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ARTICLE

The impact of skin color and tone on histamine iontophoresis and Doppler flowmetry measurements as a pharmacodynamic biomarker

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Abstract

The phenotypical manifestations of asthma among children are diverse and exhibit varying responses to therapeutic interventions. There is a need to develop objective biomarkers to improve the characterization of allergic and inflammatory responses relevant to asthma to predict therapeutic treatment responses. We have previously investigated histamine iontophoresis with laser Doppler flowmetry (HILD) as a potential surrogate biomarker that characterizes histamine response and may be utilized to guide the treatment of allergic and inflammatory disease. We have identified intra-individual variability of HILD response type among children and adults with asthma and that HILD response type varied in association with racial classification. As laser Doppler flowimetry may be impacted by skin color, we aimed to further validate the HILD method by determining if skin color or tone is associated with observed HILD response type differences. We conducted an observational study utilizing quantification of skin color and tone obtained from photographs of the skin among participants during HILD assessments via the RGB color model. We compared RGB values across racial, ethnic, and HILD response type via the Kruskal-Wallis test and calculated Kendall rank correlation coefficient to evaluate the relationship between RGB composite scores and HILD pharmacodynamic measures. We observed that RGB scores differed among racial groups and histamine response phenotypes ($p < 0.05$). However, there was a lack of correlation between the RGB composite score and HILD pharmacodynamic measures (r values 0.1, $p > 0.05$). These findings suggest that skin color may not impact HILD response variations, necessitating further research to understand previously observed differences across identified racial groups.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

It has previously been described that variation exists in biomarkers and devices that rely on the transmission and measurement of light across the skin surface. It is also now known that many devices and biomarkers are not validated within diverse populations. We have previously demonstrated that histamine iontophoresis with laser Doppler (HILD) assessments appear to vary across racial groups.

WHAT QUESTION DID THIS STUDY ADDRESS?

We aimed to determine if HILD response types are associated with skin color/tone among a racially diverse pediatric group.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

We did not observe that histamine pharmacodynamic quantitative variables were associated with skin color or tone. This suggests that HILD is a valid measure of histamine pharmacodynamic response across diverse skin tones/colors.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This discovery may lead to further validation of HILD as a pharmacodynamic biomarker that may be useful across diverse patient groups to assess therapeutics that target the histamine and allergic pathways. The use of this biomarker may ultimately improve drug development and clinical practice for a more diverse patient population.

INTRODUCTION

Asthma is one of the most frequently diagnosed diseases globally and is commonly associated with allergic rhinitis and eczema, creating what is often noted as the atopic triad.[1] According to the Centers for Disease Control and Prevention, 8.4% of children in the United States have asthma.[2] Asthma is a heterogeneous disease of varying phenotypes that are associated with different responses to therapeutic treatments. Current biomarkers used to better direct therapy to specific disease phenotype include IgE level, blood and/or sputum eosinophil counts, and exhaled nitric oxide. However, there remains a need to develop objective biomarkers to better characterize allergic responses in the body and to predict treatment responses to therapies that target the allergic pathways.

We have investigated the use of histamine iontophoresis with laser Doppler flowmetry (HILD) as a potential biomarker to objectively measure histamine response in the body. We have studied HILD as a potential surrogate endpoint to evaluate therapies targeting the histamine and allergic pathway.[3,4] Histamine is a central mediator within the allergic and inflammatory response cascade. Histamine is released as a pre-formed mediator from mast cells, resulting in an acute inflammatory and immediate hypersensitivity response within the body. This response typically manifests as itching, sneezing irritation, shortness of breath, vomiting, and other symptoms.[5] Histamine plays a critical role in asthma and allergic rhinitis as the amine is described to cause airway obstruction by causing smooth muscle contraction, increased bronchial secretions, and airway mucosal edema via type 2 immune response mechanisms related to IgE antibody-mediated mast cell degranulation.[6] Allergic asthma is among the most common asthma phenotype, and the histamine pathway is known to play an important role in allergic asthma pathogenesis. Therefore, biomarkers capable of differentiating variation in histamine response may be useful for personalized approaches to asthma treatment.

