

# Serum endocannabinoids and N-acyl ethanolamines and the influence of simulated solar UVR exposure in humans in vivo

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**Serum endocannabinoids and N-acyl ethanolamines and the influence of simulated solar UVR exposure in humans *in vivo***

**Short title: Impact of UVR on human serum endocannabinoids**

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**Abstract**

Solar ultraviolet radiation (UVR) exposure of human skin has beneficial and harmful effects on health, including impact on immune function, inflammation and reportedly mood, but these are not fully elucidated. Since the endocannabinoid system is implicated in many activities including mood alteration, our objective was to (i) determine and quantify circulating levels of a wide range of endocannabinoid and N-acyl ethanolamine (NAE) species (ii) evaluate whether these are modulated by cutaneous UVR exposures, as attained through repeated low level summer sunlight exposure. Wearing goggles to prevent eye exposure, 16 healthy volunteers (23-59y; 10 light skin, phototype II, and 6 dark skin, phototype V) received the same UVR exposures (1.3 SED, 95% UVA/5% UVB) thrice weekly for 6 weeks, whilst casually dressed to expose ~35% skin surface area. Blood samples were taken at baseline, days 1, 3 and 5 of week one, then at weekly intervals, and analysed by LC-MS/MS. Eleven endocannabinoids and NAEs were detected and quantified at baseline, with N-palmitoyl ethanolamine the most abundant (30% of total). Levels did not vary according to phototype ($p>0.05$), except for the NAE docosapentaenoyl ethanolamide, which was higher in phototype II than V ($p=0.0002$). Level of the endocannabinoid, 2-AG, was elevated during the UVR exposure course ($p<0.05$ vs baseline for all subjects; $p<0.01$ for each phototype group), with maximum levels reached by week 2-3, while NAE species did not significantly alter. These findings suggest differential involvement of the cutaneous endocannabinoid system in low dose solar UVR responses in humans.

**Abbreviations**

| | |
|---|---|
| 2-AG | 2-Arachidonoyl glycerol |
| AEA | N-Arachidonoyl ethanolamine (anandamide) |
| CB | Cannabinoid receptor |
| DGLEA | N-Dihomo-γ-linolenoyl ethanolamine |
| DHEA | N-Docosahexaenoyl ethanolamine |
| DPEA | N-Docosapentaenoyl ethanolamine |
| EPEA | N-Eicosapentaenoyl ethanolamine |
| LC-MS/MS | Liquid chromatography coupled to tandem mass spectrometry |
| LEA | N-Linoleoyl ethanolamine |
| MED | Minimal erythemal dose |
| MEA | N-Myristoyl ethanolamine |
| NAE | N-Acyl ethanolamine |
| OEA | N-Oleoyl ethanolamine |
| PEA | N-Palmitoyl ethanolamine |
| STEA | N-Stearoyl ethanolamine |
| UVR | Ultraviolet radiation |

## INTRODUCTION

Solar ultraviolet radiation (UVR) exposure of the skin has a range of beneficial but also harmful effects on health, with vitamin D synthesis, sunburn, skin cancer induction, photosensitivity and photoageing being well documented,[1] while further impacts including production of other hormones and modulation of immune and inflammatory status are less well elucidated. UVR is pro-inflammatory and immunomodulatory, reducing cell-mediated immunity while augmenting innate responses, and in predisposed individuals activates the Herpes simplex virus. It is also observed that sunlight exposure causes a 'feel-good factor' or euphoria, which could be mediated by UVR.[2-4]

Mood enhancement is observed in indoor tanning, where skin is exposed to the UVR component of sunlight alone; many individuals continue to self-expose despite knowledge of the adverse consequences, leading to the term 'tanorexia' or addictive-like tanning behaviour.[2,3,5] Whilst this phenomenon has been suspected to be attributable to circulating endorphins, akin to mood enhancement after intense exercise,[6] β-endorphin is unable to cross the blood-brain barrier[7] and investigations for a role of endorphins in tanorexia proved inconsistent.[8-11] Whilst a potential opioid role continues to be explored,[12] and induction of withdrawal –like symptoms was observed after opioid blockade in frequent tanners,[13] recently, the endocannabinoid system has been implicated in 'runner's high,' with increased circulating levels of anandamide (AEA), which can cross the blood-brain barrier, detected after intense aerobic exercise.[14-17]

Recent studies evidence the extensive cutaneous profile of lipid mediators in human skin, encompassing the endocannabinoids, NAE, sphingolipids and eicosanoid families.[17-21] Some of these lipid species are known to be modulated

by UVR and to play a role in photobiological effects in humans[22, 23] whilst the potential involvement of the endocannabinoids and their congeners in UVR-induced effects, awaits further exploration. The main endocannabinoids anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), and a range of N-acyl ethanolamine (NAE) species, derive from membrane lipids (Fig. 1).[24-26] AEA, 2-AG and some NAE species are physiological ligands for the G-protein coupled cannabinoid (CB) receptors, originally identified as the target for biologically active components of the cannabis plant.[27-29] They are active in neurotransmission in the central and peripheral nervous systems, including reduction in pain perception via $CB_1$ and transient receptor potential vanilloid-1 receptors (TRV-1),[30] and show anti-inflammatory/immune-modulatory effects via peripheral $CB_2$ receptors, including reduced IFN-δ and increased IL-10 secretion.[31] Although $CB_1$ receptors were traditionally described in the central nervous system and $CB_2$ peripherally as in immune cells, it has become evident their distribution is more variable and widespread throughout organ systems, including skin[21,32-33] which has been shown to possess a functional endocannabinoid system.[17] $CB_1$ and $CB_2$ receptors are expressed by keratinocytes and melanocytes, and also identified in sebocytes and hair follicles. Recent evidence also suggests that the endocannabinoid system helps skin maintain homeostasis and respond to UVR challenge, with $CB_1$/$CB_2$-deficient mice experiencing increased allergic contact dermatitis[34] and cutaneous carcinogenesis.[35]

Despite increased interest in roles of endocannabinoids in human physiology and disease, including mood, information on individual mediators and their responses to cutaneous UVR exposure is sparse. Skin is a large organ

that may substantially contribute to circulating endocannabinoids and NAEs; this may have consequences for mood, immune, inflammatory and other functions. In this study, we used a UVR protocol (including UVR emission, dose and skin site) mimicking a summer's repeated low-level, sunlight exposures, to examine potential influence on circulating endocannabinoids and NAEs in daily life, with particular interest in AEA and 2-AG. Detection and quantification of a wide range of circulating species, was by LC-MS/MS. Different phototypes were included as melanisation may affect UVR responses.

