

# Visible-Near Infrared Spectroscopic Assessment of Urogenital Tissue in Premenopausal and Postmenopausal Women

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## ABSTRACT:

**BACKGROUND:** A clinical study was conducted to evaluate the feasibility of using visible and near-infrared (NIR) spectroscopy as a potential noninvasive measure of genital skin health in premenopausal and postmenopausal women.

**METHODS:** A total of 45 female subjects (aged 21-70 years), all of whom gave fully informed consent to participate, were enrolled in the study and assigned to 1 of 3 groups: 15 premenopausal (Pre-M), 15 postmenopausal receiving hormone replacement therapy (Post-M HRT), and 15 postmenopausal receiving no form of hormone replacement therapy (Post-M non-HRT). Spectral measurements were taken at the vaginal mucosa, and spectral data were evaluated for the erythema index (EI), hemoglobin index (HI), bilirubin/ $\beta$ -carotene, and melanin. The color index (CI; calculated as the ratio of absorbance at 480 nm/540 nm) was also determined. Results were compared with previously published results on biomarkers and physical characteristic of genital tissue measured on the same groups of women.

**RESULTS:** Spectral measurements from the Post-M Non-HRT subjects indicated a significant reduction in HI compared with the Pre-M group ( $P = .0003$ ) and to the Post-M HRT group ( $P < .0001$ ). Similarly, EI was reduced in the Post-M Non-HRT ( $P < .0001$  and  $P = .0041$  for the Pre-M and Post-M HRT groups, respectively). In contrast, the Post-M Non-HRT subjects exhibited a significant increase in  $\beta$ -carotene compared with the Pre-M subjects ( $P = .0098$ ). Bilirubin and melanin were not significantly affected. The Post-M Non-HRT group exhibited a significant increase in CI, indicating a shift away from the hemoglobin absorption region (510-620 nm wavelength) and toward the bilirubin/ $\beta$ -carotene absorption region (450-490 nm wavelength). This change was significant when compared with both the Pre-M group ( $P < .0001$ ) and the Post-M HRT group ( $P = .0048$ ). The changes in spectral measurements were consistent with previously reported changes in physical parameters (vaginal atrophy, increased pH, decreased skin temperature) and with decreased concentrations of the biomarkers histamine and histidine.

**CONCLUSIONS:** Hemodynamic spectral characteristics differ in postmenopausal vaginal tissue compared with tissue in premenopausal women, with decreased absorbance in the hemoglobin absorption region (510-620 nm wavelength) and an increased absorbance in the bilirubin/ $\beta$ -carotene absorption region (450-490 nm wavelength). A change in absorbance in the visible and NIR wavelengths is a promising, additional measure of genital skin health related to menopause and vulvovaginal atrophy.

**KEYWORDS:** Near-infrared spectroscopy, urogenital tissue, vulvovaginal atrophy, postmenopausal, hormone therapy, microvasculature indicators, urogenital biomarkers

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## Introduction

For decades, it has been known that skin can be evaluated spectrophotometrically, and that blood flow and the level of oxygenation of blood can have major influences on the spectral absorption wavelengths of the skin. As early as 1929, Brunsting and Sheard reported that oxygenated blood near the surface of the skin tends to shift the dominant absorption wavelength toward the red end of the spectrum (620-660 nm), whereas venous blood shifts the dominant absorption wavelength toward the blue end of the spectrum (490-500 nm).<sup>1</sup> Understanding this basic principal enabled the development of the first practical, lightweight oximeter in the early 1940s to measure hypoxia in pilots.<sup>2</sup> The development of pulse oximetry

as a noninvasive means to continuously monitor oxygen levels in patients was developed in the 1970s and early 1980s.<sup>3</sup>

In addition to oxygenation, spectroscopy using absorption bands in the visible and near-infrared (NIR) spectral region can be used in the monitoring and diagnoses of a variety of inflammatory and disease processes, including tissue edema, hydration, and the degree of tissue perfusion.<sup>4</sup> The technology can be used to evaluate the severity of burn wounds based on the oxygen saturation of the tissue and regional blood flow<sup>5</sup> or to monitor real-time changes in regional oxygen saturation of cerebral and somatic tissues following hypoxic neurologic injuries.<sup>6</sup> Near-infrared spectroscopy has a history of successful use



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**Table 1.** Summary of demographics and baseline vaginal atrophy scores.