The HILD procedure allows for a fixed dose of histamine to be delivered by iontophoresis into the superficial surface of the skin whereby histamine delivery and binding to H1 receptors in the microvasculature lead to a vasodilatory response that can be measured by Doppler flowmetry of blood flow at the site. The HILD technique provides an objective and quantitative assessment of individual histamine pharmacodynamic response.[3] The Doppler flowmetry uses a laser light beam to transmit through epicutaneous tissue, allowing for backscattered light to be converted into a quantified signal that is transmitted and recorded via a computer software. This signal is used to calculate a value of blood flow at the site described as "flux."[7] Iontophoresis and laser Doppler flowmetry have been used as a tool within various areas such as burn research in evaluating tissue vascularization, in

evaluating endothelial function in cardiovascular and sickle cell disease research.[8,9]

In our previous investigations of HILD, we observed that histamine response varies among adults and children including those with allergic disease and asthma.[3,4] We also noted that among children with asthma, histamine microvasculature response can be grouped into response types of "hypo-responsive" and "hyper-responsive" based on HILD flux measurements and model fit. We further observed differences in classification in different response types (hyper-responsive vs. hypo-responsive) between different racial identities in our studies.[10,11] These findings have led us to explore potential reasons for these differences. As race is understood to be a derived social construct that often categorizes people based on phenotypic characteristics and cultural identity, race is understood to be not indicative of unique biological processes or differences in biological processes.[12,13] However, racial identity groups may share certain physical characteristics such as skin color or tone that may influence performances of devices that function based on light transmission through the skin.[12] During the COVID-19 pandemic, pulse oximetry device measurements, which are a biomarker of blood oxygenation, were revealed to be less accurate in patients with darker skin pigmentation as these devices have primarily been developed and validated among those with lesser or lighter skin pigmentation. These findings have a significant clinical impact because overestimation among patients with darker skin pigment can lead to a lack of recognition of hypoxia and subsequently increased morbidity and mortality among these patients.[14,15] Due to these observations for the pulse oximeter, which uses technology similar to that used with HILD as a surrogate measure obtained to measure light reflection through the skin, we aimed to further validate the HILD technique among varying skin colors/tones. We sought to determine if HILD values and our observed response types were associated with skin color/tone.

METHODS

We used readily available data from participants included in a research protocol that aimed to evaluate HILD in children with allergic rhinitis. The protocol was approved by the Children's Mercy Institutional Review Board (IRB # 11120477). Children 8–19 years of age were recruited via convenience sampling from the Children's Mercy Allergy/Asthma/Immunology clinics after obtaining parental permission and, where appropriate (i.e., age ≥7 years), child assent. Race and ethnicity were determined by participant self-identification. HILD was performed in an identical fashion in all participants according to previously published methods.[10,16] Photographs were taken with a handheld camera (Olympus Stylus® 1200) of the volar surface of the participants' forearm simultaneously on the opposite arm from the HILD assessment. An automated zoom function was used when taking photographs. Multiple photographs were taken with the intention of capturing the histamine skin prick wheal and flare response used for initial validation of HILD. However, photographs also included skin areas without wheal and flare response. All participant study visits were conducted during the day and were conducted in similarly designed rooms with similar outside and indoor light sources within the pediatric clinical research unit at Children's Mercy. For each participant, the best quality photo was chosen by our team based on photo clarity, shadows, brightness, and available areas of skin without erythema from histamine application to be used in the skin color/tone analysis.

RGB color model was used to convert skin color into a quantifiable measurement for comparison among the participants. An RGB value is made up of three-color components. The red, green, and blue components each represent the amount of red, green, and blue that are present in an overall color. Each component is a number between 0 and 255. The numbers represent the amount of red, green, and blue mixed to form the color. Pure black is represented by [0,0,0], which demonstrates the absence of all colors. Pure white is represented by [255, 255, 255], which demonstrates the full presence of all colors.[17] The three numbers can represent every color, which can be useful for analyzing the depth of skin tone. RGB values were generated from each selected photograph.

Each individual image was divided into similar-sized quadrants. A color picker (Image Color Finder) was used to select an area within each quadrant for color sampling, avoiding areas with visible veins, erythema, shadows, or markings.[18] The color picker generated an RGB that was recorded (Figure 1). An RGB value was obtained from each quadrant to account for the intra-individual variance in skin tone.

