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Impact of data averaging strategies on $\dot{V}O_{2max}$ assessment: mathematical modelling and reliability

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ABSTRACT

Background: No consensus exists on how to average data to optimise $\dot{V}O_{2max}$ assessment. Although the $\dot{V}O_{2max}$ value is reduced with larger averaging blocks, no mathematical procedure is available to account for the effect of the length of the averaging block on $\dot{V}O_{2max.}$

Aims: To determine the effect that the number of breaths or seconds included in the averaging block has on the $\dot{V}O_{2max}$ value and its reproducibility and to develop correction equations to standardise $\dot{V}O_{2max}$ values obtained with different averaging strategies.

Methods: Eighty-four subjects performed duplicate incremental tests to exhaustion (IE) in the cycle ergometer and/or treadmill using two metabolic carts (Vyntus and Vmax N29). Rolling breath-averages and fixed time-averages were calculated from breath-by-breath data from 6 to 60 breaths or seconds.

Results: $\dot{V}O_{2max}$ decayed from 6 to 60-breaths averages by 10% in low fit ($\dot{V}O_{2max}$<40 mL·kg^{-1}·min^{-1}) and 6.7% in trained subjects. The $\dot{V}O_{2max}$ averaged from a similar number of breaths or seconds were highly concordant (CCC>0.97). There was a linear-log relationship between the number of breaths or seconds in the averaging block and $\dot{V}O_{2max}$ (R^2>0.99, P<0.001), and specific equations were developed to standardise $\dot{V}O_{2max}$ values to a fixed number of breaths or seconds. Reproducibility was higher in trained than low-fit subjects and not influenced by the averaging strategy, exercise mode, RR_{max} or IE protocol.

Conclusions: The $\dot{V}O_{2max}$ decreases following a linear-log function with the number of breaths or seconds included in the averaging block and can be corrected with specific equations as those developed here.

Keywords: *maximal oxygen uptake, reproducibility, breath-by-breath, metabolic cart, endurance training, aerobic performance*

INTRODUCTION

The maximal oxygen uptake ($\dot{V}O_{2max}$) is the highest flow of oxygen (O_2) that can be used by the tissues and is measured to assess cardiorespiratory and aerobic fitness.[1-3] Since the improvement in $\dot{V}O_{2max}$ achieved by short (i.e. weeks) and long-term (i.e. months/years) endurance training is only 10-30% [4], an accurate and reproducible assessment is mandatory to properly determine the effects of any intervention on this variable. During the last 50 years, the automated gas analysis systems have improved their precision and accuracy markedly. The development of faster computers and gas sensors, almost matching mass spectrometers, based the transition from mixing-chamber sampling to breath-by-breath analysis.[5,6] Breath-by-breath (BxB) analysis determines the flow of O_2 and carbon dioxide (CO_2) in each breath by measuring continuously inspiratory and expiratory flows in parallel with the concentrations of O_2 and CO_2.[7] Given the inherent variability in breath-by-breath data, it is puzzling that there is no universal consensus on how to average BxB data [6] to optimise the assessment of $\dot{V}O_{2max}$. Few studies have examined the impact that different averaging strategies may have on the validity [8-14] and reproducibility [10,12] of $\dot{V}O_{2max}$. However, no single study has tried to model mathematically the influence of the averaging strategy on the value of $\dot{V}O_2$ imputed as $\dot{V}O_{2max}$. This is further complicated by the effect that different exercise protocols and exercise modes may have on the highest $\dot{V}O_2$ value attainable and the time during which $\dot{V}O_{2max}$ can be maintained.

Several averaging strategies are used to calculate $\dot{V}O_{2max}$ from time intervals or number of breaths. Breath-averaging strategies can be applied by calculating a "rolling average" (also named "smoothing," "moving" or "running" average) [6,15] or on consecutive fixed (also named "stationary") time intervals, with the former being the most extensively used [6]. The rolling breath-average is obtained by continually removing the first breath value of the previous block and adding the first breath of the next block to calculate a new average, always with the same number of breaths.

The "data processing unit" in pulmonary gas exchange is the single breath, which reaches very high frequencies in the latest stages of incremental exercise to exhaustion (IE). The maximal respiratory rate (RR_{max}) during IE varies widely across populations and can be modified by several factors such as exercise training, ageing, and diseases.[16-20] The majority of the studies assessing $\dot{V}O_{2max}$ have used time-based sampling intervals, disregarding the influence that the number of breaths included in the averaging block might have on the reliability of the measure.[6,15,21]

Therefore, the main aim of this study was to assess the impact that the number of breaths or seconds included in the breath-averaging strategy has on the absolute value and the reproducibility of $\dot{V}O_{2max}$, accounting for, fitness, sex, metabolic cart used, exercise mode, RR_{max}, and IE protocol. Another aim was to determine which of the two strategies, breath- or time-averaging is more reliable and to develop correction equations that could be used to standardise $\dot{V}O_{2max}$ values obtained with different averaging strategies.

