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Assessment of the peripheral ventilatory response to CO_2 in heart failure patients: reliability of the single-breath test

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Abstract
The assessment of chemoreflex sensitivity in heart failure patients is gaining increasing interest since recent studies demonstrated that augmented chemosensitivity is an independent predictor of mortality and represents an important pathogenic factor in the development of Cheyne–Stokes respiration. The single-breath CO_2 test is a well-established method to quantify peripheral hypercapnic chemoreflex sensitivity. As the original criteria for the computation of the chemoreflex sensitivity in healthy subjects need to be modified in heart failure patients to take into account impaired cardiac function, the effects of such modifications on measurement reliability deserve investigation. Hence, we devised this study to assess the reliability of the single-breath CO_2 test in heart failure patients. In 27 clinically stable, mild-to-moderate heart failure patients (age (mean $\pm$ SD): 64 ± 10 years, left ventricular ejection fraction: $34 \pm 7\%$, NYHA class: 2.7 ± 0.4), the test was administered on two consecutive days in the same conditions. Reliability was assessed by the standard error of measurement (SEM) and by the intraclass correlation coefficient (ICC). The mean value of the chemoreflex sensitivity on the two days was: 0.25 ± 0.12 and 0.24 ± 0.12 l min^{-1} mmHg^{-1} ($p = 0.45$), respectively. The SEM was 0.05 l min^{-1} mmHg^{-1}, indicating large intra-subject variability. Consequently, in order to be 95% confident that a real change has occurred between two measurements taken on the same individual (test–retest), the observed difference must be higher than ± 0.15 l min^{-1} mmHg^{-1}, which is about 60% of the mean value across our population. The ICC was 0.71, indicating that

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intra-subject variability, although high, is a limited (29%) portion of inter-subject variability. Intra-subject variability should be carefully taken into account when using the single-breath CO_2 test in assessing changes in individual patients. The observed ICC indicates that this test may provide useful information for diagnostic/classification purposes.

Keywords: chemoreflex sensitivity, single-breath CO_2 test, reliability, reproducibility

Introduction

Increased sensitivity of chemoreceptors to both hypoxia and hypercapnia has been shown to be a powerful and independent predictor of mortality in heart failure patients (Giannoni *et al* 2009, Ponikowski *et al* 2001). Moreover, in heart failure patients with Cheyne–Stokes respiration—a frequent comorbidity characterized by waxing and waning of ventilation with hyperpneic phases alternating with apneas or hypopneas—both peripheral and central chemoreflex sensitivity are elevated as compared to patients without Cheyne–Stokes respiration and healthy controls (Solin *et al* 2000, Ponikowski *et al* 1999), supporting the notion that enhanced ventilatory response to chemoreceptor stimulation represents an important pathogenic factor in the development of the cyclic breathing pattern.

A simple and well-established method to quantify peripheral hypercapnic chemoreflex sensitivity is the single-breath CO_2 test, which is based on the assessment of the ventilatory response to a single breath of a gas mixture containing a high concentration of CO_2 (Solin *et al* 2000, McClean *et al* 1988, Chua *et al* 1996). Although the reliability of this test has been assessed in normal subjects (McClean *et al* 1988, Chua and Coats 1995), published results cannot be generalized to patients with heart failure, as the criteria for the computation of the ventilatory response (McClean *et al* 1988) need to be changed to take into account the much longer circulatory delay of the patients (Solin *et al* 2000, Hall *et al* 1996). It is also likely that the hypercapnic stimulus travelling from the lungs to carotid chemoreceptors is subject to a much higher distortion in these patients than in normal subjects, due to greater mixing effects in the enlarged left atrial and ventricular chambers and augmented mitral regurgitation (Khoo *et al* 1982, Javaheri *et al* 2007). This distortion may blunt the ventilatory response (Fitzgerald and Lahiri 1986) and, possibly, increase its variability.

Therefore, the aim of this study was to assess the reliability of the single-breath CO_2 test in heart failure patients, using the state-of-the-art methodology to estimate both absolute and relative reliability.

Methods

Study population

The subjects for the study were recruited among stable, optimally treated, mild-to-moderate heart failure patients referred to our Heart Failure Unit for periodic follow-up evaluation. Exclusion criteria were: chronic obstructive or other pulmonary disease, peripheral or central nervous system disorders and cognitive impairment.

