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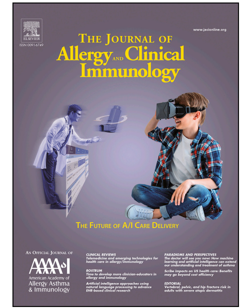

Unique skin abnormality in patients with peanut allergy but no atopic dermatitis

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1 **Unique skin abnormality in patients with peanut allergy but**
2 **no atopic dermatitis**
3
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36 **Abstract**

37 **Background:** The nonlesional skin of atopic dermatitis (AD) children with peanut allergy
38 (PA) is associated with increased transepidermal water loss (TEWL), low urocanic
39 (UCA) and pyroglutamic (PCA) acids (filaggrin [FLG] breakdown products), and reduced
40 ratio of esterified ω-hydroxy fatty acid sphingosine ceramides (EOS-CER) to non-
41 hydroxy fatty acid sphingosine ceramides (NS-CER) in the skin. The skin barrier of PA
42 without AD (AD-PA+) subjects has not been studied.

43 **Objective:** To explore whether AD-PA+ is associated with skin barrier abnormalities.

44 **Methods:** 33 participants were enrolled including 13 AD-PA+, 9 AD+PA+, and 11 non-
45 atopic participants (NA).

46 **Results:** The content of PCA in the stratum corneum (SC) of AD-PA+ subjects was
47 significantly reduced in comparison to NA (Median: 67 vs 97 μg/mg protein; p=0.028).
48 The ratio between *cis*- and *trans*-UCA significantly decreased from being the highest in
49 NA group (1.62) to the lowest in AD+PA+ group (0.07, p<0.001 vs NA; p=0.006 vs AD-
50 PA+ group), with AD-PA+ group having intermediate *cis/trans*-UCA ratio (1.17, p=0.024
51 vs NA group). The TEWL in AD-PA+ subjects did not differ from NA skin. Interestingly,
52 AD-PA+ subjects had increased EOS/NS-CER ratio vs NA (1.9 vs 1.3; p=0.008), while
53 AD+PA+ group had decreased proportion of EOS-CER (0.8, p=0.001 vs AD-PA+
54 group).

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55 **Conclusion**: Our data demonstrate that, irrespective of AD, PA is associated with
56 decreased skin cis-UCA and PCA content. An increase in skin EOS/NS-CER ratio
57 separates AD-PA+ from AD+PA+ and NA groups.

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74 **Clinical Implications**

75 Food allergy, without overt atopic dermatitis, is associated with skin barrier dysfunction
76 and may require targeted therapy to prevent food allergy.

77

78 **Capsule Summary**

79 Skin in peanut allergy (PA) without atopic dermatitis (AD) is associated with low levels
80 of filaggrin (FLG) breakdown products. Increased long chain lipids distinguish this group
81 from PA with AD and normal skin.

82

83 **Key Words:** atopic dermatitis, food allergy, skin barrier, peanut allergy

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93 **Abbreviations**

94 AD: atopic dermatitis

95 AD-PA+: peanut allergy without AD

96 AD+PA+: peanut allergy with AD

97 AD+PA-: AD without peanut allergy

98 BSA: bovine serum albumin

99 AUC: area under the curve

100 EOS-CER: esterified ω-hydroxy fatty acid sphingosine ceramides

101 FA: food allergy

102 FLG: filaggrin

103 IgE: immunoglobulin E

104 LC-ESI-MS/MS: liquid chromatography electrospray ionization tandem mass

105 spectrometry

106 NA: non-atopic control

107 NMF: natural moisturizing factor

108 NS-CER: non-hydroxy fatty acid containing sphingosine ceramides

109 PA: peanut allergy

110 PCA: pyrrolidone carboxylic acid

111 SC: stratum corneum

112 STS: skin tape strip

113 TEWL: transepidermal water loss

114 UCA: urocanic acid

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116 **INTRODUCTION**

117 Food allergy (FA) affects nearly 7% of children and is associated with major
118 health burdens (1). Peanut allergy (PA) is the most common form of FA and is thought
119 to be associated with skin barrier dysfunction. Skin barrier abnormalities allow allergen
120 penetration through the skin, immune cell sensitization, and systemic immunoglobulin E
121 (IgE) responses to allergens (2,3). This scenario has been most convincingly
122 demonstrated in filaggrin (FLG) deficient mice (4) and humans with atopic dermatitis
123 (AD) (5). Risk factors for individuals who have concurrent AD with PA (AD+PA+) include
124 early onset of AD, severity of AD, and duration of AD (6,7). Importantly, a recent study
125 reported that early intervention in the treatment of AD reduces the occurrence of PA (8).
126 This suggests that early detection of infants with skin barrier abnormalities may
127 represent a window of opportunity for prevention of PA and other forms of FA.

128 There is also a subset of children who do not not have any history of AD but
129 develop peanut allergy (AD-PA+) (9). It is not known if AD-PA+ subjects have a normal
130 skin barrier. Recently, we have presented evidence that AD+PA+ children can be
131 assigned to a distinct endotype and separated from children who have AD but not PA
132 (AD+PA-) by a combination of parameters that include transepidermal water loss
133 (TEWL), decreased skin urocanic acid (UCA) (surrogate marker for low FLG
134 expression), increased type 2 immune activation, and reduced long chained esterified
135 ω-hydroxy fatty acid sphingosine ceramides (EOS-CER) expression (10). Here we
136 present evidence that the skin of AD-PA+ individuals has a distinct profile of polar and
137 lipid components and that AD-PA+ subjects represent a distinct endotype different from
138 AD+PA- or AD+PA+ subjects. Altogether, our findings provide a strong support for the

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139 hypothesis that the PA, with or without AD, is associated with an imbalance of stratum
140 corneum (SC) lipid and protein components known to facilitate sensitization to allergens
141 through the skin.

