

Creatine

Revitalizing Of Skin Cells' Energy Metabolism:

The physiological fatigue of skin evidenced by the early signs of aging is caused by stress and environmental influences. The consequence is a decrease of the energy amount stored in the cells followed by a suppressed capability to synthesize proteins such as collagen, elastin, and keratin. Additionally, cells experience increased oxidative stress as they age, increasing the amount of energy required for internal defenses. So, a well-working energy metabolism is essential for skin cells vitality. Creatine is very effective at stimulating the cells' energy metabolism.

The "cell energizing" effect of Creatine was assessed in- vitro in a cell culture (Human keratinocytes, NHEK cells). A pre-screen cytotoxicity study was conducted using the Neutral Red assay, showing the non-cytotoxicity of Creatine.

Keratinocytes were incubated with Creatine for 24 hours at 37 °C. After this the cell vitality was measured with XTT, a water-soluble Formazan dye. The results are summarized in Fig. 1.

The diagram indicates that the vitality of the cells treated with 0.01 % Creatine was about 60 % higher than the control vehicle.

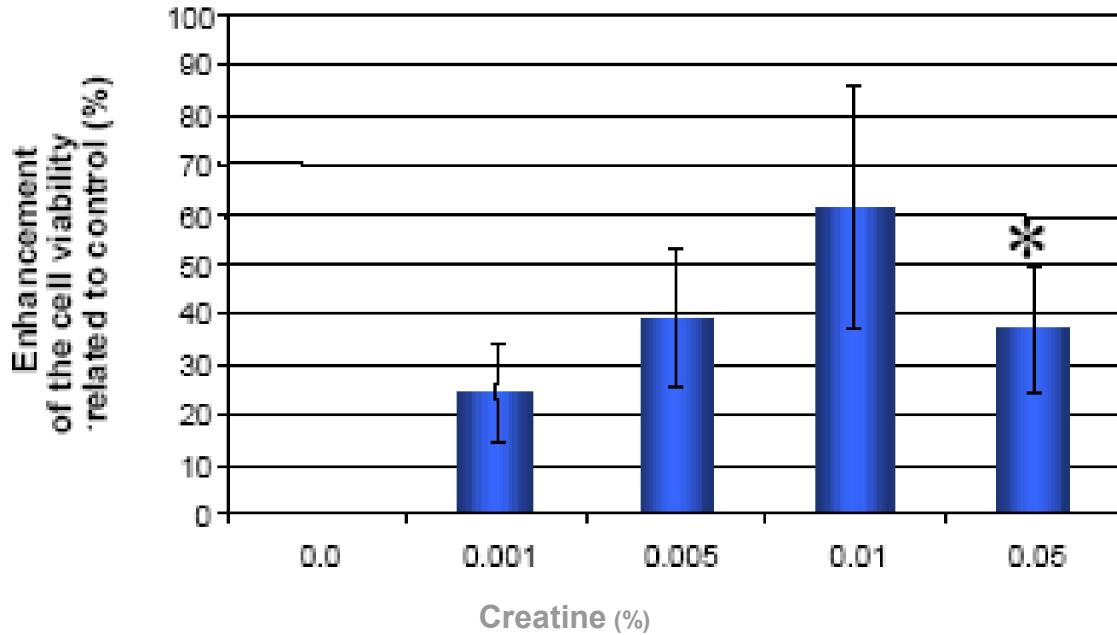
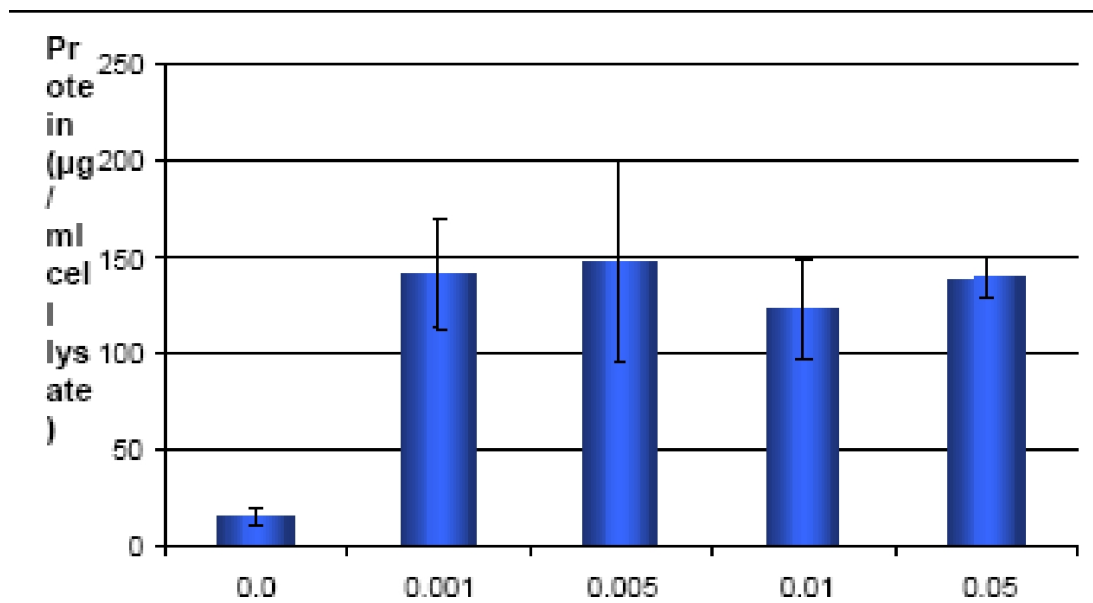


