

# Effect of anti-aging peptide on age-related changes in N-glycosylation process in fibroblasts and skin elasticity *in vivo*

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Aging results mainly in the loss of dermal collagen and the accumulation of unorganized collagen and elastin fibers in the dermis, however it is also contributed to glycoproteins and proteoglycans deficiency. Protein N- and O-glycosylation of eukaryotic secretory and membrane-bound proteins, is fundamental protein modification, serving many intra- and extracellular functions. The group of disorders caused by alteration of this process display severe phenotypes which can affect also skin tissue, like skin laxity, severe wrinkling, dryness or psoriatic lesions. So far changes in the activity of protein glycosylation during skin aging were not reported. Thus the aim of the study was to evaluate the efficacy of anti-ageing peptide on protein N-glycosylation process and impact of cosmetic formulation containing this peptide on skin elasticity *in vivo*. The activity of Dpm1 (dolichyl-phosphomannose synthase) was checked before and after anti-aging peptide treatment in the fibroblast derived from individuals in different age. The *in vivo* study of cream was conducted on 26 volunteers. Skin condition was analyzed after 4 weeks of product usage by VISIA and Cutometer. The *in vitro* assay showed decrease of Dpm1 activity in older cells. This effect was reversed by addition of anti-ageing peptide. In the *in vivo* studies we observed that skin elasticity improved by 108% (R0 measurements). In addition to this we observed improvement in skin elasticity displaying the level characteristic for younger subjects. This result suggest that N- and O-glycosylation might be altered with age and select cosmetic active compounds may reduce symptoms of skin aging.

## Materials and Methods

### Cell lines and cell cultures

The fibroblasts from donors - age 38 (M38) and 58 (P58) were maintained at 37°C under 5% CO<sub>2</sub> in Dulbecco's modified Eagle's medium containing 10% FCS and antibiotics: 100 U/ml penicillin, 100 µg/ml streptomycin. Cells were treated with cosmetic actives for 21 days followed by enzymes activity estimation. Cosmetic active used for the experiment: A – anti-aging peptide in concentration. Preparing microsomal fraction from fibroblasts according to Thiel et al. (Thiel et al. 2002, Biochem. J. 367, 195-201).

### Enzyme activity

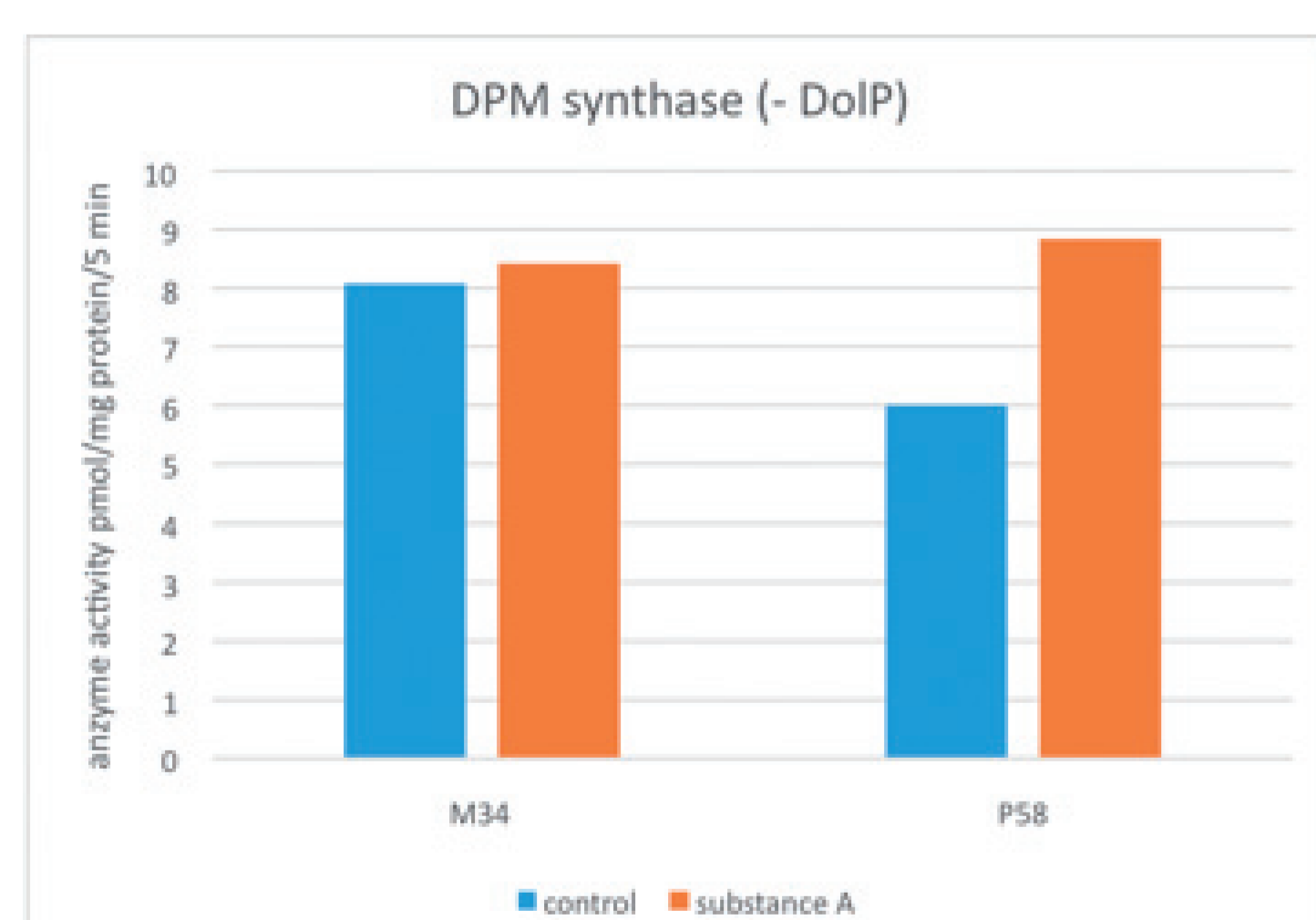
The activity of DPM was measured by using <sup>14</sup>C radioactive substrate, GDP-mannose. Reaction was run without dolichol phosphate (DolP). In this reaction, sugars from GDP-mannose were transferred to endogenous DolP presented in microsoms. Activity of DPM was measured in buffer containing 7mM Tris-HCl pH=7,2, 7mM MgCl<sub>2</sub>, 0,1% Nonidet P40, 100 000 cpm <sup>14</sup>C – GDP-mannose and 500 µg protein from microsomes. After 5 min of incubation in 28°C the reaction was terminated by adding 4 ml of chloroform:methanol 3:2 (v/v). After washing out unreacted substrate, the amount of radioactive product – DPM was measured in scintillator.

