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Short-time UVA exposure to human keratinocytes instigated polyunsaturated fatty acid without inducing lipid peroxidation

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ABSTRACT

Short-term exposure to ultraviolet A (UVA) radiation can directly injure our skin through inflammatory response and indirectly through oxidative stress, triggering polyunsaturated fatty acid (PUFA) peroxidation in skin cell membrane and formation of DNA adduct, 8-hydroxy-2'-deoxyguanosine (8-OHdG). It is known that UVA exposure leads to photoaging, immunosuppression and skin cancer. However, the changes in PUFA and its oxidized metabolites, and cell cycle after short UVA exposure, are debatable. In this study, human keratinocytes (HaCaT) were exposed to low dose (5 J/cm²) and high dose (20 J/cm²) of UVA and assessed immediately, 8 h, 12 h, and 24 h post-treatment. Both doses showed a transient suppression in S-phase after 8 h of UVA exposure, and G₂/M phase arrest after 12-h UVA exposure in the cell cycle but subsequently returned to normal cycle. Also, no observable DNA damage took place, where 8-OHdG levels were below par after 24-h UVA exposure. A dose of 20 J/cm² UVA stimulated significant amount of arachidonic acid, n-3 docosapentaenoic acid, and docosahexaenoic acid (DHA) but lowered adrenic acid and eicospentaenoic acid after 24-h exposure. Among the 43 oxidized PUFA products determined, enzyme-dependent oxidized PUFAs, namely, 14-hydroxy-DHA (HDoHE) level reduced, and 8- and 13-HDoHE levels elevated significantly in a linear trend with post-treatment time. Out of the non-enzymatic oxidized PUFAs, a significant linear trend with post-treatment time was shown on the reduction of 5-F_{2t}-Isoprostane (IsoP), 15-F_{2t}-IsoP, Isofurans, 5-F_{3t}-IsoP, Neurofurans, and 20-HDoHE. Our observations indicate oxidative stress through short UVA exposure on human keratinocytes did not have detrimental consequences.

KEYWORDS

Isoprostanes; HaCaT; UVA; skin peroxidation; polyunsaturated fatty acids

Introduction

Numerous epidemiologic evidences suggest ultraviolet radiation (UVR) is a major environmental cause of skin disorder. The key source of UVR is solar such as ultraviolet A (UVA, 320–400 nm) which is the primary component to be in contact with human skin [1] and ultraviolet B (UVB) which is the major contributor to sunburn. Both UVA and UVB can lead to a wide range of adverse effects [2,3] such as photoaging, immunosuppression, and skin cancer [4–6], but the degree of damage by UVA is lesser than UVB. The risk concern of UVA is especially important in Asia compared to Europe due to higher level of UVA radiation exposure in winter season [7].

UVA activates reactive oxygen species (ROS) and in excess could overwhelm intra- and extracellular antioxidant defense mechanism, resulting in oxidative stress on the lipid raft, particularly polyunsaturated fatty acid

(PUFA) [8]. For example, UVA irradiation causes an inflammatory response in skin [9] and upregulates cyclooxygenase and lipoxygenase enzymes to release prostaglandin-F_{2α} (PGF_{2α}) and 12-HETE from arachidonic acid (AA), respectively. The generation of these metabolites is found to be associated to immune responses, pigmentation, cutaneous wound healing and inflammatory diseases [10–13].

PUFA is prone to nonenzymatic oxidation by ROS and free radicals due to the vulnerable skipped diene double bonds in the structure, to produce oxidized lipid metabolites namely, isoprostanooids such as F₂-isoprostanes (F₂-IsoPs) from AA. F₂-IsoPs is a well-validated biomarker for oxidative damage by free radicals and have shown to possess activities related to inflammation and immunity in different biological systems [9]. Furthermore, increased F₂-IsoPs level is reported in sunburnt skin and wound fluid of damaged skin [14–17].

Aside from AA, other PUFAs exist such as docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids that can be peroxidized to generate neuroprostanes (NeuroPs) and F₃-Isoprostanes (F₃-IsoPs), respectively [18–20]. However, under high level of oxygen, the formation of isofuranoids, e.g. isofurans and neurofurans, dominates with minor amount of isoprostanoids. Nevertheless, consumption of DHA and EPA is suggested to contribute to healthy skin and prevent skin inflammation [21,22], and more recently their oxidized metabolites are reported to protect cardiovascular system and have anti-inflammatory properties [23,24].

UVA-induced oxidative damage may also occur at nuclear level, causing DNA damage. This results in the formation of numerous oxidative DNA adducts [25] specifically, 8-hydroxy-2'-deoxyguanosine (8-OHdG) which is the most abundant and widely studied due to its mutagenicity and utility as a reliable marker of oxidative DNA damage [5]. Oxidative DNA damage triggers cell cycle machinery to arrest its progression for cell repair [26]. If the DNA damage is beyond repair, it undergoes permanent cell cycle arrest or apoptosis to avoid mutation in the genome [27] and accordingly serve as the indicator of cell damage.

Although exposure to UVA is less detrimental to skin compared to UVB, several reports showed UVA could be harmful, leading to inflammation and lipid peroxidation. It is unclear if short daily UVA exposure to skin can trigger cell damage by free radical-oxidative stress, and if recovery is possible. In this study, human keratinocytes (HaCaT cells) were exposed to short-time UVA to investigate the etiology of the cell cycle and PUFA lipid profiles including its oxidized metabolites.

Materials and methods

Cell culture

Human keratinocytes (HaCaT) (gift from Professor GSW Tsao, Li Ka Shing Faculty of Medicine, The University of Hong Kong) were maintained in a monolayer culture at 5% CO₂, 37 °C in RPMI-1640 supplemented with fetal bovine serum (10% FBS) and penicillin – streptomycin (5%). The cells were sub-cultured every 3 days at a 1:3 split ratio, and the experiments were performed at approximately at 70%–80% confluence unless stated.

