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TITLE:

Precision Implementation of Minimal Erythema Dose (MED) Testing to Assess Individual Variation in Human Inflammatory Response

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Inflammation, Ultraviolet, Erythema, Spectrophotometry, MED, Psychoneuroimmunology

SUMMARY:

Minimal erythema dose (MED) testing is used to establish dosage schedules for ultraviolet radiation phototherapy. It can assess individual variation in inflammatory response but lacks methodology for achieving reproducible results. Here, we present a precision implementation of MED and demonstrate its ability to capture individual variation in inflammatory response.

ABSTRACT:

Minimal erythema dose (MED) testing is frequently used in clinical settings for determining the smallest amount of ultraviolet (UV) irradiation necessary to produce erythema (inflammatory reddening) on the surface of the skin. In this context, the MED is regarded as a key factor in determining starting doses for UV phototherapy for common skin conditions such as psoriasis and eczema. In research settings, MED testing also has potential to be a powerful tool for assessing within- and between-persons variation in inflammatory responses. However, MED testing has not been widely adopted for use in research settings, likely owing to a lack of published guidelines, which is a barrier to obtaining reproducible results from this assay. Also, protocols and equipment for establishing MED vary widely, making it difficult to compare results across laboratories. Here, we describe a precise and reproducible method to induce and measure superficial erythema using newly designed protocols and methods that can easily be adapted to other equipment and laboratory environments. The method described here includes detail on procedures that will allow extrapolation of a standardized dosage schedule to other equipment so that this protocol can be adapted to any UV radiation source.

INTRODUCTION:

Minimal erythema dose (MED) testing is an FDA-approved procedure to evaluate cutaneous sensitivity to radiation typically in the UVB range, although the MED can be determined at other wavelengths in the UV and visible spectrum[1]. Erythema is defined as superficial reddening on the surface of the skin caused by engorgement of capillaries (later stages of erythema are more commonly known as sunburn). MED testing has been used extensively in the dermatology literature and clinical phototherapy settings to identify the minimal amount of ultraviolet (UV) radiation that will produce the smallest unit of measurable change in the redness of the skin. MED testing can be accomplished with a commercially available UV lamp, equivalent to what is used in most commercial tanning facilities.

MED testing involves continuous dispersal of UV radiation or light from the visible spectrum onto the surface of the skin for a predetermined length of time, with dosage schedules depending primarily on pigmentation of the skin and the intensity and type of radiation. This procedure is commonly used in clinical settings to determine dosage schedules for patients receiving UV radiation therapy for skin conditions such as psoriasis and eczema[2,3]. Basic procedures for determining the MED in clinical settings have been described elsewhere[4], and can be used to adjust the total dosage of UV radiation upward or downward, depending on individual variation in skin sensitivity.

Skin pigmentation is perhaps the most important subject-specific variable in conducting and measuring results from the MED procedure[6]. This is because the duration of UV exposure required to evoke the minimal erythema response is principally determined by the lightness or darkness of the participant's skin, as defined by the participant's Fitzpatrick skin type (FST). FST[7] is a numerical scheme for classifying human skin color. The Fitzpatrick scale is a recognized tool for dermatological research into human skin pigmentation[8,9], and classifies human skin into one of six categories from lightest (FST I) to darkest (FST VI).

Darker FST typologies require longer UV duration, therefore accurate classification of FST is important. There is an extensive literature on methods for accurate assessment of FST, using a wide variety of approaches including self-report, dermatologist interview and instrumentation-based assessment. Observer ratings of FST have been shown to be correlated with current, but not natural skin color[10], however FST can be determined subjectively[11] using self-report via questionnaire[12] and/or objective assessment via spectrophotometry. Fitzpatrick typing by spectrophotometry has been shown to correlate closely to participant self-report in a number of studies[10,13-15].

Despite the utility and widespread use of MED testing in clinical services, this procedure has not been widely adopted in laboratory settings for measurement of individual variation in response to pro-inflammatory stimulation. The purpose of the methodology outlined here is to provide techniques and step-by-step procedures that increase the precision and reproducibility of the MED testing procedure, in order to facilitate future work in laboratory settings focused on fine-grained quantification of intra-individual variability in inflammatory response. We further provide representative results that illustrate the capability of this standardized protocol to

accurately capture person-to-person variation in inflammation.

PROTOCOL:

All methods described below including the use of human volunteers have been reviewed and approved by the local Institutional Review Board (IRB), and are in accordance with the Declaration of Helsinki and Belmont Report. All participants (N=72) signed informed consent as proscribed by the IRB protocol. Inclusion/exclusion criteria and discontinuation procedures were designed to maximize participant safety, and any deviation from these procedures should be considered in light of their impact on risk and tolerability to human subjects. In the context of the work presented here, the exclusionary criteria restricted participation to individuals with no personal or family history of inflammatory conditions, or any licit or illicit substances. The justification for doing so is that these factors may influence responses to the MED testing procedure.

