Associations of Retinal Oximetry in Healthy Young Adults

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PURPOSE. To assess factors associated with retinal oximetry values in healthy young adults.

METHODS. Retinal oximetry readings were assessed using the oximetry module of the Vesselmap System in 100 eyes of 50 healthy subjects aged 18 to 58 years. Generalized estimating equation models were used to estimate the associations of candidate variables (age, sex, retinal capillary flow, HbA1c, triglyceride, total cholesterol, ocular perfusion pressure, and finger oxygen saturation [SO2]) with retinal oximetry measures (arteriolar SO2, venular SO2, and the arterio-venous [A-V] difference).

RESULTS. Of the candidate factors assessed, only age and finger SO2 were found to be significantly associated with one or more measures of retinal oximetry in unadjusted analyses. After adjusting for age, sex, and significant factors from unadjusted analyses, age and finger SO2 values remained significant. Age was associated with retinal arteriolar and venular SO2 values (per year increase in age, β = 0.31, 95% confidence interval [CI]: 0.15–0.48 and β = 0.26, 95% CI: 0.08–0.43, respectively), but not associated with the A-V difference. Finger SO2 values were associated with retinal arteriolar SO2 and A-V difference (per percentage change in finger SO2, β = 1.34, 95% CI: 0.40–2.28 and β = 0.74, 95% CI: 0.36–1.11, respectively), but not with venular SO2.

CONCLUSIONS. In healthy young adults, age was positively associated with the retinal arteriolar and venular SO2 values, whereas finger SO2 was positively correlated with greater arteriolar SO2 and A-V difference. Our findings serve as a basis for future studies assessing retinal oximetry values in young adults under normal and pathophysiological conditions.

Keywords: retinal arteriole, retinal venule, oxygen saturation, retinal oximetry

The recent advent of modern spectrophotometric retinal oximetry techniques has gained increasing interest. It is based on the fact that oxygenated and deoxygenated hemoglobin have different light absorption spectra, and by using these differences in absorption wavelengths, the oxygen saturation (SO2) in retinal blood vessels can be estimated noninvasively.1,2 Using these oximetry techniques, researchers have demonstrated changes in retinal SO2 with flicker light stimulation,3 changes in illumination,4 diabetes,5,6 and retinal vein occlusions.7,8 However, little is known about the variables that might influence oximetry values under normal physiological conditions.

Previous work has evaluated the effects of age, sex, and cardiovascular parameters (e.g., finger SO2 values and ocular perfusion pressure [OPP]) on retinal oximetry measures (i.e., arteriolar, venular, and the arterio-venous [A-V] difference in SO2).9 However, the associations of hemodynamic parameters (i.e., retinal blood flow) and serum hemoglobin and lipid measures (e.g., glycated hemoglobin [HbA1c], total cholesterol, and triglyceride levels) with these retinal oximetry values have not been investigated. Given that these factors have been implicated in the pathogenesis of retinal metabolic disorders, such as diabetic retinopathy,10,11 it is important to understand the correlation between these hemodynamic and serum parameters with retinal oximetry measures in healthy adults to enable an appropriate assessment of retinal vessel SO2 and better utilization of retinal oximeters in research and clinical practice.

In this study, we aimed to assess associations of age, sex, retinal capillary flow (RCF), HbA1c, triglyceride, total cholesterol, OPP, and finger pulse SO2 values with retinal arteriolar, venular, and the A-V difference in SO2 measured in a sample of healthy Caucasian adults, using the oximetry module of the Vesselmap System (Imedos US, Jena, Germany). Our results will serve to describe a more complete normative profile for retinal oximetry data in the Caucasian population.