UVA irradiation treatment

The culture medium of HaCaT cells was replaced by a thin layer of phosphate-buffered saline (PBS). The cells were then exposed to of 5, 10, or 20 J/cm² (100 Watt, 365 nm, high-intensity UV Lamp, UVP® Blak-Ray®,

Upland, CA) UVA dose. The irradiated cells were immediately re-incubated into the new culture medium and samples were taken at various time points: immediately after treatment (0h), and 8h, 12h, 24h post-treatment and then harvested using trypsin-EDTA (Sigma, St. Louis, MO). Nonirradiated control cells were treated accordingly.

MTT assay

MTT assay was used to quantitatively determine the rate of cellular proliferation by Mosmann's method with slight modifications [28]. Briefly, the cells were seeded at 1.2×10^4 cells/well in 96 well-plates. Following UVA treatment, the cells were incubated at 37 °C, 5% CO₂ for 24 h before the MTT test was performed. The culture medium was supplemented with 100 µl of MTT reagent ($c = 800 \mu\text{g/ml}$) to each well and incubated for 4 h at 37 °C. Afterward, MTT solution was removed and 100 µl of acidified isopropanol was added. The plates were left in the dark and shaken for 30 min. Absorbance readings were performed at 595 nm with the reference of 750 nm using iMark microplate reader (Bio-Rad, Hercules, CA). Inhibition rate of cell proliferation was calculated by $1 - (\text{OD of treated cells} / \text{OD of controls}) \times 100\%$.

Cell cycle analysis

After UVA treatment, HaCaT cells were harvested and fixed with 5 ml of ice-cold 70% ethanol and 5 mM butylated hydroxytoluene (BHT) and 5 mM indomethacin to prevent lipid peroxidation. For cell cycle analysis, the cell samples were prepared by Leung et al. [29] method with slight modifications. The fixed cells were centrifuged at $800 \times g$ for 5 min at 4 °C and washed with PBS. Then, the cells were incubated with propidium iodide (PI) staining solution containing PI (50 µg/ml), RNase (10 µg/ml), Tris-base (0.01 M), NaCl (10 mM), and Nonidet P-40 (0.1%) (Sigma-Aldrich, St Louis, MO). The stained cells were incubated in the dark for 30 min at room temperature and thereafter, DNA content was determined by flow cytometry (Beckman Coulter, Brea, CA) and analyzed using FlowJo software (TreeStar, Ashland, OR).

Extraction of polyunsaturated fatty acids and oxidized lipid products

PUFA and oxidized lipid products were extracted from cell samples ($\sim 1 \times 10^6$ cells in PBS with BHT and indomethacin) collected as described previously [29]. First, the cells were freeze-thawed and then homogenized in 10 ml of Folch solution (chloroform/methanol 2:1 v/v + 0.05% BHT) using blade homogenizer (T25, ULTRA-TURRAX, IKA, China). After centrifugation at

800 × *g* for 10 min at 4 °C, the lower organic phase was transferred to a glass vial and the solvent was evaporated under a stream of nitrogen gas. The dried extract was re-suspended in 1 M potassium hydroxide in methanol (1:1). The samples were then hydrolyzed overnight, in dark and at room temperature (25 °C). After hydrolysis, the samples were cooled and neutralized by hydrochloric acid. Finally, formic acid (pH 4.5) was added and mixed together with heavy labeled internal standards 4-(*RS*)-4- F_{4t} -NeuroP- d_4 and 10- F_{4t} -NeuroP- d_4 synthesized by Durand's group (IBMM, Montpellier, France), 5- F_{2t} -IsoP- d_4 , 15- F_{2t} -IsoP- d_4 , PGF $_{2\alpha}$ - d_4 , 5(S)-HETE- d_8 , 12(S)-HETE- d_8 , 15(S)-HETE- d_8 , 20-HETE- d_6 , arachidonic acid- d_8 , eicosapentaenoic acid- d_5 , and docosahexaenoic acid- d_5 (Cayman Chemical, Ann Arbor, MI). The samples were then cleaned and extracted using anionic exchange solid-phase extraction columns (60 mg, Oasis MAX, Waters, Milford, MA) as described in our previous reports [29–31].

Lipids and oxidized lipid products analysis by LC-MS/MS analysis

The extracted PUFA and oxidized lipid products were analyzed following our previous studies with modifications [29–32]. Liquid chromatography tandem mass spectrometry system consisting of 1290 Infinity LC system (Agilent, Santa Clara, CA) with a C_{18} column (2.6 μ m particle size, 150 × 2.1 mm, Phenomenex, Torrance, CA) maintained at 30 °C was used. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The flow rate was set to 200 μ l/min, and the injection volume was 10 μ l. The gradient was first maintained at 10% B from 0 to 1 min, then a linear gradient from 10% B to 98% B for 7 min, then 98% B was held for 4 min and finally linear gradient from 98% B to 10% B for 0.1 min. Afterward, the column was re-equilibrated to initial condition. A QTrap 3200 triple quadrupole mass spectrometer (Sciex Applied Biosystems, Framingham, MA) coupled to the LC was operated at negative atmospheric pressure chemical ionization (APCI) mode. The spray voltage was set to –4000 V, and nitrogen gas was used as curtain gas. The scan mode was multiple reaction monitoring (MRM) and the MS/MS transition was monitored as shown in Supplementary information [29,31,33]. Quantitation of each analyte was determined by relating the peak area with its corresponding deuterated internal standard peak. For the analytes without their corresponding deuterated internal standards i.e. ALA, AdA, n-3 DPA, 8(S)-, 9(S)-, and 11(S)-HETE, 5- F_{2t} -IsoP, 8- F_{2t} -IsoP, Isofurans, 7- F_{2t} -DihomolsoP, 17- F_{2t} -DihomolsoP, 7- F_{2t} -DihomolsoF, 17- F_{2t} -DihomolsoF 9- D_1 -PhytoP, 9- F_1 -

PhytoP, *Ent*-16- F_{1t} -PhytoP, 9- L_1 -PhytoP, 16-*epi*-16- B_1 -PhytoP, 16(*R,S*)-13-*epi*-ST- Δ^{14} -9-PhytoF, 5- F_{3t} -IsoP, 8- F_{3t} -IsoP, 15- F_{3t} -IsoP, 4-HDoHE, 7-HDoHE, 8-HDoHE, 10-HDoHE, 11-HDoHE, 13-HDoHE, 14-HDoHE, 16-HDoHE, 17-HDoHE, 20-HDoHE, resolvins E1, resolvins D1, neurofurans, 4(*RS*)-ST- Δ^5 -8-NeuroF, 14(*RS*)-14- F_{3t} -IsoP, and 4- F_{3t} -IsoP, quantitation was performed by using the following deuterated internal standards, 5(S)-HETE- d_8 , 15- F_{2t} -IsoP- d_4 , and 4-(*RS*)-4- F_{4t} -NeuroP- d_4 . A total of 43 oxidized lipid products were quantitated in this study.