The RGB value data from the four quadrants sampled were calculated as a composite score to represent a participant's overall skin color/tone. The average of the red, green, and blue components of the recorded RGBs from each quadrant was used to create an overall average RGB. For an individual component, the average was taken using squares. We used a previously published method, as reflected in Equation 1, to calculate the individual participant RGB representative values.[19]

The equation to find the average of an RGB component where x is the component value from quadrant I, y is the component from quadrant II, z, is the component from quadrant III, and b is the component from quadrant IV.

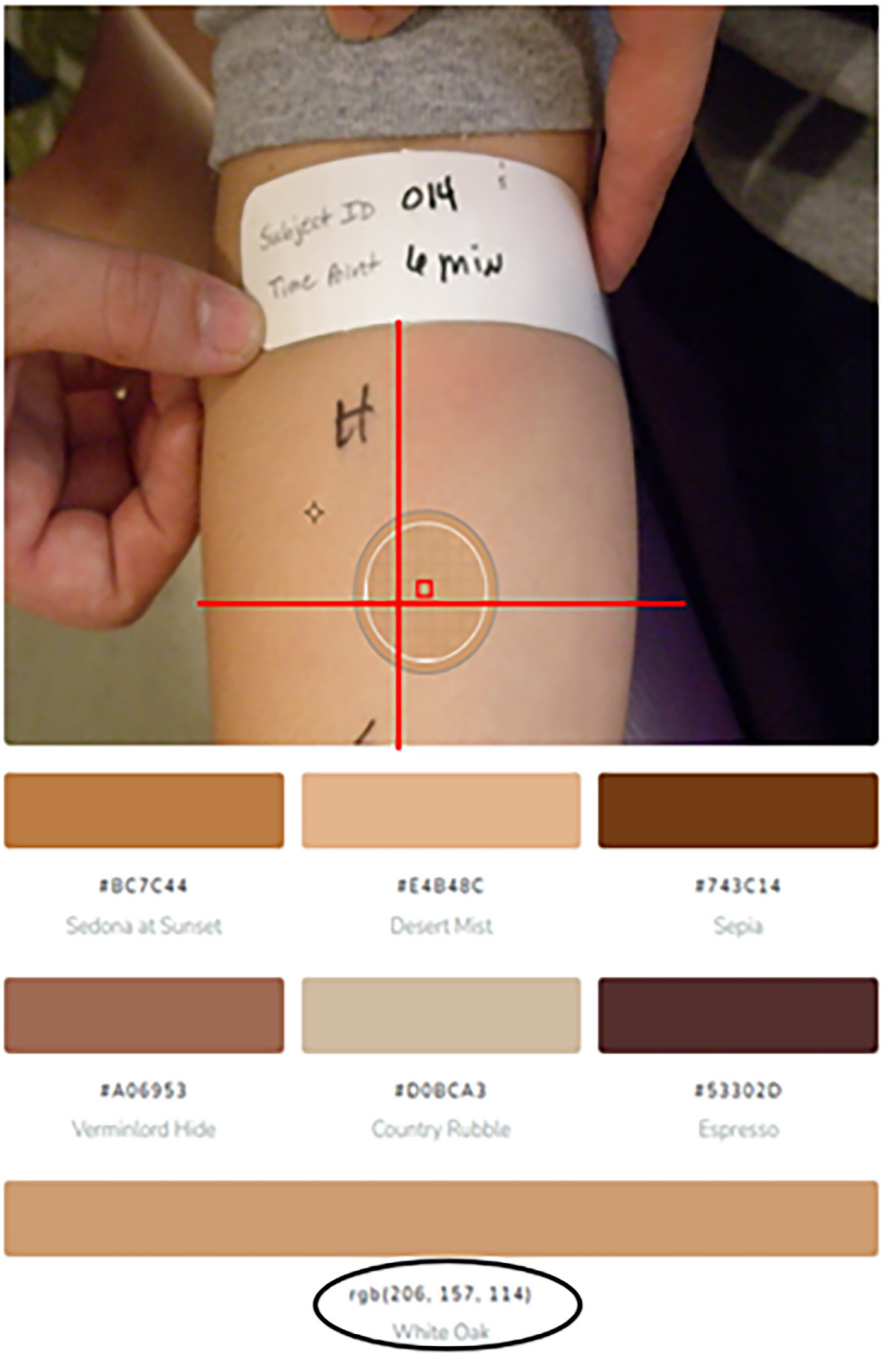

FIGURE 1 Illustration of method for identifying areas for color sampling and RGB generation. Images were divided into similar size quadrants. A color picker (Image Color Finder) was use to select an area within each quadrant for color sampling, avoiding areas with visible veins, erythema, shadows or markings. The color picker was used to obtain an RGB value, with the RGB value being circled in black. Color Picker is being used to obtain the RGB value, with the RGB value being circled in black.