METHODS

Study Population

Fifty healthy Caucasian individuals aged 18 years or older without self-reported medical history of any systemic diseases that might affect the retinal microvasculature (i.e., diabetes and hypertension) were recruited. This was further confirmed with information obtained from blood pressure measurements (≤140/80 mm Hg)12 and biochemistry analysis (e.g., HbA1c [≤6.5%]13 and random glucose [≤11.1 mM]).14 All participants had a best-corrected logarithm of minimum angle of resolution visual acuity of 0.00 (equivalent to 6/6 on the Snellen chart) or
better. Subjects were excluded if they had IOP higher than 21 mm Hg, cataracts or other media opacities, or any history or signs of retinal or optic nerve degeneration/disease. All subjects were also advised to refrain from consuming caffeinated products and alcohol for at least 12 hours before the study. The study was approved by the human ethics committee of the Royal Victorian Eye and Ear Hospital (Ethics Approval Number 11/103-1H) and abided by the tenets of the Declaration of Helsinki.

Blood Biochemistry Measures

Nonfasting blood samples were collected for analysis of blood glucose, HbA1c, and lipids (total, high-density lipoprotein cholesterol, LDL-cholesterol, and triglycerides). All blood analyses were performed at Melbourne Pathology, Melbourne, Australia, with individual results electronically delivered through a password-protected program. The laboratory is accredited to the International Standard ISO15189 (Medical Laboratories) and is certified by the National Association of Testing Authorities. The reference ranges for healthy subjects of the serum markers analyzed in our study were: 4.4% to 5.6% for HbA1c, lower than 1.5 mM for triglycerides, and 3.5 to 5.5 mM for total cholesterol.

Image Capture for Retinal Capillary Flow

Retinal capillary flow was captured using the Heidelberg Retinal Flowmeter (HRF; Heidelberg Engineering, Inc., Heidelberg, Germany). Briefly, the examination was performed in a sitting position at room temperature with diffuse natural light on undilated pupils. An optic disc–centered scan was obtained, together with the regions within 1.5 disc diameters to either side of the optic disc margin. A total area of 2.56 × 0.64 mm (horizontal and vertical orientation, respectively) was scanned within 2 seconds at a resolution of 256 points × 64 lines × 128 times with the default 780-nm wavelength laser head installed in the HRF camera. During the data acquisition, the participant was asked to fixate on an adjustable mounted artificial light field (of the right eye) were then taken using a fundus camera together with the regions within 1.5 disc diameters to either side of the optic disc margin. A total area of 2.56 × 0.64 mm (horizontal and vertical orientation, respectively) was scanned within 2 seconds at a resolution of 256 points × 64 lines × 128 times with the default 780-nm wavelength laser head installed in the HRF camera. The magnification and flash settings were not changed between images, and that the background illumination remained constant.

Assessment of Retinal Vessel SO₂ and A-V Difference

Retinal vessel oxygenation measurements in both retinal arteries and veins were estimated using the Oximetry module of the Vesselmap System. In brief, two monochromatic fundus images were obtained using a double-band pass filter (light transmission at 548 ± 10 nm and 610 ± 10 nm) inserted in the illumination path of a fundus camera FF450 (Carl Zeiss). Only one observer (REKM) was assigned to take the images. The ratio of the optical densities at 610 nm to that at the isosbestic wavelength 548 nm (known as the optical density ratio) is proportional to the vessel hemoglobin SO₂ after compensation for vessel diameter and fundus pigmentation, as retinal oximetry values were inversely associated with vessel diameter and fundus pigmentation.

For each image, a peripapillary annulus (specifically designed by Imedos UG to be adjustable to account for different optic disc sizes as per our request) was used to mark an area in the image for analysis (Fig. 2). Within this area (inner radius of 1.0 and outer radius of 1.5 disc diameters), the O₂ saturation was measured for all arterioles and venules. For each vessel, approximately 10 to 20 SO₂ values, depending on the length of the vessel within the measurement area, were calculated and averaged by the algorithm. Any SO₂ values that were more than 20% over the mean SO₂ value of that vessel were excluded to eliminate the confounding effects of specular reflection.