Sample preparation for 8-OHdG analysis

Measurement of 8-OHdG was made in the medium of the cell culture ($\sim 5 \times 10^6$ cells) after 24 h with and without UVA treatments since it can be metabolized and readily excreted as free by-product. Sample preparation was performed using Lee et al. [34] method with slight modifications. Briefly, the medium samples were thawed at room temperature and mixed thoroughly by vortex (30 sec). After centrifugation at 5000 × *g* for 5 min at 4 °C, 25 μ l of the sample medium was transferred into 2 ml glass vials with inserts for LC-MS/MS measurement.

LC-MS/MS analysis for 8-OHdG

Levels of 8-OHdG were analyzed by Lee et al. [34] method with modifications. The 1290 Infinity LC system (Agilent, Santa Clara, CA) with a C_{18} column (2.6 μ m particle size, 150 × 2.1 mm, Phenomenex, Torrance, CA) was maintained at 30 °C. The mobile phase consisted of ammonium acetate (10 mmol/l, adjusted to pH 4.3 with acetic acid) and acetonitrile (96.4:3.6 v/v). The flow rate was set to 500 μ l/min, and the injection volume was 10 μ l. A QTrap 3200 triple quadrupole mass spectrometer (Sciex Applied Biosystems, Framingham, MA) was operated in a positive APCI mode. The spray voltage was set to –4000 V, and nitrogen gas was used as curtain gas. The scan mode was MRM, and the MS/MS transition (Supplementary information) was monitored by Lee et al. [34] method. To quantify the content of 8-OHdG, a series of calibrators was prepared (Supplementary information). The calibrators were prepared by spiking known amount of 8-OHdG standard into freeze-thawed cell free culture medium without any treatment.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6.0 (GraphPad Prism, La Jolla, CA). All values were expressed as mean ± SD. Data were analyzed by 2-way analysis of variance (ANOVA) including linear trend

in post-treatment time, and $p < .05$ was noted as statistically significant.

Results

Survival rate after exposure to physiological dose of UVA

HaCaT cells were exposed to 5 J/cm², 10 J/cm², and 20 J/cm² to test for a suitable low and high dose of UVA (Figure 1). The survival rate indicates the damage by physiological UVA dose to HaCaT cells was mild. Levels of 5 J/cm² and 10 J/cm² of UVA exposure reduced approximately 20% of the viable cells after treatment. On the other hand, doubling the dose (20 J/cm²) reduced viable cells by 50% approximately. To investigate the severity of UVA on HaCaT cells, 5 J/cm² (equivalent to 15 min sunlight exposure) was chosen as low and 20 J/cm² (equivalent to 1 h sunlight exposure) as high physiological dose in our study.

Effect of UVA irradiation on cell cycle

No significant changes were observed in the cell cycle of viable HaCaT cells immediately after exposure of low (5 J/cm²) and high (20 J/cm²) UVA doses compared to control (Figure 2). The adverse effect on the cells was noticeable after 8 h for both UVA doses. A significant reduction of cells in S-phase compared to control was observed, showing that there is decreased DNA synthesis of the HaCaT cells after UVA treatment but this inhibition was only transient as we observed recovery after 12 h and 24 h. However, the cell cycle of UVA-treated cells was not at homeostasis as G₂/M-phase arrest occurred after 12 h recovery for both UVA doses. The G₂/M checkpoint potentially blocked the damaged cells to proceed to mitosis and was in transition from recovering by UVA damage [35]. However, after 24 h of

UVA exposure, the S-phase and G₂/M-phase were comparable to control but G₀/G₁-phase was significantly high compared to control for both doses. During this period, it appears the HaCaT cells could reinstate homeostasis and return to normal cell cycle.

Effect of UVA irradiation on DNA damage

Since there were increased HaCaT cells in G₀/G₁-phase after 24 h high-dose (20 J/cm²) UVA exposure, we postulated that there may be DNA damage and potentially the release of the DNA adducts i.e. 8-OHdG into the cell medium. Since the excision, repair-damage and excretion process of the damaged DNA is long (usually 24–48 h in human urine), we measured the concentration in cell medium of HaCaT cells after 24 h from UVA exposure. Interestingly, no 8-OHdG was detected in the treated HaCaT cell medium, indicating UVA doses used in this study was insufficient to cause major DNA damage in HaCaT cells.

Effect of UVA irradiation on the lipid profile

Exposure of low dose

Low (5 J/cm²) UVA dose exposed to HaCaT cells showed no significant impact on the levels of n-6 PUFA, and all enzymatic and nonenzymatic oxidized PUFA products immediately after exposure (0 h). However, n-3 PUFAs were stimulated, as ALA levels were significantly elevated immediately after exposure compared to control (nontreated), and DHA levels were elevated at 8 h after exposure but returned to control level thereafter (Figure 3).

Treatment of 5 J/cm² UVA on HaCaT cells appeared to have a weak effect on the peroxidation of PUFA, and only 21 out of 43 oxidized PUFA products were detectable in the HaCaT cells and not all showed a significant change. Only some LOX-mediated oxidation of PUFA was activated after exposure, namely 8-HETE and 12-HETE levels from AA and 8-HDoHE from DHA were significantly increased whereas 7-HDoHE level decreased after 8 h compared to control. These changes were temporary and gradually returned to baseline level. We also observed in a linear manner a significant reduction of 14-HDoHE level and elevation of COX-mediated 13-HDoHE level with post-treatment time (Table 1).