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143 **METHODS**

144 **Study design**

145 In this article, we report our observations from a prospective, clinical mechanistic
146 study approved by The National Jewish Health Institutional Review Board. Written
147 informed consent was provided by the parent/legal guardian and written assent was
148 provided by the participant, as applicable, before participation. Importantly, all laboratory
149 data were analyzed without knowledge of study participant diagnostic group in order to
150 eliminate any investigator bias. Endpoints measured in this study included TEWL area
151 under the curve (TEWL AUC) assessed in nonlesional skin prior to skin tape stripping
152 (STS) and repeated after 5, 10, 15, and 20 STS. STS samples were also assessed for
153 FLG breakdown products and lipid profiles.

154

155 **Study participants**

156 A total of 33 participants were enrolled including 13 AD-PA+ participants, 9
157 AD+PA+ participants, and 11 non-atopic (NA) participants. The summary of study
158 subjects clinical characteristics is shown in Table E1. NA controls were defined as those
159 without a personal history of atopic diseases and negative skin prick tests to common
160 foods and aeroallergens. All participants in the AD-PA+ and AD+PA+ groups had a
161 history of immediate clinical reactions to peanut and a positive skin prick wheal size to
162 peanut of 8 mm or greater (Table E1). Peanut wheal size was significantly greater in
163 AD+PA+ subjects as compared to AD-PA- subjects; no significant difference in peanut
164 specific IgE was observed. The AD-PA+ group had no history of previous skin rash. The

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165 AD+PA+ had AD as described in reference 10. Peanut-specific IgE was detected in
166 serum samples from both AD-PA+ and AD+PA+ groups.
167
168 **Skin Tape Strip (STS) collection**
169 D-Squame tape strips (22 mm diameter, CuDerm) were collected from the upper
170 extremity of each subject. Skin lesions were in the region of the antecubital fossa of all
171 AD subjects. Nonlesional skin, which had a normal clinical appearance and no visible
172 excoriations, was obtained 5 cm from the skin lesion. In all subjects without AD (AD-
173 PA+ and NA), the nonlesional STS was just below the antecubital fossa. The D-Squame
174 pressure instrument D500 was used to apply all tape strips with equivalent pressure
175 (e.g. 225 g/cm2). On application of the first tape disc, four marks were placed around
176 the disc with a pen so that subsequent discs could be applied to the same location.
177 Each tape disc was applied to a designated surface area of a D-Squame Disc Storage
178 Card (CuDerm) and then placed into a plastic pouch and stored at -80°C until STS
179 analysis was conducted for FLG breakdown products, lipids, and by electron
180 microscopy.
181
182 **Skin barrier assessments**
183 TEWL was assessed using the AquaFlux AF200 (Biox). All skin barrier
184 measurements for TEWL AUC were made on nonlesional, non-flexural, non-sun
185 exposed skin from the upper extremity in the region of the antecubital fossa. The
186 baseline lesional TEWL was also assessed for AD participants. All TEWL
187 measurements were made in temperature and climate-controlled conditions. SC

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188 integrity of nonlesional skin was measured by TEWL, at the baseline before tape
189 stripping and after 5, 10, 15, and 20 tape strips. TEWL AUC was calculated as
190 previously described (10).
191

192 **FLG skin measurements**

193 FLG breakdown products, *cis/trans*-urocanic acid (total UCA) and pyrrolidone
194 carboxylic acid (PCA), also known as pyroglutamic acid, were quantified via a liquid
195 chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS)
196 approach on a Sciex 6500QTRAP mass spectrometer coupled with a Shimadzu Nexera
197 X2 UHPLC system as previously described (10). Briefly, SC from STS #15-16 was
198 removed by scraping STS in 2 ml water-methanol (9:1, v/v) solution in a Petri dish with
199 a rubber cell scraper. Floating SC particles were carefully transferred into the glass
200 screw cap tubes. Petri dishes were washed twice with 1 ml methanol, which was
201 combined with primary SC suspension. Final SC particle suspension was further
202 subjected to a modified Bligh and Dyer extraction (11). A known amount of U-
203 [^{13}C,^{15}N]proline was added at this step to ensure absolute quantitation of targeted
204 molecules. Extraction was performed overnight by adding 0.25 ml chloroform, then
205 phase separation was achieved by adding 2.0 ml chloroform and 0.45 ml 2% formic
206 acid, followed by intensive vortexing and centrifugation (2,000g x 10 min). After
207 centrifugation, the bottom chloroform phase was collected and kept for lipid analyses
208 while the upper water-methanol phase was re-extracted by adding 2.25 ml chloroform,
209 followed by additional intensive vortexing and centrifugation. At the end, the upper
210 water-methanol phase was carefully collected, dried under a nitrogen stream, re-

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211 dissolved in methanol/water (1:1, v/v), and subjected to the LC-ESI-MS/MS analysis.
212 The chloroform layers were combined and processed for lipid analyses. The protein
213 interface was subjected to hydrolysis with 1N NaOH at 80°C for 3 hours, then
214 neutralized with 1N HCl. The sample protein content was measured using a DC Protein
215 Assay kit (Bio-Rad) with bovine serum albumin (BSA) as a protein standard.