PARAMETER	PREMENOPAUSAL GROUP (PRE-M) N = 15	POSTMENOPAUSAL WITH HRT GROUP (POST-M HRT) N = 15	POSTMENOPAUSAL NON-HRT GROUP (POST-M NON-HRT) N = 15
	MEAN ± SD	MEAN ± SD	MEAN ± SD
Age, y	33.0 ± 6.4	60.5 ± 3.6	60.7 ± 3.6
Height, inch	64.7 ± 3.7	63.9 ± 2.5	63.5 ± 2.8
Weight, lb	156.0 ± 22.8	147.8 ± 23.7	149.2 ± 27.0
BMI, %	26.2 ± 3.3	25.5 ± 4.3	25.9 ± 3.6
Years since last period	NA	14.2 ± 8.2	15.8 ± 9.3
Average time on HRT	NA	5 y, 2 mo	NA
	NO. (%)	NO. (%)	NO. (%)
Ethnicity			
African-American	6 (40)	0 (0)	2 (13)
White	8 (53)	15 (100)	13 (87)
Other	1 (7)	0 (0)	0 (0)
	MEAN ± SE	MEAN ± SE	MEAN ± SE
Vaginal atrophy scores	0.29 ± 0.12	0.71 ± 0.22	6.98 ± 0.44 <sup>a</sup>
Vaginal pH	4.72 ± 0.12	4.36 ± 0.20	6.77 ± 0.25 <sup>a</sup>

Abbreviations: BMI, body mass index; HRT, hormone replacement therapy; NA, not applicable.

<sup>a</sup>Post-M Non-HRT group significantly different from Pre-M and Post-M HRT groups ( $P < .0001$ ).

in evaluating oxygen saturation in the retinal vessels<sup>7</sup> and as a diagnostic tool for periodontal diseases.<sup>4,8-10</sup>

The specific objective of this study was to evaluate the feasibility of using visible and NIR spectroscopy as a potential noninvasive measure of genital skin health, vaginal inflammation, and other skin conditions related to vulvovaginal atrophy and menopause-related genitourinary symptoms. We conducted a study of biomolecular and physical measures in the urogenital skin of 3 groups of women in different life stages: premenopausal, postmenopausal with benefit of hormone replacement therapy (HRT), and postmenopausal taking no HRT. Recently, we reported that skin temperature was lower in both postmenopausal groups indicating reduced blood perfusion.<sup>11</sup> Histamine and histidine levels were also reduced significantly. This current report presents results of the visible and NIR spectroscopic evaluation conducted during this study.

## Materials and Methods

Testing was conducted in compliance with the Good Clinical Practice Regulations (21 Code of Federal Regulations [CFR] 50) and in accordance with the Declaration of Helsinki.<sup>12</sup> Test protocols were approved by the test facility's Institutional Review Board (Liberty IRB, Deland, FL, USA). Details of the study inclusion/exclusion criteria and demographic information for the subjects were detailed in an earlier publication.<sup>11</sup>

Briefly, 45 female subjects, aged 21-70 years, who fully gave their informed consent to participate and met all entrance criteria, were enrolled. A summary of the test group demographics is presented in Table 1. The groups consisted of 15 premenopausal women (Pre-M), 15 postmenopausal women receiving HRT for at least 12 consecutive months (Post-M HRT), and 15 postmenopausal women who were not receiving any type of HRT (Post-M Non-HRT). A number of end points were measured in this study including physical measures (such as vaginal atrophy, skin surface pH, and temperature), histamine/histidine levels, cytokines, and natural moisturizing factor. The collection and analyses of these data were described previously.<sup>11</sup>

For this phase of the study, spectral measurements were obtained from 4 different body sites: vagina, introitus, labia minora, and labia majora. The specifics of the instrument used to measure reflectance spectra have been described previously by Liu and colleagues.<sup>4</sup> Spectra were collected using a portable PDA512-ISA spectrograph (Control Development Inc., South Bend, IN, USA) interfaced to a customized bifurcated fiber optic probe designed for use in the oral cavity (Fiberguide Industries, Stirling, NJ, USA). The probe consisted of 7 central fiber optic bundles to deliver light to the measured site and an outer ring of 22 fiber optic bundles arranged in a circular pattern to collect light emitted from the tissue. Relative absorbance was recorded

**Table 2.** Spectral data acquisition.

GROUP	SUCCESSFUL DATA COLLECTION			
	VAGINA	INTROITUS	LABIA MINORA	LABIA MAJORA
Pre-M (N=15)	15	8	9	6
Post-M HRT (N=15)	15	12	15	7
Post-M Non-HRT (N=15)	15	10	11	6
All combined (%)	45 (100)	30 (67)	35 (78)	19 (42)

Abbreviation: HRT, hormone replacement therapy.

for the spectral range of 420 to 700 nm wavelengths. Table 2 shows the effectiveness of data acquisition. Measurements taken at the vaginal mucosa were successful in all study participants. As the anatomic site of the measurement moved from mucosal to stratified squamous epithelium, there was a higher fraction of rejected spectra, likely due to the attenuation by melanin. Because data acquisition was most reliable at the vaginal site, this was the focus of subsequent data evaluation.