1. Participant selection

1.1. Use the following inclusion criteria: 18-55 years old; in good general health as determined by Medical Symptom Checklist (MSCL)[5]; can understand and communicate about the lab safety protocol presented in English; can provide written consent.

1.2. Use the following exclusion criteria: Fitzpatrick skin type I, as determined by self-report; uses commercial tanning equipment regularly; skin wounds or lesions at the planned site of exposure; current skin cancer, or personal history of skin cancer; family history of skin cancer; diabetes; psoriasis or other inflammatory skin condition; peripheral vascular disease, peripheral arterial disease, Raynaud's disease, or any other diagnosed circulatory disorders; any involuntary motor disorders; allergic to adhesive tape; takes inhaled steroids for asthma (e.g., Fluticasone); takes any corticosteroids; 2 or more of the following (diagnosed hypertension, hyperlipidemia, high cholesterol, smoke cigarettes, family history of coronary or atherosclerotic disease (parents/siblings prior to age 55)); active substance dependence - legal or illicit; people with substance dependence new to recovery (less than one year); use of medications that affects CNS function, including psychotropics, opiate medication or corticosteroids, during the last 3 months; any prescribed psychotropic medications, currently or during the last 3 months (These include medications for anxiety, depression, or other psychological problems).

2. **Scheduling and preparation for the MED**

2.1. Schedule participants for two appointments: the first, the MED exposure event (approximately 45 minutes), and the second, a follow-up to gather spectrophotometry readings (approximately 10 minutes). Schedule the follow-up appointment for 24 hours subsequent to the first appointment.

2.2. Before participants arrive, lay out and set up essential equipment including two dose testing cuffs and safety equipment. Have a variety of UV-protective clothing (such as UV-

protective sports sleeves, UV-protective gloves, long sleeve medical scrubs, UV-protective sheets and tape to affix the sheets) for both the participant and the researcher to cover all skin exposed to UV radiation.

2.3. Calibrate the spectrophotometer according to manufacturer specifications. Do this for each subject and each session.

3. Determining Fitzpatrick skin type (FST)

3.1. When the participant arrives for the MED exposure event (Visit 1), identify FST either through self-report or spectrophotometry. To maximize participant safety, do not conduct MED testing on participants categorized as FST 1. For all other Fitzpatrick Skin Types (2—6), use the FST score to determine which exposure schedule should be used.

4. Cuff 1 application

4.1. Explain to the participant how MED testing works and solicit questions before proceeding.

4.2. Generally, perform the MED procedure on the inside of the non-dominant forearm.

4.3. Place Cuff 1 (with all aperture coverings removed) avoiding freckles, moles, scars, hair (to the extent possible), and any cuts, bruises or lesions on the skin. Remove only the protective wax paper backing from the lateral (not central) portions of Cuff 1. It is important that the wax paper backing from the central portion of Cuff 1 not be removed, as the adhesive has a strong potential to irritate the skin when peeled off after baseline readings, causing reddening of skin proximal to the apertures.

4.4. After situating Cuff 1 at the intended exposure site, place landmarks using a permanent marker to ensure that Cuff 2 will be situated at precisely the same location. Mark the skin at four points outside of the creases of each of the side flaps of Cuff 1, the upper right, upper left, lower right and lower left points.

4.4.1. Make these marks dark enough to survive approximately 24 hours, as they will also be used to place Cuff 3 in precisely the same location at the follow-up appointment 24 hours later.

5. Baseline reading: cuff 1 application

5.1. Using a spectrophotometer that has been calibrated according to manufacturer specifications, obtain and permanently record readings at each of the six open apertures in sequence.

5.2. Ensure that the spectrophotometer is placed the center of the cuff apertures while avoiding moles, scars, or other blemishes to the extent possible.

5.3. Permanently record all "SCI" values (L, A, B). To ensure consistent readings with the same calibration point, keep the spectrophotometer in the **ON** for the duration of the MED procedure and do not turn off until the post-exposure readings have been completed.

5.4. After baseline spectrophotometry measurements have been recorded, remove Cuff 1. To minimize participant discomfort, apply medical adhesive solvent to the perimeter of Cuff 1 as it is peeled off, which will prevent painful removal of hair on the arm.

6. Pre-Exposure reading: cuff 2 application

6.1. After removal of Cuff 1, situate Cuff 2 at the same location using the landmarks drawn on the skin for Cuff 1. The full adhesive backing may be exposed and applied to ensure that Cuff 2 is sufficiently sealed to prevent cross exposure among apertures due to insufficient adhesion to the skin.

6.2. Have both the participant and the researcher don UV-protective clothing and safety accessories. At minimum, the participant, the technician administering the procedure and any other parties in the room must don UV-protective glasses. Technicians should wear long sleeves or use a UV-protective sleeve.

6.3. Prior to activating the lamp, have the technician assist the participant in covering all exposed skin, including the arm above the patch, the arm and wrist below the patch, the hand, and parts of the front or back of the arm that may be exposed on the sides of the patch (UV-protective sheets and tape to affix the sheets may be helpful for this). Additionally, some participants wearing shirts with open necklines may want to drape UV-protective cloth over their neck and chest if these areas will be close to the UV source.