216 LC separation of *cis/trans*-UCA, PCA, and proline was achieved using an Acquity
217 UPLC BEH Amide (2.1 x 100 mm, 1.7 μm particle size) column using a gradient from
218 acetonitrile (Solvent A) to methanol:water:formic acid (65:35:0.5, with 5 mM ammonium
219 formate) (Solvent B) and the following elution program: hold at 5%B until 0.5 min, then
220 linear increase to 20%B at 1 min, then increase to 60%B at 3 min, hold at 60%B until
221 4.1 min, then decrease to 5%B by 4.5 min, and hold at 5%B until 5 min. All amino acids
222 were detected in positive ion mode using the following transitions: mass to charge (m/z)
223 139.1 > m/z 121.1 (UCA), m/z 130.2 > m/z 83.9 (PCA), and m/z 122.1 > m/z 75.0 (U-
224 [^{13}C,^{15}N]proline). Exact quantitation of PCA and *cis/trans*-UCA was achieved by creating
225 standard curves of responses of variable amounts of analytes versus a fixed amount of
226 the internal standard (U-[^{13}C,^{15}N]proline). Authentic standards of *cis*-UCA, *trans*-UCA,
227 and PCA were from MilliporeSigma (Burlington, MA); U-[13C,^{15}N]-proline was from
228 Cambridge Isotope Laboratories (Tewksbury, MA).

229

230 **Analysis of SC lipids**

231 ***STS processing for lipid extraction***

232 The bottom chloroform layer from the extraction procedure was used for lipid
233 analysis by mass spectrometry. A fixed amount of the internal standard (*N*-palmitoyl-D-

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234 erythro-sphingosine [d7], D7-ceramide) was added at the beginning of the extraction
235 process. D7-ceramide as well as other standards of NS-ceramides (*N*-16:0-, *N*-18:0-, *N*-
236 20:0-, *N*-24:1-, and *N*-24:0-D-erythrosphingosines) were from Avanti Polar Lipids, Inc
237 (Alabaster, AL). Data were normalized to the total amount of hydrolyzed protein,
238 determined as described above.
239
240 ***Lipid analysis by targeted lipid chromatography-tandem mass spectrometry***
241 EOS-CER and NS-CER were identified and quantified using a targeted LC-ESI-
242 MS/MS approach on a Sciex 6500QTRAP mass spectrometer coupled with a Shimadzu
243 Nexera X2 UHPLC system as previously described (12). All molecules were detected in
244 positive ions mode. EOS-CER and NS-CER were detected as a transition from
245 molecular ions to the m/z 264, m/z 292, and m/z 320 as our work has identified all three
246 sphingoid bases (C18-, C20-, and C22-sphingosine) being present in human skin
247 ceramides. Chromatography was performed on an Ascentis Express RP-Amide 2.7 µm
248 2.1 x 50 mm column using gradient elution from methanol:water:formic acid (65:35:0.5,
249 5mM ammonium formate) to methanol:chloroform:water:formic acid (90:10:0.5:0.5, 5
250 mM ammonium formate). Absolute amounts of NS-CER were determined in a
251 quantitative and semi-quantitative way by using correction factors from standard curves
252 that were created using variable amounts of *N*-14:0-24:0 ceramides with C18-
253 sphingosine as a base versus a fixed amount of D7-ceramide. Correction factors for
254 molecular species for which there are no available standards were used with best
255 possible approximation to the closest available molecular species of ceramide
256 standards. Absolute amounts of EOS-CER were determined in a semi-quantitative way

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257 by using a correction factor from a standard curve created using variable amounts of *N*-
258 24:0-D-erythro-sphingosine (24:0-CER) versus D7-ceramide.
259

260 ***Analysis of protein-bound ceramides by targeted lipid chromatography-tandem***
261 ***mass spectrometry***

262 Protein-bound ceramides were extracted from neutralized protein hydrolysates
263 using Bligh and Dyer extraction (11). D7-ceramide internal standard was added again
264 before initiation of extraction to allow semi-quantitative estimation of ω-hydroxy
265 ceramides that formed upon basic hydrolysis of proteins. Separation of ω-hydroxy
266 ceramides was achieved using same chromatography conditions as described above.
267 The identification of ω-hydroxy ceramides was performed against the authentic standard
268 of ceramide 1 (d18:1/26:0/18:1) N-[26-oleoyloxy hexacosanoyl]-D-erythro-sphingosine)
269 (Avanti Polar Lipids, Inc) subjected to the same hydrolysis procedure as protein, thus
270 providing the standard of ω-hydroxy *N*-26:0-sphingosine (ω-hydroxy ceramide).
271

272 **Statistical analysis**

273 To compare differences in the demographic characteristics between groups, chi-
274 square tests were used for categorical variables and Mann-Whitney or Kruskal-Wallis
275 tests were used for two group or three group comparisons, respectively. Comparisons in
276 FLG breakdown products, lipid ratios, free- and protein-bound EOS-CER between the
277 three study groups were done using one-way ANOVA tests with multiple comparisons.
278 $P<0.05$ was considered statistically significant.

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279 **RESULTS**

280 **Skin TEWL measurements**

281 TEWL was measured in nonlesional skin at the baseline before STS and after 5,
282 10, 15, and 20 STS. At the baseline, TEWL was similar for all subjects. AD+PA+
283 subjects were found to have impaired skin barrier as demonstrated by progressively
284 increasing TEWL with consecutive STS (Fig 1, A). However, AD-PA+ subjects did not
285 demonstrate an impaired skin barrier as measured by the TEWL following 20 STS (Fig
286 1, A, red line). This is consistent with the lack of visible skin abnormalities and no
287 personal history of AD in these subjects. Integration of TEWL measurements over all 20
288 STS layers (TEWL AUC) also did not reveal TEWL difference between NA and AD-PA+
289 groups (Fig 1, B).
290

291 **Skin FLG breakdown products**

292 It has previously been demonstrated that breakdown of FLG protein gives rise to
293 skin components of Natural Moisturizing Factor (NMF), urocanic acid (UCA) and
294 pyroglutamic acid (PCA) (13). The *trans*-UCA isoform is the direct product of histidine
295 transformation, but under UV-light *trans*-UCA is converted to *cis*-UCA isoform. A
296 previous study demonstrated a significant decrease in UCA and PCA levels in the skin
297 of AD+PA+ subjects (10). In this study, this observation was confirmed: we found a
298 significant decrease in the content of both total UCA and PCA in AD+PA+ subjects as
299 compared to NA subjects (Fig 2, A and B). Surprisingly, the skin of AD-PA+ subjects
300 also revealed the following trends of a decrease in UCA content (Fig 2, A) and a
301 significant decrease in PCA content (Fig 2, B). Moreover, the individual analysis of the