Our aims were to assess the range and quantity of endocannabinoids and related NAE in human sera and to examine their responses to multiple low-level UVR exposures, as could be experienced incidentally in summer. Our research calls attention to the possibility that the endocannabinoid system may play a role in responses to sunlight/UVR in healthy humans, thus opening novel avenues of research.

## MATERIALS AND METHODS

### Study subjects and design

Healthy volunteers were recruited (January 2010). Exclusion criteria were pregnancy, breastfeeding, taking photoactive medication or supplements that contained vitamin D, a history of skin cancer or a photosensitivity disorder, and use of a sunbed or sunbathing in the 3 months prior to or during the study. Body mass index (in $kg/m^2$) was calculated as weight/height$^2$. Ethical approval was obtained from the North Manchester Research Ethics Committee (reference 09/H1014/73), as part of a study examining additional UVR outcomes.[36] Informed consent was obtained and the study adhered to the principles of the Declaration of Helsinki. Participants proceeded through the study process as outlined in the protocol overview (Fig 2). Participants were white Caucasians of Fitzpatrick[37] sun-reactive skin type II (i.e. usually burns, sometimes tans) or of South Asian ethnicity with skin type V (brown skin).

### Minimal erythemal dose (MED) assessment

The MED, defined as the lowest dose of UVR that produced a visually discernable erythema at 24 hours, was assessed in each subject prior to the exposure course, as a precaution. A geometric series of 10 doses (7–80mJ/cm$^2$ for phototype II, 26.6–271mJ/cm2 for phototype V) of erythemally weighted UVR was applied over 2 horizontal rows of buttock skin with a Waldmann UV 236B unit containing Waldmann CF-L 36W/UV6 lamps (peak emission: 313nm; range: 290–400nm; Waldmann GmbH, Villinge-Schwenningen, Germany).

Simulated summer sunlight UVR exposures

Volunteers were given a six-week course of UVR exposures 3x weekly (Monday, Wednesday and Friday at approximately the same time of day), concordant with the length of the summer school holiday period when the population is most exposed to sunlight, as previously described.[38] They wore opaque UVR-blocking eye protection goggles (4-eyez, Scottsdale, AZ, USA), and standardised T-shirts and knee-length shorts to expose approximately 35% skin surface area. A Philips HB588 whole body horizontal irradiation cabinet (Eindhoven, The Netherlands) fitted with Arimed B (Cosmedico GmbH, Stuttgart, Germany) and Cleo Natural (Philips, Eindhoven, The Netherlands) fluorescent tubes provided an UVR emission close to summer sunlight (95% UVA: 320–400nm, 5% UVB: 290–320nm), which was characterised and monitored by spectroradiometry, as described.[38] The course of simulated solar UVR was given simultaneously to all volunteers in wintertime (January/February) when ambient UVB is negligible, with a low dose UVR exposure of 1.3 standard erythemal dose[39] at every visit. The time to deliver this dose was 6.5 minutes; a constant UVR dose was maintained throughout the study by adjusting for decrease in irradiance by increasing delivery time. Using radiative-transfer modeling to translate this to real-life exposures, this equates to ~13-17 minutes of unshaded sunlight exposure on a clear June midday in Manchester, UK (53.5N) 6x weekly, which takes account of (i) ventral and dorsal surfaces are not irradiated simultaneously in sunlight and (ii) postures may range from the horizontal to the vertical randomly orientated to the sun.[40]

Endocannabinoid and NAE analysis

Blood samples were taken pre-UVR exposures on Monday, Wednesday and Friday of the first week of irradiation at the same time of day (i.e. 10 am) to within 60 minutes on each occasion, to look for any shorter-term changes in levels, and each subsequent Monday until course-end (i.e. 3 days after last irradiation of the week) to identify any cumulative effects, and serum was stored at −20°C until study completion. Samples were defrosted on ice and 3 ml of ice-cold 2:1 (v/v) chloroform/methanol added. Anandamide-*d8* (20ng/sample) and 2-arachidonoyl glycerol-*d8* (40ng/sample) (Cayman Chemicals, Ann Arbor, MI, USA) were added as internal standards. Samples were mixed and incubated on ice for 30min. 500µl of water was added to each sample before centrifugation (5000rpm, 4°C, 5min). The organic phase was dried under a steam of nitrogen and the lipid extract reconstituted in 100µl HPLC-grade ethanol, and stored at -20°C awaiting LC-MS/MS analysis.

LC-MS/MS was performed on a UPLC pump (Acquity, Waters) coupled to an electrospray ionisation triple quadrupole mass spectrometer (TQ-S, Waters). Analytes were separated on a C18 column (Acquity UPLC ® BEH Phenyl C18, 1.7µm, 21 x 5mm; Waters) using a gradient of solvent A (water:acetic acid; 99.98:0.02; v/v) and solvent B (acetonitrile:acetic acid; 99.98:0.02; v/v) as follows: 22-28 % B (0-3 min), 28-55% B (3-3-1min), 55-80% B (3.1-11min), 80% B (11-12.5 min), 80-22% B (12.5-12.51min) and 22% B (12.51-15min), at a flow rate of 0.6ml/min. The instrument was operated in the positive ionisation mode and, for all compounds, the MS/MS settings were as follows: capillary voltage 1800V, source temperature 100°C, desolvation temperature 400°C, dwell time 0.025s. Mass Lynx™ V 4.1 was used as operating software to control the

instrument and acquire data. Calibration lines using commercially available standards (Caymen Chemicals, USA) were generated to cover a range of 1-20pg/µl, which showed a linear response and samples were analysed within this range prior to normalisation against volume. The limit of detection for each compound was <0.16pg on the column.

Outcome measures

Primary outcome measures were baseline levels of serum endocannabinoids and NAE, and their changes during the simulated summer UVR exposures of the skin. Comparisons were additionally made between white Caucasian and south Asian individuals.