The study was approved by the local Ethical Committee and all subjects gave their written informed consent before participation.

Single-breath CO_2 test

The test was implemented as originally proposed by McClean and colleagues (1988), with modifications for heart failure patients to take into account the increased circulatory delay (Solin *et al* 2000). A detailed description of the custom-made complete system can be found elsewhere (Maestri *et al* 2013).

All tests were administered between 10:00 and 12:00 AM in a quiet laboratory at a comfortable temperature. Before the study, all subjects fasted for more than 2 h and refrained from coffee or tea for 12 h.

The CO_2 and O_2 partial pressures at the mouth and lung volume were continuously monitored during the test using a device for pulmonary function testing (Quark PFT, Cosmed, Pavona di Albano, Rome, Italy) equipped with a multi-use pneumotach (Lilly-based technology), paramagnetic O_2 analyser and digital infrared CO_2 analyser. Breath-by-breath end tidal CO_2 ($PetCO_2$), respiratory rate and minute ventilation were computed and displayed in real time. After stable ventilation was achieved, a valve was automatically switched during the expiratory phase to let the subject take a single breath of a gas mixture containing 13% CO_2, 21% O_2 and balance N_2 from a non-diffusing gas bag.

A transient increase in ventilation was expected in a response interval between 13 and 33 s after gas inhalation (Solin *et al* 2000). The increase in $PetCO_2$ (i.e. the stimulus for chemoreceptors) was computed taking the difference between the $PetCO_2$ at the CO_2-enriched breath and the average value of $PetCO_2$ over five breaths preceding CO_2 inhalation (McClean *et al* 1988). The response to the stimulus was computed as the difference between the maximum value of minute ventilation in the response interval and its average value over the five breaths preceding CO_2 inhalation. The ratio between the response and the stimulus was taken as the estimated ventilatory response for that test (McClean *et al* 1988). A test was deemed to be properly carried out if: (1) minute ventilation was stable in the breaths preceding CO_2 inhalation ($\pm$20% of median value), (2) the volume of inspired CO_2 enriched mixture was close to the values of pre-bolus inspired volume ($\pm$20% of median value) and (3) end-expiratory CO_2 after bolus showed a clear plateau.

The test was repeated after a $\geqslant$2 min interval, provided ventilation returned to baseline values. Each single test was analysed after its completion and, if not properly carried out, excluded from further analysis. Measurements were stopped after 6–8 successful repetitions of the test.

Negative values of the response were considered artifactual and excluded from further analysis as well as values classified as outliers according to the Tukey (1977) and the median absolute deviation statistic (MAD) (Wilcox 2001) criteria. Fulfilment of one of these criteria was sufficient for exclusion. This choice of joint use of both criteria is a conservative one, which takes into account that both outlier detection methods have strengths and weaknesses.

The ventilatory response for the examined patient was finally computed as the average response of all accepted tests.

An identical session was repeated on the next day at the same time of the day.

Within $\pm$1 day from chemoreflex sensitivity tests, all patients underwent the assessment of respiratory function and arterial blood gases.

Statistical analysis

To visually explore the test-retest data, we plotted the difference between the two measurements as a function of their average (Bland–Altman plots) (Bland and Altman 1999). These plots allow us to detect possible systematic changes between the two tests and to explore the

relationship between the random component of the measurement and the magnitude of the characteristic being measured.

This qualitative investigation was followed by a formal verification of the assumptions underlying the assessment of reliability. Specifically, we tested the hypothesis of normality (Shapiro–Wilk test for normality) and zero mean of the difference between the two tests (two-sided paired *t*-test at the 0.05 significance level); we also verified the homoscedasticity assumption (i.e. the random component of the measurement has a fixed standard deviation) by regressing the absolute differences between the two measurements against their average (Atkinson and Nevill 1998, Bland and Altman 1999).

To assess absolute reliability, we computed the SEM (standard error of measurement), which estimates the magnitude of intra-subject variability. This was obtained as the square root of the within-subject mean square (WMS) from the ANOVA table (Fleiss 1986, Dunn 1992). From the SEM, we obtained the 95% limits of random variation between the two measurements. These limits express the range of values within which 95% of the differences between two measurements are expected to lie due to pure random variation, and therefore provide a picture of measurement repeatability within individuals.