Only a few nonenzymatic oxidized PUFA products were modified, mainly, 16-HDoHE level from DHA significantly increased after 8 h, and 17-Dihomo-IsoP level from AdA and 15-F_{3t}-IsoP level from EPA increased significantly after 12 h exposure compared to control.

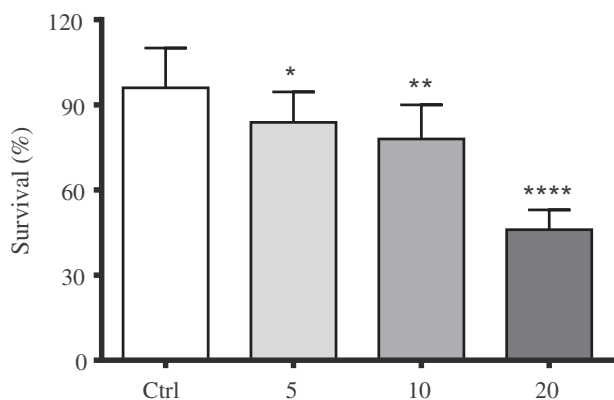


Figure 1. Cell viability of HaCaT cells exposed to different UVA treatment. Values are mean \pm SD, $n = 10$, 1.2×10^4 cells per set. * $p < .05$, ** $p < .01$, and **** $p < .0001$ versus control (Ctrl).

However, all returned to control level at 24 h, indicating the HaCaT cells could recover from oxidative stress after low-dose UVA exposure (Table 1, Figure 3). We further found a noticeable linear trend with post-treatment time on the suppression of 15-F_{2t}-IsoP from AA.

Exposure of high dose

High-dose (20 J/cm²) UVA exposure to HaCaT cells showed no significant impact on the levels of PUFA, and enzymatic and nonenzymatic oxidized PUFA products immediately after exposure and after 8 h and 12 h (Figure 4). Only AA level decreased compared to controls after 8 h and 12 h of exposure (Figure 4, Table 2). However, after 24-h exposure, AA, n-3 DPA and DHA were significantly elevated whereas AdA and EPA decreased compared to control, and consistently, these findings showed a significant linear trend with post-treatment time (Figure 4).

As like low UVA dose exposure, only 21 out of 43 oxidized PUFA products were detected in the HaCaT cells after high-dose exposure. Mixed observations were made for the oxidized products of DHA mediated by enzymatic pathway after high UVA exposure.

Unlike other reports, products released via LOX such as resolvin D1 and its precursor 17-HDoHE were not detected [36]. However, 4-HDoHE and 14-HDoHE levels were reduced after 8 h. Notably, 8-HDoHE level increased and 14-HDoHE level acutely reduced after 24 h, and both showed significant decrease in linear trend with post-treatment time. Furthermore, only LOX-mediated 12-HETE level from AA increased immediately after exposure but returned to control level (Table 2). Moreover, not all nonenzymatic oxidized PUFA products detected were significantly altered compared to control and only 5-F_{3t}-IsoP level from EPA was significantly lower compared to control at 12 h and 24 h post-treatment. Our observations also showed a significant linear trend with post-treatment time in 5-F_{2t}-IsoP, Isofurans, 5-F_{3t}-IsoP and Neurofurans, and 20-HDoHE level reduction (Figure 4).

Discussion

UVA exposure to the skin can penetrate deep and be absorbed by the epidermis including the inner dermis whereas UVB is mainly absorbed by the stratum