Typically, about 20 measurements consisting of approximately 8 to 10 arterioles and 8 to 10 venules that were above 50 μm in diameter were averaged to give the mean arteriolar and venular SO₂ for each eye. This vessel diameter threshold was chosen as it was observed that the algorithm of the Vesselmap System had difficulty tracing and measuring the O₂ saturation of vessels smaller than 50 μm in diameter without repeated efforts by the observer to mark the edges of the vessel walls accurately. The A-V difference was then calculated as the difference between the arteriolar and venular SO₂ values. A reliability and reproducibility study we did on the same sample using the above technique produced extremely good intraobserver reliability (i.e., the ability to obtain consistent oximetry values when analyzing the same image twice by the same observer), intraobserver reproducibility (i.e., the ability to obtain consistent values from images of the same subject taken at different times by the same observer), and interobserver reproducibility (i.e., the ability to obtain consistent oximetry values when analyzing the same image was undertaken by different observers) in all three measures of retinal oximetry.
Assessment of Other Key Covariables

Standardized questionnaires were used to assess basic demographic details (age, sex), history of ocular and systemic conditions, and medication use. Systolic blood pressure and diastolic blood pressure measurements were taken using the Omron Automatic Blood Pressure Monitor (Model IAIB; Omron Healthcare Co., Kyoto, Japan). IOP was assessed using Goldmann applanation tonometry. OPP was then derived from the formula
\[ OPP = \frac{2}{3}(\text{mean arterial pressure}) - \text{IOP} \]
where mean arterial pressure
\[ \text{MAP} = \frac{2}{3}\text{diastolic blood pressure} + \frac{1}{3}\text{systolic blood pressure} \].

Finger SO\(_2\) values (measure of finger arteriolar SO\(_2\)) were obtained using the Rossmax Pulse Oximeter, Model SB100. Key covariables were age, sex, finger pulse SO\(_2\), and OPP. Two-field fundus images of the right eye were also taken using the Canon CR-6–45NM camera (Canon, Inc., Tokyo, Japan). The images were graded at the Centre for Eye Research Australia, and eyes with any degenerative changes other than tessellated fundi or slight crescents at the optic disc were excluded from the study.

Statistical Analysis

Analyses were performed using Intercooled STATA version 12.1 for Windows (Stata Corp., College Station, TX). First, we tested for differences and correlations in the outcome variables (arteriolar SO\(_2\), venular SO\(_2\), and the A-V difference) between the left and right eyes using the Wilcoxon signed-rank test/paired t-test where appropriate, as well as Pearson’s correlation coefficient. Eye-specific data (arteriolar, venular, and the A-V difference in SO\(_2\), RCF, OPP) from both eyes of each subject and person-specific data (age, sex, HbA1c levels, triglyceride levels, total cholesterol levels, OPP, and finger SO\(_2\)) with the outcome variables while controlling for the correlations between two eyes. We undertook the analyses using the inverse Gaussian function, which was the closest fit to the highly skewed data (arteriolar and venular SO\(_2\))\(^{19}\). Multivariable-adjusted models, including age, sex, and significant variables in unadjusted analyses, were then used to assess the associations between candidate variables and the SO\(_2\) values of arterioles, venules, and the A-V difference.

RESULTS

Data from 100 eyes of 50 subjects were included in the analysis. The median age (interquartile range [IQR]) was 26 (23–31) years and 28 (56%) were female. The median (IQR) for arteriolar and venular SO\(_2\) of all 100 eyes were 96.02% (90.62%–98.57%) and 61.99% (56.68%–64.08%), respectively. The mean (SD) for the A-V difference was 33.83% (3.36%). Table 1 summarizes the demographic and clinical parameters of the sample.