Fig.1: "Cell energizing" test with keratinocytes.
* Saturation of the cells with in vitro concentrations > 0.01 % .

Increased Cellular Protein Content

The cell energizing effect of Creatine causes an increase of the cellular protein content.

(Fig. 2): The effect was demonstrated after a 24 hour incubation with several concentrations of Creatine. Keratinocytes were lysed followed by an analysis of protein amount (Method of Bradford).

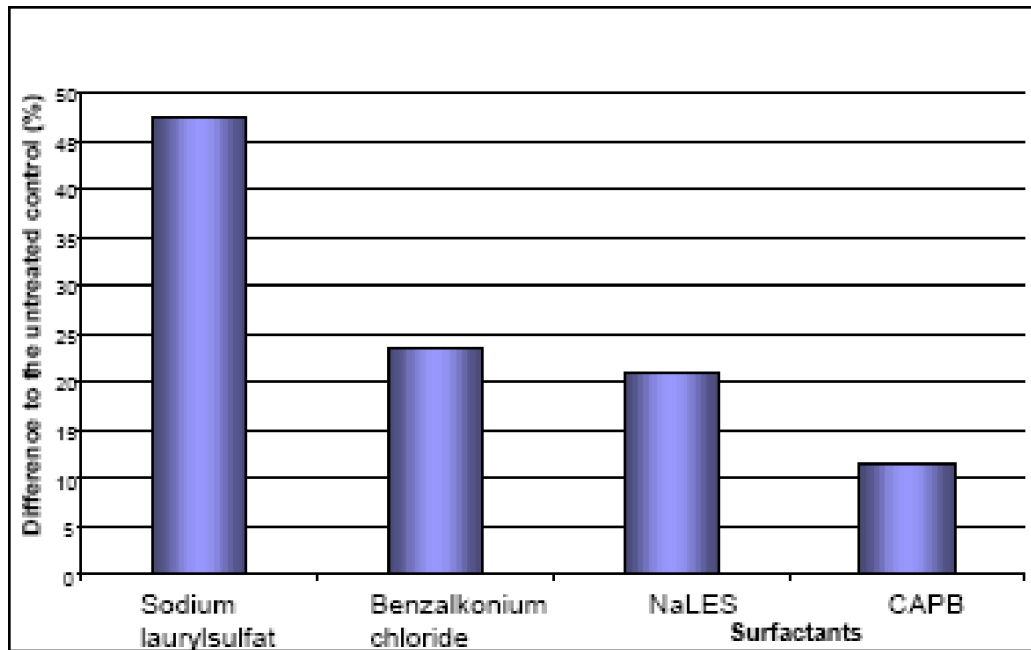


(Fig. 2: Protein assay of cell lysates.) (Enhancement of the cell viability % related to control %.)

Because Creatine **stimulates both the mitochondrial activity and the protein synthesis** of the skin, it provides longterm protection from premature aging and environmental stress, which is important for skin soothing and anti-aging formulations.

Membrane stabilization by Creatine:

Creatine helps to ameliorate skin irritation from various environmental factors. A modified red blood cell (RBC) test was used to evaluate this effect on cell membrane stabilization.



(Fig.3: Modified red blood cell (RBC) test.)

