



Title of the paper

Topical niacinamide enhances hydrophobicity and resilience of corneocyte envelopes on different facial locations

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Conflicts of interests and disclosures

RV is an employee of DSM, AVR is a consultant to DSM and MC is an employee of Newtone Technologies. BS, DG and MEL have no conflict of interest.

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The stratum corneum (SC) undergoes a variety of catabolic and anabolic reactions towards its outer surface layers in preparation for its external assault from the terrestrial environment [1]. These events are essential for the formation of a healthy barrier. Key is the maturation of the corneocyte envelope (CE) (Fig. 1). Morphologically CE's appear fragile in the deeper layers of the SC and more rigid in the outer layers, the mechanics of which have been confirmed with several biomechanical approaches [2]. Early approaches to assessing the maturity of the CE's were based upon staining with tetramethylrhodamine isothiocyanate (TRITC) where rigid CEs stain more intensely than fragile ones [3]. Later their protein content was assessed by involucrin immunochemistry and lipid content was assessed by Nile red staining which was then expressed as a ratio [4-9]. Using these approaches improvements in CE maturity of the volar forearm and the legs have been observed with moisturisers containing glycerol [3, 10] and niacinamide [11].

We have recently published on the regional complexity of facial skin using a variety of bio-instrumental approaches [12, 13] and also developed new CE maturation assays to further probe the corneocyte lipid envelope (CLE) hydrophobicity and corneocyte protein envelope (CPE) structural integrity as independent parameters [14]. These findings prompted us to assess the effect of niacinamide in a 4 week placebo controlled, randomized topical study on these biochemical and physical parameters on several facial skin sites in young (n=24, 20.8±1.7 years old) and old Caucasian study participants (n=24, 57.5±2.9 years old) living in Pretoria, South Africa. The study was approved by the School of Health Care Sciences

Research Committee (SRC) together with the Medunsa Campus Research and Ethics Committee (MREC), South Africa and took place from July to September 2016. All participants provided written informed consent before start of the study.

After a three day wash-out phase either the vehicle or the test cream including 3% of niacinamide (Table I) were applied twice daily (morning and evening) onto the face after cleansing. The subjects were randomly assigned to use either the vehicle or the test cream. A simple but panellist-acceptable vehicle was chosen with a suitable penetration enhancer, in this case 1-2 propanediol. 2% [15] and 5% [11] concentrations of niacinamide have been used in the past to evaluate its clinical efficacy. 3% was selected as an intermediary concentration in these studies.

The application dosage was approximately 2 mg cm^{-2} of skin. Single tape-strippings (D-Squames) were taken on four pre-defined facial sites (central forehead (CF), cheek, 3 cm vertically beneath the outer edge of the eye (CH), top nasolabial sulcus (NT), and midpoint nasolabial sulcus (NM)) before the first product application and after the four weeks treatment phase (Fig. 2) as previously reported [14]. The CLE and CPE were assessed using our recently published methods [14].

Microsoft Excel Office 2016 was used to analyze the data. Normality was assessed using the Kolmogorov-Smirnov statistical test. Paired T-test was used to compare means and investigate differences between sites and probability of $p < 0.05$ was considered statistically significant. All results are presented as mean and standard error of the mean.

We first performed baseline comparisons of the effect of ageing on CE maturation parameters (size, hydrophobicity, rigidity and relative CE maturity (RCEM)). Others have observed an ageing-related increase in corneocyte size that we also observed except for the NT site (Fig. 3a). More pronounced changes were observed for hydrophobicity (Fig. 3b) and sonication-stress resilience (Fig. 3c). Interestingly for the former, increases were observed on the CF and CH areas with age whereas the other two sites were less hydrophobic (Fig. 3b). These results further demonstrate the complexities in facial aging and the sensitivity of our assays as previous studies have found no age-related changes in the Nile red to involucrin comparison [16]. A decrease in hydrophobicity was expected as decreased covalently bound lipids have been observed on forearm SC as well as decreased levels of substrate and associated enzymes necessary for their attachment in photoaged skin sites. This could be the reason for the decline in the hydrophobicity in the aged nasolabial areas as these facial sites are those recently reported to have the highest levels of oxidative stress [17]. The aging related

decreases in CE rigidity were consistent across all four sites (Fig. 3c). When considering all three measures as the RCEM (Fig. 3d) only the cheek had a small increase in maturation with ageing whereas the nasolabial areas were consistently immature. These results highlight the complexity of facial site CE maturation status and the impact of aging. The changes we observe with aging relative to the lack of change in other studies may highlight the increased sensitivity of our methodology.