Statistical analyses

Data analyses, specifically paired and unpaired t-tests, linear regression and repeated measures ANOVAs with Greenhouse-Geisser corrections and Bonferroni *post-hoc* tests, were performed using SPSS statistical software (version 21.0.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism (version 6; GraphPad Software, La Jolla, CA, USA). Serum concentrations were logarithmically transformed to make them normally distributed. Results were considered statistically significant if $p<0.05$.

**RESULTS**

Volunteer characteristics

Of the 18 recruited subjects, two of the eight South Asians dropped out early for personal reasons unrelated to the study; their results were not analysed. Table 1 displays baseline characteristics of participants.

The presence of AEA, 2-AG and NAE in human serum at baseline

AEA, 2-AG and nine NAE species were detected and quantified in human serum. These comprised myristoyl-ethanolamine (MEA), N-palmitoyl ethanolamine (PEA), N-linoleoyl ethanolamine (LEA), N-oleoyl ethanolamine (OEA), N-stearoyl ethanolamine (STEA), N-eicosapentaenoyl ethanolamine (EPEA), N-dihomo-γ-linolenoyl ethanolamine (DGLEA), N-docosahexaenoyl ethanolamine (DHEA) and N-docosapentaenoyl ethanolamine (DPEA).

Endocannabinoid and NAE species' concentrations varied widely at baseline. Prior to UVR exposure, median serum AEA concentration for all subjects was 318.6 (range 62.5 to 636.0)pg/ml and 2-AG was 1018.0 (312.6 to 5025.0)pg/ml. PEA was the most abundant NAE quantified (median 2824.0 [range 2282.6 to 4506.7]pg/ml) followed by LEA and OEA (median values around 1000pg/ml), then STEA and DHEA, (median values around 600pg/ml), while EPEA and DGLEA were undetectable in some individuals or at values <100pg/ml when present (Table 2). Figure 3A displays baseline levels of these eleven compounds.

When participants were analysed according to their skin type (II or V), baseline serum endocannabinoid and NAE levels were not statistically different between

the two groups, apart from DPEA, which was higher in the phototype II cohort at 73.6pg/ml (61.1 to 103.3pg/ml) than the phototype V cohort (median 39.8 [30.4 to 60.2]pg/ml); p=0.0002 (Tables 2, 3).

Changes in serum endocannabinoids and NAE over the first week of UVR-exposures

Serum samples collected prior to cutaneous UVR exposures on Monday, Wednesday and Friday during the first week of the study showed variation in 2-AG, the median value for all subjects apparently increasing from 1018.0 [312.6 to 5025.0]pg/ml at baseline to 1713.0 [637.6 to 9039.3pg/ml] following two irradiations although this did not reach statistical significance (repeated measures ANOVA, p=0.067; Fig 4A). No changes in serum levels of AEA or NAE species were detected over week one for all participants combined ($p>0.05$). Similarly, when participants were analysed according to their skin type, levels did not vary significantly between the two groups, ($p>0.05$ for all).

Changes in serum endocannabinoids and NAE species over the six weeks' repeated UVR exposures

Serum 2-AG concentration for all subjects increased significantly over the six weeks of simulated summer sunlight exposures (one-way repeated measures ANOVA, $p<0.05$). Levels reached a peak around week 4 with a median value of 1704.0 pg/ml (range 300.1 to 4850.6pg/ml) before returning towards baseline (median 1157. 7 [275.1 to 2283.9]pg/ml, Table 3A, 3B, Fig 4B). No relationship was seen between either baseline AEA or change in serum AEA concentration

over the six weeks' simulated summer sunlight and body mass index (data not shown). The remaining NAE were unaffected by the repeated, low-level UVR exposures ($p>0.05$ for all).

Analysis of the endocannabinoids and NAE species over the six-weeks' irradiation for both skin type groups showed only 2-AG to vary significantly, reaching a maximum of 1609.4 (range 587.6 to 4246.3)pg/ml at week 3 in phototype II and 2257.3 (range 319.8 to 4850.6)pg/ml at week 4 in phototype V (two-way repeated measures ANOVA, $p<0.01$; Table 3; Fig 4).

## DISCUSSION

This study makes a novel examination of healthy human *in vivo* endocannabinoid and NAE responses to cutaneous UVR exposures that simulate incidental summer sunlight exposures, in people of light and dark skin types. The protocol, with UVR emission close to summer sunlight (95% UVA, 5% UVB), subjects wearing informal clothing (T-shirt and shorts) to expose only commonly - exposed skin sites, and brief as opposed to prolonged times, reflects the exposures occurring in everyday life rather than deliberate sunbathing. The UVR doses were equivalent to ~15 minutes June midday exposure (53.5°N), gained on most days of the week.[40] Circulating level of 2-AG, a NAE, significantly increased during the UVR exposure course, thus implicating an *in vivo* role for UVR modulation of the skin endocannabinoid system even at these low doses. In view of the reported activities of 2-AG, health implications may include mood alteration and wider aspects such as UVR-induced inflammation and immunomodulation.

At baseline we detected the endocannabinoids (AEA and 2-AG) and nine NAE species (MEA, PEA, LEA, OEA, STEA, EPEA, DGLEA, DHEA and DPEA) in human sera (Tables 3A, 3B). All major organ tissues may be contributing to these levels, including brain, liver and skin. Studies examining circulating levels in healthy humans are scarce. We found that prior to UVR exposure, median serum levels for most species were similar to recently reported values for healthy subjects,[41-43] including for AEA and 2-AG, while PEA was the most abundant NAE quantified. LEA, OEA and DHEA showed similar levels, while EPEA, DGLEA and DPEA had the lowest serum concentrations. However, STEA showed a median concentration of 697.1pg/ml, contrasting with ~6000ng/ml reported for

"healthy controls" by Pavon *et al.*[42] The reason for the difference is unknown, although in the Pavon *et al* study it is not clear if those taking regular medications affecting fatty acid metabolism were excluded, and 17% subjects had received psychiatric treatment.[42] Interestingly, our ethnically different subject groups showed a DPEA level that was significantly higher in white Caucasians than South Asians. This may be attributable to the higher omega-3 fatty acid (as found in fish oil) dietary intake observed in white Caucasians than south Asians,[44,45] as consumption of the omega-3 fatty acid eicosapentaenoic acid (EPA) leads to increased DPEA.