Previously generated reference spectra for oxygenated and deoxygenated hemoglobin (HbO<sub>2</sub> and Hb, respectively) were used for comparison,<sup>4</sup> and relative absorption was in the 510 to 620 nm wavelength region. Absorbance of bilirubin was in approximately the 450 to 490 nm wavelength region.<sup>13–15</sup>

$$EI = 100 * \left( OD_{560} + 1.5 * (OD_{545} + OD_{575}) - 2 * (OD_{510} + OD_{610}) \right) \quad (1)$$

The HI was based on measurements at wavelengths in the absorbance spectra at which absorbance is independent of the state of oxygenation, ie, the isosbestic points.<sup>18</sup> Differences between absorbances at the isosbestic points at wavelengths 527.5, 544, and 573 nm define the HI, calculated from the following expression:

$$HI = 100 * \left( \frac{(OD_{544} - OD_{527.5})}{16.5} \right) - \left( \frac{(OD_{573} - OD_{544})}{29} \right) \quad (2)$$

The relative amounts of bilirubin/ $\beta$ -carotene, and melanin in skin were determined by fitting spectra from subjects to a linear combination of reference spectra of the major chromophores in skin. These reference spectra for bilirubin,<sup>19,20</sup>  $\beta$ -carotene,<sup>21,22</sup> melanin,<sup>19,23</sup> and oxygenated and deoxygenated hemoglobin<sup>23</sup> were obtained from previous literature reports. Classical least squares (CLS) were used to quantify the relative amounts of each component in skin spectra from subjects.<sup>24,25</sup> Both reference and subject spectra were processed using standard normal variate normalization followed by an offset correction producing processed spectra with zero absorption in the spectral region >650 nm that corresponds to a spectral region of skin that does not absorb light. Nonnegativity constraints on the regression coefficients were used when the CLS model was applied to spectra from the subjects. This approach allows

Absorbance of  $\beta$ -carotene was also in the 400 to 500 nm range.<sup>16</sup> Melanin absorbs over a broad range of wavelengths with absorption increasing linearly in the range of 620 to 720 nm and exponentially toward shorter wavelengths (300–600 nm).<sup>17</sup>

Equations used to calculate the erythema index (EI) and hemoglobin index (HI) were adapted from Feather and colleagues.<sup>18</sup> The EI was determined by establishing an arbitrary baseline joining points 510 and 610 nm in the spectrum of oxy-hemoglobin and calculating the area under the curve above this baseline. It was determined by Feather and colleagues that this approach reduces the effect of the tissue matrix in the measurement. The EI is calculated from the following expression:

comparison of relative amounts of each chromophore in the skin between subjects. However, the absolute amount of each component in skin is unknown.

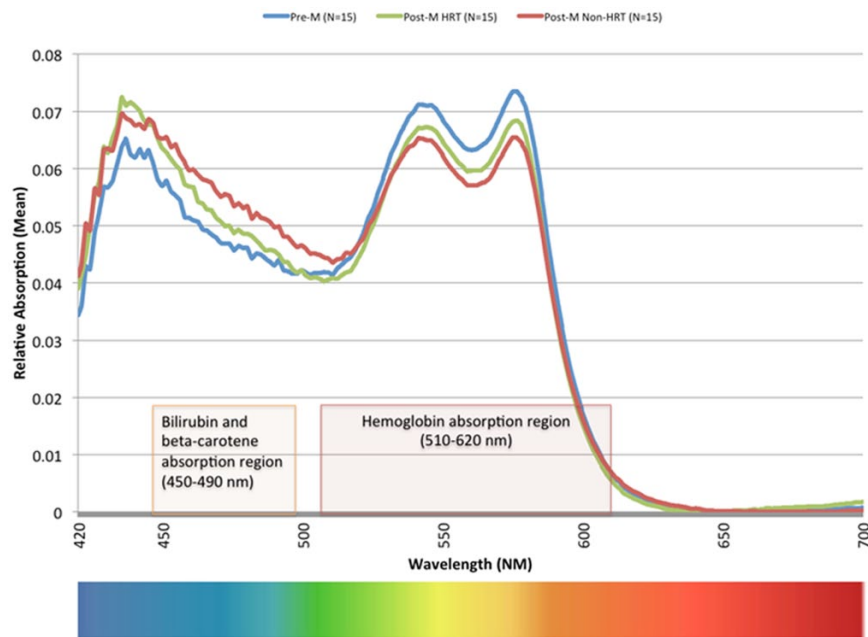
A color index (CI) was calculated to provide a metric for characterizing the impact of bilirubin and  $\beta$ -carotene absorbance (450–490 nm) relative to the region of the spectra dominated by hemoglobin absorption (510–620 nm). The CI was calculated using the following expression:

$$CI = \frac{OD_{480}}{OD_{540}} \quad (3)$$

Group means for each end point of interest were determined using a single result from each subject. Statistical significance was determined using paired comparisons of the means in a *t* test.

## Results

Overall optical spectra measured in the vaginal region were generated for the 3 test groups (Figure 1). Relative absorption in the 510 to 620 nm wavelength region reflects the absorption from oxygenated and deoxygenated hemoglobin (HbO<sub>2</sub> and Hb, respectively). When group means were compared, the Pre-M group demonstrated the highest absorption in this region, with the Post-M Non-HRT group demonstrating the