Comparison of Oximetry Measures Between Eyes

All oximetry readings were not significantly different, and were highly correlated, between the left and right eyes. The median (IQR) of the arteriolar SO\(_2\) values was 95.94% (91.53%–98.49%) for the right eye, and 96.54% (89.55%–98.91%) for the left eye (correlation coefficient: 0.83, \(P < 0.001\)). The median (IQR) of the venular SO\(_2\) values was 62.35% (57.65%–64.17%) for the right eye, and 62.28% (56.60%–64.05%) for the left eye (correlation coefficient: 0.77, \(P < 0.001\)). Finally, the mean (SD) of the A-V difference was 33.79% (3.37%) for the right eye, and 33.57% (3.45%) for the left eye (correlation coefficient: 0.78, \(P < 0.001\)) (Table 2).
Table 1. Demographic and Clinical Parameters of Participants (n = 100 Eyes)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean (SD) or Median (IQR)</th>
<th>Range of Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>26 (23–31)</td>
<td>18–58</td>
</tr>
<tr>
<td>Female, %</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Arteriolar SO2, %</td>
<td>96.02 (90.62–98.57)</td>
<td>77.03–100.82</td>
</tr>
<tr>
<td>Venular SO2, %</td>
<td>61.99 (56.68–64.08)</td>
<td>41.15–71.65</td>
</tr>
<tr>
<td>A-V difference, %</td>
<td>33.83 (3.36)</td>
<td>24.13–42.47</td>
</tr>
<tr>
<td>Blood flow, arbitrary units</td>
<td>247.66 (39.50)</td>
<td>126.22–345.92</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.24 (0.31)</td>
<td>4.50–5.80</td>
</tr>
<tr>
<td>Triglycerides, mM</td>
<td>1.00 (0.80–1.30)</td>
<td>0.40–3.50</td>
</tr>
<tr>
<td>Total cholesterol, mM</td>
<td>4.94 (1.02)</td>
<td>2.60–8.00</td>
</tr>
<tr>
<td>Finger SO2, %</td>
<td>98 (97–99)</td>
<td>92–99</td>
</tr>
<tr>
<td>OPP, mm Hg†</td>
<td>45.48 (6.30)</td>
<td>32.00–60.33</td>
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* Glycerated hemoglobin.
† Formula = 1/3(mean arterial pressure) + IOP, where mean arterial pressure = 1/3 diastolic blood pressure + 1/3 systolic blood pressure.

Table 2. Comparison and Correlation of Retinal Arteriolar, Venular, and A-V SO2 Values Between the Right and Left Eyes (n = 50 Subjects)

<table>
<thead>
<tr>
<th>Outcome Parameters</th>
<th>Medain (IQR) or Mean (SD)</th>
<th>P Value</th>
<th>Correlation Coefficient</th>
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<tbody>
<tr>
<td>Arteriolar SO2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Right eye, %</td>
<td>95.94 (91.53–98.49)</td>
<td>&lt;0.001</td>
<td>0.83</td>
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<tr>
<td>Left eye, %</td>
<td>96.54 (89.55–98.91)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venular SO2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right eye, %</td>
<td>62.35 (57.65–64.17)</td>
<td>&lt;0.001</td>
<td>0.77</td>
</tr>
<tr>
<td>Left eye, %</td>
<td>62.28 (56.60–64.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-V Difference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right eye, %</td>
<td>33.79 (3.37)</td>
<td>&lt;0.001</td>
<td>0.78</td>
</tr>
<tr>
<td>Left eye, %</td>
<td>33.57 (3.45)</td>
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**Associations Between Age and Gender With Retinal Oximetry Measures**

Unadjusted analyses revealed that increasing age was associated with greater arteriolar and venular SO2 (per year increase in age, β = 0.33, P < 0.001, and β = 0.25, P = 0.003, for arteriolar and venular SO2 respectively, Table 3), but was not associated with the A-V difference. Scatter plots of age versus arteriolar and venular SO2 confirmed a linear pattern of the associations, although most study subjects were aged between 18 and 40 years (Figures 3 and 4). These associations persisted after additional adjustment of other cofactors (per year increase in age, β = 0.31, P < 0.001; and β = 0.26, P = 0.002, for arteriolar and venular SO2 respectively, Table 4).

**Associations Between RCF and Serum Markers With Retinal Oximetry Measures**

Neither RCF, nor any of the serum markers, were associated with any of the three retinal oximetry measures in unadjusted analyses (Table 3). Additional adjustments for age, sex, and significant factors found in unadjusted analyses confirmed that no associations exist between RCF and serum markers with the SO2 measurements of arterioles, venules, and the A-V difference (Table 4).