$$f(x) = \sqrt{\frac{\left(x^2 + y^2 + z^2 + b^2\right)}{4}} \quad (1)$$

This process was repeated for the other two components. The average red, green, and blue components were then used to compose an overall average RGB for the patient's skin color in the following format: [average Red component, average Green component, average Blue component]. This method was used for averaging because the RGB values [0,255] originate from larger numbers that were compressed, using square roots, to save space. To account for this issue when averaging, squares must be used Vanga.[20] After RGB values were derived for each participant, a composite RGB score was calculated (Equation 2).

The equation to generate a composite score for patient's skin color where R is the red component of the average RGB, G is the green component of the average RGB, and B is the blue component of the average RGB.

$$f(x) = \frac{R + G + B}{3} \quad (2)$$

Statistical analysis

Non-parametric summary distributions of RGB composite scores were compared across groups (e.g., race

and ethnicity categories, histamine response type) using the Kruskal-Wallis test. Histamine response was categorized as hypo-responsive, normo-responsive, or hyper-responsive. Kendall rank correlation coefficients were calculated to determine associations between HILD pharmacodynamic measures HDTmax, HDCmax, HDAUC, and HDEC50 and RGB composite score. All statistical analyses were completed using Stata software.

RESULTS

Evaluable data were available for 42 children with asthma and/or allergic rhinitis. Participants' ages ranged from 8 to 18 years; 65% were male. 57% of participants identified as White, 33% identified as African American or Black, and 10% identified as Hispanic. We did not collect race data for participants who identified as Hispanic ethnicity.

Skin color and histamine response

As shown in Table 1, differences in RGB composite scores were observed across the three racial and ethnic groups, with participants identifying as African American or Black with lower RGB values (i.e., deeper tones), followed by White and then Hispanic ($p < 0.01$).

RGB scores differed across histamine response groups ($p = 0.028$), with the hypo-responsive histamine group having a lower median score, and the normo-responsive and hyper-responsive groups having similar scores (Figure 2). The hypo-responsive group ($n = 27$) had a median RGB of 120 [IQR range 85.3–147], while the normo-responsive ($n = 8$) and hyper-responsive ($n = 7$) groups had medians of 153 [IQR range 141.3–178] and 150 [IQR range 115.3–156], respectively.

Correlation among the RGB composite score and HILD pharmacodynamic variables HDTmax, HDCmax, HDAUC, and HDEC50 was generally considered weak and did not meet statistical significance (Figure 3). HDTmax and RGB composite had a correlation of 0.081 ($p = 0.47$). HDCmax and RGB composite had a correlation of 0.129 ($p = 0.23$). HDAUC and RGB composite had a correlation of 0.137 ($p = 0.20$). Finally, HDEC50 and RGB composite had a correlation of 0.022 ($p = 0.85$).

TABLE 1 Median RGB composite score by race and ethnicity.

	Median [IQR]
White ($n = 24$)	148.8 [137, 164.2]
African American/Black ($n = 14$)	86.5 [76, 94.7]
Hispanic ($n = 4$)	168.8 [129.2, 192.5]

Note: $p > 0.01$ reflects comparisons among all three racial and ethnic groups.

DISCUSSION

We observed that RGB composite scores differ between racial and ethnic groups, which is not surprising given that racial and ethnic groups often share phenotypic characteristics such as skin color or tone. This finding suggests that the RGB method used is appropriate for quantifying skin color or tone. RGB composite scores differed across histamine response phenotype. The hypo-responsive phenotype group demonstrated lower RGB composite scores relative to the normo-responsive and hyper-responsive response types. We have previously described that histamine pharmacodynamic response type, as measured by HILD, differed between racial groups in our previous studies. In those studies, we observed that children who identified as African American/Black were more frequently represented among the hypo-responsive response phenotype group, which somewhat parallels our findings in the current study related to RGB values and distribution across the histamine response type groups. However, in evaluating the relationship between skin tone/color and HILD values, we did not observe a significant association between RGB skin tone/color composite and individual quantitative histamine response pharmacodynamic parameters such as HDTmax, HDCmax, HDAUC, and HDEC50. We believe that these findings suggest that skin color or tone may not impact measured histamine pharmacodynamic response parameters via the HILD method. However, there is a further need to investigate our findings for group classification. Our previous findings related to the histamine response type group may be related to underlying biological responses relevant to disease pathophysiology, which may differ among racial and ethnic groups due to differences in the causes/drivers (e.g., environmental exposures) of disease.