We discovered that circulating concentration of 2-AG was significantly raised during the course of UVR treatments ($p<0.05$), with the highest levels overall (median 1704pg/ml) achieved after 3 weeks, and no significant changes observed in other fatty acids after these low-level simulated summer exposures. A lack of further increase in 2-AG levels after 3-4 weeks of 3x weekly exposures could imply saturation of endocannabinoid biosynthesizing enzymes, depletion of their precursors, saturation of CB receptors and/or photoadaptation.

Despite the sub-erythemal doses being fixed, i.e. 1.3 SED rather than individually MED-related, the same response was seen in both phototype II and V subjects, i.e. it occurred regardless of skin pigmentation. The differential increase in 2-AG and not other species may relate to their different biosynthetic pathways (Fig 1).[46] Since UVR influences lipolytic enzymes including phospholipase C (PLC),[47] modifications could include increased release of diacylglycerol (DAG) from membrane phospholipids,[48] resulting in increased availability of DAG as 2-AG substrate. Indeed UVR exposure to keratinocyte cultures has been shown to increase endogenous DAG production.[47] Additionally, the catabolising enzymes

FAAH and MAG lipase, found in many tissues, may reduce the concentration of metabolites produced post low-dose UVR, limiting the detection particularly of metabolites present at lower concentration than 2-AG.[49]

Potentially, further UVR effects on endocannabinoids/NAEs might be observed with an increasing UVR-dosing schedule (more hazardous to skin), as can be found with deliberate sunbathing, indoor tanning or phototherapy regimes. A recent exploratory study by our group examined cutaneous endocannabinoid and NAE levels in skin biopsies taken 24 hours after UVR exposure to a localized area of the buttock, and found no alteration.[50] However, that study employed only a single exposure of 2xMED of principally UVB (275-380, peak 305nm) implying that repeated UVR exposures may be necessary for endocannabinoid and NAE responses. In-keeping with this hypothesis, Magina *et al* detected changes in plasma endocannabinoids after six weeks of whole-body narrowband UVB (311nm) therapy.[51] However, in contrast to our results, Magina *et al* report a decrease in AEA with 2-AG remaining unchanged. Potential reasons for these differences include their escalating UVR-dose (from 0.3 to 2 J/cm$^2$), the very different UVR emission employed, and their study population being psoriasis patients. Levels of endocannabinoids may be influenced by skin conditions including psoriasis and cutaneous itching, in addition to comorbidities of diabetes and hypertension[52-55] that are prevalent in psoriasis,[56] thus confounding observations compared with healthy volunteer studies.

Implications of our study may include involvement of sunlight in mood control via the endocannabinoid system. Support for endocannabinoid activity in mood control includes studies in rats where depressive models had reduced AEA levels and differential changes in $CB_1$ receptor binding density in the

brain;[57] moreover, activation of the endocannabinoid system had anti-depressive effects mediated through $CB_1$ receptors.[58] Human studies demonstrated reduced serum 2-AG and AEA levels in patients with untreated depression compared to controls.[59,60] The endocannabinoids are hypothesized to activate $CB_1$ receptors on brain GABA-ergic neurons, thereby increasing dopamine release in central reward centres,[61] while the neutrophin brain-derived neurotrophic factor,[17] and peripheral $CB_1$ and $CB_2$ receptor activation[62] may be involved. Responses of the skin endocannabinoid system might also contribute to mediation of UVR-induced skin inflammation, possibly mediated via alterations in arachidonic acid and prostaglandin levels,[46] and immunomodulation, including of cell-mediated immunity.[63] In mouse studies, genetic deletion or pharmacologic blockade of keratinocyte $CB_1$ and $CB_2$ receptors enhances allergic contact dermatitis,[34] potentially mediated through endocannabinoid regulation of monocyte chemotactic protein 2 (MCP-2)/chemokine ligand 8 (CCL8) expression.[64]

Strengths of the study include the originality of this work, the examination of a range of serum endocannabinoids and NAEs in healthy human volunteers *in vivo*, and assessment of their responses to carefully performed low-level simulated summer solar UVR exposures, with UVA/UVB emission close to midday sunlight, and exposures whilst wearing casual clothing, as it cannot be assumed that responses of normally unexposed skin are the same as routinely exposed sites. Protective goggles were worn throughout exposures, eliminating possible impact on endocannabinoid or NAE levels of transmission through the eyes. Invasive (skin biopsy) assessment to quantify endocannabinoids/NAEs and $CB_1$/$CB_2$ expression directly in the skin following multiple UVR exposures, circulating changes in DAGL expression, as the synthesising enzyme of 2-AG, and

assessment of health outcome measures, including mood questionnaires, were not performed but would be appropriate for future studies. Future investigations could also examine these novel findings in larger numbers of volunteers and include a control group. In addition to incidental exposures seen in everyday life in summertime, the impact of deliberate sunbathing on endocannabinoids/NAEs in healthy individuals could be insightful to explore.

In summary, repeated low-dose simulated sunlight exposure, as may be gained incidentally in summer-time, is associated with activation of the endocannabinoid system with elevation in serum 2-AG. This may contribute to health effects of UVR exposure of human skin, including influence on mood, inflammation and immunity, and warrants further study.

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**Table 1: Subject demographics**

| Ethnicity | White Caucasian | | South Asian | |
|---|---|---|---|---|
| Phototype | II | | V | |
| Participants (n) | 10 | | 6 | |
| Sex (n): | | | | |
| Male | 2 | | 4 | |
| Female | 8 | | 2 | |
| | Median | Range | Median | Range |
| Age (years) | 47 | 30-59 | 42 | 23-51 |
| BMI ($kg/m^2$) | 25 | 22-35 | 25 | 24-31 |
| MED ($mJ/cm^2$) | 30 | 22-54 | 125 | 104-271 |

