Purpose. To compare the results from manifest refraction using trial lenses and a standard visual acuity protocol to results from autorefraction for obtaining refractive error and best corrected visual acuity in patients enrolled in a randomized clinical trial.

Methods. During a 4-month period, 29 patients with subfoveal choroidal neovascularization (CNV), who were enrolled in the Submacular Surgery Trials (SSTs) Pilot Study at the Wilmer Ophthalmological Institute, gave verbal consent to participate in this study. Best corrected visual acuity was obtained using Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity charts and standardized room lighting after performance of manifest refraction, according to the SST protocol, and autorefraction. Refractive error (spherical equivalent) and visual acuity scores were obtained in both eyes of all patients.

Results. On average, manifest refraction gave a spherical equivalent that was 1.04 D more plus than autorefration (95% limits of agreement = 0.74, 1.34). On average, the visual acuity score was 1.5 letters better after manifest refraction than after autorefraction (95% limits of agreement = 0, 3.0). The comparison of the two methods of refraction was subdivided according to visual acuity level and eye disease (age-related macular degeneration or ocular histoplasmosis syndrome).

Conclusions. Despite large differences in spherical equivalent between manifest refraction and autorefraction, the visual acuity scores were close (mean difference, 1.5 letters). Other studies comparing subjective refraction and autorefration have shown similar results. Autorefration in patients with subfoveal CNV may be a satisfactory alternative to manifest refraction in clinical trials and field studies in which best corrected visual acuity is of interest. (Invest Ophthalmol Vis Sci. 2001;42:447–452)

Many ophthalmic clinical trials use best corrected visual acuity as the primary outcome in the investigation of treatment efficacy.1-6 Methods used to obtain best corrected visual acuity are either primarily subjective, such as manifest refraction with trial lenses or a phoropter, or objective, such as retinoscopy or autorefration.7 In this article we report a comparison of manifest refraction with trial lenses, in accordance with a standard protocol, with autorefration for obtaining best corrected visual acuity for patients enrolled in a randomized clinical trial.

The two refraction methods require different levels of examiner education, training, and time to perform each procedure. Manifest refraction requires a basic understanding of ophthalmic optics. Typically, months of practical experience are needed for the clinician to perform manifest refraction satisfactorily and reproducibly. In clinical trials, manifest refraction typically is performed by following a prescribed sequence of steps outlined in a study protocol or manual of procedures. This technique must be practiced on many patients with varying levels of visual acuity and types of refractive error before it is mastered.1,9 In contrast, autorefration does not require knowledge of ophthalmic optics or practical experience in refraction. It requires only a basic understanding of how to operate the autorefractor, which can be learned from reading the instruction manual that comes with the autorefractor and from minimal practice with patients.8,9

Obtaining an objective refraction in a patient with reduced vision due to refractive error usually takes only approximately 5 minutes per eye, whereas subjective refraction in the same patient using the phoropter or trial frames usually requires approximately 15 minutes.10 In the experience of two authors (PRO, NMB), refraction in a patient, with reduced vision and inability to fixate centrally because of macular disease, performed with the autorefractor or manifest refraction often requires more than 15 minutes. The difference in time to perform both refraction techniques, whether in patients with good or poor vision, becomes significant when large numbers of patients are screened for inclusion in a study.

As an example, the Submacular Surgery Trials (SSTs), a set of multicenter, randomized clinical trials, were designed to evaluate the role of submacular surgery for treatment of subfoveal choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD), ocular histoplasmosis syndrome (OHS), and idiopathic causes. Patients with AMD eligible for this trial must have visual acuity of 20/100 or worse in the study eye, and patients with CNV due to OHS or idiopathic causes must have visual acuity of worse than 20/50 or worse in the study eye.1

Change in best corrected visual acuity is the primary outcome variable of the SST. Therefore, all SST vision examiners are trained and certified in manifest refraction and visual acuity testing as described in the SST Manual of Procedures. In addition, periodic observation of the vision examiners at each participating clinical center is necessary to ensure that all are performing in accordance with the protocol.1

Training and monitoring of vision examiners in large multicenter studies is both time consuming and costly. In addition, manifest refraction and visual acuity measurement in patients who have visual acuity of 20/100 or worse with eccentric fixation and poor central vision are very time consuming for the vision examiner. Autorefration was suggested as a possible solution to both problems.