Next we examined the effect of niacinamide on these parameters. Only a small increase in corneocyte size was observed following treatment on the NT site (not shown). Numerical increases in size were observed on the other test sites and are in agreement with other studies [11]. Hydrophobicity and CE rigidity increased in all facial sites and both age groups which was then reflected in the RCEM (Figs. 4a-c). Nevertheless there was a marked vehicle effect suppressing CE maturation that has also been observed in previous studies [11]. Despite the vehicle effect the biggest changes in hydrophobicity were observed on the CF for the young subjects and the NT for the older subjects. The NM had the least change in both age groups. The biggest changes in CE rigidity were observed in the older subjects probably reflecting their lower starting levels. Clearly niacinamide treatment improves CE maturation as measured by our methodology but CE hydrophobicity is not improved as much in the NM area.

Our data on the effects of niacinamide on facial skin are largely consistent with another publication using the Nile red/involucrin ratio following treatment of the volar forearm [11]. However, using our new methods we specifically demonstrated the differences in increased CE hydrophobicity and increased mechanical resilience following treatment with niacinamide.

Increase in CE hydrophobicity from ceramide attachment results from a variety of enzyme processing steps on intercellular ceramide EOS-linoleate and one of the key enzymes in that processing is 12R-lipoxygenase (12R-LOX) [18], as it is the first maturation enzyme and acts as a primer for the enzyme cascade forming the CLE. We have previously reported its reduced mass and activity levels in facial SC [19, 20]. Also transglutaminase (TG) activity which is believed to be involved in the final attachment of ceramides to the CE is also known to be decreased in facial SC [19]. Other enzymes are possibly involved in lipid attachment in this cascade such as short-chain dehydrogenase/reductase family 9C member 7 (SDR9C7),

however, our proteomic work showed no deficiency in its mass levels in facial SC but we have not measured activity levels [19,21]. Niacinamide has been reported to increase keratinocyte ceramide biosynthesis [15] and, although it is speculative, it is highly likely that it also increases the expression of 12R-LOX and TG especially as niacinamide is reported to increase the gene expression of peroxisomal proliferator activated receptor-alpha (PPAR α), a key nuclear hormone receptor involved in keratinocyte differentiation and corneocyte formation [22].

The development of a healthy SC involves a well-orchestrated series of enzymatic steps enabling lipid, natural moisturising factor and CE formation and maturation [1]. The effects of niacinamide on CLE hydrophobicity and CPE integrity is consistent with improved CE maturation, a key event in healthy skin. However, using the forearm as a surrogate testing site does not reveal the full picture of the effect of niacinamide on different facial skin sites [11-13]. The negative effect of the vehicle in this study and previous studies, most likely its 1-2 propanediol content, shows many similarities to the effects of aqueous cream and its use in cosmetic products should be minimised [9, 23]. It was shown that 1-2 propanediol can increase the activity of the desquamatory serine protease kallikrein 7 (KLK7) [24] and the negative effect of increasing protease activity on CE maturation has been shown to be mitigated with protease inhibitors [25]. However, these study results support further investigations into the effect of niacinamide on ceramide EOS-linoleate levels together with enzymes involved in its processing and attachment to the CE. It is possible that the differences in skin permeability may reflect differences in barrier function as facial barrier function is complex [26].

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Captions

Table I: INCI list of test creams

Vehicle (pH 5.7): Aqua, caprylic/capric triglyceride, coco-caprylate, decyl cocoate, propanediol, glyceryl stearate, stearic acid, cetearyl glucoside, polyacrylamide, C13-14 isoparaffin, laureth-7, phenoxyethanol, ethylhexylglycerin, xanthan gum, parfum, sodium hydroxide

Active (pH 5.8): Aqua, caprylic/capric triglyceride, coco-caprylate, decyl cocoate, propanediol, niacinamide, glyceryl stearate, stearic acid, cetearyl glucoside, polyacrylamide, C13-14 isoparaffin, laureth-7, phenoxyethanol, ethylhexylglycerin, xanthan gum, parfum, sodium hydroxide

Figure 1: Model of the corneocyte envelope. An early step in the cornification process is the formation of the intercellular cytoskeleton mainly composed of keratin filaments and filaggrin. Cross-linking of proteins, mainly of loricrin and involucrin form the rigid inner CPE. Then a lipid monolayer, the CLE is covalently attached to the CPE. The CLE serves as a scaffold for the lamellar organization of the extracellular lipid matrix. Courtesy of Thomas Schmitt, Halle University, DE.