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302 content of *cis*-UCA and *trans*-UCA in the skin revealed a noteworthy phenomenon. The
303 ratio between *cis*- and *trans*- isoforms of UCA dramatically declined in AD+PA+ skin
304 samples, and was also significantly decreased in the skin of AD-PA+ subjects as
305 compared to NA subjects (Fig 2, C). Interestingly, the content of *trans*-UCA was not
306 influenced by either AD or PA, but its conversion to *cis*-UCA was progressively affected
307 in AD-PA+ and AD+PA+ subjects (Fig 2, D). This observation is especially important in
308 view of previous data that demonstrated the immunosuppressive properties of *cis*-UCA
309 but not *trans*-UCA (14-16). Our data suggests that the skin of AD-PA+ people has
310 diminished amounts of FLG protein, given the decreased levels of FLG breakdown
311 products, UCA and PCA, in the skin of these subjects. Although the study groups were
312 somewhat imbalanced by gender (Table E1), sex did not have any effect on the skin
313 tape measurements (data not shown).
314
315 **Skin lipids**
316 One characteristic of ceramides, and all lipids in AD skin, is the overall
317 shortening of their fatty acid chain length, which is a result of type 2 immune activation
318 in the AD skin (12). Here we performed targeted lipidomic analysis of STS layers 15-16
319 in NA, AD-PA+ and AD+PA+ subjects to determine if this phenomenon is recapitulated
320 in AD-PA+ subjects. Remarkably, AD-PA+ subjects did not show evidence of any
321 changes in the chain length of sphingosine-linked fatty acids, as shown by the absolute
322 amount of three major molecular species of NS-ceramides with palmitic (16:0),
323 lignoceric (24:0), and behenic (26:0) fatty acids. In contrast, AD+PA+ subjects
324 demonstrated a clear shift towards a prevalence of short-chained NS-ceramide with

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325 palmitic acid (Fig 3, A). Furthermore, the calculated ratio between the short-chain *N*-
326 16:0-sphingosine ceramide (16:0-NS-CER) and the sum of the long-chain *N*-24:0- and
327 *N*-26:0-sphingosine ceramides (24:0-, 26:0-NS-ceramides) confirmed that the AD+PA+
328 group is clearly distinct from AD-PA+ and NA groups (Fig 3, B), while AD-PA+ group did
329 not differ from NA subjects for this parameter.

330 EOS-CER are uniquely expressed in the skin and are critical for skin barrier
331 function, including water retention in the skin. EOS-CER are present in the skin not only
332 in free form, but they are also precursors to the oxidized and protein-bound ceramides
333 (17,18). It was previously reported that the relative proportion of free EOS-CER within
334 all ceramides is diminished in the skin of AD patients (19-21). In the current study, our
335 semi-quantitative targeted EOS-CER analysis has shown that protein-normalized
336 content of free and protein bound EOS-CER is decreased in AD+PA+ STS as
337 compared to STS from NA subjects (Fig 4, A-C). On the contrary, both free and protein-
338 bound EOS-CER were significantly increased in the SC of AD-PA+ subjects relative to
339 both NA and AD+PA+ groups (Fig 4, A-C). Furthermore, the proportion of EOS-CER
340 was significantly increased in the skin of AD-PA+ subjects in comparison to the skin of
341 both AD+PA+ and NA subjects, as shown by the ratio of total EOS/NS-CER in the STS
342 analysis (Fig 4, D). These data suggest that the skin of AD-PA+ people has increased
343 biosynthesis of EOS-CER as a potential mechanism to compensate for the loss of FLG
344 and its degradation products. This compensatory increase in EOS-CER may account for
345 lack of skin TEWL changes in AD-PA+ subjects.
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348 **DISCUSSION**

349 Our current findings provide evidence that the stratum corneum of children with
350 allergy to peanut is not normal and bears a distinctive epidermal signature. While the
351 exact causation link between observed abnormalities in stratum corneum composition
352 and the development of food allergy is yet to be defined, our data support the
353 hypothesis that skin barrier abnormalities, i.e. low FLG, may facilitate the onset of PA
354 irrespective of a past or present history of AD. This hypothesis is most strongly
355 supported by animal model observations that the epicutaneous application of protein
356 allergens to nonlesional skin of FLG-deficient mice results in the activation of type 2
357 cytokine production and generation of protein-specific IgE and IgG1 immunoglobulins
358 (4). It has been further suggested that genetic or environmental factors lead to an
359 impaired skin barrier with activation of skin proteases that facilitate allergen penetration
360 (5, 22). Until now, there has been no information about the normal appearing skin of PA
361 patients, specifically whether it is associated with impaired skin barrier function. In our
362 study, we demonstrate that a routine measure of skin barrier function such as TEWL
363 does not reveal skin impairment in PA only people (Fig 1), similiar to the lack of such
364 demonstration in AD subjects without food allergy (10). This is consistent with the lack
365 of AD in such individuals, since increased TEWL has primarily been associated with AD
366 codiagnozed with allergy to multiple foods.