This study was designed to compare manifest refraction and autorefration within a subset of patients participating in the SST Pilot Study to determine whether the refractive errors and visual acuity measurements from the two methods were similar, to estimate the actual savings in time with autorefration, and to identify situations in which one method was preferable.

From 1The Johns Hopkins University School of Medicine, and 3Wilmer Clinical Trials and Biometry, The Johns Hopkins University, Baltimore, Maryland; and the 2Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, New York.

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One of the goals of the Pilot Study was to evaluate the feasibility of various methods, such as autorefration, under consideration for the full-scale SST.

**Materials and Methods**

**Subjects**

During a 4-month period, from July through October 1995, all SST Pilot Study patients who were seen at the SST clinical center at the Wilmer Ophthalmological Institute for follow-up examinations were invited to participate in a comparison of the two refraction methods. Written informed consent, approved by the Johns Hopkins Joint Committee on Clinical Investigation (JCCI) and in accordance with the Declaration of Helsinki agreement for research involving human subjects, was obtained from all patients when they enrolled in the SST. The JCCI approved the use of verbal consent to obtain autorefration measurements in a subset of SST participants at Johns Hopkins.

All refractions and vision measurements were performed by one SST-certified vision examiner (PRO). A typical examination involved manifest refraction followed by visual acuity measurement and measurement of other aspects of vision with both the refraction and acuity testing performed according to the SST protocol. Refraction and visual acuity data then were recorded on a SST visual acuity data form. Patients who agreed to autorefration in addition to routine manifest refraction had visual acuity remeasured after autorefration, again according to SST protocol. The data were recorded on separate copies of the SST data form without reference to the original measurement. Visual acuity testing for all patients was performed in an SST vision of the SST data form without reference to the original measurement. According to SST protocol. The data were recorded on separate copies of the SST data form with visual acuity remeasured after autorefration, again according to SST protocol. The data were recorded on separate copies of the SST data form.

**Vision with Best Correction (Refraction Distance)**

<table>
<thead>
<tr>
<th>Sphere Power (a)</th>
<th>Increment</th>
<th>Cylinder Axis (b)</th>
<th>Power (c)</th>
<th>Increment</th>
<th>Sphere Refinement Power (d)</th>
<th>Increment</th>
</tr>
</thead>
<tbody>
<tr>
<td>20/20–20/80 (2 m)</td>
<td>+0.50</td>
<td>+0.50</td>
<td>0.50*</td>
<td>+0.25</td>
<td>+0.37</td>
<td>+0.25</td>
</tr>
<tr>
<td></td>
<td>−0.37</td>
<td>−0.25</td>
<td>0.25*</td>
<td>−0.25</td>
<td>−0.37</td>
<td>−0.25</td>
</tr>
<tr>
<td></td>
<td>+0.50</td>
<td>+0.50</td>
<td></td>
<td></td>
<td>+0.37</td>
<td>+0.25</td>
</tr>
<tr>
<td>20/200–20/320 (2 m)</td>
<td>+2.00</td>
<td>+2.00</td>
<td>1.00*</td>
<td>+1.00</td>
<td>+1.00</td>
<td>+1.00</td>
</tr>
<tr>
<td></td>
<td>−2.00</td>
<td>−2.00</td>
<td>1.00*</td>
<td>−1.00</td>
<td>−1.00</td>
<td>−1.00</td>
</tr>
<tr>
<td></td>
<td>+2.00</td>
<td>+2.00</td>
<td></td>
<td></td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>20/320 (0.5 m)</td>
<td>+2.00</td>
<td>+2.00</td>
<td></td>
<td></td>
<td>No cylinder power or axis adjustment</td>
<td>No refinement</td>
</tr>
</tbody>
</table>

Based on four levels of best corrected visual acuity. Sequence of refraction steps: (a) through (d).