6.4. Spread a UV-protective cloth under the participant's arm (to reduce reflectance off the table surface).

7. MED procedure: pre-exposure

NOTE: The rays from the lamp must be perpendicular to the exposure site. In general, physical movement of the lamp is less possible than movement or arrangement of the angle of participants' arm.

7.1. Prior to activating the lamp, arrange the participant's arm such that the UV rays from the lamp will be perpendicular to the angle of Cuff 2 on the participant's arm.

7.2. Identify the proper distance between the lamp and Cuff 2 on the participant's arm. Place the radiometer's sensor facing the UV lamp parallel to the surface of the skin and as close as possible to the location of Cuff 2.

7.3. Cover the participants arm with a UV proof cloth to prevent exposure, and briefly activate the lamp to adjust the distance to Cuff 2 until the radiometer's sensor reads 270 $\mu W/cm^2$.

7.3.1. To achieve this reading, adjust the distance between the lamp and the surface of the skin until the radiometer reads 270 $\mu W/cm^2$ (± 10 μW). Once the proper distance has been determined, deactivate the lamp.

NOTE: It is important to note here that small differences in the angle of the radiometer *will greatly affect* the reading. Thus, the angle of the radiometer should be as close to parallel to the surface of the skin as possible.

7.4. Make further adjustments in distance throughout the exposure session to prevent drift in the location of the arm. At each reading, confirm the distance and readjust as necessary to maintain radiometer readings at approximately 270 $\mu W/cm^2$ (± 10 μW).

8. MED procedure: exposure

8.1. Use a stopwatch to implement the MED schedule. Remove the first aperture covering before activating the UV source. Activate the source and the stopwatch simultaneously and remove each aperture covering on Cuff 2 according to the schedule specified below, based on FST.

8.2. At the point of removal for each aperture covering, record the radiometer reading when the radiometer is held parallel to the surface of the skin and pointed at the lamp. If the distance has changed, adjust the distance to lamp to ensure that the radiometer once again reads 270 (± 10) $\mu W/cm^2$.

8.3. Have the technician monitor the participant's arm to ensure consistent positioning. In particular, readjust the arm if the arm rotates, as many participants will rotate the arm as they relax. Subsequent to any adjustments, re-confirm that the radiometer reads 270 $\mu W/cm^2$ (± 10 μW).

8.4. Turn off the lamp at precise time specified by the dosage schedule in **Table 1**. Do not deactivate the stopwatch, as an additional series of spectrophotometer readings should be gathered exactly 7 minutes subsequent to deactivation of the lamp, as described below.

9. 7-Minute post-exposure reading

9.1. Exactly 7 minutes subsequent to the deactivation of the lamp, record the final spectrophotometer readings from each aperture in Cuff 2. The purpose in collecting data immediately subsequent to the exposure procedure is to first confirm no adverse reaction to the UV radiation, and second to evaluate initial responses, which in some cases can be slight,

but measurably different from baseline (pre-exposure) values. Any increase in redness after 7 minutes is likely to be a thermal effect and not erythema.

NOTE: An adverse reaction to UV radiation exposure after 7 minutes is likely related to the minimal urticarial dose of solar urticaria, an acquired photosensitivity disorder. Photosensitivity disorders are assessed prior to the MED procedure and subjects with these disorders should be ruled out. However, if this is observed at any point during testing the exposure protocol should be discontinued immediately.

9.2. After exposure to UV radiation, Cuff 2 can be particularly difficult to remove. Use medical-grade adhesive solvent, if necessary, to minimize discomfort to participants during removal of Cuff 2. Participants with particularly hirsute or otherwise sensitive skin may find it helpful to apply either olive oil or alcohol-based adhesive remover under the edge of Cuff 2, as they remove the patch slowly. After Cuff 2 is removed participants may have residual adhesive on their skin, which can also be removed with either olive oil or medical adhesive solvent.

9.3. Prior to departure from the exposure session, remind participants not to wash the landmarks and not apply any lotions to the exposure site.

10. Follow-Up appointment: cuff 3 application

10.1. Before the participant arrives, calibrate the spectrophotometer according to manufacturer specifications.

10.2. Prepare Cuff 3 by removing all of the aperture coverings (leaving the white wax paper backing on the central portion of the patch). When placing Cuff 3 on the participant's arm, remove the white wax paper backing from the two side flaps of the patch. Using the landmarks on the participant's forearm, place Cuff 3 in the same location as the previous two patches.

10.3. Take a reading at each of the six open apertures in sequence. Additionally, visually inspect each aperture and record whether there appears to be visual evidence of an erythema response in each of the six apertures (red or pink skin indicates erythema). After permanently recording spectrophotometer readings, remove Cuff 3, using solvent if necessary.