MATERIALS AND METHODS

Participants

Eighty-four volunteers from a wide range of ages, fitness and body compositions were included in the study for a total of 282 incremental tests to exhaustion (IE) from which 91 were tests carried out in duplicate (Table 1 and Fig. 1). Of the total number of volunteers, 62 were part of previous research projects and performed their tests on a Vmax N29 SensorMedics (Yorba Linda, California, USA) (hereafter so-called Vmax Group). The remaining 22 participants were specifically recruited for this study and were split into a group tested on a Vyntus CPX (Jaeger-CareFusion, Hoechberg, Germany) (so-called Vyntus Group), and a group that performed one test on a Vmax N29 and the replicate on a Vyntus CPX metabolic cart (Combined Group).

Before the start, all participants received full oral and written information about the potential risks and benefits of the study and gave written informed consent. The procedures for all tests included in this article were approved by the local ethical committee.

Procedures

All subjects underwent several visits to the laboratory to complete duplicate IE while continuously recording gas exchange using an automated metabolic cart. The same protocol was applied to individual participants for repeated tests. All protocols were designed to elicit exhaustion in no less than 6 min and no more than 16 min.[22] For familiarisation purposes, participants performed an IE on the cycle ergometer (Vmax Group and Combined Group) or the cycle ergometer and the treadmill, in the latter case separated by a 2-h recovery period (Vyntus Group). Subjects were instructed to take their last meal at least 4 h before the IE and to refrain from carbonated, caffeinated and alcohol-containing beverages. No intense physical activity was allowed during the 24 h-period preceding the tests. Repeated measurements were performed at a similar time of the day and subjects were requested to record the meal preceding the first test and reproduce the same meal for the subsequent tests.

Vmax Group

The 62 participants composing this group performed IE on the cycle ergometer and were assessed with the Vmax N29 metabolic cart (Fig. 1). Duplicate tests were performed with at least 48 h of rest in between. Three different IE protocols were used in this group with increments calculated to elicit a test duration between 6 and 16 min based on previous studies carried out in our laboratory. Based on these different protocols, three subgroups were analysed (Fig. 1). Forty-one subjects belonging to subgroups 1 and 2 participated in a 6-week high-intensity interval training (HIT) protocol aimed at increasing $\dot{V}O_{2max}$. [23] After the training period, a single $\dot{V}O_{2max}$

test was performed. This was followed by a 3-week detraining period [23] after which another single $\dot{V}O_{2max}$ test was performed.

Vyntus Group

The 11 participants composing this group were assessed with the Vyntus CPX metabolic cart (Fig. 1). Subjects in this subgroup performed one duplicate of IE in each visit (cycling and running visits), except for two subjects that only performed a duplicate on the cycle-ergometer. Each of the duplicate tests was carried out within the same day separated by a 2-h rest period, which is sufficient to elicit reproducible $\dot{V}O_{2max}$ values in subjects of a wide fitness range during cycling [24] and running [25].

Combined Group.

Eleven subjects (8 men and 3 women) underwent duplicate IE, each of them performed using a different metabolic cart (Vmax N29 and Vyntus CPX) in random order and separated by 2 h (Fig. 1).

The metabolic carts were calibrated before each test following manufacturer instructions with high-grade calibration gases (Carburos Metálicos, Las Palmas de Gran Canaria, Spain and CareFusion, Hoechberg, Germany for Vmax N29 and Vyntus CPX, respectively). All tests were performed using the breath-by-breath mode. Mask size was individually fitted before the first test, and the same size was maintained for subsequent tests.

Cycling tests were performed on a Lode cycle ergometer (Groningen, The Netherlands) while running tests were carried out on a motorised treadmill (PowerJog, EG30, Birmingham, UK). During the bicycling tests, participants maintained pedalling rates at 70 rpm (Vmax Group, subgroups 1 and 2) or 80 rpm (Vmax Group subgroup 3, Vyntus Group and Combined Group). Seat and bar adjustments were fit to the subject anthropometric characteristics and remained unchanged for subsequent days. Verbal encouragement was provided throughout the last phases of every IE.