Link text: These events are essential for

Figure 2: Facial test sites, central forehead (CF), cheek (CH) (3 cm vertically beneath the outer edge of the eye), top nasolabial sulcus (NT), midpoint nasolabial sulcus (NM).

Figure 3: Baseline data, differences of CE maturation parameters (size, hydrophobicity, rigidity, RCEM) between young and aged facial SC. Data are mean \pm SEM, statistical comparison young vs. old, * $p < 0.05$, ns not significant.

Link text 3a: corneocyte size that we also o

Link text 3b: changes were observed for hydr

Link text 3c: sonication-stress resilience

Link text 3d: When considering all three measure

Figure 4

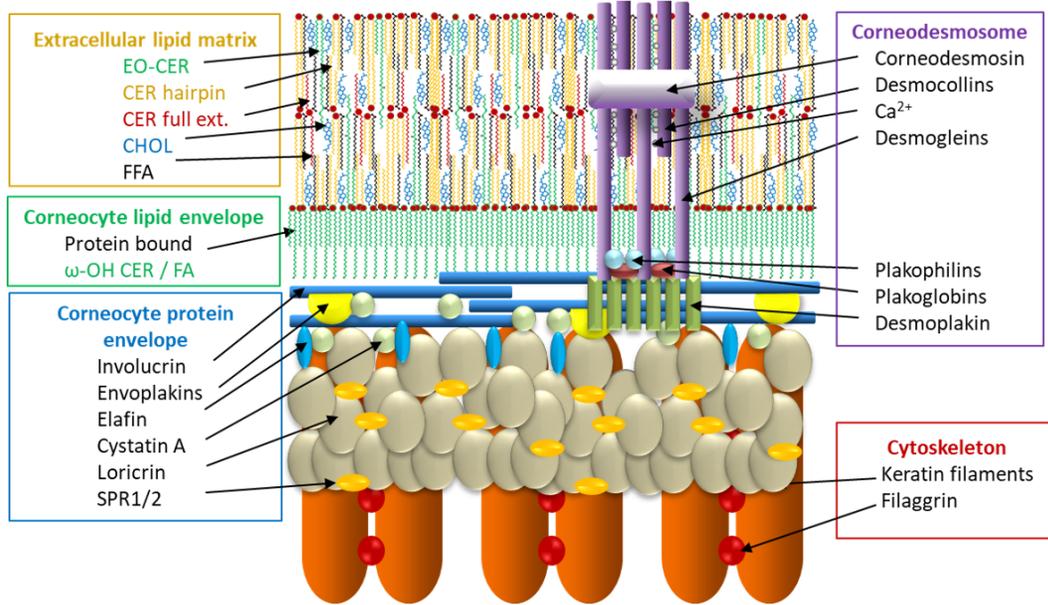
Baseline corrected data of the impact of topically applied niacinamide on CE maturation parameters (hydrophobicity, rigidity, RCEM) in young and aged facial SC. Data are mean \pm SEM, statistical comparison baseline vs. treatment, * $p < 0.05$, ns not significant.