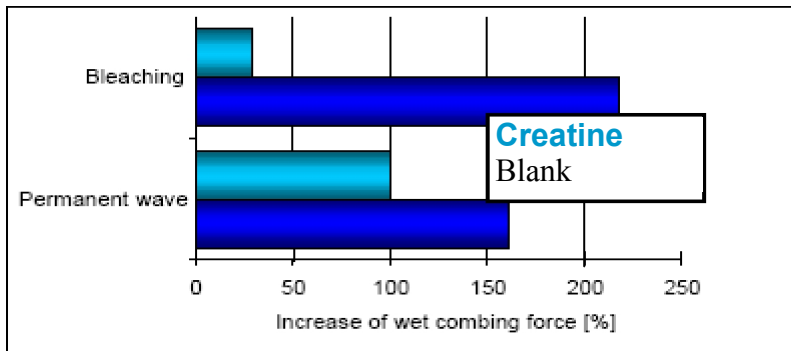
A defined number of erythrocytes (red blood cells) were treated for 1 hour with phosphatebuffered saline PBS as a standardized biological buffer (control) or PBS containing 1 % of Creatine. Afterwards, blood cells were stressed for 10 minutes with surfactants at defined concentrations. Damaged, or lysed, cells can be determined by a spectroscopic determination of free hemoglobin. Free hemoglobin is proportional to the number of lysed cells.

The efficacy of Creatine to reduce hemolysis (as compared to the blank) is clearly demonstrated in Fig.3. A higher percentage difference indicates greater protection.

It can be shown that damaging effects caused by surfactants are mitigated in cells preconditioned with Creatine.

Hair Conditioning Properties:

Creatine has a conditioning effect on hair. It improves body/volume and the dry feel of hair treated with Creatine before a bleaching or a permanent wave. Creatine smoothes the surface of the cuticle (Fig.5) and as a consequence the increased combing forces by permanent waving or bleaching are minimal (Fig.4). **This in turn reduces the physical damage which results from combing damaged hair.**

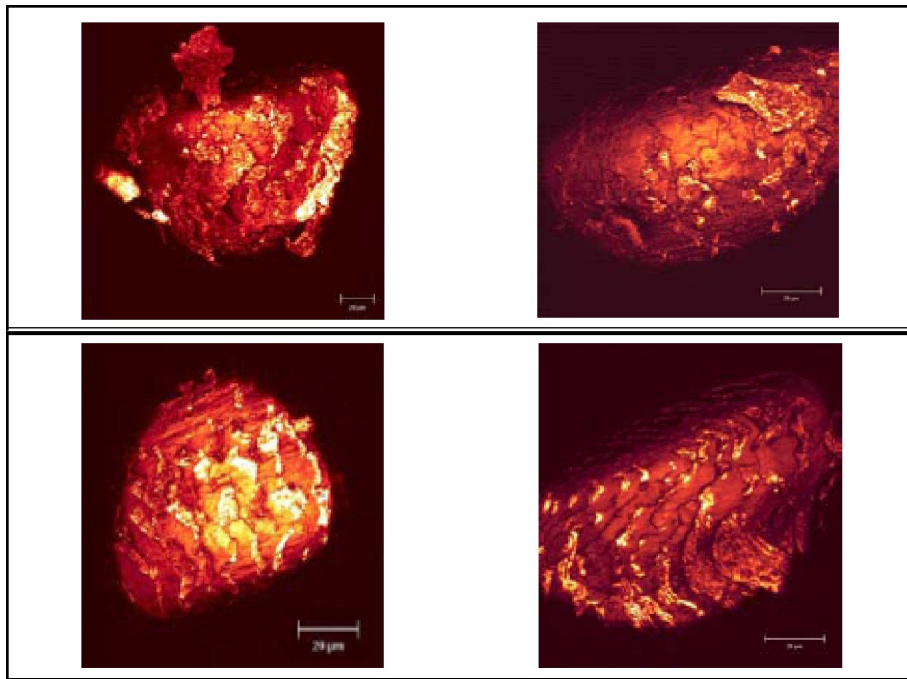


(Fig. 4: Wet combing measurements.)

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In Figure 5: Predamaged (permed and bleached) European brown hair was treated with

Creatine followed by a further damaging step (bleaching or perming). Afterwards the wet combing forces were measured.



Placebo.

1. Damaged by permanent waving+ bleaching.
2. Treated 5 times with **Placebo** formulation.
3. Damaged again by bleaching.

Creatine (2 % solution).

1. Damaged by permanent waving + bleaching.
2. Treated 5 times with **Creatine (2 % solution)**.
3. Damaged again by bleaching.

(Fig. 5: Effect of **Creatine** on the hair surface.)