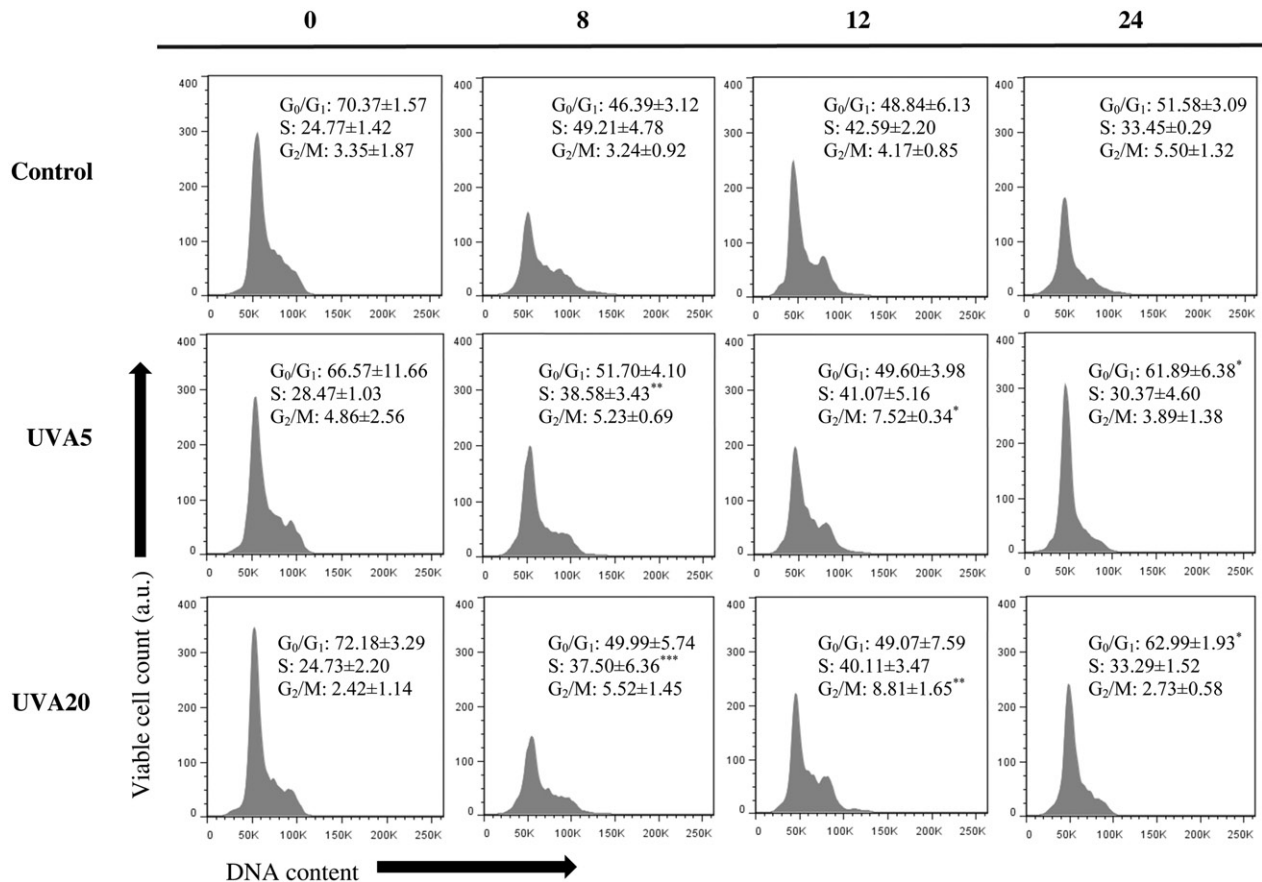
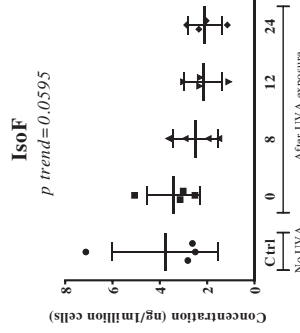
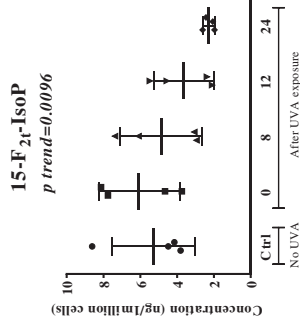
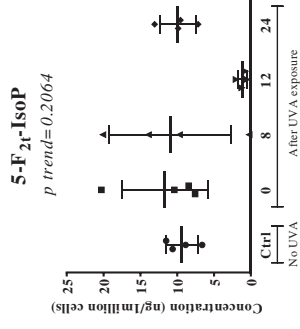
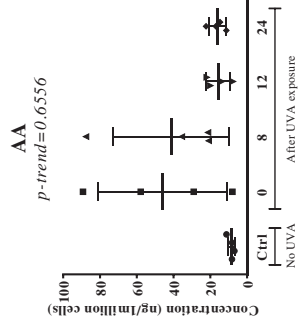
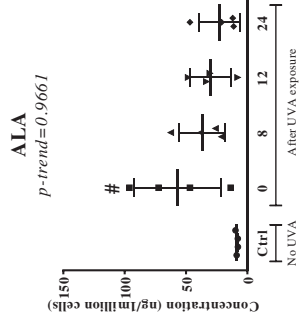


Figure 2. Cell cycle distribution of HaCaT cells before and after 5 J/cm² and 20 J/cm² UVA exposure. Values are mean ± SD, *n* = 4. **p* < .05, ***p* < .01, and ****p* < .001 versus control. G₀: Gap phase; G₁: G₁ checkpoint; S: synthesis phase; G₂: G₂ checkpoint; M: mitosis.

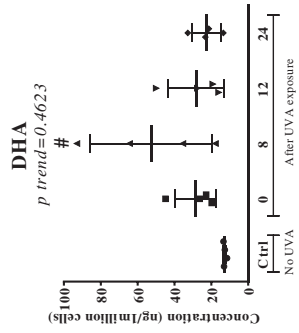
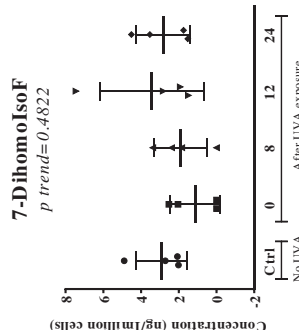
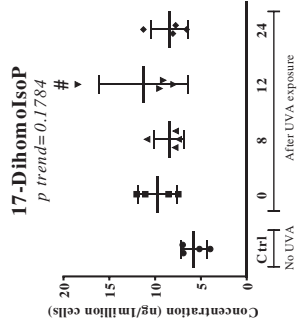
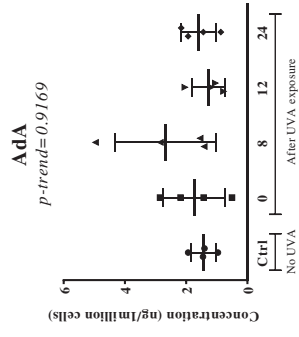
AA



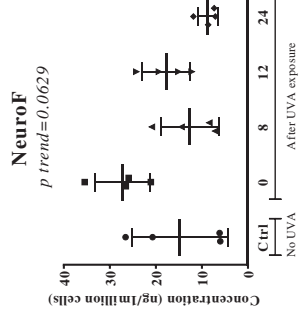
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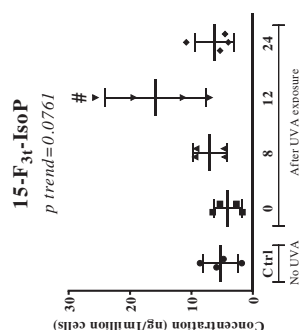
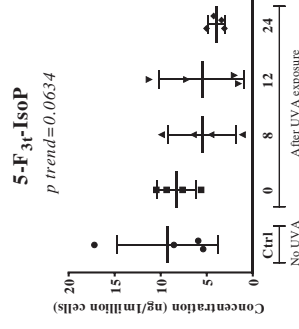
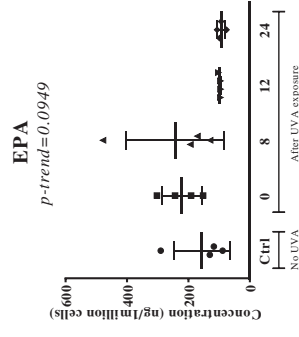
AdA



DHA



EPA



n-3 DPA

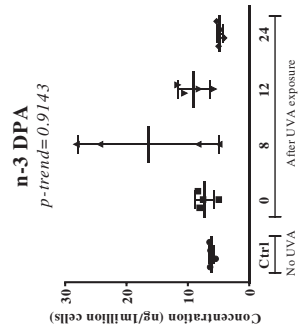


Figure 3. PUFA and nonenzymatic metabolites in HaCaT cells before and after 5J/cm² UVA exposure. Values are mean \pm SD, $n = 4$. # $p < .05$ versus control. AA: arachidonic acid; ALA: α -linolenic acid; AdA: adrenic acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; IsoP: isoprostane; IsoF: isofurans; NeuroF: neuroprostane; NeuroF: neurofurans.