For the sake of comparison with results from other studies, we also computed the coefficient of variation (CV) as the mean value across individual CV (Atkinson and Nevill 1998).

Relative reliability was assessed by the intraclass correlation coefficient (ICC), computed from the mean square value of the ANOVA table (Fleiss 1986, Dunn 1992). Ninety-five per cent confidence intervals for ICC were also calculated (Shrout and Fleiss 1979). The ICC expresses the proportion of measurement variability of a given characteristic in a given population that is due to between-subject variation in the 'true value' of the characteristic, the remaining part of variability being due to random intra-subject variability. Hence, in the context of the ICC, a measurement is reliable if it mostly reflects the true value relative to random variability. The ICC ranges from 0 to 1: the higher the value, the lower the intra-subject variability relative to inter-subject variability. Values greater than 0.8 are usually considered as indicating good to excellent reliability, while values between 0.6 and 0.8 represent substantial reliability (Donner and Eliasziw 1987).

All analyses were carried out using the SAS/STAT statistical package, release 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Out of the 30 patients enrolled in the study, 1 was unable to complete the test due to difficulties with the mouthpiece and 2 were lost at the second test due to organizational reasons. This led to a final sample of 27 heart failure patients.

Demographic and clinical characteristics of the study population are reported in table 1. All patients were in NYHA (New York Heart Association) functional class II or III. Reported values confirm that studied patients have normal pulmonary function and normal arterial blood gases parameters.

A representative example of the signals recorded during a single-breath CO_2 test is given in figure 1. Figure 2 shows the scatterplot of all repetitions of the test in a typical session. The range for outliers detection according to both the Tukey (dashed lines) and MAD (dash-dotted lines) criteria are shown. In this example one of the tests (marked by an asterisk) was an outlier and accordingly was excluded from the final average (solid line).

Figure 3 shows the Bland–Altman plots for chemoreflex sensitivity measurements in all studied patients. No relationship can be observed between the random component of the

Table 1. Patients' demographic and clinical characteristics.

Gender (male/female)	26/1
Age (years)	64 ± 10
NYHA class	2.7 ± 0.4
BMI (kg/m^2)	27 ± 5
Heart rate (bpm)	67 ± 11
SBP (mmHg)	109 ± 15
DBP (mmHg)	70 ± 9
LVEF (%)	34 ± 7
LVEDD (mm)	64 ± 18
LVESD (mm)	52 ± 18
BUN (mg/dl)	70 ± 47
Sodium (mEq/l)	137 ± 3
Creatinine (mg/dl)	1.47 ± 0.56
VC% pred (%)	98.03 ± 15.6
FEV1% pred (%)	90.12 ± 13.97
FEV1/VC (%)	74.26 ± 11.15
FRC% pred (%)	98.57 ± 20.86
RV% pred (%)	95.16 ± 21.24
pH	7.44 ± 0.03
$PaCO_2$ (mmHg)	33.83 ± 3.51
PaO_2 (mmHg)	87.0 ± 10.9
SaO_2 (%)	96.7 ± 1.6

NYHA: New York Heart Association; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LVEF: left ventricular ejection fraction; LVEDD: left ventricular end diastolic diameter; LVESD: left ventricular end systolic diameter; BUN: blood urea nitrogen; VC% pred: per cent predicted vital capacity; FEV1% pred: per cent predicted forced expiratory volume in 1 s; FEV1/VC: FEV1/VC ratio; FRC% pred: per cent predicted functional residual capacity; RV% pred: per cent predicted residual volume; $PaCO_2$: arterial partial pressure of CO_2; PaO_2: arterial partial pressure of O_2; SaO_2: oxyhaemoglobin saturation.

Table 2. Descriptive and agreement statistics for the CO_2 single-breath test.