**Figure 1.** Overall optical spectra measured in the vaginal region. HRT indicates hormone replacement therapy.

**Table 3.** Results of visible/near-infrared spectroscopy measures.

	PRE-M	POST-M HRT	POST-M NON-HRT	P VALUES FROM STATISTICAL ANALYSIS PAIRWISE COMPARISONS		
	MEAN ± SE	MEAN ± SE	MEAN ± SE	PRE-M VS POST-M HRT	PRE-M VS POST-M NON-HRT	POST-M HRT VS POST-M NON-HRT
Hemoglobin index (HI)	0.090 ± 0.0049	0.103 ± 0.0048	0.065 ± 0.0038	.06	<b>.0003</b>	<b>&lt;.0001</b>
Erythema index (EI)	18.1 ± 0.63	16.7 ± 0.62	14.0 ± 0.76	.12	<b>&lt;.0001</b>	<b>.0041</b>
Bilirubin	0.0013 ± 0.0009	0.0041 ± 0.0018	0.0037 ± 0.0017	.19	.27	.84
β-carotene	0.094 ± 0.0091	0.114 ± 0.0074	0.130 ± 0.0114	.14	<b>.0098</b>	.23
Melanin	0.56 ± 0.022	0.58 ± 0.012	0.58 ± 0.012	.41	.49	.9
Color index (CI) (480/540)	0.66 ± 0.027	0.74 ± 0.031	0.89 ± 0.046	.11	<b>&lt;.0001</b>	<b>.0048</b>

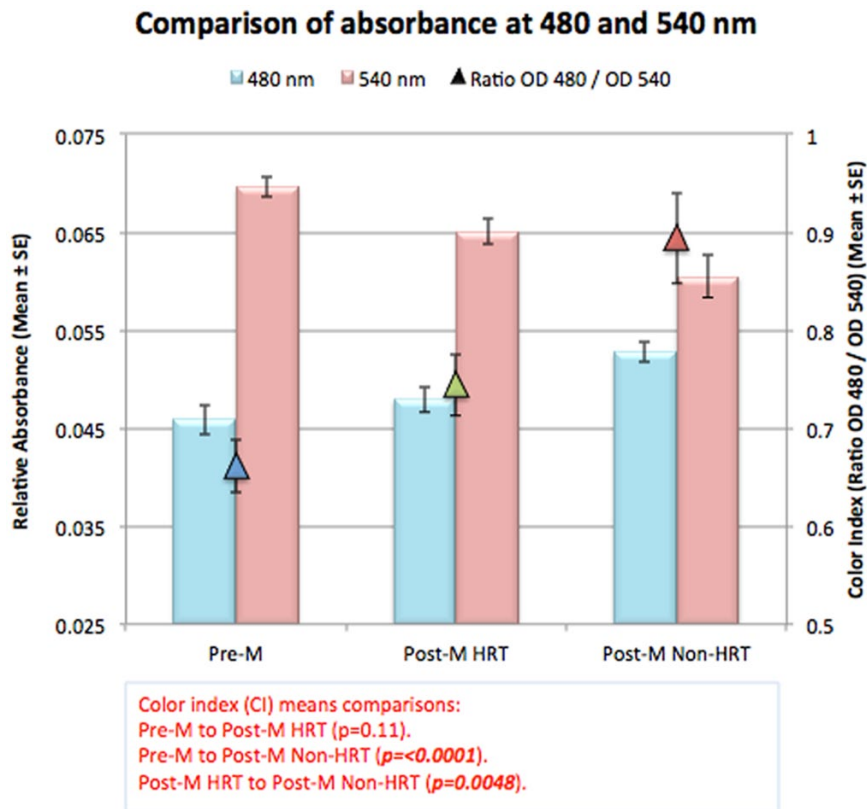
The mean absorption at selected wavelengths for HI, EI, bilirubin/β-carotene, and melanin were determined as described in the “Materials and Methods” section. The CI is defined as the ratio of absorbance of at 480 and 540 nm. Bold numbers means a statistical significance.

lowest. The Post-M HRT group showed an intermediate relative absorption in this wavelength range. In contrast, absorption in the 450 to 490 nm region was lowest in the Pre-M group, intermediate in the Post-M HRT group, and highest in the Post-M Non-HRT group. This wavelength region reflects absorption from bilirubin and β-carotene.

Using a fiber optic probe designed for use in the oral cavity as described in the “Materials and Methods” section, overall optical spectra were measured in the vaginal region for 3 different test groups: premenopausal women (Pre-M), postmenopausal women receiving HRT (Post-M HRT), and postmenopausal women receiving no form of HRT (Post-M Non-HRT). Relative absorbance was recorded for the spectral range of 420 to 700 nm wavelengths.

Based on absorption at selected wavelengths, end points of interest were determined as described in the previous section. Results are presented in Table 3. For the HI, the mean absorptions for the Pre-M and Post-M HRT groups were not significantly different ( $P = .06$ ). However, the Post-M Non-HRT group had a mean absorption that was significantly lower than the Pre-M group ( $P = .0003$ ) and the Post-M HRT group ( $P < .0001$ ). A similar pattern was observed for EI, with no difference between the Pre-M and Post-M HRT groups ( $P = .12$ ), but a significant lower mean absorption for the Post-M Non-HRT group ( $P < .0001$  when compared with the Pre-M group, and  $P = .0041$  for the Post-M HRT).