Body composition

Body composition for Vmax Group and Vyntus Group subgroup 1 was determined by dual-energy x-ray absorptiometry (Lunar iDXA, General Electric, Wisconsin, USA) as described elsewhere.[26] For Vyntus Group subgroup 2 and Combined Group, body fat percentage was estimated by an anthropometric equation [27] from skinfold thickness (Holtain skinfold calliper, Holtain Ltd, Crosswell, UK).

Data processing for $\dot{V}O_{2max}$ determination and profiling of $\dot{V}O_2$ signal at the end of IE

Breath-by-breath data were recorded without the application of filters. Anomalous breaths (i.e. due to swallowing, talking, coughing or incomplete breaths with respiratory exchange ratio ((RER) < 0.65 or > 1.6) were manually identified and discarded.

The rolling breath-averaging strategy utilised was defined as the averaging of a fixed number of single-breath measurements (i.e., breath 1 to 6 for a 6 breath-average strategy: 6-b), then discarding the first breath and adding the subsequent breath to obtain a new breath-averaged block (i.e., breaths 2 to 7). The analysis was carried out using averaging blocks from 6 to 60 breaths (i.e. 6-b, 7-b, and so forth up to 60-b) computed from the start to the end of the IE. The calculated value for the breath-averaging block was assigned to the starting time of the breath-averaging block to maintain the length of the block until the end of the IE.

The stationary time-averaging data were calculated from breath-by-breath data being time-averaged from 6 to 60-s periods (i.e. 6-s, 7-s, and so forth up to 60-s) from the start to the end of the IE, with all breaths within the time block averaged and assigned to the starting time of the time-averaged block . The highest averaged value was reported as the $\dot{V}O_{2max}$ of the corresponding breath- or time- averaging strategy.

The $\dot{V}O_2$ near the end of the IE was classified as downward or plateau, linear and upward trajectories, as proposed by Poole and Jones [28], using 30-s time-averages of the $\dot{V}O_{2max}$ value and the two preceding data points.[13] First, the difference (in $mL \cdot min^{-1}$) between $\dot{V}O_{2max}$ and the first preceding data point (i.e. first difference, d_1) and the difference between this first preceding value and the previous one (i.e. second difference, d_2) was calculated. When d_1 was lower than 150 $ml \cdot min^{-1}$, the test was classified as plateau or downward trajectory. When the subtraction of these two differences (i.e. $d_2 - d_1$) in absolute value was lower than 150 $ml \cdot min^{-1}$, the test was classified as linear trajectory, and if higher or equal to 150 $ml \cdot min^{-1}$, upward trajectory.

Tests were considered as valid if at least two of the following secondary criteria were met: 1) volitional exhaustion and/or incapacity to maintain a pedalling rate of 50 rpm despite strong verbal encouragement; 2) heart rate within 10 $beats \cdot min^{-1}$ of the age-predicted maximal, and 3) respiratory exchange ratio (RER) ≥ 1.10; and otherwise the test was repeated. The procedure used to obtain the maximal $\dot{V}O_2$ value with the different breath-averaging strategies was also applied, regardless of simultaneous occurrence to $\dot{V}O_{2max}$, for the following variables: carbon dioxide production ($\dot{V}CO_{2max}$), respiratory exchange ratio (RER_{max}), pulmonary ventilation ($\dot{V}_{Emax}$), alveolar ventilation ($\dot{V}_{Amax}$), end-tidal O_2 pressure ($P_{ET}O_{2max}$), end-tidal CO_2 pressure ($P_{ET}CO_{2max}$), fraction of expired O_2 (F_EO_{2max}), fraction of expired CO_2 (F_ECO_{2max}), fraction of inspired O_2 (F_IO_{2max}), tidal volume (VT_{max}), respiratory rate (RR_{max}) and heart rate (HR_{max}).

Statistical Analysis

The reproducibility of $\dot{V}O_{2max}$ between each averaging method was reported as the within-subject coefficient of variation (CV) (average of individual CV calculated by dividing the within-subject standard deviation by the within-subject mean), the CV as proposed by Forkman (CV_f) [29] and its 95% confidence intervals, the standard deviation of the difference for repeated measures (SDd) and the concordance correlation coefficient (CCC) [30]. The agreement between averaging methods (breaths versus time) was tested by using the CCC, by calculating the bias in absolute values and the 95% limits of agreement (upper limit of agreement (ULA) = bias + 1.96 * SD; lower limit of agreement (LLA) = bias − 1.96 * SD).