Response day 1 (l min^{-1} mmHg^{-1})	Response day 2 (l min^{-1} mmHg^{-1})	Difference (l min^{-1} mmHg^{-1})	SEM (l min^{-1} mmHg^{-1})	95%LoV (l min^{-1} mmHg^{-1})	CV (%)	ICC (95% CI)
0.25 ± 0.12	0.24 ± 0.12	−0.01 ± 0.08	0.05	−0.15–0.15	20.3 ± 15.2	0.71 (0.44,0.86)

Descriptive statistics are reported as mean ± SD.

measurement and the magnitude of the chemoreflex sensitivity ($r = 0.13$, $p = 0.22$). This statistical property is referred to as homoscedasticity. It can be seen that the limits of random variation (dashed lines) describe quite well the dynamics of random changes between first and second day measurements. It also appears that there was no systematic change (i.e. bias) between the two tests ($t = 0.76$, $p = 0.45$).

Descriptive and agreement statistics for the measurements of peripheral chemoreflex sensitivity on the two consecutive days are reported in table 2.

The individual coefficients of variation obtained from the two tests showed a mean value of 20.3% and ranged between 0% and 47%. Since CV is a measure of variability relative to the magnitude of the mean, the highest values were observed at low mean values of the single-breath CO_2 response.

The ICC was 0.71 (95%CI: 0.44–0.86), indicating substantial relative reliability.

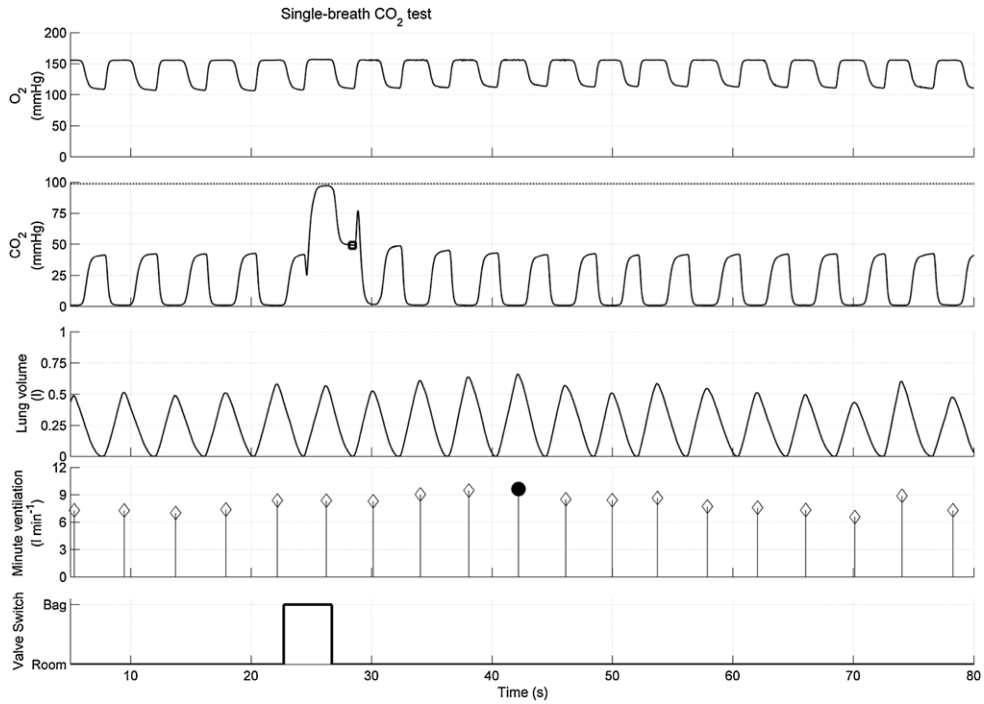

Figure 1. Signals recorded during a typical single-breath CO_2 test. From top to bottom: O_2 partial pressure, CO_2 partial pressure, lung volume, breath-by-breath minute ventilation and valve switch. Switching from room air to the bag containing the gas mixture is done during the expiratory phase and lasts a single inspiratory cycle. During inspiration, the CO_2 signal reaches the value of 99 mmHg (13%), the value of the gas mixture in the bag, and corresponding $PetCO_2$ (square marker) (50 mmHg in this example) is increased with respect to baseline value (mean value over five breaths preceding CO_2 inhalation: 41 mmHg). The reflex change in ventilation is estimated by the difference between the maximum value of minute ventilation in the 13–33 s response interval (9.8 l min^{-1} in this example) and the average value of ventilation over the five breaths preceding CO_2 inhalation (7.3 l min^{-1}). The ratio between the change in ventilation and the change in $PetCO_2$ gives the estimate of the ventilatory response.