367 Our current study of the skin components that control skin pH and moisturization
368 (FLG breakdown products: PCA and UCA) and provide hydrophobic barrier (lipids) has
369 identified abnormalities in the skin composition of AD-PA+ subjects (Figs 2-4). These
370 changes have never been previously observed when studying the skin of AD subjects

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371 with or without FA (10,12). What separates the skin of peanut-allergic people who do
372 not have AD from those who do is the lack of particular changes in lipid chain length
373 that are driven by type 2 cytokines in AD (12) and the increase in the proportion of ultra
374 long-chain EOS-CER versus much shorter NS-CER (Fig 4 and references 10,12).
375 Increased levels of EOS-CER are likely a compensatory response to an impaired FLG
376 expression in the skin of AD-PA+ patients. It is not known what drives the decline in
377 FLG expression in the skin of AD-PA+ patients (as measured by the content of its
378 breakdown products PCA and UCA). Aside from *FLG* null mutations and type 2 cytokine
379 regulation of FLG, there are many environmental causes of low FLG in the skin,
380 including detergents, pollution, immune cytokines, and stress (23).

381 Our current study has revealed another phenomenon that has not been
382 previously described in the literature. We have observed a unique decline in *trans*- to
383 *cis*-UCA conversion in the skin of not only AD subjects with PA, but also in people with
384 PA without AD (Fig 2, D). This observation has, potentially, a very important
385 consequence for the development of allergic responses in the skin, as *cis*-isoform of
386 UCA and not its *trans*-isoform possesses immunosuppressive properties through the
387 ability to bind to serotonin 5-HT_{2A} receptor (24). It is known that *trans*-UCA is initially
388 formed from histidine, an amino acid that is enriched in FLG. Then it is converted to *cis*-
389 UCA by UV-light (14-16). A recent study published in The Journal of Allergy and Clinical
390 Immunology demonstrated that UV-light is more beneficial than vitamin D
391 supplementation as an eczema prevention strategy (25), suggesting that along with skin
392 vitamin D production, UV-light regulates *cis*-UCA levels in the skin, an additional
393 immunoregulatory substance in the skin. Reduced levels of *cis*-UCA observed in the

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394 skin of PA subjects in this study suggests that they may be less exposed to UV-light, or
395 there are additional factor(s) that are involved in *trans*- to *cis*-UCA conversion in the skin
396 that are yet to be identified.

397 FLG is critical for skin barrier function not only due to its importance as a major
398 source of NMF for the skin and the involvement of its breakdown product, *cis*-UCA, in
399 local skin immunosuppression, but also by functioning as a core for the assembly of
400 skin proteo-lipid complexes during corneocyte maturation (17,18). It is logical to expect
401 that proper SC lamellar structure formation will be affected if the lamellar complexes
402 assembly is impaired due to FLG insufficiency, even if the expression of other proteins,
403 lipid components, and their binding to proteins are not affected. However, we did not
404 find clear indications of the disturbance of lamellar structures in SC STS samples from
405 AD-PA+ subjects (data not shown). Further investigation of a larger cohort of AD-PA+
406 subjects is required to characterize the skin epithelial barrier in AD-PA+ subjects.

407 We have recently described the mechanism driven by type 2 cytokines, IL-4 and
408 IL-13, that shortens lipid chain length in the skin of people with AD (12). Clearly, lipids in
409 the skin of people with PA only, but no AD, do not bear the signature of being affected
410 by type 2 cytokines (Fig 3). Surprisingly, we have observed an increase in the
411 proportion of EOS-CER (free and protein-bound) in the skin of these people (Fig 4).
412 This could be a sign of a potential, yet to be identified, compensatory mechanism for the
413 lack of FLG expression that is observed only without hyperactivation of type 2 immune
414 responses. This upregulation of EOS-CER biosynthesis might be responsible for the
415 lack of abnormalities in skin TEWL in AD-PA+ subjects (Fig 1). However, this might not

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416 be enough to protect from allergen penetration through the skin that has decreased
417 content of FLG as measured by skin content of NMF (Fig 2).

418 In summary, the current study demonstrates that individuals with PA have
419 reduced expression of immunosuppressive *cis*-UCA and PCA, the products of FLG
420 breakdown, irrespective of AD, but the subtype of AD-PA+ can be distinguished from
421 AD+PA+ by a unique SC lipid signature. Further development of STS methodology and
422 mass spectrometric analyses of SC components has the potential to become a clinical
423 application to identify people at risk for developing PA and AD in early childhood. The
424 results from this study may provide future approaches, using skin tape stripping, to
425 monitor epidermal changes during atopic match progression and its responses to
426 targeted prevention therapies.

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532 **FIGURE LEGENDS**

533 **Figure 1. TEWL and TEWL AUC after sequential STS by group. (A)** TEWL
534 measurements at the baseline and after 5, 10, 15, and 20 STS. The line figure
535 represents Mean±SE for TEWL measurements at each STS layer (black line represents
536 NA, red line represents AD-PA+, and blue line represents AD+PA+ groups). #,& -
537 significant differences ($p<0.05$) between AD+PA+ and NA (#) or between AD+PA+ and
538 AD-PA+ (&). **(B)** TEWL AUC box plots: here and in all subsequent figures, the box
539 margins are the 25-75%-interquartile range (50% of the observations), whisker lines are
540 minimal and maximal observations, and the annotations are the p values from one-way
541 ANOVA with the multiple comparison analysis.

542

543 **Figure 2. FLG breakdown products in nonlesional skin.** Comparisons between
544 groups for FLG breakdown products, total UCA **(A)**, PCA **(B)**, *cis/trans*-UCA ratio **(C)**
545 and *cis-* and *trans*-UCA, were all assessed at skin tapes 15 and 16 collected from
546 nonlesional skin areas. (**D**) *Cis*-UCA and *trans*-UCA levels in NA, AD-PA+, and
547 AD+PA+ subjects.

548

549 **Figure 3. Major NS-ceramides in nonlesional skin.** (**A**) Comparisons between groups
550 for major NS-ceramide molecular species (C18-sphinosine with amide-linked 16:0, 24:0,
551 and 26:0 fatty acids) and (**B**) the ratio between *N*-16:0- and the sum of *N*-24:0- and *N*-
552 26:0-NS-ceramides. Ceramide levels were assessed at STS 15-16. Data for three major
553 species of NS-ceramides (*N*-16:0-, *N*-24:0, and *N*-26:0-sphingosines) are presented.

