Skin aging in different ethnicities and treatment effects by a hydrophobically modified dipeptide in Caucasians, Asians and Black Africans

R. Campiche*, S. Boudon, R. Voegeli, D. Paxton, A. Rio, D. Imfeld

DSM Nutritional Products Ltd., Personal Care, Kaiseraugst, Switzerland

* correspondance to: remo.campiche@dsm.com

Keywords: multi-ethnicity study, anti-aging, collagen maturation

Introduction

Skin aging is of global concern as it is linked with social acceptance and status. However, perception and manifestation of skin aging differs among different ethnicities and regions. Behavioural and constitutional differences can lead to a different level of skin condition and appearance. Recent studies showed significant facial differences in terms of pores (1), wrinkles and sagging (2-4) and transepidermal water loss (TEWL) and skin capacitance (5). In addition, extrinsic aging varies among different ethnic groups and domiciles (6-7).

Hence, our aim was to develop a cosmetic active dedicated to the specific needs and aging differences of distinct ethnic groups. We therefore investigated consumer perception in different locations for differences of beauty aspiration. In addition, we measured these differences in Caucasian, Asian and African volunteers and the treatment effects with a dipeptide (INCI: Dipeptide Diaminobutyroyl Benzylamide Diacetate), assuming an anti-wrinkle and pore-size reduction activity. In parallel we also wanted to understand the underlying molecular mechanism of the peptide *in-vitro* using a human dermal fibroblast model.

Summary

Recent research concerning facial skin aging revealed differences in wrinkles and pores among distinct ethnic groups. In addition, perception and concerns of facial aging differ among various ethnic groups. In line with this we observed ethnicity- and facial site-dependent differences in wrinkle surface of Caucasian, Asian and Black African volunteers. Our results suggest that facial aging is not a clear cut ethnicity-dependent process. We, and a very recent publication (7), found evidence that skin aging may also depend on facial site and that this varies in different ethnic groups. Complex facial distributions of TEWL and skin hydration were found previously in different ethnic skin (5). Regarding treatment with the dipeptide we found distinct in-vivo activity for wrinkle reduction and a decrease in facial pores. The biggest improvements were found in Caucasian skin which correlated with the volunteer's perception and the overall most pronounced pattern of wrinkles for this group. We could also observe specific effects for Asian and Black African skin.

Material & Methods

Consumer insight study

18 qualitative interviews in Beijing and Wuhan with Chinese middle class women; 19 qualitative interviews in New York and Los Angeles with Hispanic and Black African American women; quantitative online study with 2000 women globally with Chinese, Caucasian, Hispanic and Black African American women on skin care behaviours.

In-vivo study

Placebo controlled, full face, and parallel groups study. Volunteers had to apply the cosmetic formulations twice daily for 28 days.

Subjects

50 female subjects minimum per cultural group participated in the study. Group A1(placebo/control): 25 subjects minimum. Group A2 (product containing active group): 25 subjects minimum (Table 1).

Table 1: The study took place at the following locations with the following volunteers:

Cohort:	Location:	Phototype*:	Age in years:
Caucasian	Lyon (France)	II-III	40-55 (median 46)
Asian	Bangkok (Thailand)	III-IV	41-55 (median 47.5)
Black African	Mauritius Island	V-VI	41-64 (median 55)

*Fitzpatrick skin phototype

Wrinkles analysis

Of Caucasian and Black African volunteers two photographs of half-face (45°), left and right angles and one photograph of full face were taken with the Color Face System (Newtone Technologies, Lyon, FR), and the Asian volunteers were photographed with the standard setting of Visia CR® device (Canfield, Parsippany, US) for wrinkle and pore analysis. To be able to compare the wrinkle data among all three ethnic groups we re-calculated length and surface of wrinkles into mm and mm2.

In-vitro study:

Quantification of gene-expression in cell culture: Normal human dermal fibroblasts from female donors (18 and 63 years old) were grown for 24hrs in culture medium in 24 well plates. Medium was changed to assay medium and further incubated for 24hrs before treatment with the dipeptide or TGFß1. The cells were washed with PBS, frozen at -80°C after 7 and 24hrs and processed for gene-expression analysis. Gene-expression was measured on a LightCycler system (Roche Molecular System Inc, Pleasanton, US). Two technical measurements were taken and averages calculated.

Results

Figure 1: Our consumer insight research revealed that contrary to Caucasian women, women in Asia and specifically in China were less concerned about wrinkles, but more about fine lines, age-spots and uneven skin tone. Furthermore, we learned that while cutaneous research revealed differences in ethnic skin (1-7), people with darker skin tones in North America focus more on specific skin needs, for example hyperpigmentation and bump free skin, rather than responding to targeted ethnic communication.