1. Jackson Cross Cylinder.

The overall strategy of the SST refraction protocol was to “push” plus power and add minus power only when objective improvement was demonstrated. In this way, the highest plus and lowest minus prescription would be obtained so that accommodation was minimized.1

In accordance with the SST protocol, best corrected visual acuity was measured, beginning at 2.0 m. The Lighthouse Distance Visual Acuity Chart 1 was used to measure vision for the right eye, and Chart 2, for the left eye. The lens correction obtained from the SST protocol refraction for each eye was placed in the trial frames. The patient was seated at a distance of 2.0 m from the chart, regardless of whether the refraction was performed at 0.5 m, and instructed to read the letters from left to right, beginning with the top line of the chart and continuing to the smallest line. With the fellow eye well occluded with the black occluder lens and tissue placed behind the trial frames, the patient was encouraged to use eccentric fixation and to guess.

When the patient read at least 15 letters correctly at 2 m, 30 was added to the total number of letters read at 2 m to determine the visual acuity score for that eye. Patients who read fewer than 15 letters on the chart at 2 m were positioned at 0.5 m from the chart. A −0.75 sphere was added to the refraction obtained at 2 m. Again, the patient was asked to read the letters on the top line and to proceed to the smallest line of letters. The visual acuity score was then calculated as the total number of letters read at 0.5 m.

If the patient could not read any letters at 0.5 m, the visual acuity score was recorded as 0, and the patient was checked for light-perception vision using the indirect ophthalmoscope according to the SST protocol.1

After manifest refraction and best corrected visual acuity measurement, the patient was escorted by the same examiner who performed manifest refraction to another examination room to have autorefration performed on both eyes. Because of its convenience and availability, the AR-1600G Auto Refractometer (Marco, Sunnyvale, CA) was used. The autorefractor was checked at the beginning of the study and continuing to the smallest line of letters. The visual acuity score was then calculated as the total number of letters read at 0.5 m.

If the patient could not read any letters at 0.5 m, the visual acuity score was recorded as 0, and the patient was checked for light-perception vision using the indirect ophthalmoscope according to the SST protocol.1

After manifest refraction and best corrected visual acuity measurement, the patient was escorted by the same examiner who performed manifest refraction to another examination room to have autorefration performed on both eyes. Because of its convenience and availability, the AR-1600G Auto Refractometer (Marco, Sunnyvale, CA) was used. The autorefractor was checked at the beginning of the study and before each use to confirm that it was operating properly.

Brief instructions were given to the patient while he or she was seated at the autorefractor. The patient was asked to focus on the internal target. In cases in which the patient could not fixate centrally because of macular scarring or other lesions in the visual axis, the patient was instructed to look straight ahead toward the sound of the examiner’s voice.

Three independent measurements were taken for the right eye first. An average of the three readings for each eye was automatically calculated by the autorefractor at the end of the third reading.

**Table 1. SST Refraction Protocol Summary**

<table>
<thead>
<tr>
<th>Distance</th>
<th>Power (a)</th>
<th>Increment</th>
<th>Axis (b)</th>
<th>Power (c)</th>
<th>Increment</th>
<th>Power (d)</th>
<th>Increment</th>
</tr>
</thead>
<tbody>
<tr>
<td>20/20–20/80</td>
<td>+0.50</td>
<td>+0.50</td>
<td>0.50*</td>
<td>+0.25</td>
<td>+0.37</td>
<td>+0.25</td>
<td></td>
</tr>
<tr>
<td>&lt;20/80–20/160</td>
<td>+1.00</td>
<td>+1.00</td>
<td>1.00*</td>
<td>+1.00</td>
<td>+1.00</td>
<td>+1.00</td>
<td></td>
</tr>
<tr>
<td>20/200–20/320</td>
<td>+2.00</td>
<td>+2.00</td>
<td>1.00*</td>
<td>+1.00</td>
<td>+1.00</td>
<td>+1.00</td>
<td></td>
</tr>
<tr>
<td>&lt;20/320</td>
<td>+2.00</td>
<td>+2.00</td>
<td>No cylinder power or axis adjustment</td>
<td>No refinement</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Jackson Cross Cylinder.
After autorefraction, the patient returned to the SST visual acuity examination room. The lens correction obtained from the average reading for each eye was placed in the trial frames, and the left eye was well occluded with both the occluder lens and tissue behind the trial frames. In the manner described for manifest refraction, the patient was seated at 2 m from the Lighthouse Distance Visual Acuity Chart. To minimize possible memorization of letters, Chart 2 was used to record visual acuity for the right eye and Chart 1 for the left eye.