There were no significant differences in the mean absorption when the 3 groups were compared for bilirubin. However,



**Figure 2.** Color index (CI) change in the vaginal region associated with menopause. HRT indicates hormone replacement therapy.

the absorption reflecting the  $\beta$ -carotene was significantly higher for the Post-M Non-HRT group when compared with the Pre-M group ( $P=.0098$ ). The absorption reflecting melanin was not significantly different when the groups were compared.

The CI was defined as the ratio of absorbance at 480 nm (ie, in the bilirubin/ $\beta$ -carotene region) to 540 nm (ie, in the hemoglobin region) using equation 3. When the CI was calculated for the 3 groups (Table 3), there was no significant difference between the means for the Pre-M and Post-M HRT groups ( $P=.11$ ). However, the mean for the Post-M Non-HRT was significantly higher compared with both the Pre-M group ( $P<.0001$ ) and the Post-M HRT group ( $P=.0048$ ) indicating a higher absorption at the 480 nm region and a lower absorption at the 540 nm region (Figure 2). The means for each wavelength are shown on the primary vertical axis. The mean ratios of these 2 values are shown in the secondary vertical axis.

A summary of the NIR measures is provided in Figure 3 to illustrate the relative absorbance for the 3 test groups. With the exception of the absorption indicative of bilirubin, all other end points of interest demonstrated a significant difference between the Post-M Non-HRT group and the Pre-M group (ie, means for  $\beta$ -carotene, CI, HI, and EI). In addition, the Post-M Non-HRT group differed significantly from the Post-M HRT group in the means for CI, HI, and EI.

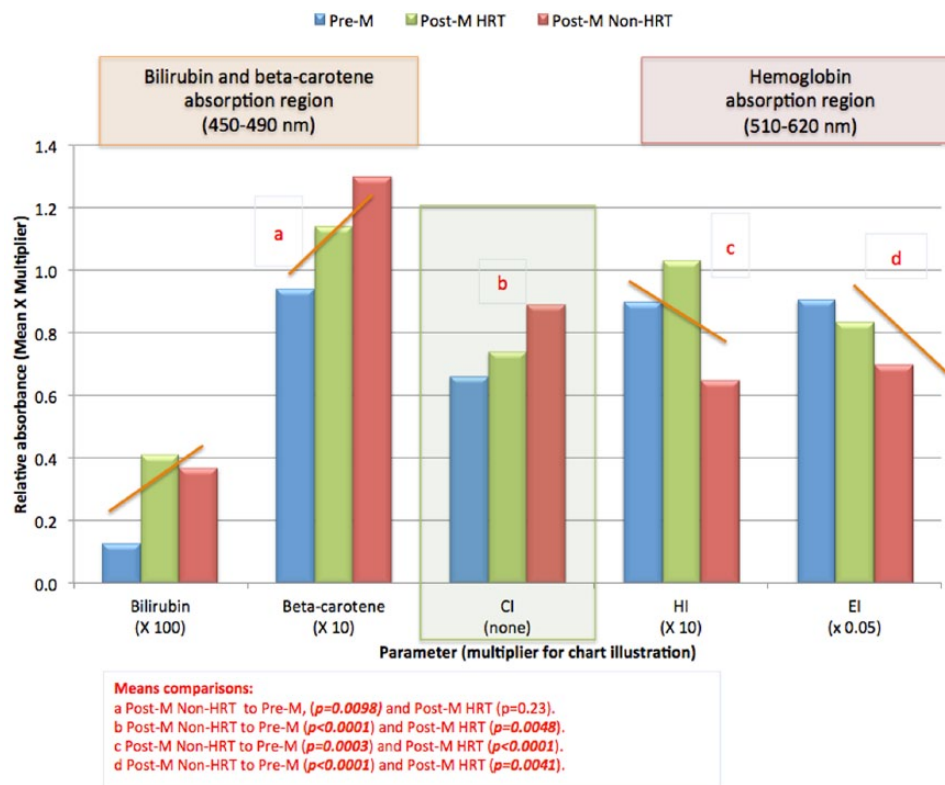
The calculated values for each variable determined in the study are summarized. To allow the data to be presented on a single graph, each variable from Table 3 was subjected to a

multiplication factor:  $100 \times$  bilirubin,  $10 \times \beta$ -carotene,  $1 \times$  CI,  $10 \times$  HI, and  $0.05 \times$  EI.