**Associations Between Cardiovascular Parameters With Retinal Oximetry Measures**

Finger SO2 values were found to be associated with a greater arteriolar SO2 and the A-V difference (per percentage change in finger SO2, β = 1.42, P = 0.02, and β = 0.80, P < 0.001, for arteriolar SO2 and the A-V difference respectively, Table 3), but not with the venular SO2. These associations remained after multivariable adjustments (per percentage change in finger SO2, β = 1.34, P = 0.005 for arteriolar SO2, and β = 0.74, P < 0.001, for the A-V difference, respectively, Table 4). No significant association was found between OPP and arteriolar SO2, venular SO2, and the A-V difference.

**DISCUSSION**

In this study, we demonstrated that in healthy young adults, increasing age was significantly associated with increased arteriolar and venular SO2 values but not the A-V difference, whereas increasing finger SO2 values were significantly associated with increased arteriolar SO2 and the A-V difference, but not venular SO2 values. Importantly, we established that there were no associations between retinal capillary flow, OPP, and any of the serum markers (HbA1c, total cholesterol, triglycerides), with the SO2 values of arterioles, venules, and the A-V difference in this sample.

One only previous study has evaluated the effect of age on retinal oximetry values. Geirsdottir and colleagues found that increasing age did not affect retinal arteriolar SO2 values, but was associated with decreasing venular SO2 values, as well as an increased A-V difference. The difference in sample population between our sample and that of Geirsdottir’s group may be a major factor that could have contributed to the differences in association between age and retinal oximetry values. First, Geirsdottir’s group did not exclude subjects with systemic hypertension (albeit they were well controlled), which may have influenced their results due to hypertension-related capillary nonperfusion and sheathing of vessel walls. More importantly, our sample consisted predominantly of younger subjects (90% of subjects < 45 years), whereas Geirsdottir and colleagues’ sample had a much larger age range (18–80 years). Therefore, the difference in results may have arisen from the effects of cataract and other media opacities that may have been present in participants of the latter study, as well as the possibility that age affects retinal oximetry differently after the age of 40 years.

A distinction also should be made between the SO2 assessed from retinal oximeters with the actual SO2 in retinal vessels, especially with regard to age. The actual SO2 may differ from measured SO2 values, with increasing age as a result of influence from several factors, such as age-related lenticular and vascular changes. Hence, caution needs to be taken when extrapolating the results of age-related SO2 changes from our study sample to older subjects (aged > 40 years). Consequently, further investigations into the effects of age on retinal SO2 values in the older population are needed.

Geirsdottir and colleagues further demonstrated that the OPP was associated with a higher arteriolar SO2 value, with a similar trend seen in venules. They also established that the finger SO2 values were not associated with any of the oximetry values. This was in contrast to our study, where we discovered that OPP was not correlated with any of the SO2 values, whereas the finger SO2 values were strongly associated with the arteriolar SO2 and A-V difference values, but not the venular SO2. Given the tight autoregulation of retinal metabolism, small changes within the range of OPP values should not have influenced O2 metabolism, which is consistent with our results. The positive correlation between finger SO2 values and the retinal arteriolar and A-V difference in SO2 in our study is also plausible, as finger SO2 is essentially a
measurement of SO$_2$ in the finger arterioles, and the SO$_2$ value of arteriolar blood before reaching the small vessels (i.e., capillaries) should be consistent across all end organs of the body, barring the presence of local vascular abnormalities. Our results, therefore, suggest a close correlation between local (retinal) SO$_2$ and that of the systemic circulation. The difference in results between our study and the study by Geirsdottir and colleagues could have arisen because of the differences in study sample: subjects with systemic hypertension were included in the study by Geirsdottir and colleagues, whereas they were excluded in our study. The vascular changes inherent to hypertension (such as sheathing of vessel walls, and capillary nonperfusion) might have confounded the SO$_2$ measurements.