10.4. To further enhance participant comfort and safety, provide 4-6 single-use burn gel or aloe vera, and indicate to the participant that if the exposure site becomes itchy or uncomfortable, it may be treated like a sunburn with these or similar over the counter products.

REPRESENTATIVE RESULTS:

The timing schedule presented in **Table 1** is a novel dosage schedule that was calculated to capture the MED, on average, at the mid-point of the exposure event (i.e., aperture 3 or 4) for each FST. The basis for the calculated schedule is as follows.

Prior work has established that for individuals with FST 2, the median MED for radiation in the UVB range is 66.9 milliwatts (mW) per cm^2, 77.429 mW/cm^2 for FST 3 and 85.0 for FST 4[16]. Under an assumption of a constant UVB energy of 270 $\mu W/cm^2$, we extrapolated this constant into the temporal domain to determine the number of seconds required to capture the MED at the mid-point of a given dosage schedule for each FST based on these median values. We further incorporated an expanding schedule, such that each aperture (2 through 6) receives 25% more energy than the one before it, similar in principle to expanding ratio series that are typically used in MED testing. Although no reference ranges exist for the MED within FST 5 and 6, we calculated schedules for these skin types by extrapolating the differences among reference ranges from 2 through 4 to determine the constant for multiplication of the expanding time series to a theoretically correct estimate of the required energy to reach the MED in these individuals, if it exists. It should also be noted that prior work has illustrated that statistically significant differences in the MED may only emerge when comparing FST I to FST IV[20]. Consequently, in the context of the methods described here, statistically significant differences between contiguous FST categories are not necessarily expected to emerge.

Figure 1 depicts complete data for a single, representative subject, broken down by assessment period (Pre-exposure, 7 minutes post-exposure and 24 hour follow up). **Figure 2** provides the complete data for all subjects in this study, in order to provide a comprehensive overview of the individual patterns and variation that are likely to be encountered. **Figure 3** provides summary statistics representing the aggregated results for all subjects, to illustrate overall patterns of variability within each aperture. Raw data from this procedure could be correlated with other variables of scientific interest, depending on the context and nature of research applications employing precision MED testing as described here.

FIGURE AND TABLE LEGENDS:

T**able 1. Calculated dosage schedule according to FST.** Units represent duration of exposure in min:s)

Figure 1. Representative Results for one subject.

Figure 2. Aggregated results for 72 subjects illustrating A* change from baseline to follow-up.

Figure 3. Box plot results summarizing variability for 72 subjects illustrating A* change from baseline to follow-up.

DISCUSSION:

Precision implementation of MED testing as described here could offer several advantages over other extant lab-based inflammatory challenges that have achieved popular use. For example, suction blister protocols[17-19] raise a fluid-filled blister on the skin that is subsequently aspirated with a syringe to gain direct access to the cytokine microenvironment. Although skin blistering is a well-known tool for studying skin immunology and inflammation[20], and may be particularly effective for gaining access to rare populations of cells and proteins[21], such procedures require specialized personnel and are often prohibitively invasive and uncomfortable for general use in

research subjects thus presenting both ethical and practical challenges. Along these same lines, superficial inflammatory challenge procedures such as capsaicin cream-induced vasodilation are effective in stimulating cutaneous inflammation, but quantification of the localized inflammatory response (flare) relies on manual tracing and human rating which can be subject to error, thus reducing the reliability of this procedure in the laboratory.

In phototherapy settings the purpose in determining the MED before UVB phototherapy is to calculate the individual starting dose for a UV radiation protocol. In the case of the methods outlined here, we do not seek identify the MED for this purpose, but instead provide a replicable method for assessing intra-individual variation in responses to UV radiation. In a similar sense, it should also be noted that the results from this procedure cannot be used in the calculation of the erythema threshold under the sun, which instead would require a solar simulator and not a UV lamp. Along these same lines, it is important to assess previous tanning and sun exposure status on the test site. In this study, we excluded subjects who reported consistent use of commercial tanning equipment. These factors, if not accounted for, could interfere with accurate determination of skin phototypes.

We used a handheld spectrophotometer to objectively define baseline skin color prior to exposure, and to measure variation in skin color induced by UV radiation. Spectrophotometers are lightweight, handheld devices that measure hue or red versus green (a* scale), lightness or black versus white (L* scale), and saturation or yellow versus blue (b* scale). Previous work has demonstrated that increases in b* and decreases in L* scale components better indicate skin darkening caused by cumulative UV exposure[22], whereas the a* scale measures skin erythema or redness (i.e., sunburn). Although the MED is, by definition, an increase in visible erythema, the use of spectrophotometry provides an additional quantitative metric that complements visual inspection of the site of exposure. The use of spectrophotometry to measure erythema simultaneously reduces risk to subjects and increases precision in the measurement of erythema. This is because the spectrophotometer is able to measure changes in skin redness with considerably greater precision than visual observation alone. Using instrumentation such as spectrophotometry to measure superficial responses to UV radiation thus has the added benefit of reducing the amount of UV radiation required to produce a perceptible change. Observational ratings to measure erythema (typically done with a Likert-type visual rating scale)[23] are highly subjective and limit the utility of MED testing. Consistent with prior work that has established MED procedures for clinical settings[4], we report the a* metric displayed directly from the spectrophotometer, which is a measure of redness. The use of spectrophotometry (specifically the a* factor) for measuring cutaneous erythema has a substantial precedent in the scientific literature[4,14,24,25], and is considered to be more accurate than visual rating alone.