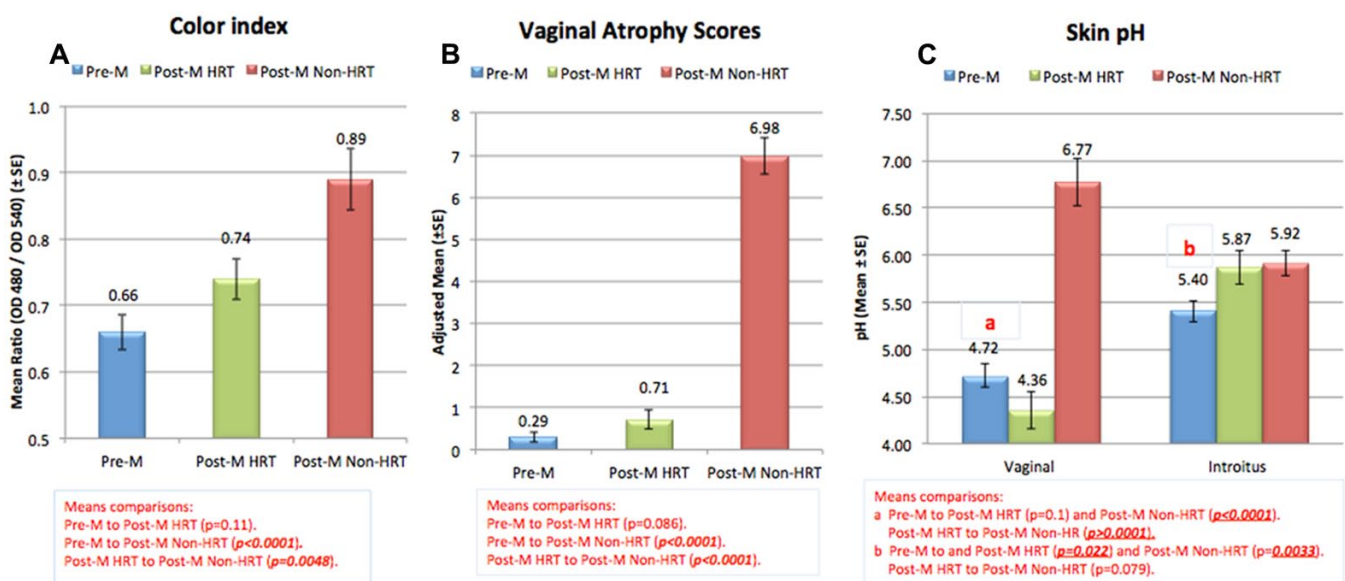
Results of the spectral measurements were compared with physical parameters evaluated on the same subjects. The change in CI in the 3 test groups (Figure 4A) was consistent with increased scores for vaginal atrophy (Figure 4B) with significant differences in the mean when the Pre-M and Post-M HRT groups were compared with the Post-M Non-HRT group. A similar pattern was observed with vaginal pH (Figure 4C), with the Post-M Non-HRT group demonstrating a significantly higher mean than either the Pre-M group or the Post-M HRT group. In contrast, the pH measures of skin at the introitus resulted in a significant increase in both the Post-M HRT ( $P=.022$ ) and the Post-M Non-HRT ( $P=.0033$ ) groups (Figure 4C).

Indicators of blood perfusion (HI and EI) calculated from spectroscopy results (Table 3) were compared with skin surface temperatures (Figure 5). As discussed earlier, both the HI and EI decreased significantly in the Post-M Non-HRT group compared with the Pre-M and the Post-M HRT groups (Table 3 and illustrated in Figure 5A). Skin temperatures at the labia minora and labia majora sites also decreased significantly in the Post-M Non-HRT group compared with the Pre-M group ( $P=.0087$  at the labia minora and  $P=.0025$  at the labia majora; Figure 5B). The Post-M HRT group showed an intermediate skin temperature at the labia minora and a skin temperature similar to the Post-M Non-HRT group at the labia majora. At the introitus, the Pre-M group exhibited the highest skin





**Figure 3.** Visible/near-infrared spectroscopy results summary. HRT indicates hormone replacement therapy.

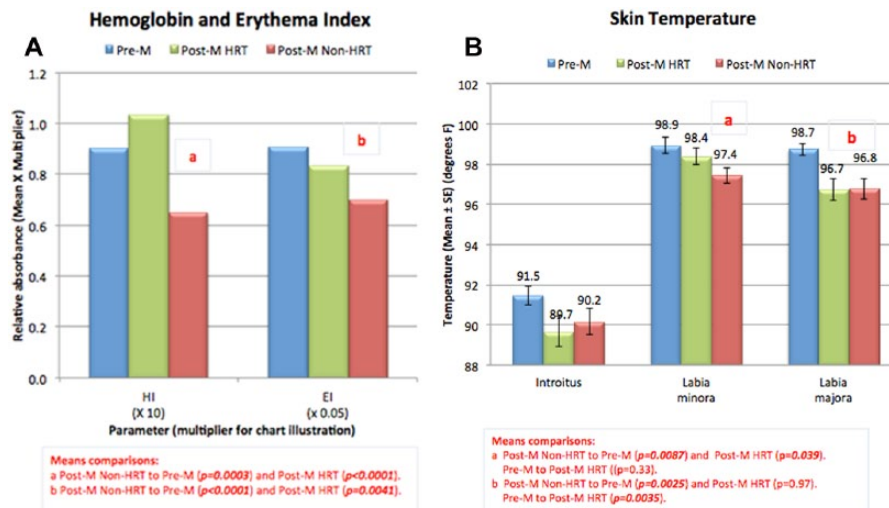


**Figure 4.** Comparison of color index (CI) to physical measures. The (A) mean CI for each test group was compared with (B) mean vaginal atrophy scores and (C) skin pH measured at the vagina and introitus. HRT indicates hormone replacement therapy.

temperature, but the difference was not significant compared with either Post-M group.