Data Analysis
To perform a comparison between manifest refraction and autorefraction, the statistical method of Bland and Altman was used.12,13 To explore the relationship between measurement error and the true value (estimated by the mean), the difference between measurements taken with the two refraction methods was plotted against the average measurement taken with the two methods. This type of plot allows for the display of both the size of the measurement difference and the distribution of the differences around 0. If the plot shows no apparent pattern in the scatter of the points around 0, then the bias, or non-agreement may be estimated by the mean difference. Then, 95% of the differences are expected to lie within 2 SDs of the mean difference. This interval represents the 95% limits of agreement between the two methods. To test whether the mean difference was significantly greater than 0, a one-sample t test of the difference against 0 was performed.

For each method of refraction, the spherical equivalent of the refractive correction was calculated by adding one half the cylindrical correction to the spherical correction. The bias, or mean difference, between the two methods of refraction was estimated by subtracting the measurement obtained from autorefraction from that obtained from manifest refraction. Because the difference between the two measurements of visual acuity of one eye was not correlated with the difference between the measurements of the fellow eye of the same individual (Pearson correlation coefficient = 0.007), all eyes were combined for this analysis.

A previously published study of replicate refractions and visual acuity measurements in patients with CNV from AMD, OHS, or idiopathic causes demonstrated that the reliability of such measurements may be dependent on visual acuity level and disease process. The study reported greater differences between measurements for patients with visual acuity worse than 20/100 and for patients with AMD.14 Because SST patients are similar in disease process and range of visual acuity, the comparison of manifest and autorefraction methods was stratified by study visual acuity measurement (with manifest refraction), as well as by disease process. Subgroups of measurements by visual acuity were defined by study visual acuity of 20/100 or better (good vision), acuity between 20/100 and 20/320 (moderate vision), and acuity of 20/320 or worse (poor vision).

RESULTS
From July through October 1995, 29 SST patients participated in this study, for a total of 31 examinations on 62 eyes. (Two
patients were examined twice during this period.) Of the 62 eyes tested, 5 eyes with reduced vision (from three patients) could not fixate for autorefraction; thus, 57 pairs of refraction and visual acuity measurements from 27 patients were analyzed to compare manifest refraction and autorefraction. The 27 patients (including 10 men) ranged in age from 28 to 83 years, with visual acuities ranging from 20/20 to 20/1600. Seventeen patients had AMD, and 10 had OHS.

The median spherical equivalent score obtained by manifest refraction was 0.5 D, with scores ranging from −2.75 to 8.375 D. The median spherical equivalent score obtained by autorefraction was −0.5 D, with scores ranging from −5.625 to 3.625 D. A scatterplot of autorefracted versus manifest refracted spherical equivalent scores is shown in Figure 1B. Observations tended to fall near or below the line of equality, indicating similar corrections from both methods or a more positive correction from the manifest refraction, respectively.

Figure 1B is a plot of the difference between the spherical equivalent scores obtained by the two methods versus the mean of the two scores. The points are similarly distributed above and below the mean, and there is no apparent pattern to indicate that the size of the differences is related to the mean. Most of the differences are above 0, indicating a more positive correction from manifest refraction. Table 2 displays the mean difference, or bias, and the 95% limits of agreement for this bias between refraction methods. On average, manifest refraction gave a spherical equivalent that was 1.04 D more positive than autorefraction (95% limits of agreement = −1.19, +3.24). This difference was significantly different from 0 (P < 0.001). Less than half (45.6%) of the spherical equivalent measurement pairs differed by 1 D or less. Only 31.5% of the measurement pairs differed by 0.5 D or less.

