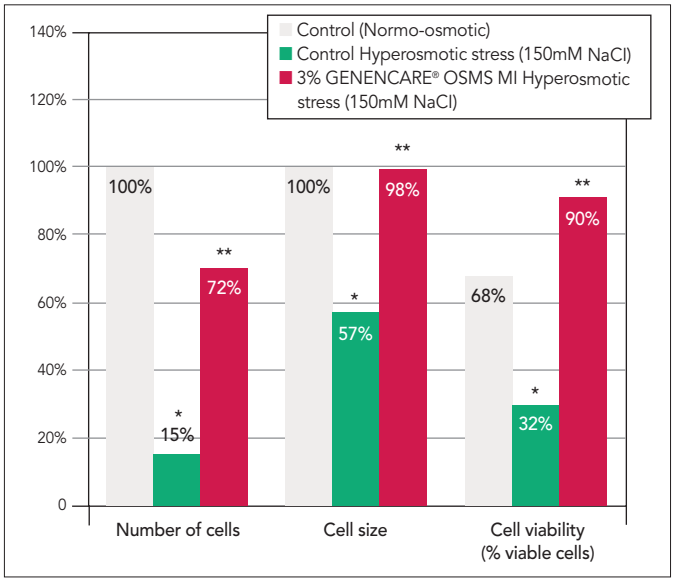

Figure 7: Cell survival and morphology assessment of keratinocytes under hyperosmotic stress. Flow cytometry.

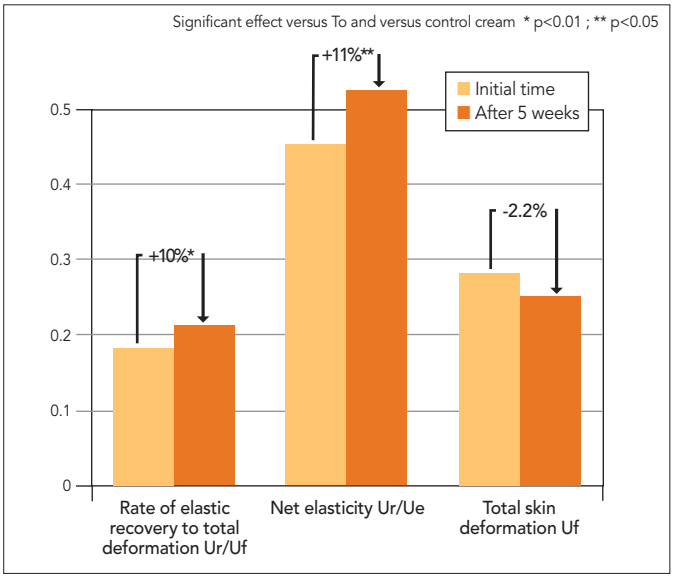

Figure 8: Improved biomechanical properties of the skin of face by 3% myo-inositol.

shows its efficacy in mitigating the signs of ageing. PC

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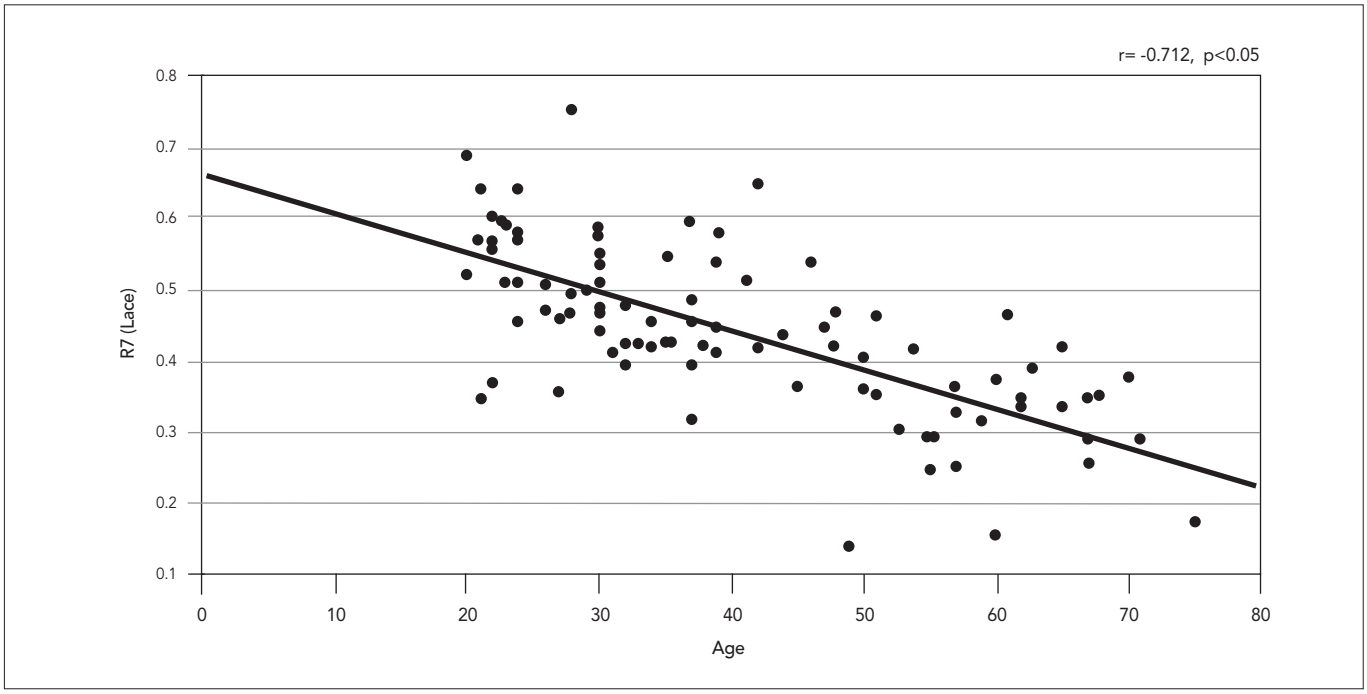

Figure 9: Correlation between elastic recovery and age on the face (from ref. Ryu, 2008).