Currently, the evaluation of antihistamine pharmacodynamic response in clinical trials is conducted via the epicutaneous skin prick test. This test involves administering a histamine solution to the epicutaneous layer of the skin and assessing response via measurement of the "wheal and flare" response of erythema and swelling at the site. This technique employs the manual application of histamine via a "prick" device and measurement by the human eye using a handheld ruler type of device. Variability in this technique has been described that may impact the validity of pharmacodynamic assessments using this method. Studies have also described differences in histamine prick skin test results across different racial and ethnic groups.[21] It is plausible that this described difference may be due to operator error in accurately assessing erythema in varying skin tones. Similar errors related to the ability to perceive erythema or other skin changes in darker skin tones have been described across various dermatologic conditions and states (e.g., eczema, infectious skin rashes, psoriasis).[22] Therefore,

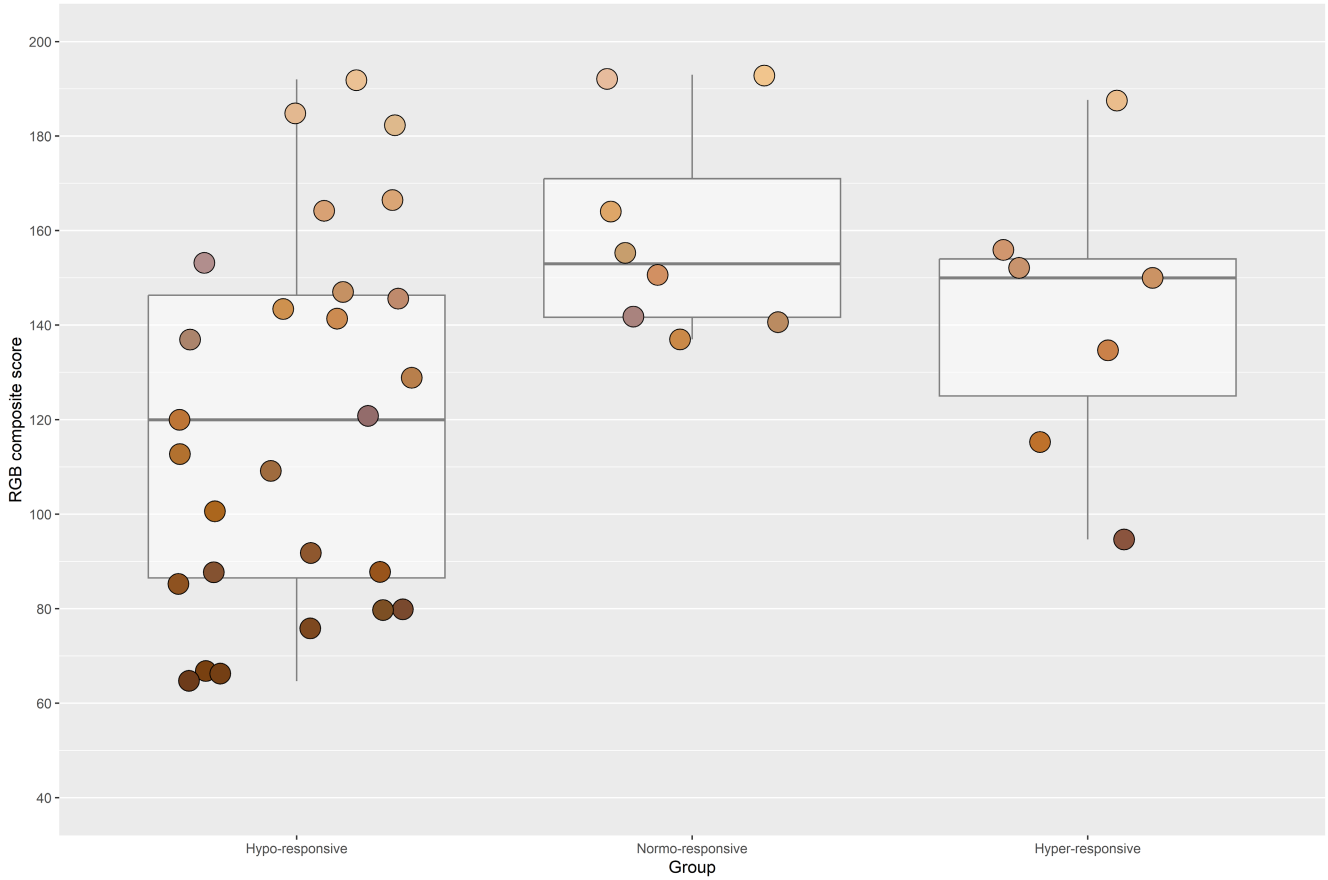

FIGURE 2 RGB values versus histamine response groups. Box-plots were created to depict RGB scores at different histamine response groups ($p = 0.028$), with the hypo-responsive histamine group having a lower median score, and the normo-responsive and hyper-responsive groups having similar scores.

our data suggest that the HILD method may be more appropriate for assessing histamine pharmacodynamic response across varying skin tones than currently established methods in clinical research.

Previous studies have characterized the laser measurement depth for laser Doppler flowmeter (LDF) methodologies across different skin tones. Melanin is largely responsible for the variation in skin pigmentation and tone. Differences in optical properties of lighter skin tones and darker skin tones are largely informed by melanin concentration in the skin. Higher concentrations of melanin have been shown to affect how deep laser light travels in skin layers.