**Table 2: Serum endocannabinoid and NAE levels during week one of UVR-exposure* for white Caucasians (n=10) and South Asians (n=6).**

| | White Caucasians (Phototype II) | | | South Asians (Phototype V) | | |
|---|---|---|---|---|---|---|
| | **Monday** | **Wednesday** | **Friday** | **Monday** | **Wednesday** | **Friday** |
| | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) |
| **AEA** | 343.1 (106.7 to 636.0) | 264.9 (571. to 832.9) | 299.0 (99.7 to 653.4) | 285.6 (62.5 to 583.7) | 187.1 (27.8 to 376.4) | 161.4 (106.4 to 196.5) |
| **2-AG** | 860.3 (312.6 to 2283.2) | 1197.8 (212.5 to 2112.9) | 1279.8 (637.6 to 9039.3) | 1086.1 (577.1 to 5025.0) | 1940.3 (442.8 to 5568.1) | 1985.7 (1109.9 to 3194.2) |
| **MEA** | 318.4 (198.8 to 825.2) | 305.9 (156.7 to 862.7) | 326.4 (165.0 to 900.2) | 315.4 (118.0 to 642.8) | 288.1 (151.9 to 1042.3) | 417.8 (134.8 to 3890.3) |
| **PEA** | 3054.0 (2282.6 to 4506.7) | 2941.9 (2141.0 to 6663.8) | 3259.0 (2087.5 to 9376.9) | 2695.0 (2314.4 to 4112.3) | 3022.9 (1949.3 to 4015.6) | 2413.0 (2188.8 to 3231.6) |
| **LEA** | 845.8 (550.1 to 1269.3) | 818.1 (418.1 to 1837.9) | 880.2 (546.4 to 1650.7) | 1006.5 (550.4 to 1591.5) | 886.9 (795.6 to 1777.2) | 1007.1 (526.9 to 1112.0) |
| **OEA** | 1288.7 (562.6 to 2802.3) | 1564.7 (475.1 to 2659.0) | 1478.8 (450.1 to 3272.8) | 740.0 (362.2 to 1283.2) | 1048.8 (596.3 to 1148.4) | 556.7 (238.4 to 1614.4) |
| **STEA** | 650.1 (271.8 to 1425.3) | 693.9 (315.5 to 1500.3) | 800.7 (247.4 to 2750.6) | 599.4 (350.2 to 1288.9) | 540.8 (396.2 to 575.4) | 417.7 (313.3 to 555.1) |
| **EPEA** | ND | ND | ND | ND | ND | ND |
| **DGLEA** | 25.0 (21.0 to 50.0) | 23.2 (11.1 to 87.5) | 25.0 (12.5 to 25.3) | 19.3 (14.8 to 29.7) | 20.9 (11.1 to 25.4) | 24.9 (15.1 to 40.8) |
| **DHEA** | 662.8 (144.1 to 1123.8) | 489.9 (317.0 to 979.7) | 524.6 (172.9 to 1095.0) | 605.1 (410.4 to 1066.2) | 893.3 (605.1 to 1239.1) | 734.8 (327.7 to 1037.4) |
| **DPEA** | 73.6 (61.1 to 103.3) | 82.6 (25.0 to 95.6) | 72.4 (25.0 to 104.2) | 39.8 (30.4 to 60.2) | 58.6 (41.8 to 73.3) | 46.2 (35.3 to 64.5) |

ND not detected. *Bloods were sampled prior to UVR exposures on Monday, Wednesday and Friday

**Table 3: Serum endocannabinoid and NAE levels at baseline (week 0 prior to irradiation) and during the six weeks of simulated summer UVR-exposures* for A. white Caucasians (n=10; upper) and B. South Asians (n=6; lower).**

A.

| | Week | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) |
| **AEA** | 343.1 (106.7 to 636.0) | 209.3 (103.0 to 728.5) | 354.7 (69.2 to 530.7) | 325.4 (10.6 to 5549.0) | 258.6 (78.5 to 901.7) | 354.1 (179.0 to 624.7) | 496.4 (145.3 to 766.1) |
| **2-AG** | 860.3 (312.6 to 2283.2) | 1182.7 (325.1 to 1853.2) | 1609.4 (587.6 to 4246.3) | 1269.6 (300.1 to 3382.1) | 1476.5 (350.1 to 3453.2) | 1452.5 (250.1 to 3213.8) | 1375.7 (275.1 to 2283.9) |
| **MEA** | 318.4 (198.8 to 825.2) | 414.3 (125.0 to 2360.1) | 246.5 (165.0 to 900.2) | 246.8 (164.9 to 2425.5) | 348.4 (148.0 to 2557.6) | 364.7 (172.3 to 962.7) | 366.2 (226.6 to 737.9) |
| **PEA** | 3054.0 (2282.6 to 4506.7) | 2914.9 (1920.6 to 4963.5) | 2811.6 (2350.2 to4963.5) | 3201.4 (1710.4 to4151.5) | 3301.6 (1845.8 to5326.1) | 2916.4 (2045.1 to 4988.5) | 3040.2 (2279.9 to4655.5) |
| **LEA** | 845.8 (550.1 to 1269.3) | 825.0 (422.2 to 1487.8) | 797.5 (409.2 to 1700.3) | 797.3 (555.5 to 1213.2) | 1046.2 (354.6 to 1750.4) | 887.2 (475.1 to 1787.9) | 928.3 (490.5 to 1345.7) |
| **OEA** | 1288.7 (562.6 to 2802.3) | 1191.3 (650.1 to 2518.5) | 1529.0 (672.9 to 2696.7) | 1121.0 (587.6 to 2975.7) | 1637.8 (454.6 to 3198.0) | 1370.6 (825.2 to 2170.3) | 1402.1 (525.1 to 4173.6) |
| **STEA** | 650.1 (271.8 to 1425.3) | 626.5 (334.6 to 1675.3) | 651.7 (300.2 to 1412.8) | 656.4 (274.3 to 1137.7) | 686.5 (399.8 to 1447.9) | 649.7 (242.6 to 1262.8) | 769.9 (324.8 to 1445.7) |
| **EPEA** | ND | ND | ND | ND | ND | ND | ND |
| **DGLEA** | 25.0 (21.0 to 50.0) | 25.4 (12.5 to 40.8) | 25.0 (14.7 to 37.5) | 25.0 (13.4 to 42.4) | 27.0 (14.4 to 38.3) | 27.4 (16.8 to 50.0) | 21.2 (14.5 to 27.3) |
| **DHEA** | 662.8 (144.1 to 1123.8) | 691.6 (403.4 to 896.3) | 648.4 (201.7 to 1066.2) | 561.9 (201.7 to 1095.0) | 763.6 (230.5 to 1210.3) | 561.9 (259.3 to 1037.4) | 764.9 (259.3 to 886.6) |
| **DPEA** | 73.6 (61.1 to 103.3) | 77.1 (39.1 to 112.5) | 78.1 (40.8 to 106.6) | 66.9 (50.0 to 122.6) | 87.9 (36.4 to 114.3) | 73.6 (37.2 to 125.0) | 72.0 (50.0 to 93.5) |