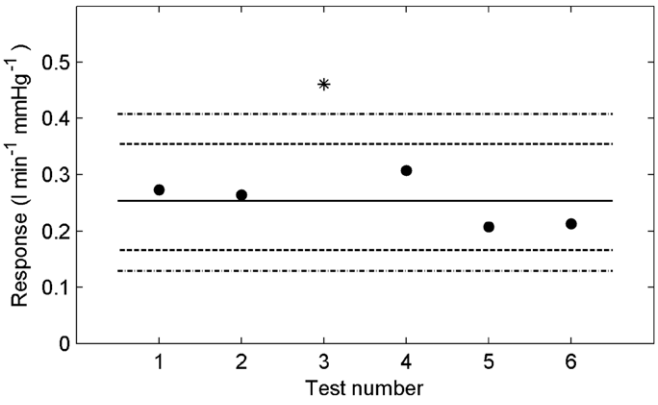

Figure 2. Scatterplot of six repetitions of the single-breath CO_2 test in a typical session. The dashed lines represent the range for outliers detection according to the Tukey criterion, the dash–dotted lines represent the range for outliers detection according to the MAD criterion and the solid line represents the average value. Tests number 3 (asterisk) was considered an outlier and excluded from the final average.

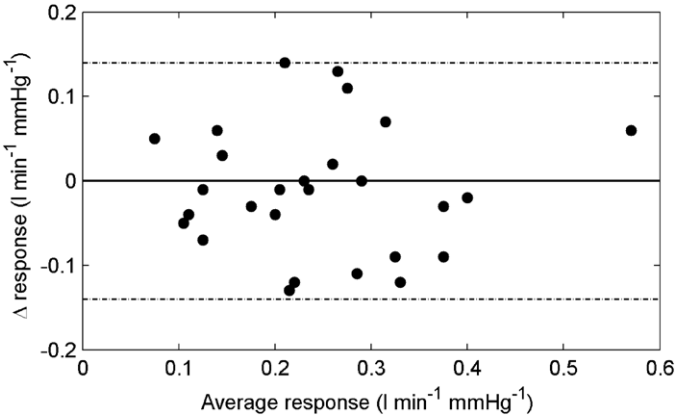

Figure 3. Bland–Altman plot of the difference between the two chemoreflex sensitivity measurements (day 2–day 1) against their average. The scatter of points is symmetrical around the zero line, indicating the absence of systematic change (i.e. bias) between the two tests. The magnitude of the scatter around the same line is pretty homogeneous, indicating homoscedasticity.

Discussion

This is the first study providing a thorough investigation of the reliability of peripheral hypercapnic chemoreflex sensitivity as assessed by the single-breath CO_2 test in heart failure patients. The assessment of chemoreflex sensitivity in these patients is likely to gain increasing interest in the light of recent studies showing that augmented sensitivity of chemoreceptors to both hypoxia and hypercapnia is a powerful and independent predictor of mortality (Giannoni *et al* 2009, Ponikowski *et al* 2001) and that enhanced ventilatory response to chemoreceptor stimulation represents an important pathogenic factor in the development of Cheyne–Stokes respiration (Solin *et al* 2000). Chemoreflex overactivation has also been indicated as a potential specific therapeutic target in heart failure, to be treated by novel pharmacological, device-based, or behavioural approaches (Giannoni *et al* 2009).

Absolute and relative reliability of the single-breath CO_2 test

Both absolute and relative reliability were considered in this investigation. The former refers to the degree of random variability of the measurements in any given subject (i.e. intra-subject variability), and therefore is relevant to the assessment of individual changes in test-retest experiments (e.g., before and after a treatment). We found that the single-breath CO_2 test is characterized by large day-to-day random variations. Indeed, the 95% limits of random variation indicate that increases or decreases as great as 0.15 l min^{-1} $mmHg^{-1}$ may occur between measurements taken on two consecutive days. This value, which is about 60% of the mean value across our population, should be carefully taken into account if one wants to use this test in assessing changes in individual patients.

Relative reliability assesses a different aspect of reliability, indicating how much of the variation of a measurement among patients is explained by actual differences in the true value of the characteristic being measured. We found that the ICC was 0.71; this implies that 71% of measurement variation was due to variability in the true value of the measured characteristic and 29% to random intra-subject variability. Since an ICC value of 0.7 is commonly considered acceptable for clinical purposes, the single-breath CO_2 test may provide useful information for diagnostic/classification purposes in heart failure patients.