[5,6] Fredriksson et al. evaluated the impact of skin tone across different wavelengths emitted by the LDF device in varying models representing different tissue samples. They concluded that epidermal melanin concentration produced a change of less than 4% for measurement depth, which was determined to be a negligible difference in LDF measurement.[23] Abdulhameed et al. also conducted studies of LDF in the assessment of blood perfusion in participants with varying skin tones and did not reveal a difference in mean perfusion between darker and lighter skin tones.[12] Our findings appear to further validate the accuracy of the LDF method for measuring blood flow across varying skin tones and melanin concentrations.

We recognize potential limitations in our study to be improved upon in the future. The image quality of photos used to quantify skin tone varied across participants, and it is possible that differences in picture quality may have impacted results. We attempted to account for potential variation by shadows and other effects within the photos by using RGB data obtained from four separate quadrants within a selected skin area in each photograph. Further, variation in sampling method and area selection may have been somewhat minimized because a single investigator conducted this part of the study. Although our study appears to represent a wide variation in skin tones, we recognize that the sample size is moderate and may not reflect all skin tone variations. Overall, the study includes only a small population of participants who identify as Hispanic and may not reflect skin tone variation observed in other racial and ethnic groups. Future studies should be considered across broader racial and ethnic populations and skin tones using higher quality imaging and pre-defined standard processes for skin tone sampling.