It is also necessary to use a radiometer capable of measuring microwatts per cm^2 in the UVB range. In order to monitor the intensity of the UV radiation, and to ensure that the dosage is kept consistent between subjects, a real-time radiometer should be placed immediately adjacent to the subject's skin exposure site at all times. Among other benefits of using the radiometer (for example, in monitoring bulb fatigue over time), radiometry ensures that the distance between the bulb and the skin produces a consistent result between subjects. This also

provides an extra degree of safety for subjects, by ensuring that the source is never placed too close to the skin which would also increase the UV radiation projected onto the skin. The experimenter should record the UV intensity in real-time, or at minimum every time an aperture covering in the dose-testing patch (cuff) is removed, to confirm the consistency of dispersal across the entire exposure event. By ensuring that the intensity remains constant, further increases safety by preventing a UV dose that is larger than what is outlined in the dosage schedules provided here.

The assessment of inflammation related to erythema and its utility for the study of other types of inflammation remains a topic for future study. Specifically, future work should examine the relationship between epithelial responses indexed by the MED testing procedures described here and the biological processes that produce them in order to establish the needed conceptual and theoretical basis for further examination of these links. As such, we strongly recommend caution in the use of MED testing as a general marker of systemic inflammation, and encourage further work using this method to probe factors that regulate cellular responses to UV radiation.

MED testing is not without risks. There is a risk to study participants that the exposure site may become itchy or uncomfortable in the next 24-48 hours. Participants may experience the sensation of heat at the site, and it is possible that the skin may peel or become sore, as in the case of a sunburn. Several steps can be taken to minimize the possibility of this outcome. First, before conducting the MED procedure FST typing should be conducted in order to determine the length of time that the participant will be exposed to the UV radiation. In cases where subjects' Fitzpatrick skin type is unclear or cannot be determined, we recommend not conducting MED testing. In cases where there is disagreement between self-reported FST or observer-determined FST, default to the lower FST which will entail a lower dose of UV radiation. Also, in cases where the subjects FST is I (lightest possible skin) we recommend against conducting the procedure, because in this case subjects can approach the MED within 6-8 minutes, meaning that over-dosage may become somewhat more likely. MED testing should not be conducted if radiometer readings are unavailable (for example, due to equipment failure). Prior to leaving the lab after the UV exposure procedure, participants should be given the option to take home over-the counter remedies such as aloe packets, and should be told to monitor the site of exposure closely for the next 48 hours. Participants should be told that if any discomfort occurs, they may apply the aloe as needed, or purchase their own over-the-counter remedies. These risks also link to certain ethical considerations that must be evaluated prior to undertaking MED testing. For example, risks associated with overdosage and other unintended consequences should be clearly explicated in the informed consent document. Similarly, it should be stated clearly that while MED testing is in widespread use, the long-term consequences, whatever they may be, are incompletely understood.

The representative results presented here should be considered in light of study limitations. First, we aimed only to demonstrate the potential for precision MED testing to capture between-subjects variability in this study, and future work will be required in order to examine the test-retest reliability of this procedure. Second, we gathered data from FST 2 through 5, and

we do not present data here on FST 6 (darkest skin), as prior work has shown that individuals with very dark skin typically do not show evidence of erythema in response to UV radiation. Future work will be required in order to apply and critically evaluate results from our calculated dosage schedule for FST 6. Individuals with lighter skin (FST I and II) may also benefit from a longer follow-up period, as individuals with lighter skin may show evidence of the MED after 48 hours. Third, we report data extracted from the area of the non-dominant forearm, which is a convenient and less-invasive location for evaluation of the types of inflammatory responses of scientific interest in this study. However, this contrasts with many other studies that evaluate MED responses in regions of the body that are less exposed to natural light such as the back and buttocks. The choice of the forearm as the principal site of spectrophotometry recording was mainly for reasons of convenience, as collection of data from sensitive personal regions such as this may cause undue discomfort or embarrassment to subjects. However, areas that are typically shielded by clothing may be a better option for measurement of erythema free from the influence of prior solar exposure, and future work could consider this question by comparatively evaluating results from this procedure at different physical locations on the body. It should also be noted that the procedures outlined here are not meant to establish the MED for phototherapy settings, nor should these procedures be used for photobiological investigations such as sun protection factor (SPF) determination for commercial sunblock. Also, although we were able to achieve narrow tolerances in radiation dosage of ± 10 microwatts per cm^2, future work should assess the utility of stabilizing the physical arrangement of the UV radiometer, which in our approach is held manually. Although not necessarily a limitation, we selected 7 minutes post-exposure as a semi-arbitrary follow up point, to assess whether any immediate changes occurred - although these changes are not related to erythema, and may instead relate to localized heating or other unrelated responses. Another potential limitation is the cost of instrumentation, particularly the spectrophotometer. Lastly, we note that the large number of exclusionary criteria may limit generalizability.