ND not detected. *Bloods were sampled prior to UVR exposures on Monday; exposures were performed Monday, Wednesday and Friday

**B.**

| | Week | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) | median pg/ml (range) |
| **AEA** | 285.6 (62.5 to 583.7) | 191.3 (57.8 to 467.3) | 280.0 (114.7 to 446.5) | 148.4 (6.7 to 327.4) | 313.8 (271.8 to 418.5) | 268.4 (150.7 to 523.9) | 259.3 (137.9 to 501.7) |
| **2-AG** | 1086.1 (577.1 to 5025.0) | 1268.6 (959.3 to 4631.1) | 1797.4 (1402.2 to13166.6) | 2257.3 (319.8 to 4850.6) | 2224.7 (876.7 to 6389.6) | 1357.4 (840.8 to 3297.0) | 1099.2 (592.8 to 2151.6) |
| **MEA** | 315.4 (118.0 to 642.8) | 370.5 (115.4 to 951.3) | 565.8 (81.9 to 1769.8) | 218.2 (85.4 to 556.1) | 515.0 (237.4 to 609.9) | 402.4 (135.3 to 1069.5) | 278.1 (71.8 to 631.2) |
| **PEA** | 2695.0 (2314.4 to 4112.3) | 2466.9 (1897.2 to3115.5) | 2906.8 (2068.9 to4026.4) | 2636.0 (1866.8 to 3043.9) | 2922.7 (2722.8 to 3626.6) | 3020.5 (1942.4 to 3522.6) | 2553.1 (1922.0 to 2891.2) |
| **LEA** | 1006.5 (550.4 to 1591.5) | 759.9 (533.9 to 1569.8) | 1114.0 (691.1 to 1570.6) | 1002.4 (548.9 to 1325.1) | 1120.1 (853.0 to 1686.6) | 1120.3 (654.8 to 1597.1) | 955.8 (709.1 to 1333.3) |
| **OEA** | 740.0 (362.2 to 1283.2) | 555.6 (332.5 to 1223.9) | 460.9 (380.6 to 1732.8) | 547.1 (148.1 to 1377.7) | 548.9 (448.4 to 1778.7) | 635.7 (201.8 to1144.1) | 565.5 (422.0 to 1187.7) |
| **STEA** | 599.4 (350.2 to 1288.9) | 447.6 (348.7 to 645.7) | 558.6 (465.2 to 604.9) | 508.0 (280.6 to 621.5) | 613.9 (434.8 to 761.8) | 469.7 (340.6 to 994.2) | 502.4 (432.0 to 734.7) |
| **EPEA** | ND | ND | ND | ND | ND | ND | ND |
| **DGLEA** | 19.3 (14.8 to 29.7) | 15.0 (12.1 to 32.8) | 27.7 (12.2 to 37.7) | 18.7 (10.4 to 21.9) | 25.6 (13.8 to 34.6) | 22.3 (16.0 to 26.9) | 14.2 (8.2 to 25.9) |
| **DHEA** | 605.1 (410.4 to 1066.2) | 540.4 (317.0 to 835.7) | 1095.0 (253.0 to 1251.4) | 446.6 (288.2 to 749.2) | 806.8 (547.5 to 2253.9) | 859.7 (235.8 to 1815.4) | 547.5 (288.2 to 993.0) |
| **DPEA** | 39.8 (30.4 to 60.2) | 50.5 (33.0 to 55.0) | 50.0 (29.7 to 83.0) | 47.5 (26.5 to 56.2) | 60.7 (33.0 to 122.0) | 61.4 (50.1 to 76.1) | 42.0 (29.5 to 65.8) |

ND not detected. *Bloods were sampled prior to UVR exposures on Monday; exposures were performed Monday, Wednesday and Friday.

**Figure Legends**

**Figure 1:** Schematic of endocannabinoid and NAE metabolism. The same set of enzymes catalyse NAPE to NAE and NArPE to AEA, and their further metabolism to fatty acid and AA, respectively, while 2-AG is synthesised from DAG by DAG lipase and is also catalysed by MAG lipase. DAG= diacyl glycerol; FAAH= fatty acid amide hydrolase; MAG= monoacyl glycerol; NAPE= N-acyl phosphatidyl ethanolamine; NArPE = N-arachidonyl phosphatidyl ethanolamine; $PLA_2$= phospholipase $A_2$; PLC= phospholipase C; PLD= phospholipase D.

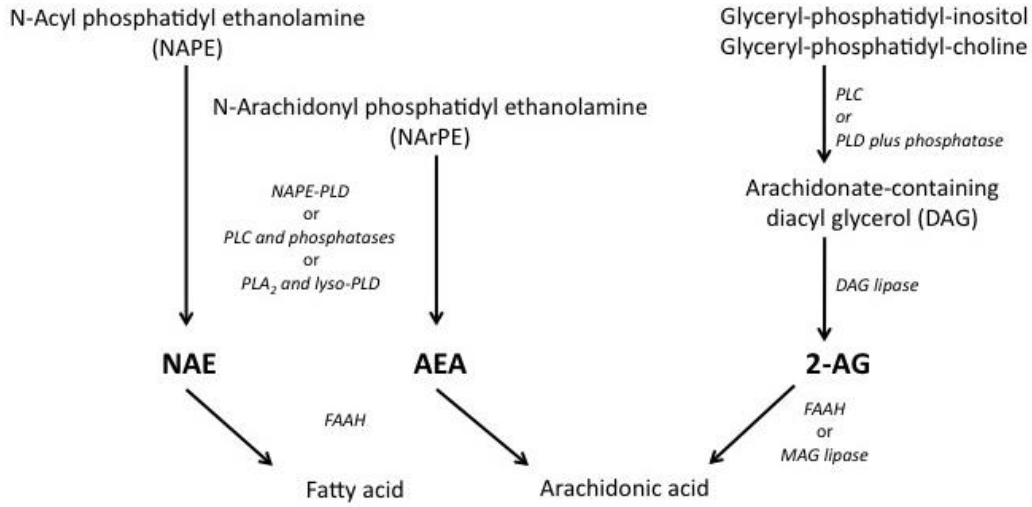
**Figure 2:** Flow chart demonstrating an individual's progression through the study protocol.