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554

555 **Figure 4. Free EOS-ceramides and protein-bound ω-hydroxy ceramides are**
556 **increased in stratum corneum of peanut allergic subjects.** Free EOS-ceramides
557 (**A,C**) and protein-bound ω-hydroxy ceramides that originate from EOS-ceramides (**B**)
558 are increased in absolute amounts, as well as in relative to NS-ceramide proportion (**D**)
559 in the skin of subjects with allergy to peanuts in comparison to healthy and atopic and
560 peanut allergic subjects. Numbers (28, 20, 32) shown on X axes represent the chain
561 length of saturated omega-hydroxy fatty acids *N*-acylating corresponding C18-C22-
562 sphingosines.

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Table E1. Characteristics of study participants by diagnostic group *

	AD+PA+ N=9	AD-PA+ N=13	NA N=11	p-value
Demographics				
Gender: Female	3 (33.3%)	3 (23.1%)	8 (72.7%)	0.04
Male	6 (66.7%)	10 (76.9%)	3 (28.3%)	
Race: White	7 (81.0%)	13 (100%)	11 (100%)	0.06
African-American	2 (19.0%)	0 (0%)	0 (0%)	
Age (yrs.)	15 [11.5; 17.0]	10 [6.0; 14.0]	11 [9.0; 16.0]	0.09
Age Group: <12 yrs.	2 (22.2%)	8 (61.5%)	6 (54.5%)	0.17
≥12 yrs.	7 (78.8%)	5 (39.5%)	5 (45.5%)	
Clinical				
SCORing Atopic Dermatitis (SCORAD)	22.3 [11.1; 26.3]			
SCORAD: Mild (<20)	3 (33%)			
Moderate (20-40)	5 (56%)			
Severe (>40)	1 (11%)			
Peanut IgE (kU/L)	4.6 [1.6; 21.4]	21.5 [3.4; 100]		0.29
Peanut Wheal Size (mm):	15.0 [10.0; 15.0]	21.5 [14.5; 33.0]	0.0 [0.0; 0.0]	<0.0001

* Medians [1st, 3rd quartile] are used for continuous variables and numbers (percents) for categorical variables. P-values for comparisons between diagnostic groups were calculated with the use of the Kruskal-Wallis test by ranks or Mann-Whitney test for continuous variables and the chi-square test for categorical variables.