The median visual acuity score obtained by manifest refraction was 50 letters (Snellen equivalent 20/200), with scores ranging from 4 to 100 letters (20/1600–20/20). The median visual acuity score obtained by autorefraction was 49 letters (20/200), with scores ranging from 10 to 100 letters (20/1280–20/20). Figure 2A is a scatterplot of the visual acuity scores obtained by autorefraction plotted against those obtained by manifest refraction. The points generally cluster around the line of equality. Figure 2B displays the difference versus the mean for visual acuity measurements. The points are reasonably scattered around both the mean and 0, unlike spherical equivalent. On average, the visual acuity score was 1.5 letters better after manifest refraction than after autorefraction (95% limits of agreement, −9.7, 12.7; Table 2). This bias estimate of 1.5 letters was not significantly different from 0. Most pairs of measurements (82%) were within five letters of each other, equivalent to one line on the ETDRS (Early Treatment Diabetic Retinopathy Study) Visual Acuity Chart.

The mean difference in visual acuity was larger in patients with AMD, whereas the mean difference in visual acuity score was smaller in those with OHS.

### Discussion

Despite the relatively large differences in spherical equivalent between the two refraction methods, visual acuity measurements were very close across a large range of visual acuities (i.e., a mean difference of only 1.5 letters). Other studies have shown similar results. A study of the repeatability of ocular component measurements of the right eyes of 40 patients aged 20 to 43 years compared cycloplegic autorefraction, using the B-1 autorefractor (Canon, Lake Success, NY), with cycloplegic subjective refraction and reported a 95% range of comparison of spherical equivalent between the two methods to be ±1.10.15 The population-based Beaver Dam Eye Study used the 550 autorefractor (Humphrey, San Leandro, CA) to obtain best corrected visual acuity in 4926 participants aged 43 to 83 years. The study reported that visual acuity scores obtained with correction given by the Humphrey autorefractor were highly correlated with scores obtained using the ETDRS protocol and

### Table 2.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean ± SD</th>
<th>95% Limits of Agreement†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherical equivalent (manifest − autorefraction)</td>
<td>+1.04 ± 1.10 D</td>
<td>−1.19, +3.24 D</td>
</tr>
<tr>
<td>Visual acuity (manifest − autorefraction)</td>
<td>1.5 ± 5.6 Letters</td>
<td>−9.7, 12.7 Letters</td>
</tr>
</tbody>
</table>

n = 57 pairs of measurements.
* Mean, bias displayed in the difference versus mean plots.
† Calculated as mean ± 2 SDs.
‡ One-sample t-test; P < 0.05.

### Table 3.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Visual Acuity</th>
<th>Mean ± SD</th>
<th>95% Limits of Agreement‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherical equivalent (manifest − autorefraction)</td>
<td>Good</td>
<td>+0.63 ± 0.68 D</td>
<td>−0.73, +1.99 D</td>
</tr>
<tr>
<td>Moderate</td>
<td>+0.91 ± 1.21 D</td>
<td>−1.51, +3.33 D</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>+1.68 ± 1.08 D</td>
<td>−0.48, +3.84 D</td>
<td></td>
</tr>
<tr>
<td>Visual acuity (manifest − autorefraction)</td>
<td>Good</td>
<td>4.1 ± 6.8 Letters</td>
<td>−9.5, 17.7 Letters</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.9 ± 4.2 Letters</td>
<td>−7.5, 9.3 Letters</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>−0.4 ± 5.1 Letters</td>
<td>−10.6, 9.8 Letters</td>
<td></td>
</tr>
</tbody>
</table>

Data defined as in Table 2. n = 57 pairs of measurements.
* One-sample t-test; P < 0.05.
logMAR charts, although they were consistently higher by an average of 4.2 letters.10

The issue of repeatability of results has not been addressed in this study because replicate measurements for each method were not taken. Bland and Altman have noted that agreement between two methods of measurement may be limited by the repeatability of the methods, where good repeatability is essential to good agreement.12-15 Studies reported by Rosenfeld and Chiu17 and Goss and Grosvenor18 have shown that replicate measurements taken for subjective and objective measurements of refraction did not reveal significant variability. The Beaver Dam Eye Study design included five examiners, among whom no significant variation was found in either refractive error or visual acuity measurements.17