We also found that the RCF was not associated with any of the retinal oximetry values (arteriolar, venular, and the A-V difference in SO$_2$). It has been proposed that blood flow and O$_2$ metabolism are closely related, as demonstrated by Riva and colleagues, who quantified the relationship between O$_2$ tension and retinal arterial and venular blood flow in porcine models. Even though the RCF is not a direct measurement of blood flow in the arterioles and venules, it should still be highly correlated with these two parameters. The nonassociation of RCF with the retinal oximetry values could be due to several possible reasons. First, this could be due to lack of statistical power as evident from the large variation in RCF readings. However, given that the $\beta$ regression coefficient of the association between RCF and all three retinal oximetry measures are very small ($\beta$ for all three associations $\leq -0.01$), even if these results were statistically significant, it would not be clinically meaningful. Second, it has been proposed that the coupling between blood flow and O$_2$ metabolism is nonlinear (i.e., it takes large changes in blood flow to bring about small changes in O$_2$ metabolism). Third, the algorithm in the oximeter analysis software corrects for vessel size when calculating SO$_2$ values, resulting in measurements that are independent of vessel diameter. However, the Hagen-Poiseuille law states that blood flow is directly proportional to the diameter (to the fourth power) of the vessel. By correcting the influence of vessel diameter on retinal SO$_2$ during analysis,
the algorithm might have invariably weakened any associations between blood flow and $SO_2$ values. Fourth, there could be possible influence from the choriocapillaries as demonstrated by Strenn et al.25 However, Strenn and colleagues25 assessed capillary flow near the macular, where there are very few retinal vessels due to proximity to the foveal avascular zone, which may allow the scanning laser from the HRF to reflect moving red blood cells in the underlying choroidal blood vessels instead. In contrast, we assessed RCF in the areas adjacent to the optic disc, where most retinal capillaries are located. Therefore, light scattering and resultant influence from the choriocapillaries is likely to be minimal in our study.

No data are currently available on the relationship between serum markers, such as HbA1c, triglycerides, and total cholesterol levels, and retinal $SO_2$ values. This is important, as these markers are risk factors in retinal metabolic disorders (e.g., increased HbA1c10 and dyslipidemia11 in diabetic retinopathy). In our study, we found no associations between these serum markers and the arteriolar, venular, or A-V difference in $SO_2$ values. These results are novel and demonstrate that the retinal oximetry values obtained with this retinal oximeter are not influenced by normal physiological variations in serum markers.

Strengths of this study include quantitative measures of RCF and one researcher (REKM) performing all fundus photography, as well as all RCF and oximetry imaging measures. Limitations include a small sample size, as well as the relatively young age group of this study sample, which might have limited generalization of our findings to samples of other age ranges. In addition, 20% of our sample population had total cholesterol and triglyceride levels exceeding the “healthy” reference range provided by the laboratory. Consequently, we performed additional analyses between triglyceride and total cholesterol with the three measures of retinal oximetry after excluding subjects who had triglyceride levels and/or total cholesterol levels exceeding the healthy range.
cholesterol levels exceeding these reference ranges (>1.5 mM for triglycerides and >5.5 mM for total cholesterol), and found that the results did not alter (see Table 5).

In conclusion, our study provides novel data on factors associated with retinal oximetry values under normal physiological conditions in healthy young adults. We have demonstrated that age was positively correlated with retinal arteriolar and venular SO2 values, whereas increased finger SO2 values were strongly associated with greater arteriolar SO2 and the A-V difference in SO2. Our results may inform future studies evaluating the associations of these factors with retinal oximetry values in older age groups, as well as in retinal metabolic dysfunction.

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References


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<tr>
<th>Study Factors</th>
<th>Arteriolar SO2 $\beta$ (95% CI)</th>
<th>Venular SO2 $\beta$ (95% CI)</th>
<th>A-V Difference $\beta$ (95% CI)</th>
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<tbody>
<tr>
<td>Triglycerides, mM</td>
<td>3.08 (–2.09–8.26)</td>
<td>4.05 (–0.91–9.02)</td>
<td>0.02 (–2.73–2.79)</td>
</tr>
<tr>
<td>Total cholesterol, mM</td>
<td>1.24 (–0.93–3.41)</td>
<td>0.42 (–2.26–3.10)</td>
<td>0.79 (–1.11–2.69)</td>
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</tbody>
</table>

* Model includes age, sex, and finger SO2.
† Model includes age and sex.