Potential applications of precision MED testing extend to any research setting in which quantification of systemic inflammatory response in humans is required. In particular, this method may be especially relevant to the field of psychoneuroimmunology, which examines the interface between psychological, neurobiological and lifestyle factors (stress, diet, hormonal status, anxiety and depression) that relate to immune responses including systemic inflammation. Future work could also examine the utility of adjusting certain physical configurations of the MED testing procedures described here. For example, adjustment of the distance from the surface of the skin to the UV radiation source could either be done manually (as was the case here) or, alternatively, real-time readings from the radiometer could be used to adjust exposure time. The influence of follow-up duration could also be evaluated, to systematically examine individual differences in the time to reach peak MED, and longer follow-up periods could be added as well, in order to evaluate intra-individual variation in recovery time. It should also be noted that the methods described here exist within a broader context of worldwide standards for sun protection test methods, cosmetics and sun protection factor (SPF) testing (e.g., ISO 24444). The methods presented here are not meant to be used in these contexts, nor should they be applied for determination of MED in phototherapy settings or SPF ratings as described by the United States Food and Drug Administration (FDA). The standards

for SPF testing are based on individual MED, and are not appropriate for intra-individual assessments as described here. Finally, future work should also take into consideration the length of the follow up period. In the current study, most subjects were evaluated at or near 24-hours post exposure, with some rare exceptions due to variation in schedules and subject availability (never exceeding 48-hours post exposure). The influence of follow-up duration could also be evaluated, to systematically examine individual differences in the time to reach peak MED, and longer follow-up periods could be added as well, in order to evaluate intra-individual variation in recovery time.

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DISCLOSURES:

The authors on this study declare no conflicts of interest, financial or otherwise.

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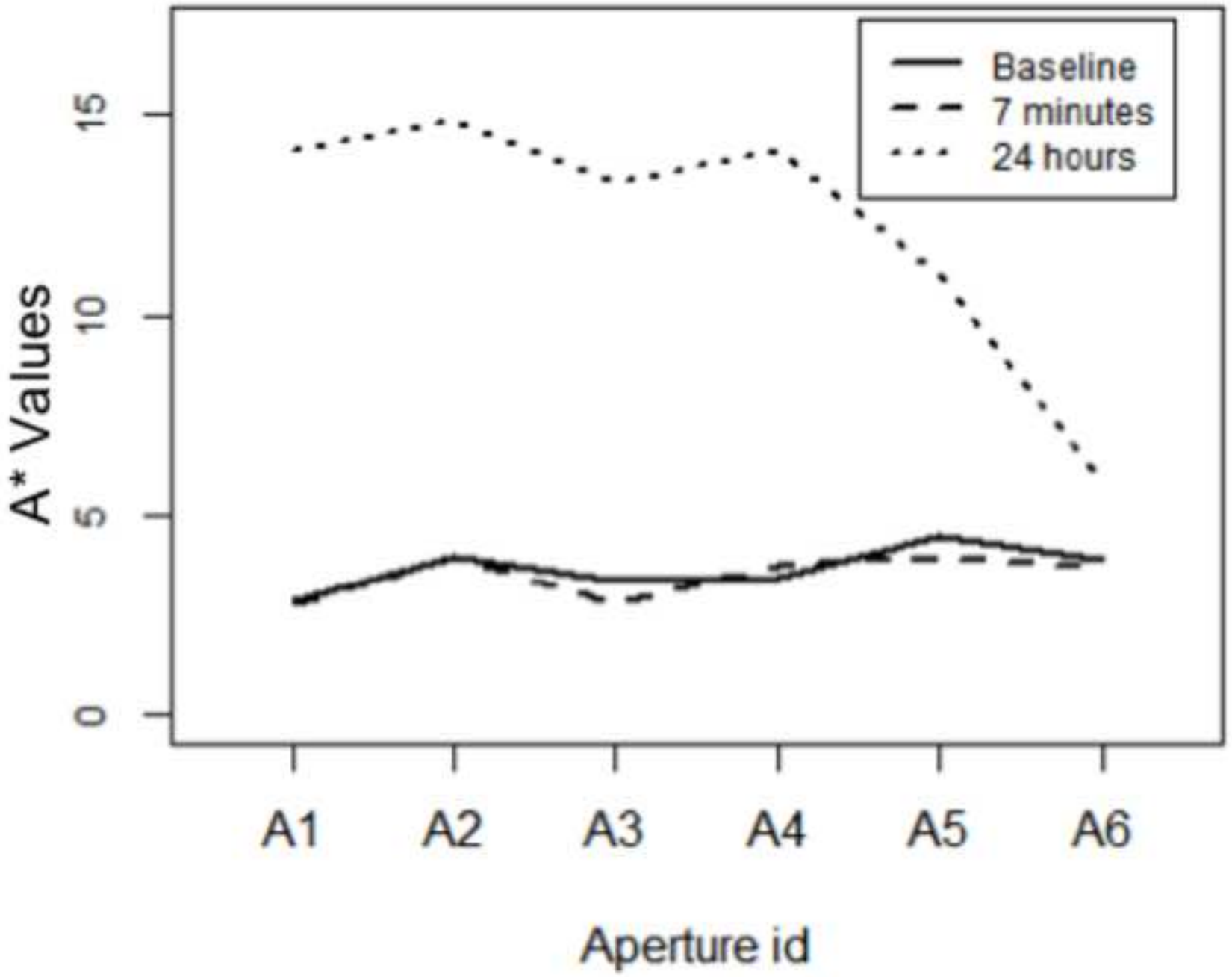