Table 1. Enzymatic and nonenzymatic metabolites in HaCaT cells before and after 5 J/cm² UVA exposure.

	Control	0 h	8 h	12 h	24 h	<i>p</i> -trend
<i>COX-mediated</i>						
PGF _{2α}	1.48 ± 0.39	1.36 ± 0.27	1.19 ± 0.32	1.81 ± 0.87	1.30 ± 0.43	0.913
13-HDoHE	6.65 ± 2.78	2.43 ± 0.34	7.12 ± 06.92	18.35 ± 6.85	10.56 ± 11.41	0.047
<i>LOX-mediated</i>						
5-HETE	1.65 ± 0.80	4.07 ± 4.78	6.25 ± 3.91	4.43 ± 5.21	11.83 ± 12.75	0.084
8-HETE	3.43 ± 2.45	4.14 ± 0.06	10.78 ± 4.74*	6.73 ± 3.36	7.66 ± 2.40	0.087
12-HETE	0.88 ± 0.20	1.20 ± 0.44	2.52 ± 1.62*	1.36 ± 0.65	0.60 ± 0.15	0.777
4-HDoHE	5.44 ± 3.09	4.43 ± 1.92	5.54 ± 1.67	4.73 ± 2.78	2.41 ± 2.28	0.192
7-HDoHE	20.47 ± 4.88	14.77 ± 4.24	13.12 ± 2.67*	19.06 ± 1.09	15.56 ± 1.49	0.294
8-HDoHE	12.97 ± 7.87	13.13 ± 6.89	33.69 ± 19.11*	16.03 ± 7.24	8.64 ± 5.72	0.732
10-HDoHE	4.94 ± 2.58	3.87 ± 1.31	5.37 ± 0.29	8.40 ± 5.10	2.75 ± 1.86	0.969
11-HDoHE	2.57 ± 1.58	3.31 ± 1.94	5.65 ± 4.33	9.01 ± 7.47	2.15 ± 0.27	0.480
14-HDoHE	38.04 ± 22.15	32.75 ± 20.48	24.73 ± 9.90	16.66 ± 14.60	17.96 ± 4.19	0.049
<i>Nonenzymatic</i>						
16-HDoHE	7.86 ± 5.32	9.25 ± 6.98	26.55 ± 12.32*	11.05 ± 10.97	5.06 ± 5.27	0.793
20-HDoHE	13.48 ± 6.91	9.65 ± 4.04	9.64 ± 1.27	10.09 ± 2.66	11.63 ± 1.87	0.620

Values are mean ± SD, *n* = 4.

**p* < .05 versus control.

COX: cyclooxygenase; LOX: lipoxygenase; PGF_{2α}: prostaglandin F_{2α}; HDoHE: hydroxyl-DHA; HETE: hydroxyeicosatetraenoic acid.

corneum and some by the epidermis [37]. As a consequence, both radiation leads to skin photoaging and photocarcinogenesis via oxidative stress. UVB radiation is known to be more harmful on human skin than UVA as it has a higher ionization energy and easily absorbed by proteins and nucleic acids to cause direct DNA damage while UVA initiates injury from cells surface.

In this study, human keratinocytes (HaCaT cells) were exposed to 5 J/cm² and 20 J/cm² of UVA which is equivalent to 15 min and 1 h of sunlight exposure in cloudless sunny day, respectively [38]. This amount of sunlight exposure is common for indoor working people. Although Lowe et al. [39] showed UVA dose required to produce minimal erythema in normal fair skin is about 29 J/cm², which is equivalent to 1.5 h of sunlight exposure, a higher dose of UVA was not considered in our study as 20 J/cm² already reduced 50% of the HaCaT cell viability; a higher dose may further decrease the viable HaCaT cells, which is too low for us to regrow for analysis.

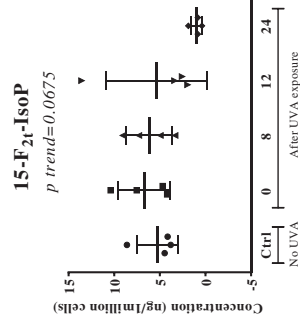
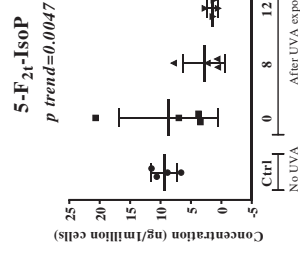
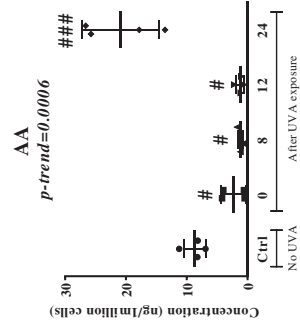
Despite the survival rate of HaCaT cells exposed to 20 J/cm² UVA to be 50% only, the cell cycle was not disrupted immediately. During the cell recovery period, transient S-phase reduction and G₂/M phase arrest were observed but both returned to homeostasis after 24 h. However, G₀/G₁-phase was still significantly high, demonstrating that the homeostasis of HaCaT cells from UVA exposure may be incomplete therefore, 8-OHdG analysis was carried in the study to identify if potential DNA damage took place and excreted to the media. In correspondence, it is also suggested that elevated 8-OHdG is associated to apoptosis [40]. We found 8-OHdG to be below the detection (<0.1 nmol/L) level when measured by LC-MS/MS indicating UVA exposure to HaCaT cells did not induce apoptosis and had the ability

to recover to homeostasis. HaCaT cell is an immortalized human cell line containing mutant p53 gene at H179Y/R282W. This mutation may cause changes in HaCaT cell compared to normal human keratinocytes which include modification in morphology, growth, and extended life span [41]. For this reason, HaCaT are susceptible to cell cycle alteration or DNA damage when exposed to UVA radiation compared to primary keratinocytes due to mutation therefore, the cell cycle analysis in this report may not align with those that use primary keratinocytes for experimentation.