Figure 1

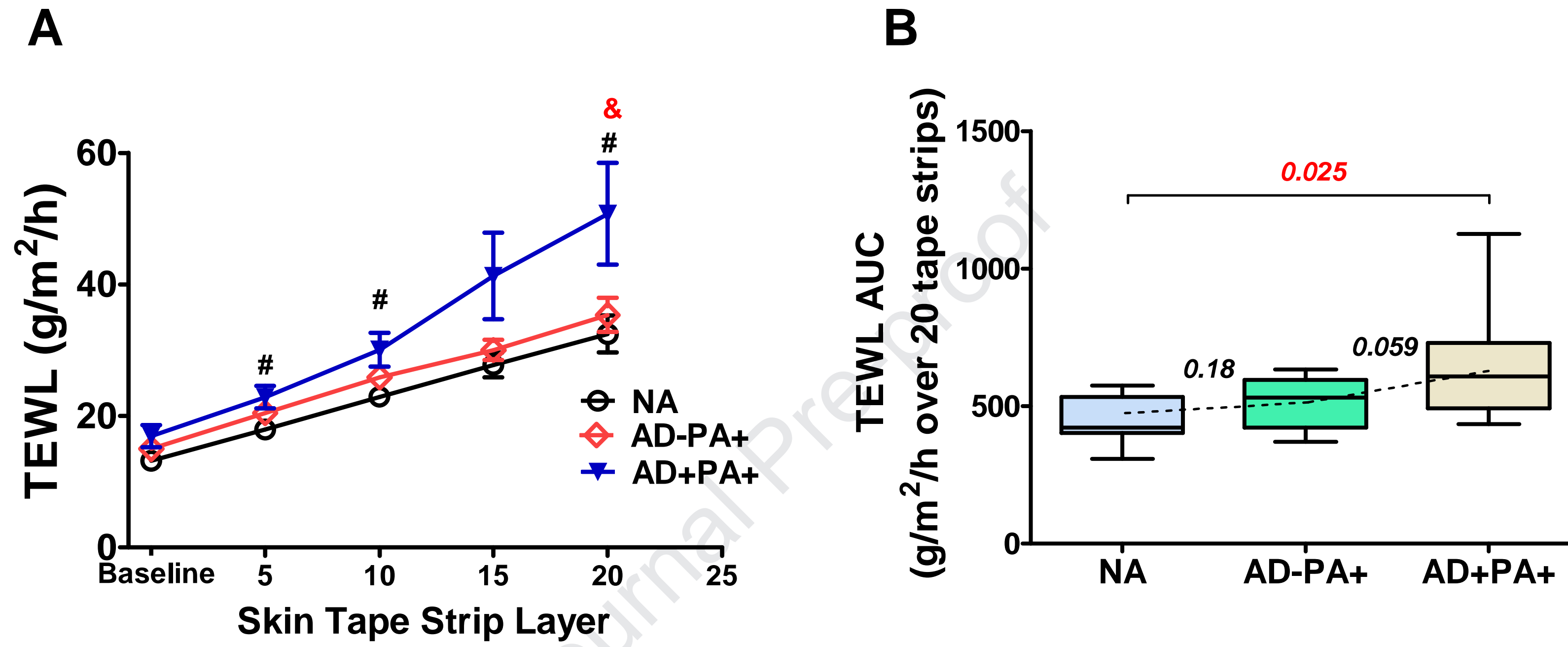

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Figure 2

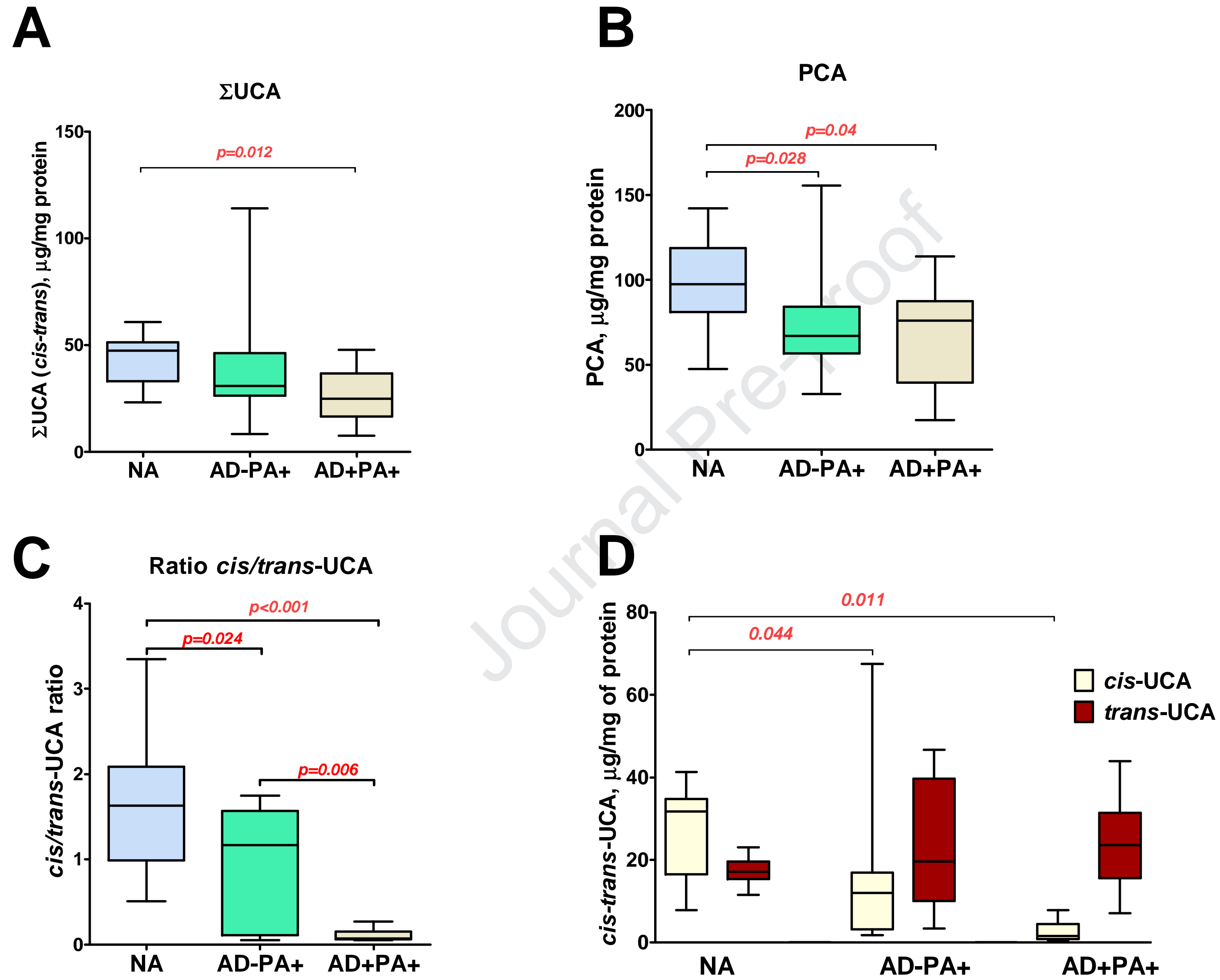

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Figure 3