Link text: age groups which was then refl

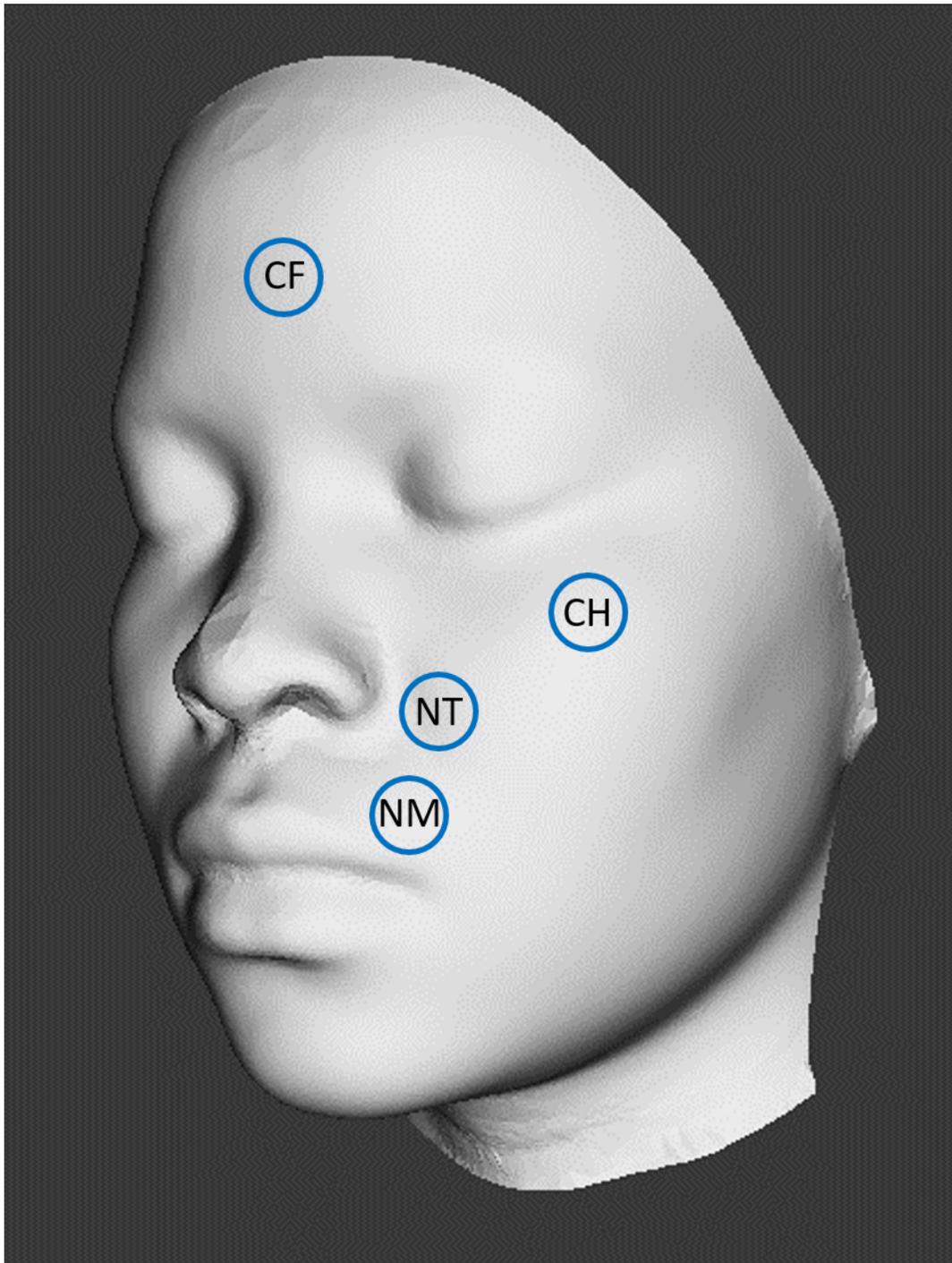
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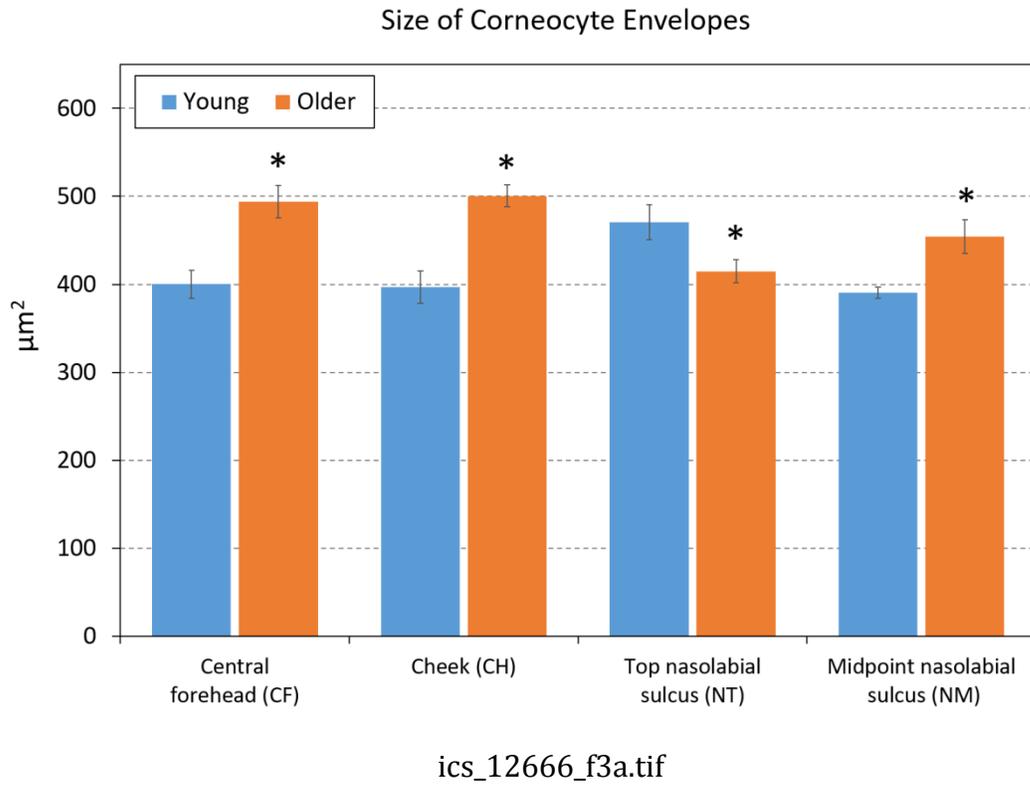
Active (pH 5.8): Aqua, caprylic/capric triglyceride, coco-caprylate, decyl cocoate, propanediol, niacinamide, glyceryl stearate, stearic acid, cetearyl glucoside, polyacrylamide, C13-14 isoparaffin, laureth-7, phenoxyethanol, ethylhexylglycerin, xanthan gum, parfum, sodium hydroxide