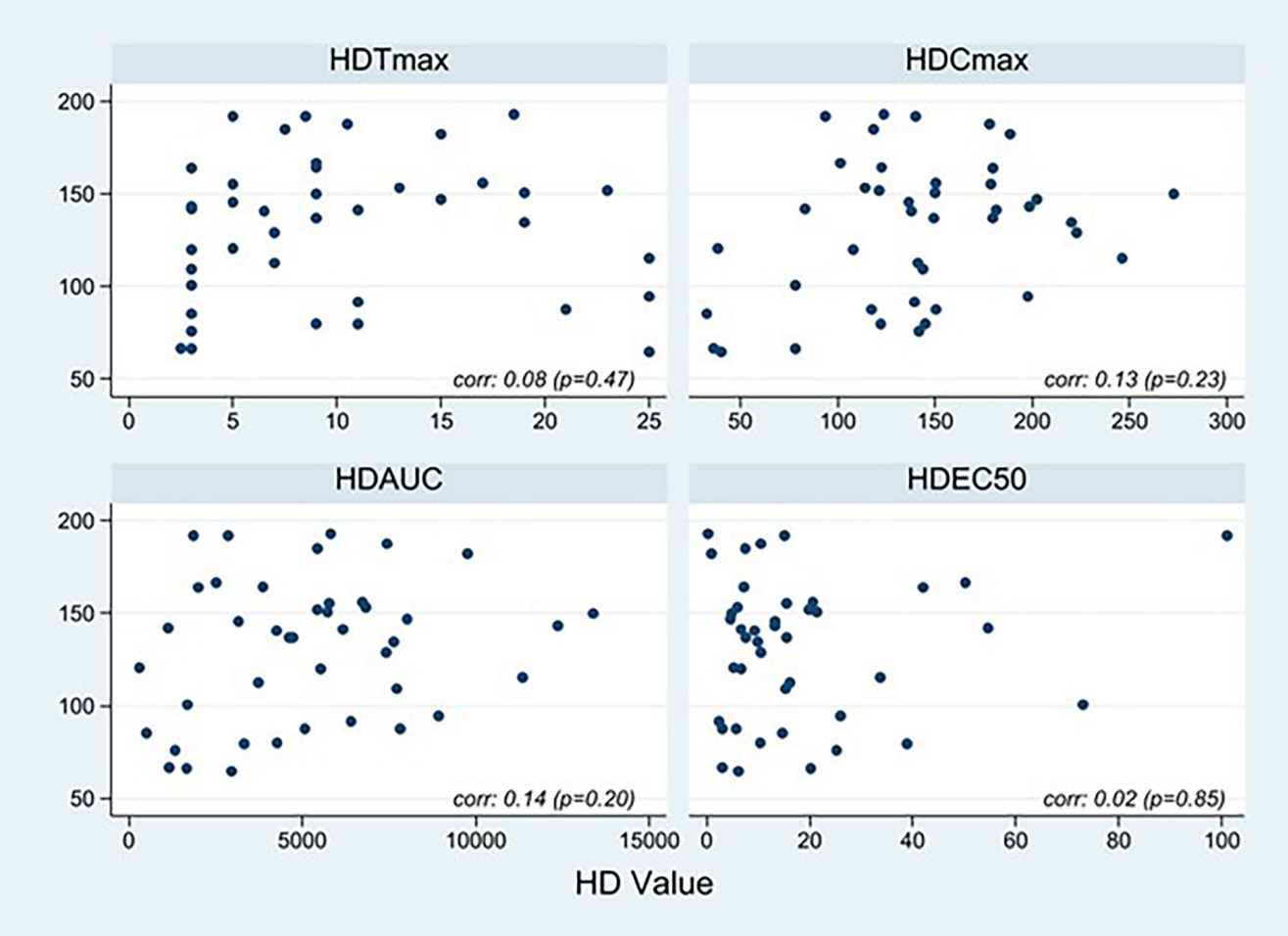

FIGURE 3 Correlation between HILD scores and RGB scores. Scatter plots were created to show the correlation between RGB composite score and HILD pharmacodynamic variables. HDTmax, HDCmax, HDAUC, HDEC50 was generally considered weak and did not meet statistical significance.

It is important that clinical and pharmacodynamic biomarkers used in clinical and/or research settings are validated for use in the general population and that variation in skin color or tone is evaluated appropriately. As previously described, pulse oximeter measurements have been found to be less accurate in those with darker skin tone. The importance of appropriately validated technological advances that may help to mitigate human error or bias is also recognized. For example, technologies such as thermography, alternative light sources, subepidermal moisture measurement, laser Doppler, and spectrophotometry have been described that could be used at the bedside by clinical staff to improve the detection and assessment of common skin injuries such as pressure sores, which may be subject to human operator bias.[24] Likewise, HILD may provide a more accurate assessment of antihistamine pharmacodynamics in clinical studies than the current skin prick test method.

In this study, we have shown that HILD may be suitable to characterize histamine pharmacodynamic response across varying skin tones. Further investigation is required to elucidate potential reasons for our previously reported differences in HILD histamine pharmacodynamic response type across racial groups. More recent literature has described epigenetic and inflammatory changes resulting from disparate exposures between racial groups (e.g., chronic stress) having secondary impacts on biological processes and functions.[25] This is an additional area to consider in our investigation of differential pharmacodynamic and clinical effects of therapies that may target potentially altered pathways.

AUTHOR CONTRIBUTIONS

A.R., A.E., B.R.L., S.B., and B.L.J. wrote the manuscript; A.R., A.E., B.R.L., and B.L.J. designed the research; A.R., A.E., B.R.L., and B.L.J. performed the research; A.R., A.E., B.R.L., and B.L.J. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

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