A one-way analysis of variance (ANOVA) for repeated measures was used to examine the differences for duplicate measurements of $\dot{V}O_{2max}$ across the main averaging strategies (10-, 20-, 30-, 40-, 50- and 60- breaths and seconds). A similar procedure was applied for the related ergospirometric variables across the main breath-averaging strategies. The Mauchly's test of sphericity was run before the ANOVA and in the case of violation of the sphericity assumption, the degrees of freedom were adjusted according to the Greenhouse-Geisser test. When a significant main effect or interaction was observed, pairwise comparisons across the different averaging strategies were adjusted for multiple comparisons with the Holm–Bonferroni procedure.

Since the coefficient of variation does not follow a normal distribution [31], a non-parametric approach was utilised to study reproducibility (i.e. CV) between groups and across averaging strategies using the Friedman test for repeated measures. When a significant main effect or interaction was found, a Wilcoxon signed-rank or Mann-Whitney-Wilcoxon test was applied for pairwise comparisons across averaging strategies for dependent and independent samples, respectively. Significance was adjusted for multiple comparisons with the Holm–Bonferroni procedure.

The relationship between the decay in $\dot{V}O_{2max}$ in absolute values and the length of the averaging block from 6 to 60 breaths and 6 to 60-s was plotted, and a linear trend was observed after logarithmic transformation of the number of breaths and seconds. Prediction equations were developed using a random-effects linear-log regression model where a random intercept was the best fit of data for both breath- and time- averaging strategies. A Gaussian distribution was assumed for the intercept and experimental error. The model was estimated using the restricted maximum likelihood method. The goodness of fit was shown by the Nakagawa and Schielzeth´s

R^2_{GLMM} [32]. Prediction errors were assessed by subtracting the estimated values within the predictive range (6 to 60 breaths or seconds) for the corresponding two equations being compared and determining the mean, the standard deviation and the 95th percentile.

The percentage decay in $\dot{V}O_{2max}$ with the length of the averaging block from 6 to 60 breaths or seconds (i.e., from 6 to 7 breaths or seconds, 6 to 8 breaths or seconds… until 6 to 60 breaths or seconds) was calculated and fitted to the linear-log model to study differences between subgroups (i.e. fitness, sex, metabolic cart used, exercise mode, RR_{max} and IE protocol). Comparisons were carried out using a linear model with interaction between a factor (the two curves compared) and a numerical variable (a natural logarithm of the number of breaths minus 5).[33] For this purpose, the mean value of each duplicate $\dot{V}O_{2max}$ value was first calculated. The influence of RR_{max} was studied by comparing the subjects with the highest and lowest RR_{max} within each metabolic cart (n=10 and 9 subjects for Vmax and Vyntus groups, respectively). Likewise, the influence of metabolic cart was assessed by comparing the subjects with matched fitness (relative-to-weight $\dot{V}O_{2max}$) in both metabolic carts (n=9 for Vmax and Vyntus groups) and also by comparing the decay within the same subjects in both metabolic carts (n=11, Combined Group).

Values are reported as the mean ± standard deviation (SD). Level of significance was set at $P \leq 0.05$. Statistical analyses were completed using SPSS v.24.0 for Windows (SPSS Inc., Chicago, IL, USA) and R v.3.4.2 (The R Foundation for Statistical Computing).

RESULTS

Impact of the number of breaths and seconds included in the averaging block on the $\dot{V}O_{2max}$ value

The absolute $\dot{V}O_{2max}$ values for the different breath- and time-averaging strategies are presented in Table 2. The $\dot{V}O_{2max}$ was similarly reduced as the number of breaths included in the averaging block increased (Fig. 2a, b, c) with a similar response for the time-averaging strategies. This was paralleled by a similar response in the rest of the ergospirometric variables (Fig. 3). The absolute $\dot{V}O_{2max}$ values obtained from breath and time averages of an equal number of breaths and seconds (i.e. 10 breaths and 10 sec, 20 breaths and 20 sec…) presented high concordance as demonstrated by CCC values above 0.97 (Table 3 and Fig. 4). The limits of agreement between the two strategies show that the deviation can reach ~ 190 mL·min^{-1}, with a significant positive bias ($P < 0.001$) towards higher values using breath-averaging strategies.

Characterisation, and factors influencing the decay of $\dot{V}O_{2max}$ with the length of the averaging block

The decay in $\dot{V}O_{2max}$ with the increase of the number of breaths or seconds included in the averaging block followed a logarithmic trend, and a linear-log model was found as the best fit following logarithmic transformation of the number of breaths or seconds ($R^2 = 0.997$ and 0.990 for breaths and time, respectively, Fig. 5). Since this behaviour could be intrinsic to our particular data, we examined the goodness of fit of our model when applied to other studies [8,11,12,14,21,34] obtaining excellent results in all cases (Table 5).