### Clinical trials

The study was conducted on 26 volunteers: cream no 2620 contained ceramides, anti-ageing peptide, oil wax, avocado oil and UV filters. Product was used once daily for a period of 4 weeks. Skin condition was analyzed by VISIA Complexion analysis as well as Cutometer probe.

## RESULTS:

The *in vitro* assay of enzyme activity showed decrease of Dpm1 activity in older cells, which was probably due to lack of available substrate in cells. This effect was reversed by addition of anti-ageing peptide, and what is more, the activity of Dpm1 in older cells increased to that same level as in younger cells. This result suggest that N- and O-glycosylation might be altered with age, which can cause skin dryness or wrinkling and select cosmetic active compounds may reduce symptoms of skin aging.



**Figure 1.** DPM synthase activity after treatment with anti-ageing peptide in 0,01% concentration. Treatment of the fibroblasts for 21 days with substance increased activity of the enzyme in cell line P58 derived from older donor. Activity also increased to the level characterized for cell line M34 obtained from younger subject.

In the *in vivo* studies we observed that skin elasticity improved by 108% (R0 measurements). In addition to this we observed improvement in skin elasticity displaying the level characteristic for younger subjects.

	Number of subjects	Before the test	Improvement in the whole group	Actual improvement
Elasticity (R2)	n=26	100%	106%	Increase of elasticity by 20 % in 69 % of subjects
Firmness (R0)			208%	Increase of firmness by 108% in 100% of subjects

**Figure 2.** Improvement in the skin elasticity and firmness after 4 weeks of using daily cream no 2620 measured by Cutometer probe.



**Figure 5.** The best result - volunteer (65 years) before and after 4 weeks of using daily cream no 2620.

ID	R2 measurement before the test	R2 measurement after 4 weeks of using daily cream no 2620	actual age of subject	Age of subject after 4 weeks of using daily cream no 2620
1	0,54	0,59	64	40-49
2	0,57	0,64	60	30-39
3	0,51	0,68	66	20-29
5	0,61	0,73	52	<20
6	0,60	0,74	60	<20
7	0,56	0,75	65	<20
8	0,60	0,65	62	30-39
9	0,46	0,47	60	50-59
10	0,41	0,61	59	40-49
11	0,52	0,55	53	40-49
13	0,60	0,62	51	30-39
14	0,57	0,61	62	40-49
15	0,52	0,68	61	20-29
16	0,46	0,65	54	30-39
17	0,46	0,54	60	50-59
18	0,49	0,75	66	<20

**Figure 3.** Changes in the level of skin elasticity after 4 weeks of using daily cream no 2620.

VISIA (n=6)	before	number of features	result
Wrinkles	100%	average reduction of wrinkles by 30% in 100 % of subjects	average reduction in the level of intensity of wrinkles by 33 % in 100 % of subjects

**Figure 4.** Average improvement in the quantity and intense of wrinkles after 4 weeks of using daily cream no 2620 measured by Visia.

## CONCLUSION:

The changes in activity of key enzymes in protein N-glycosylation process has been demonstrated. It revealed decrease in enzyme activity, such as DPM synthase, which is altered with age. Results of the *in vivo* research has shown its positive influence on the skin condition. It is suggested that certain cosmetic substance up-regulating N-glycosylation process could reduce the signs of aging.

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