The calculated HI and EI absorbance measures were also compared with histamine and histidine levels isolated via tape stripping (Figure 6). When compared with the Pre-M group, concentrations of histamine were significantly lower in both the Post-M Non-HRT group ( $P=.0003$  at the introitus and  $P=.0001$  at the labia minora) and the

Post-M HRT group ( $P=.041$  at the introitus and  $P=.003$  at the labia minora) (Figure 6B). For histidine, concentrations were directionally lower in the Post-M group (Figure 6C). The differences achieved significance at the labia minora ( $P=.045$  for Pre-M to Post-M Non-HRT and  $P=.01$  for Pre-M to Post-M HRT). The ratio of histidine to histamine was highly variable; however, it was lower in the Pre-M group compared with both Post-M groups



**Figure 5.** Comparison of hemoglobin index (HI) and erythema index (EI) with skin surface temperature. The calculated values for (A) HI and EI for each test group were compared with (B) skin surface temperature measured at the introitus, the labia minora, and the labia majora. Data on skin temperature were adapted from a previous publication.<sup>11</sup> HRT indicates hormone replacement therapy.

(Figure 6D). This difference was significant for the Post-M Non-HRT group at the introitus ( $P=.030$ ) and the labia minora ( $P=.017$ ).

## Discussion

The vulva and vagina undergo characteristic age-related changes in morphology and physiology,<sup>26,27</sup> including loss of subcutaneous fat, vaginal mucosal atrophy, and a rise in pH. Aging of the skin is associated with a regression and disorganization of capillaries and small vessels resulting in an overall reduction in blood vessel density.<sup>28</sup> This program was designed to determine whether aspects of female genital health could be evaluated noninvasively using visible and NIR spectroscopy conducted in 3 groups of women in different life stages: premenopausal, postmenopausal with benefit of HRT, and postmenopausal taking no HRT. Results were compared with previously reported biomolecular and physical measures in the urogenital skin conducted among the same test subjects.<sup>11</sup>

It should be noted that we attempted to collect spectral measurements from several anatomic sites, including the vagina, the introitus, the labia minora, and the labia majora. We found that as the anatomic sites changed from mucosal to stratified squamous epithelium, a substantial proportion of the spectra were rejected. This was likely due to the increasing presence of melanin. For the previously reported biomolecular and physical measures,<sup>11</sup> material was collected for analysis of biomarkers from anatomic sites via tape stripping, which could not be performed in the vagina. This limited evaluation of biomarkers to the introitus and external genitalia (labia minora and labia majora). Furthermore, the postmenopausal subjects in this feasibility study were predominately white. The applicability of the method to women of color will require further investigation.

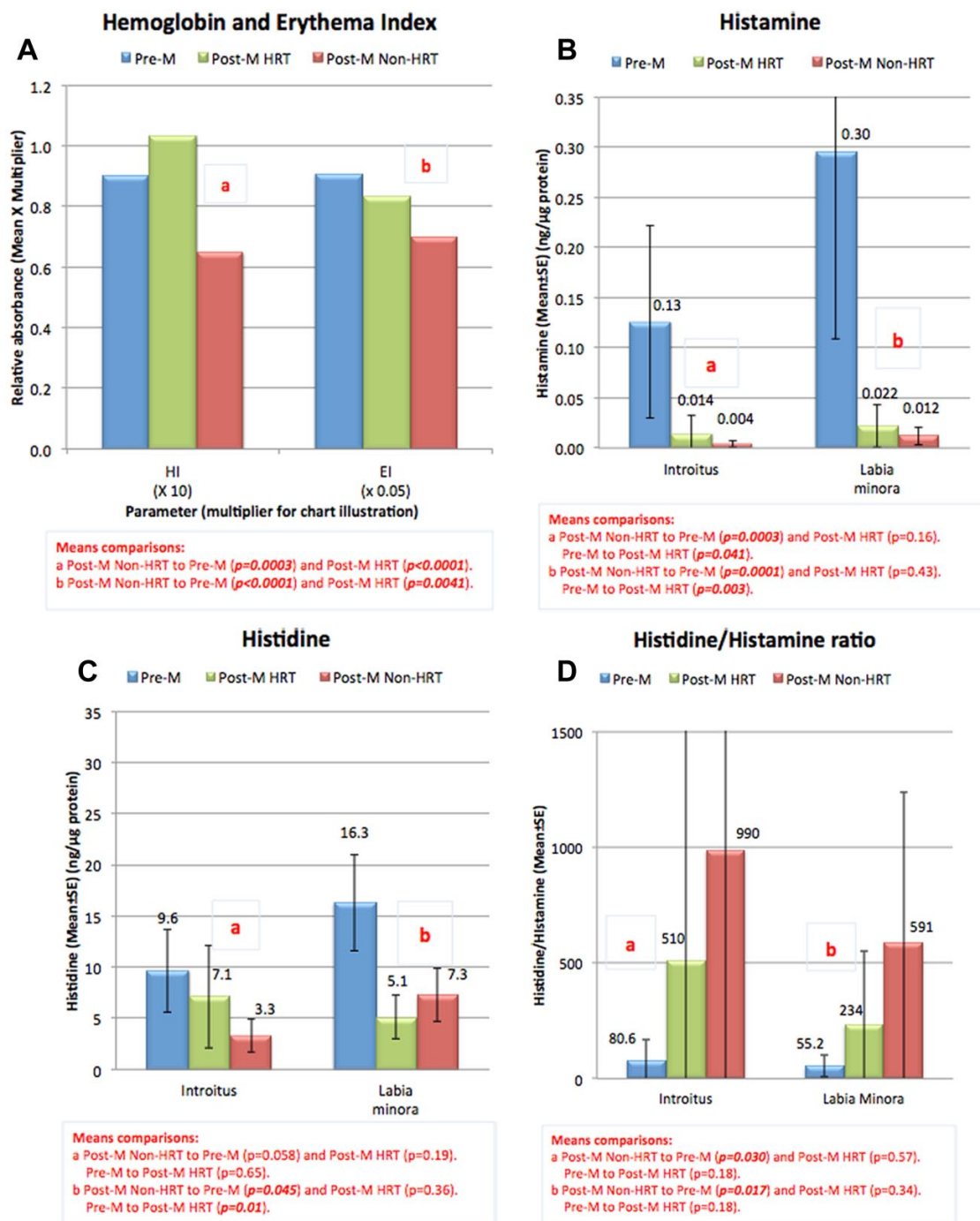
The spectroscopic assessment of urogenital tissue was consistent with the reduction in vasculature known to occur in the