Blackhurst et al.14 performed a reproducibility study of replicate visual acuity measurements after performing manifest refraction in 164 eyes in 82 patients and reported an intraclass correlation coefficient of 0.99 spherical equivalent and 0.99 for visual acuity score, despite the finding that visual acuity and disease process may affect the reliability of refraction and visual acuity measurements. Patients included in this reproducibility study are similar to SST patients in visual acuity, disease, and age. Our study confirms that the agreement between methods of measurement varies according to visual acuity level and disease. There was greater bias between the methods in the measurement of spherical equivalent for patients with poor vision and for patients with AMD. For visual acuity measurement, the method bias was greatest for patients with AMD. For visual acuity measurement, the method bias was greatest for patients with good vision and for patients with OHS. This is consistent with the findings stated by Blackhurst et al. that the diffuse disease process of AMD may affect visual acuity level and/or measurement reliability.

Another analytical limitation of this study is that neither method of refraction is considered the gold standard. It should be noted that the SST refraction protocol is a modified and attenuated method of full subjective refraction that was developed specifically for refraction in patients with low vision. It has been time tested in previous multicenter trials, particularly the Macular Photocoagulation Studies, and it has been adopted by other prominent ophthalmic clinical trials—for example, the Treatment of AMD with Photodynamic Therapy trial and the Verteporfin in Photodynamic Therapy trial. Our results reflect a comparison of one particular autorefractor with a specified method of manifest refraction; thus, extrapolation of the results to a full subjective refraction or to other autorefractor warrants further study.

A design-related potential weakness of this study is the possible bias on the data obtained by the vision examiner who performed all refraction and vision measurements. The vision examiner (PRO) is a certified SST vision examiner with approximately 23 years of refraction experience. However, there was no conscious bias on the part of the vision examiner toward patient selection or either type of refraction method. All SST patients seen during the study period were invited to participate in this study, regardless of visual acuity. In addition, maximal effort to achieve the best results for both refraction methods and vision measurements was given in an attempt to produce unbiased data. Manifest refraction and vision measurement, in accordance with the SST protocol, were always performed first to avoid possible compromise of SST vision data due to the patient’s fatigue.

A report of the Beaver Dam Eye Study attributed its successful recruitment for such a large study to reasonable participatory time demands, minimized by the use of the Humphrey refractor which required an average of 5 minutes per subject compared with the 15 minutes required for manifest refraction defined by ETDRS standard protocol.10 The present investigation supports this rationale. Except in cases in which the patient had extreme difficulty in fixating centrally, the entire process of autorefraction of both eyes averaged 10 minutes in our study. Therefore, the autorefractor can reduce staff time required to obtain best corrections.

Autorefraction may be a satisfactory alternative to manifest refraction in clinical trials and field studies for which measurement of best corrected visual acuity is of interest, at least for patients who undergo successful autorefraction. In our study of patients with subfoveal CNV, approximately 10% of the patients with impaired central vision were not able to fixate for autorefraction. Therefore, such patients would require manifest refraction. A similar, but presumably less frequent, problem has been reported in large population-based studies.16,17

Based on these findings, a more rigorous comparative study of these two methods may be warranted to define more precisely the benefits and limitations of autorefraction in multicenter studies of retinal and macular diseases.

References


<table>
<thead>
<tr>
<th>Measurement</th>
<th>Disease</th>
<th>Mean ± SD</th>
<th>95% Limits of Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherical equivalent</td>
<td>AMD</td>
<td>+1.19 ± 1.24 D*</td>
<td>−1.29, 3.67 D</td>
</tr>
<tr>
<td></td>
<td>OHS</td>
<td>+0.79 ± 0.77 D*</td>
<td>−0.75, 2.33 D</td>
</tr>
<tr>
<td>Visual acuity</td>
<td>AMD</td>
<td>0.66 ± 4.89 Letters</td>
<td>−9.12, 10.44 Letters</td>
</tr>
<tr>
<td></td>
<td>OHS</td>
<td>2.86 ± 6.40 Letters</td>
<td>−9.94, 15.66 Letters</td>
</tr>
</tbody>
</table>

Data defined as in Table 2. n = 57 pairs of measurements.

* One-sample t-test; P < 0.05.