**Figure 3:** Serum endocannabinoid and NAE levels at baseline A. Data for all participants (n=16). Data shown are median, interquartile and full range. B. A representative UPLC-MS/MS chromatogram.

**Figure 4**: Serum 2-AG levels following UVR exposures. A. For all individuals (n=16) during week 1 of UVR-exposures, blood sampled Monday, Wednesday and Friday (no statistically significant change). B. For all individuals and C. for phototype II (n=10; black) and phototype V (n=6; grey) separately, during the six weeks' simulated summer UVR-exposures with weekly samples taken, showing an increase from baseline compared with levels over the UVR course ($p<0.05$ for

all subjects; $p<0.01$ for each phototype separately; repeated measures ANOVA). Data are logged to achieve normality, and expressed as median, interquartile and full range.

**Figure 1**


N-Acyl phosphatidyl ethanolamine
(NAPE)
N-Arachidonyl phosphatidyl ethanolamine
(NArPE)
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Glyceryl-phosphatidyl-choline
PLC
or
PLD plus phosphatase
Arachidonate-containing
diacyl glycerol (DAG)
NAPE-PLD
or
PLC and phosphatases
or
PLA2 and lyso-PLD
DAG lipase
NAE
AEA
2-AG
FAAH
FAAH
or
MAG lipase
Fatty acid
Arachidonic acid

**Figure 2**

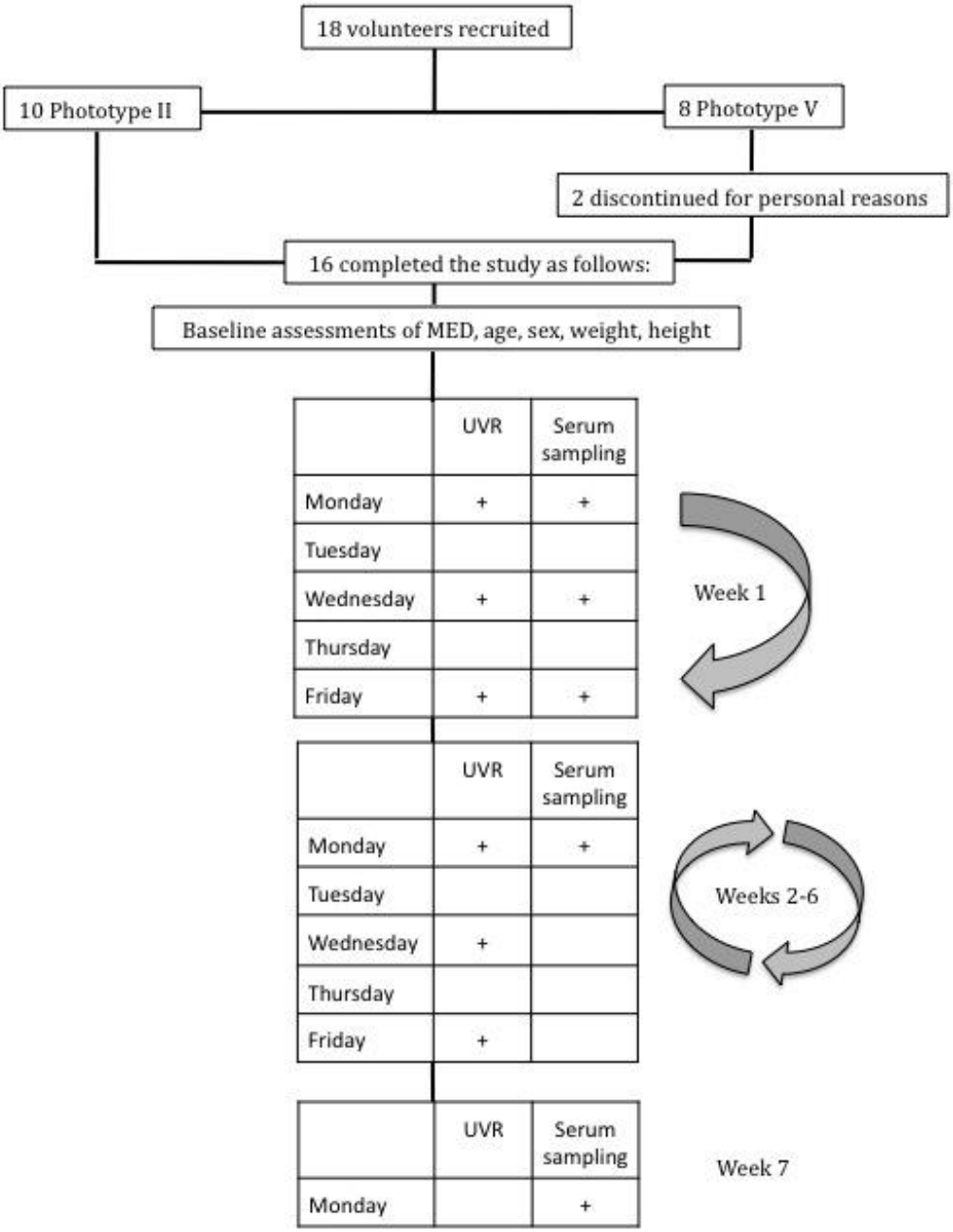
18 volunteers recruited
10 Phototype II
8 Phototype V
2 discontinued for personal reasons
16 completed the study as follows:
Baseline assessments of MED, age, sex, weight, height
UVR
Serum sampling
Monday + +
Tuesday
Wednesday + +
Thursday
Friday + +
Week 1
UVR
Serum sampling
Monday + +
Tuesday
Wednesday +
Thursday
Friday +
Weeks 2-6
UVR
Serum sampling
Monday +
Week 7