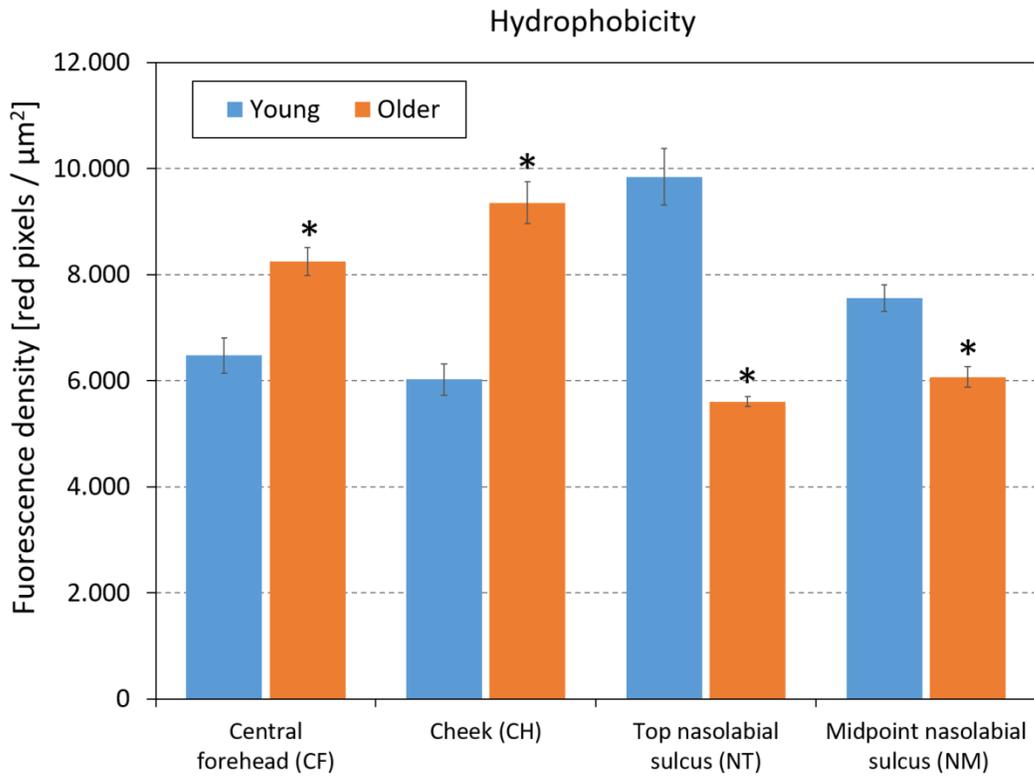


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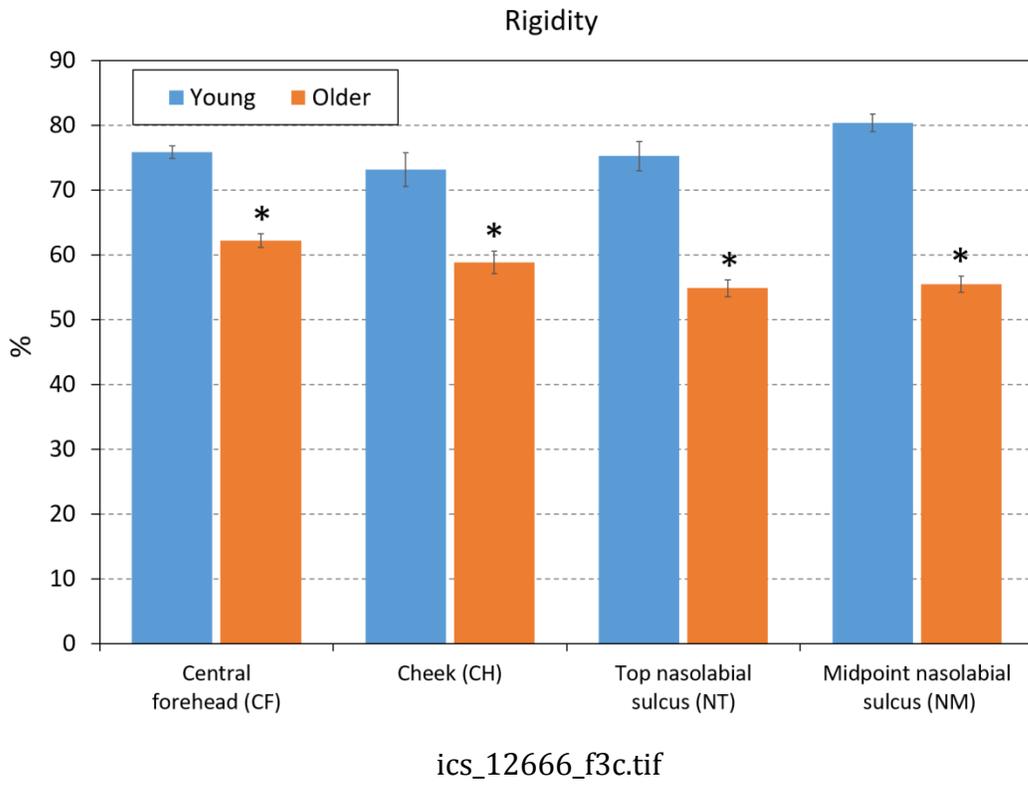