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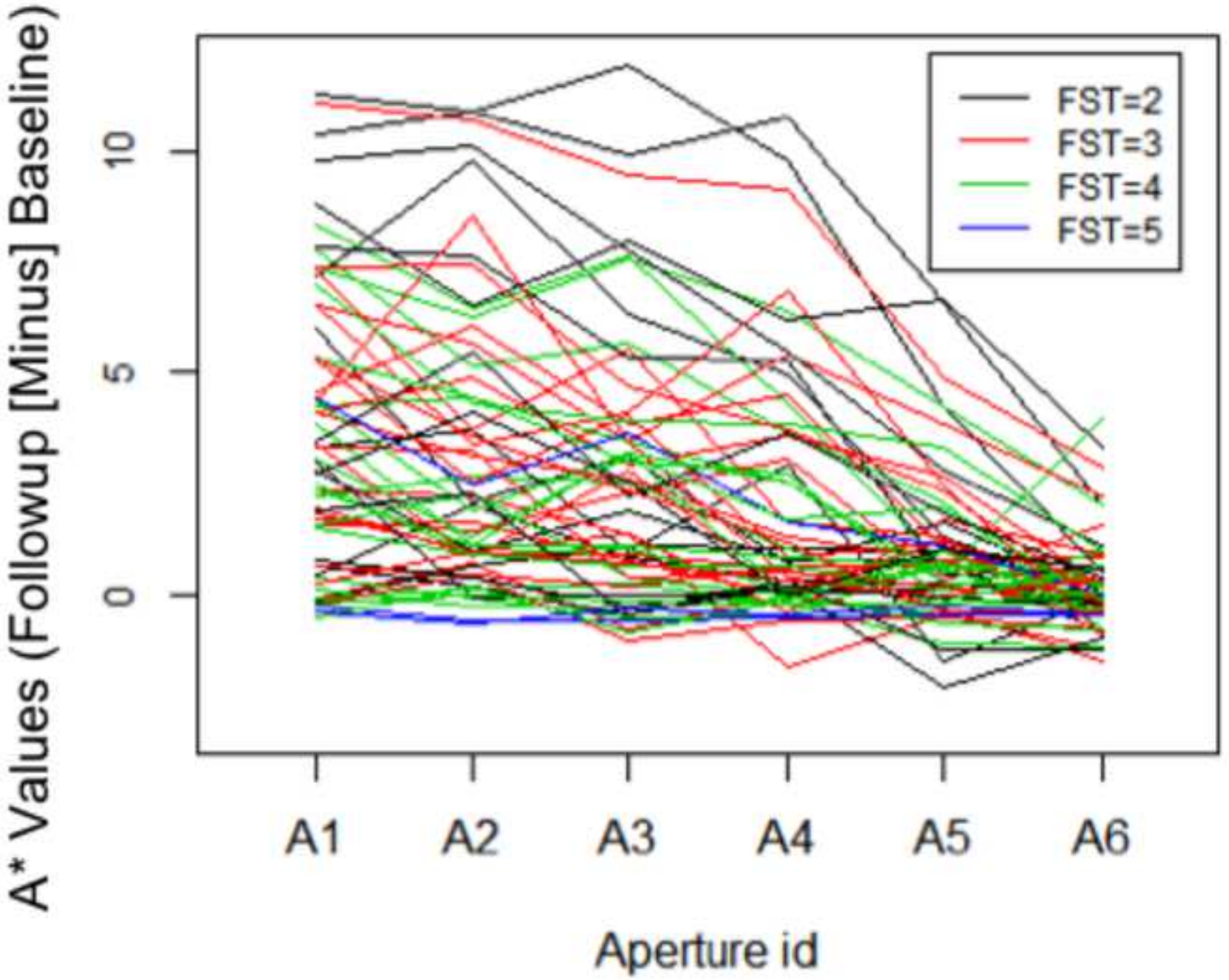