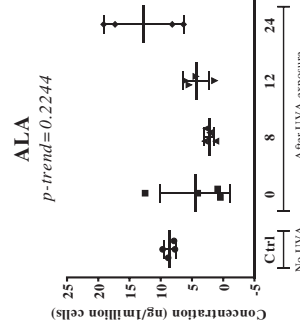
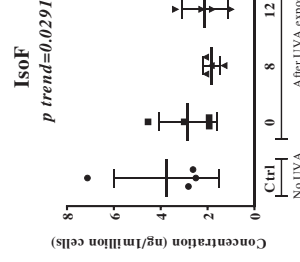
Our study showed UVA equivalent to 15 min or 1 h sunlight exposure was sufficient to alter the composition of lipid profile in the skin cells. Of note, although we aspirated the necrotic cells suspended in the cell culture medium prior to lipid extraction in the study, it does not mean the composition of the cells used for the analysis were free of dead cells. It is known that skin has low PUFA content compared to other lipids [42] but can be the initiator and propagator of lipid peroxidation under UV exposure [43–45]. We identified that high dose of UVA radiations seems to accumulate PUFA and disrupt normal PUFA metabolism even after 24 h. The accumulation of PUFA may alter the skin cells' function for example, increase the fluidity of cell membrane [45] and increase the ease of lipid peroxidation in skin [43–45] which may bring health risk to our skin. Indeed, we observed that high-dose UVA could stimulate n-3 DPA and DHA in HaCaT cells, and this group of n-3 PUFA especially DHA is known to have protective effect on skin-related ailments [21,22].

We demonstrated that UVA exposure failed to extensively propagate the generation of nonenzymatic oxidized lipid products (IsoPs) from PUFA and more so, isofurans and neurofurans from AA and

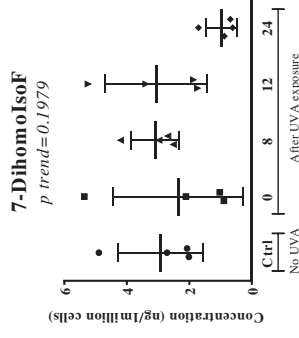
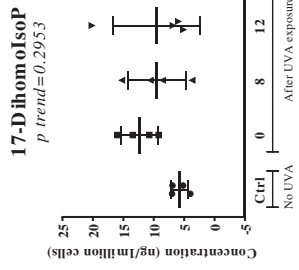
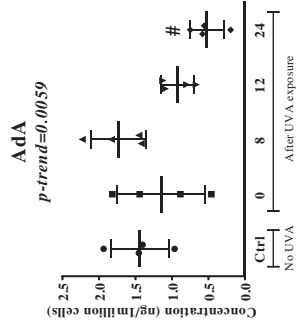
AA



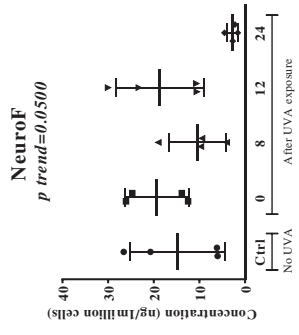
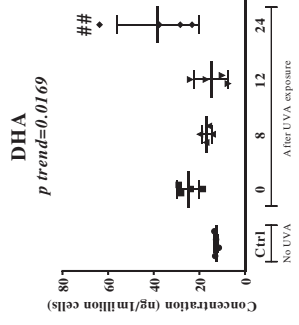
ALA



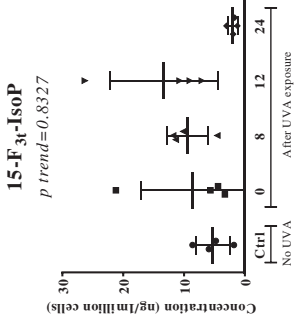
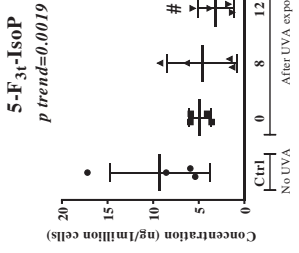
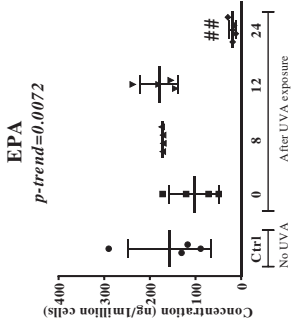
AdA



DHA



EPA



n-3 DPA

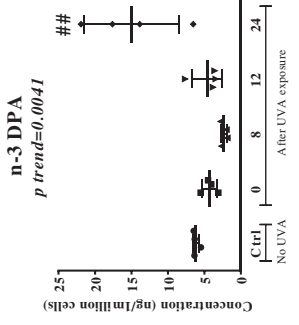


Figure 4. PUFA and nonenzymatic metabolites in HaCaT cells before and after 20 J/cm² UVA exposure. Values are mean \pm SD, $n = 4$. ## $p < .01$, and ### $p < .001$ versus control. AA: arachidonic acid; ALA: α -linolenic acid; AdA: adrenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; IsoP: isoprostane; IsoF: isofurans; NeuroP: neuroprostane; NeuroF: neurofurans.

Table 2. Enzymatic and nonenzymatic metabolites in HaCaT cells before and after 20 J/cm² UVA exposure.