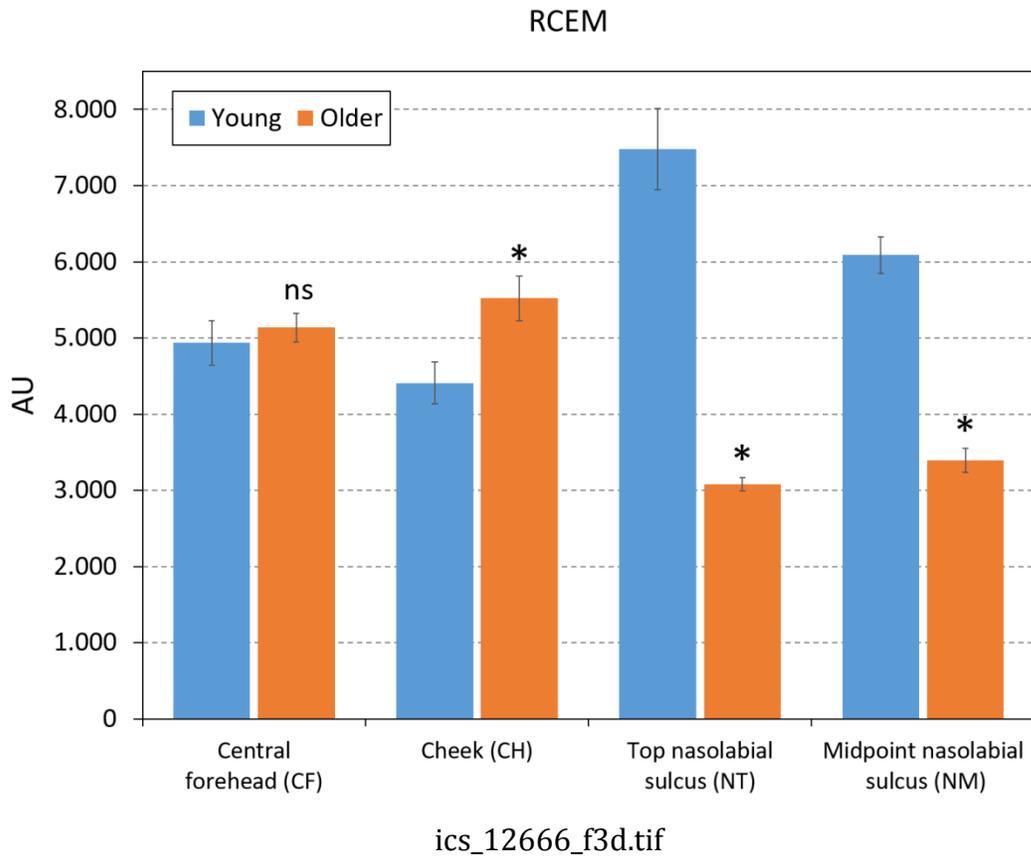
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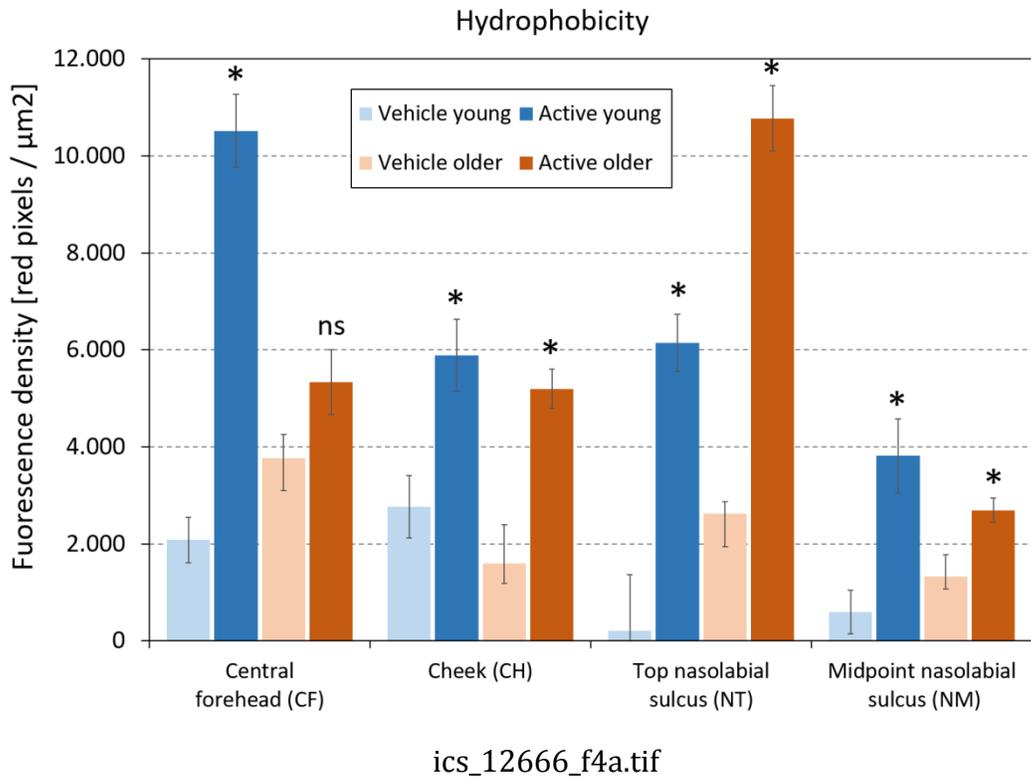