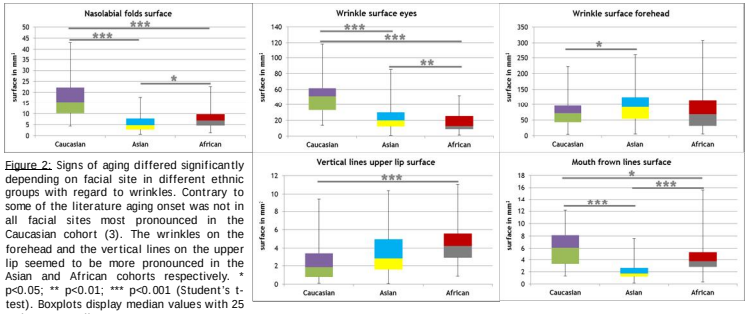

Figure 2: Signs of aging differed significantly depending on facial site in different ethnic groups with regard to wrinkles. Contrary to some of the literature aging onset was not in all facial sites most pronounced in the Caucasian cohort (3). The wrinkles on the forehead and the vertical lines on the upper lip seemed to be more pronounced in the Asian and African cohorts respectively. * p<0.05; ** p<0.01; *** p<0.001 (Student's t-test). Boxplots display median values with 25 and 75 percentile.

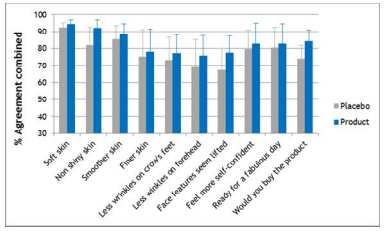

Figure 3: Consumer questionnaire of all three ethnic groups combined. The product containing the active (blue bars) outperformed the placebo for all the criteria. Interestingly, Caucasian women were most critical in assessing the product, but improvement rates of the active vs the placebo were highest in the Caucasian cohort (not shown). Error bars represent standard error of the mean of the three ethnic groups.

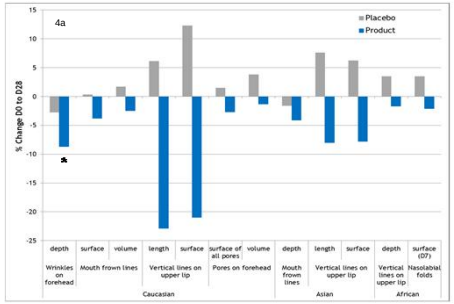

Figure 4a: Anti-aging efficacy of the active versus placebo after 28 days of treatment (except where D7 is indicated). Most changes were seen in Caucasian women. Reduction of wrinkle depth on forehead was significant (* p<0.05; by ANOVA) to D0. The only reduction in pores was measured in Caucasian volunteers, whereas a decrease in vertical lines on upper lip was measured in all three ethnicities with the most pronounced effect in Caucasian volunteers.

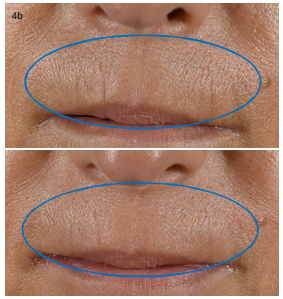

Figure 4b: Exemplary images showing vertical lines on upper lip at day 0 (upper image) and treated with active at day 28 lower image) from Caucasian volunteer #27. Blue circles highlight the area where the decrease in visible wrinkles was most apparent.

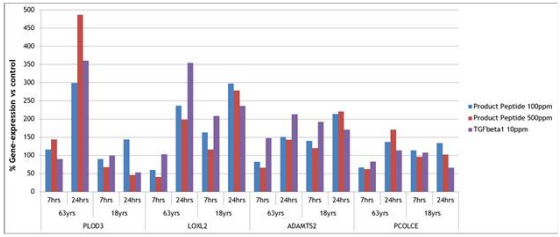

Figure 5: Gene-expression analysis of human dermal fibroblasts from a 63 year old female donor and a 18 year old female donor. Gene-expression of genes PLOD3, LOXL2, ADAMTS2 and PCOLCE (all involved in collagen fiber processing and maturation) was normalized to vehicle treated control expression (100%). The dipeptide at 100ppm and 500ppm, as well as TGFþ1 at 10ppm were incubated for 7 and 24 hours at which time points total RNA was extracted and gene-expression measured. PLOD3 could be induced in the 63 year old but not the 18 year cells. Gene LOXL2 was induced more than 2fold in both 63 year and 18 year old cells. Gene ADAMTS2 was expressed 2fold only in 18 year old cells by the peptide. Gene PCOLCE only showed minor induction of 1.5fold in 63 year old cells. The four genes considered in this dataset are all involved in collagen processing or fibril formation. In conclusion and similar to the natural cytokine TGFþ1 the results suggest a role for the dipeptide in collagen processing and maturation like lysyl hydroxylation.

References

1. Flament, F., et al., Clin Cosmet Investig Dermatol. 8:85-93, 2015
2. Tsukahara, K., et al., J Dermatol Sci. 47(1):19-28, 2007
3. Nouveau-Richard S., et al., J Dermatol Sci. 40:187-93. 2005
4. Fujimura T., et al., Int J Cosmet Sci. 31(5):361-7, 2009
5. Voegeli, R., et al., Int J Cosmet Sci. 37(6):595-605. 2015
6. Vierkötter A., et al., Dermatoendocrinol. 4(3):227-31, 2012
7. Vierkötter A., et al., J Dermatol Sci, in press, 2016

Acknowledgements

This study was fully funded by DSM Nutritional Products Ltd, Basel, CH
Dermscan, Lyon, F, conducted the *in-vivo* study
Newtone Technologies, Lyon, F, did the image analysis
Bioalternatives, Gençay, F, did the *in-vitro* study

29th IFSCC Congress, 2016, Orlando

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.