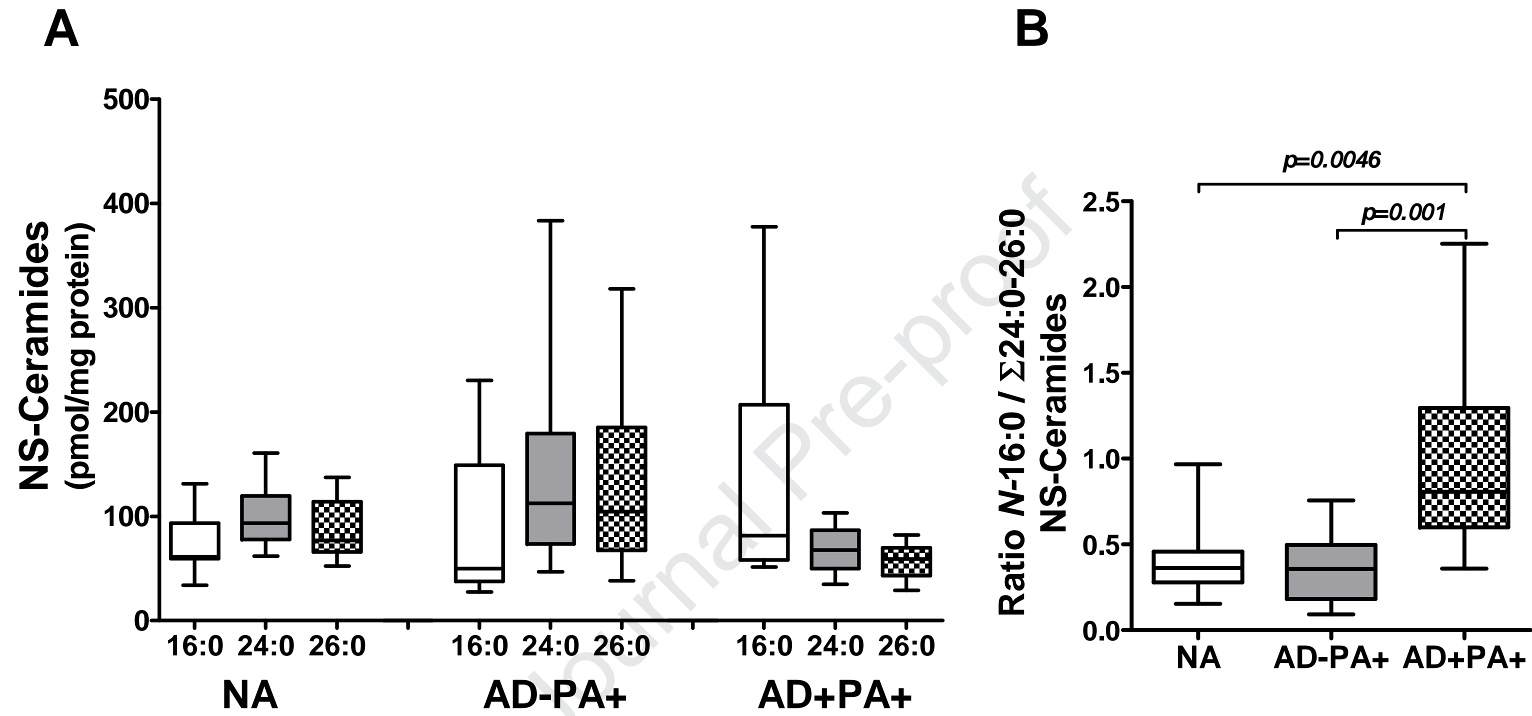

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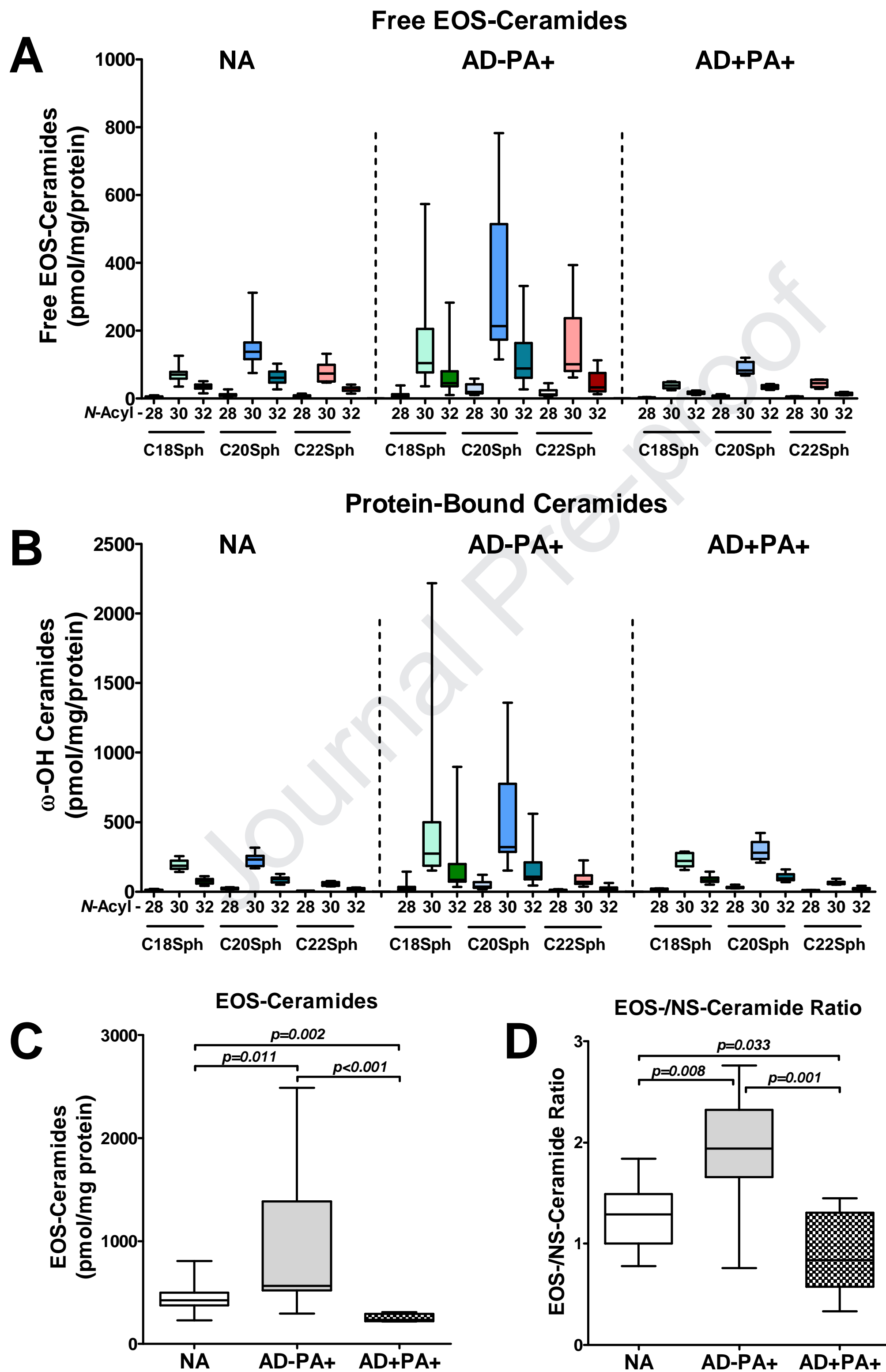
Figure 4



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