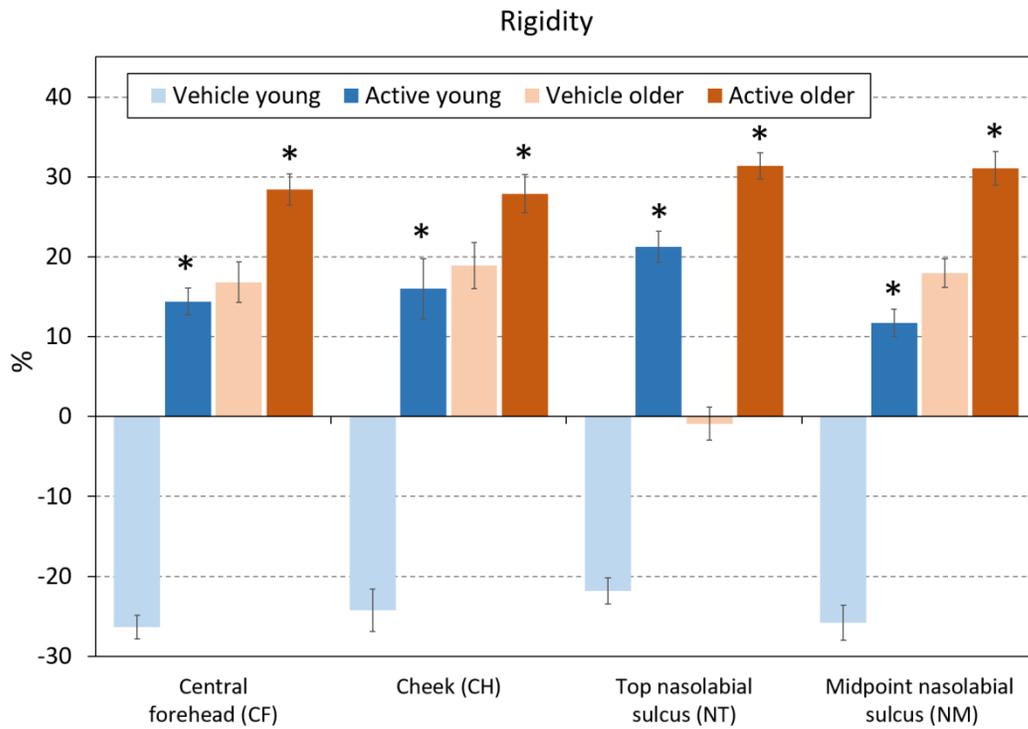


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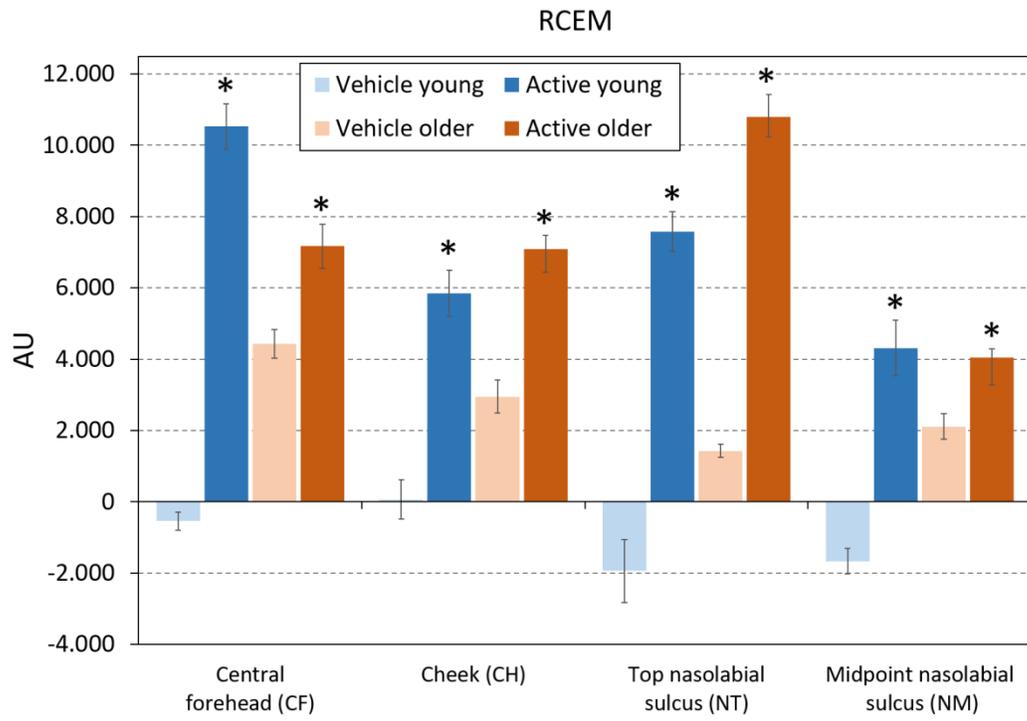








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