female genitalia with aging. The vaginal tissue of postmenopausal women exhibited a lower absorbance in the 510 to 620 nm wavelength and a higher absorbance in the 450 to 490 nm wavelength (Figures 1 and 2). This shift in color away from the orange/red region is consistent with the reduced vasculature and, therefore, reduced HI and EI calculated for the postmenopausal women (Table 3). A decrease in overall vasculature would be expected to cause a reduction in skin surface temperature (Figure 5) and lower levels of biomarkers delivered to the tissue via the circulatory system, specifically, histamine and histidine (Figure 6).

Slight increases in yellow color with aging have been reported, although it has been assumed that such changes are attributable to extrinsic aging secondary to UV exposure.<sup>29,30</sup> We detected a statistically significant increase in  $\beta$ -carotene and a directional increase in bilirubin (Table 3 and Figure 3) in the vaginal tissue of postmenopausal women. In our study, the color shift cannot be related to UV exposure because it was detected in tissues not exposed to UV light. It is unclear why the levels of  $\beta$ -carotene and bilirubin might increase in the vaginal mucosa with aging; however, our results would indicate that some element of intrinsic aging contributes to such a change.

For most of our observations, the Post-M HRT group exhibited values intermediate between the Pre-M and Post-M Non-HRT groups. Hormone replacement therapy is known to relieve many of the physical and psychological changes commonly associated with menopause, including vasomotor symptoms (hot flashes), sleep disorders, decreased sexual response, genitourinary factors, and mood changes.<sup>31</sup> Based on our observations, HRT appears to reduce the magnitude of the change in the CI in postmenopausal women (Figure 3).

As we reported in an earlier publication,<sup>11</sup> the levels of both histamine and histidine were decreased in postmenopausal women (Figure 6B and C). The ratio of histidine to histamine showed an increase (Figure 6D). Histamine is



**Figure 6.** Comparison of HI and EI to histamine and histidine levels. The calculated values for (A) HI and EI for each test group were compared with measured levels of (B) histamine, (C) histidine, and (D) the ratio of histidine/histamine. The collection of tape strip samples from the introitus and labia minora and the results of analyses for histamine and histidine were reported in a previous publication.<sup>11</sup> EI indicates erythema index; HI, hemoglobin index; HRT, hormone replacement therapy.

derived from the decarboxylation of the amino acid histidine, and an altered ratio of histamine to histidine may indicate a change in the induction of histidine decarboxylase or a shift in the equilibrium between these 2 materials. Histamine has a variety of functions in the body including effects on smooth muscle and blood vessels critical to physiological sexual arousal.<sup>32,33</sup> In our earlier publication, we proposed that the reduced level of histamine in the genital area might be related

to sexual and lubrication difficulties in postmenopausal women, making histamine an important potential biomarker for genital tissue health.

## Conclusions

Vaginal atrophy and an increase in skin pH are known to accompany menopause. A change in absorbance in the visible and NIR wavelengths is a promising, additional measure of



genital skin health related to menopause and vulvovaginal atrophy. Hemodynamic spectral characteristics differ in postmenopausal vaginal tissue compared with tissue in premenopausal women, with decreased absorbance in the hemoglobin absorption region (510–620 nm wavelength) and an increased absorbance in the bilirubin/ $\beta$ -carotene absorption region (450–490 nm wavelength).

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### Author Contributions

MAF was the clinical and project innovation leader. TC did Spectroscopy data analysis. K-ZL collected the Spectroscopic measures.

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