**Figure 3A**

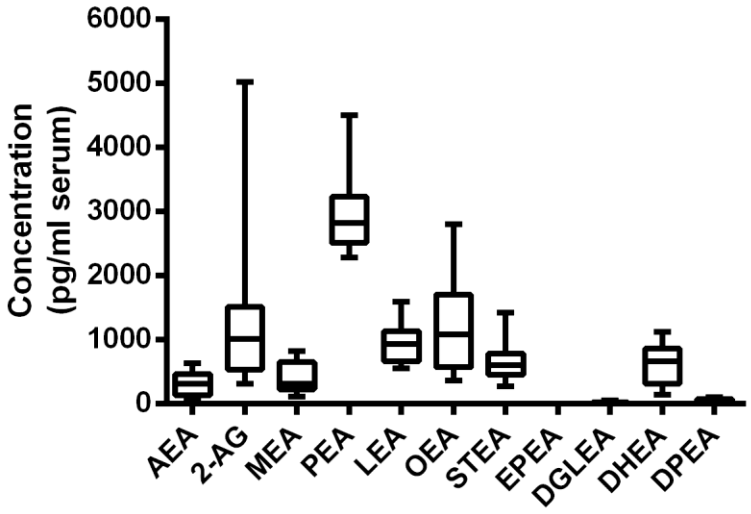
Concentration (pg/ml serum)
6000
5000
4000
3000
2000
1000
0
AEA
2-AG
MEA
PEA
LEA
OEA
STEA
EPEA
DGLEA
DHEA
DPEA

**Figure 3B**

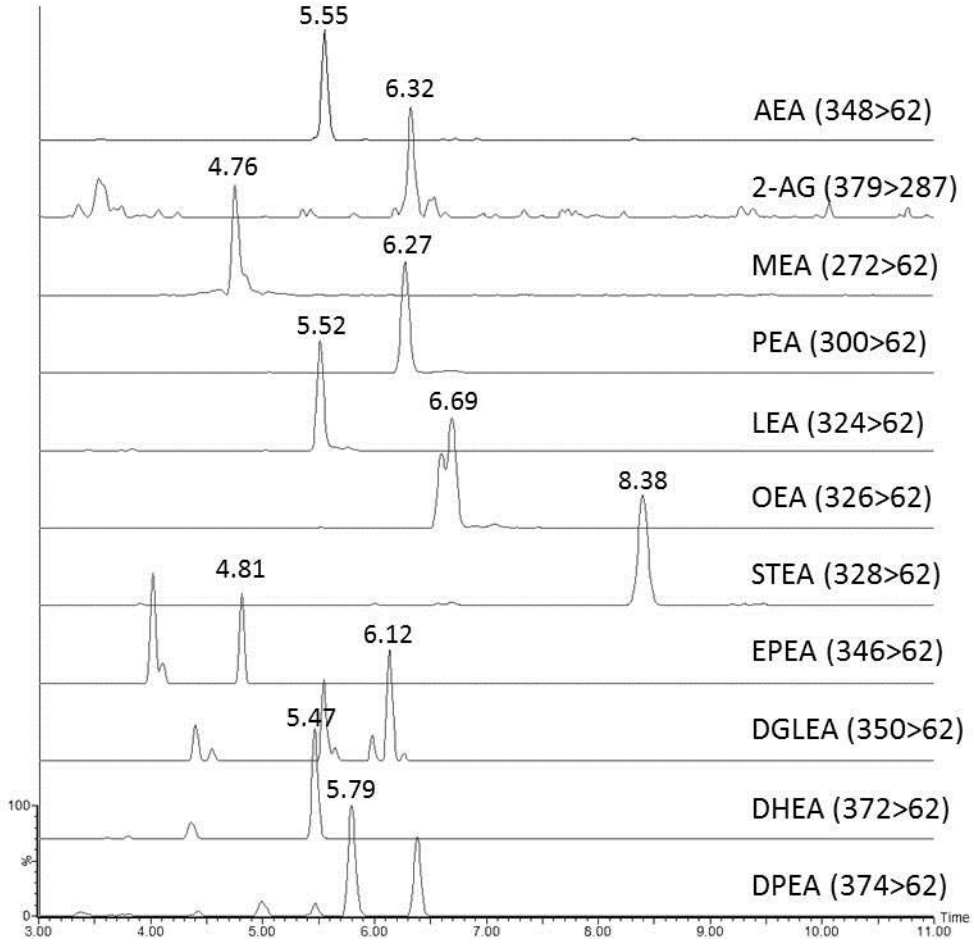
5.55
6.32
AEA (348>62)
4.76
2-AG (379>287)
6.27
MEA (272>62)
5.52
PEA (300>62)
6.69
LEA (324>62)
8.38
OEA (326>62)
4.81
STEA (328>62)
6.12
EPEA (346>62)
5.47
DGLEA (350>62)
5.79
DHEA (372>62)
DPEA (374>62)
100
%
0
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
Time

**Figure 4A**

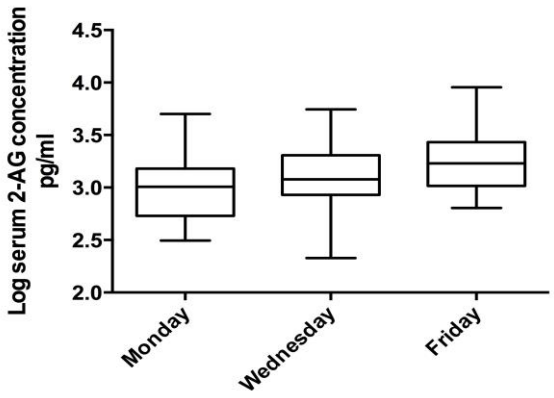


**Figure 4B**

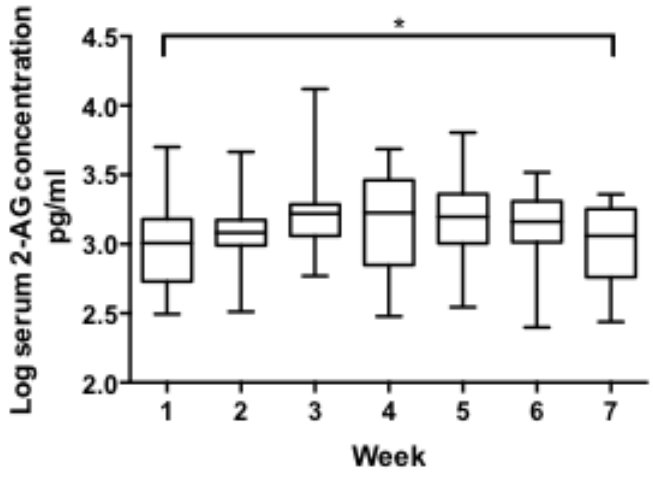


**Figure 4C**