We are aware of only one published study reporting day-to-day reproducibility data for the single-breath CO_2 test (Chua and Coats 1995). In this paper, based on a sample of seven healthy subjects, Chua and colleagues reported an average coefficient of variation of 17.7% (range 3.9–56%). Even though the use of a ratio statistics as coefficient of variation is questionable to analyse homoschedastic data (Atkinson and Nevill 1998), we also computed this index for comparison purposes and found similar results (mean value: 20.3%, range 0–47%). Instead of computing the ICC, Chua and coworkers computed the correlation coefficient—which, in the context of test-retest studies, is almost equivalent (Dunn 1989)—and found 0.84, which is higher than our result. Besides a marked dissimilarity in sample size, this difference can be attributed to the different populations studied since it is likely that measurements in heart failure patients may be characterized by higher variability, as the breathing pattern is typically more irregular than in healthy subjects.

It is noteworthy that the values of the ventilatory response observed in our population of heart failure patients are similar to those reported by Solin and colleagues for heart failure patients not suffering from sleep disordered breathing or suffering from obstructive sleep apnea, and are lower than those they observed in patients with Cheyne–Stokes respiration (Solin *et al* 2000). This might in principle limit the generalizability of our results. However, the homoscedasticity of our data suggests that the measured reliability indexes can likely be extended also to patients with higher chemosensitivity.

Factors affecting reliability of the single-breath CO_2 test

There are several uncontrollable factors which can significantly affect the estimates of peripheral chemoreflex gain derived from the single-breath CO_2 test (McClean *et al* 1988, Khoo 1990). The most important confounding factor is the natural variability in breathing, which can greatly complicate the interpretation of the test. Indeed, in our study, we often found that the increase in ventilation attributed to the reflex response to chemoreceptor stimulation was not larger than the range of spontaneous variability in the phase preceding the inhalation of the gas mixture. Another complicating factor is the variability of the arrival time of the stimulus at peripheral chemoreceptors relative to the current phase of respiration. This is important since, if the peak increase in $PaCO_2$ occurs during the expiratory phase, it will not be as effective as if it had occurred during inspiration, leading to an underestimated measurement (Fitzgerald and Lahiri 1986). Finally, the gas mixture used for the test has a distinctive taste which may potentially produce artifactual changes in ventilation in some patients.

To take into account all these confounding factors and improve the reliability of the test, we used novel analysis procedures to assure high quality data and reduce subjective judgment in the evaluation of the test. Moreover, we excluded outlier measurements using robust statistical criteria (Wilcox 2001, Dunn 1992).

An improvement of the reliability of the single-breath CO_2 test might be obtained increasing the number of replicates to be averaged. However, in our experience we observed that exceeding eight replications, besides becoming excessively time consuming, is not feasible in most heart failure patients who do not tolerate long experimental sessions. Another way to improve reliability might be to make the stimulus stronger, in such a way to increase the signal to noise ratio. Unfortunately, increasing the concentration of CO_2 beyond 13% makes almost impossible to prevent the subject knowing that the gas mixture has been inhaled and increases the likelihood of coughing as a reflex to the irritating effect of CO_2. A further option might be administering the gas mixture for two or three breaths (Leigh 1972). However, this test assumes that the ventilatory response to the transient elevation of $PaCO_2$ mediated by the

fast peripheral chemoreceptors can be disentangled from the response mediated by the much slower central chemoreceptors. Using more than one breath would increase the duration of the $PaCO_2$ elevation, with a potential overlapping of the peripheral and central chemoreceptor contribution, and a reduction of the specificity of the test.

Conclusions

In conclusion, being simple and safe, the single-breath CO_2 test is a very appealing method to quantify peripheral hypercapnic chemoreflex sensitivity. We found that it is subjected to large day-to-day random variations which should be carefully taken into account when using this test in assessing changes in individual patients. However, random variations represent a limited portion of measurement variability; therefore, observed values reflect mostly differences in the subjects' true value rather than random error, suggesting that the reliability of this test is acceptable to detect relevant differences between individuals.

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