	Control	0 h	8 h	12 h	24 h	<i>p</i> -trend
<i>COX-mediated</i>						
PGF _{2α}	1.48 ± 0.39	6.02 ± 3.34	6.24 ± 2.79	4.62 ± 2.35	0.50 ± 0.27	0.3934
13-HDoHE	6.65 ± 2.78	12.56 ± 11.61	9.64 ± 4.84	9.14 ± 3.96	2.65 ± 2.43	0.3086
<i>LOX-mediated</i>						
5-HETE	1.65 ± 0.80	14.40 ± 17.12	4.82 ± 2.73	10.63 ± 6.27	2.52 ± 1.41	0.8549
8-HETE	3.43 ± 02.45	20.57 ± 17.14	15.62 ± 5.90	9.19 ± 6.71	12.05 ± 11.70	0.7744
12-HETE	0.88 ± 0.20	3.34 ± 3.58*	1.07 ± 0.64	0.98 ± 0.35	0.82 ± 0.40	0.2168
4-HDoHE	5.44 ± 3.09	3.05 ± 31.86	1.57 ± 0.20*	4.26 ± 2.15	1.90 ± 0.57	0.0638
7-HDoHE	20.47 ± 4.88	44.54 ± 29.14	24.87 ± 4.57	26.78 ± 4.80	6.82 ± 1.21	0.1073
8-HDoHE	12.97 ± 7.87	12.06 ± 6.05	3.89 ± 0.48	7.10 ± 3.52	27.64 ± 9.89*	0.0305
10-HDoHE	4.94 ± 2.58	6.72 ± 3.22	3.32 ± 1.14	3.41 ± 0.70	4.25 ± 1.45	0.1137
11-HDoHE	2.57 ± 1.58	1.75 ± 9.79	1.13 ± 1.36	1.77 ± 1.05	4.39 ± 1.72	0.0962
14-HDoHE	38.04 ± 22.15	15.69 ± 9.55	6.22 ± 1.57**	21.75 ± 4.70	1.01 ± 0.33**	0.0043
<i>Nonenzymatic</i>						
16-HDoHE	7.86 ± 5.32	9.82 ± 8.33	6.76 ± 1.56	5.05 ± 2.48	6.08 ± 2.32	0.2012
20-HDoHE	13.48 ± 6.91	19.35 ± 8.71	8.97 ± 2.89	10.66 ± 4.13	2.53 ± 1.62*	0.0007

Values are mean ± SD, *n* = 4. Significant value is represented in bold font.

p* < .05, *p* < .01 versus control.

COX: cyclooxygenase; LOX: lipoxygenase; PGF_{2α}: prostaglandin F_{2α}; HDoHE: hydroxy-DHA; HETE: hydroxyeicosatetraenoic acid.

DHA, respectively. Moreover, there was a trend to find decreased isofurans and neurofurans after UVA treatment and the reason for this is unknown. Both metabolites are released in elevated oxygen tension and are highly associated with lung and neuronal damage under extreme oxygen condition. Although several studies reported the elevation of IsoPs in skin exposed to UVB [15,46–48], reports on UVA exposure are scarce. Insofar it is known that UVR can indirectly damage our skin via a high level of ROS [49] and lead to lipid peroxidation in cell membrane to release toxic (mainly from n-6 PUFA) and nontoxic (mainly from n-3 PUFA) products. The toxic ones tended to be lower and nontoxic ones higher after UVA exposure in this study. However, not all oxidized PUFA products were found in HaCaT in this study where only 21 out of the 43 products were observed. This may not be due to nonexistence of the other 22 products in the HaCaT cells, but rather, the concentration may be at trace level to be detected. Further, the elevation of IsoPs in skin is commonly found by UVB exposure but not UVA and may be due to higher ionization power by UVB than UVA; therefore, the level of ROS generated by the latter is lower. It agrees with Vostalova et al. [50] finding where dose-dependent increase in myeloperoxidase activity, an inflammatory marker in the skin tissues of hairless mice, was observed only by UVB but not by UVA exposure. Eminently, studies showed PUFA converted to products such as F₂-IsoPs from AA in the skin after UVB exposure could lead to cell signaling disruption, carcinogenesis, and photoaging in skin [51]. However, the property of IsoPs production by UVA in skin is still unclear.

Generation of oxidized lipid products from enzymatic pathway is commonly associated to inflammation [52–54] and those related to AA are known to be

pro-inflammatory and DHA to be anti-inflammatory [55]. For instance, it is reported cyclooxygenase (COX)-dependent PGF_{2α} and lipoxygenase (LOX)-dependent 8-HETE from AA to be pro-inflammatory agents [52,56,57] in different tissues including skin. Our study did not display a strong pro-inflammatory effect via PGF_{2α} and HETEs by UVA on HaCaT cells and instead, we found HDoHE from DHA to be activated. The role of HDoHE in keratinocytes is not well elucidated. Although no measurable amount of 17-DHoHE was found in our investigation, it is proposed to have putative anti-inflammatory effect in epidermal of guinea pig supplemented with fish oil [58].

Alteration of PUFA observed in the study showed destabilization of PUFA metabolism especially 24 h after UVA exposure. AdA and EPA were suppressed compared to control whereas AA, n-3 DPA, and DHA were elevated after 24 h and these modifications were not associated with the changes of the oxidized lipid products. In addition, it appears no desaturation and elongation of n-3 DPA and DHA from EPA took place before 24 h but then reactivated after 24 h, releasing more than control levels while simultaneously depleting EPA. This observation suggests exposure of 1 h UVA to the skin requires 24 h to return to PUFA homeostasis. Regardless, it is noted that some levels of PUFAs and oxidized lipid metabolites measured had a large deviation within the same set of sample treatments. This may be attributed by the rate of free radical generation and oxidation by UVA which could be less controllable compared to other extrinsic manipulation such as sonoporation as shown in our previous report [29].

In conclusion, short-term UVA exposure to HaCaT cells appears not as deleterious as anticipated. HaCaT cells may have a certain level of defense mechanism,

e.g. elevation of DHA when exposed to UVA thereby assisting them to recover from potential damage. Nonetheless, in our study, UVA received from 1 h duration of sunlight exposure can modify PUFA composition and its metabolism in skin cells. However, the effect on extensive exposure requires elucidation as it may be detrimental and pose a risk to skin health [59].

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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