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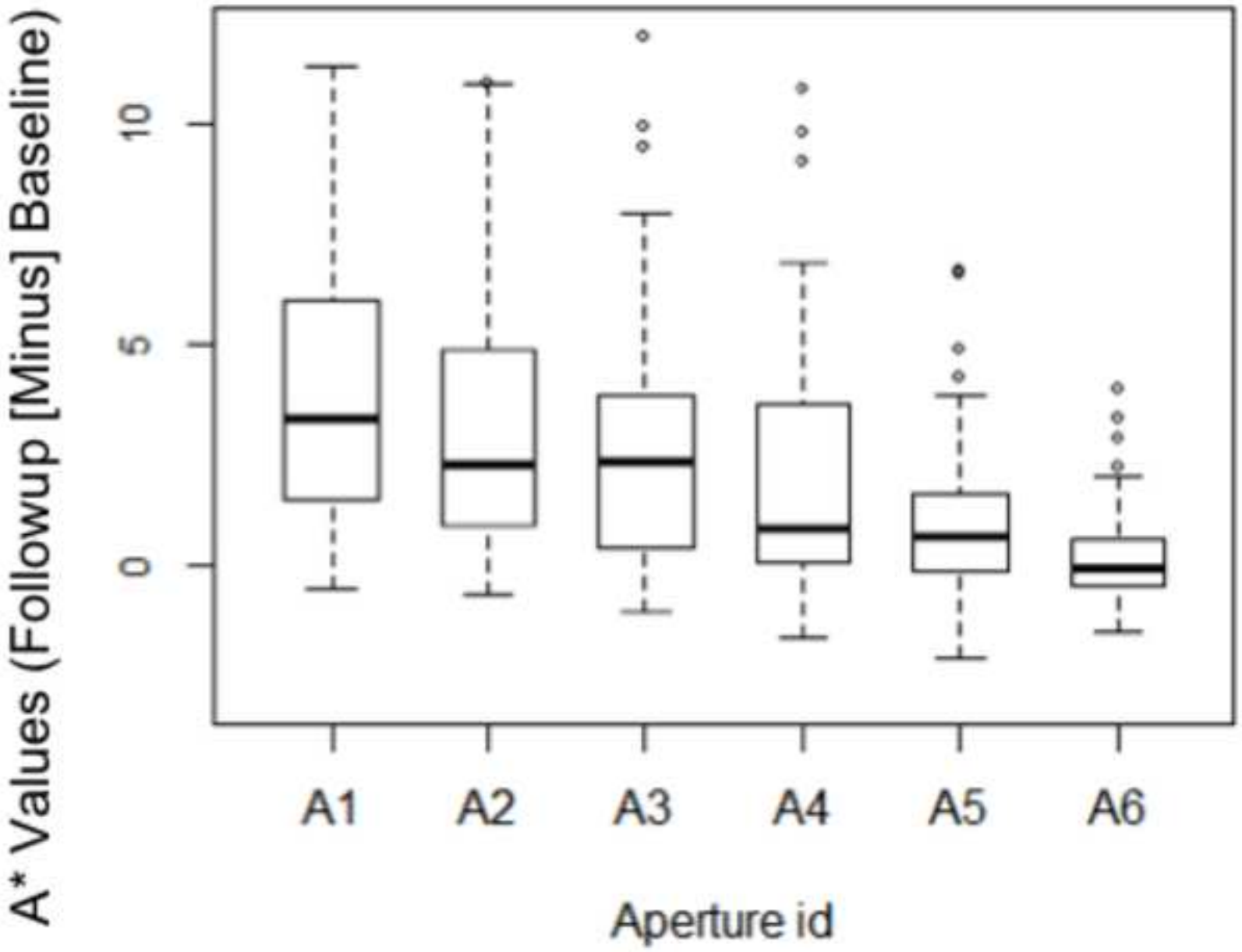

MED Schedule for Each Fitzpatrick Skin Type (FST)

	FST 2	FST 3	FST 4	FST 5	FST 6
Remove patch 2	1:22	1:38	1:55	2:11	2:28
Remove patch 3	3:05	3:42	4:19	4:56	5:33
Remove patch 4	5:13	6:16	7:19	8:21	9:24
Remove patch 5	7:54	9:29	11:03	12:38	14:13
Remove patch 6	11:14	13:29	15:44	17:59	20:14
Lamp Shutdown	15:25	18:31	21:36	24:41	27:46
7m Post-exposure	22:25	25:31	28:36	31:41	34:46

Name of Material/Equipment	Company	Catalog Number	Comments/Description
6-aperture dose testing patch ("Cuff")	Daavlin		
Medical grade adhesive solvent			
Non-reflective UV proof cloth			
Radiometer	SolarLight	Model 6.2 UVB Meter	
Single use aloe or burn gel			
Spectrophotometer	Konika-Minolta	CM-2600D	
Stopwatch			
UV lamp – Fiji Sun	Sperti		Emission spectrum 280nm-400nm, approximately 25% UVB
UV-proof safety glasses (2 pair)			
UV-proof sleeve			
White cotton gloves (2 pair)			

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