The $\dot{V}O_2$-intensity relationship presented a downward or plateau trajectory before $\dot{V}O_{2max}$ in 73 (46%) out of 182 tests (re-testing of Vmax Group after training and detraining not included), a linear trajectory in 51 (32%), and an upward trajectory in 36 (22%).

To study the influence of fitness in the percentage decay of $\dot{V}O_{2max}$ with the number of breaths or seconds included in the averaging strategy, Vmax Group subjects were divided by $\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1}) into fitness subgroups (13-20, 21-30, 31-40 and 41-60 mL·kg^{-1}·min^{-1}) and integrated into the linear-log regression model. The $\dot{V}O_{2max}$ decay was slightly higher in the groups with $\dot{V}O_{2max}$ < 40 mL·kg^{-1}·min^{-1} (1.3 to 3.3 in percentage units, shortest and largest averaging strategy, respectively) than $\dot{V}O_{2max}$ > 40 mL·kg^{-1}·min^{-1} (P<0.001). The same analysis carried out using time-averaging strategies resulted in similar effects. The decay of $\dot{V}O_{2max}$ with the number of breaths or seconds included in the averaging strategy was not significantly altered by differences in sex, metabolic cart, RR_{max}, and IE protocol used. Nonetheless, the $\dot{V}O_{2max}$ decay was greater for cycle ergometer than treadmill tests (0.7, 1.6, and 2.8 percentage units at 10, 30, and 60 breaths, $P < 0.001$). A similar effect was observed with time-averaging strategies. The difference between the 6-b and 60-b averaging strategies was reduced from 10.1 to 8.5% following a HIT training program that increased $\dot{V}O_{2max}$ by ~ 8% [23] ($P < 0.05$ and $P < 0.001$ for intercepts and slopes, respectively; n = 41). This effect was partially reverted following three weeks of detraining, i.e. the difference between the 6-b and 60-b averaging strategy increased to 9.9 % ($P = 0.35$ and $P = 0.03$ for intercepts and slopes compared to pre-training, respectively). This effect was similar when using time-averaging strategies.

Mathematical modelling of the impact of the number of breaths or seconds included in the averaging block on the $\dot{V}O_{2max}$ value

Breath- and time- specific equations were generated to standardise the $\dot{V}O_{2max}$ values to a fixed number of breaths or seconds in the averaging block, using a random-effects linear-log regression model with a random intercept and fixed slope specific for breath- and time-averaging strategies, as follows:

$$Y_f = Y_i + A \times LN\left(\frac{X_i - 5}{X_f - 5}\right); \; (R^2_{GLMM} > 0.99, P < 0.001)$$

Where A is the fixed slope for breath- or time-averaging strategies; Y_f, represents the corrected $\dot{V}O_{2max}$ value for the aimed averaging strategy (in mL·min^{-1}); Y_i, the known initial $\dot{V}O_{2max}$ value (random intercept); X_i, the number of breaths or seconds used to obtain the initial averaging strategy; X_f, the number of breaths or seconds for the aimed final averaging strategy, and LN is the natural logarithm.

The slopes of the equations specific for fitness subgroups over and below 40 mL·kg^{-1}·min^{-1} were significantly different ($P < 0.001$). Nonetheless, we determined the difference between predicted values by the two fitness-specific equations (over and below 40 mL·kg^{-1}·min^{-1}), and it was minimal (0.004 ± 0.25%, 95th percentile = 0.4% for breath- and time-averaging strategies). The same analysis applied to the three specific equations for the downward or plateau, linear, and upward $\dot{V}O_2$ trajectories near $\dot{V}O_{2max}$ deviate minimally from the values generated with the specific general equations (0.006 ± 0.42%, 95th percentile = 0.7% and 0.011 ± 0.68%, 95th percentile = 1.2% for downward or plateau; 0.002 ± 0.15%, 95th percentile = 0.3% and 0.001 ± 0.08%, 95th percentile = 0.2% for linear; and 0.007 ± 0.52, 95th percentile = 0.9% and 0.011 ± 0.67%, 95th percentile = 1.2% for upward trajectory, for breath- and time-averaging strategies, respectively). Even smaller differences were observed due to exercise mode (cycling and running). Given these almost negligible deviations in prediction due to fitness, $\dot{V}O_2$ trajectories near $\dot{V}O_{2max}$, and exercise mode, we report two general equations specific for breath- and time-averaging strategies (see appendix for application of the equations):

Equation 1,

$$Y_f = Y_i + 68.8 \times LN\left(\frac{X_i - 5}{X_f - 5}\right); \; (R^2_{GLMM} = 0.998, P < 0.001)$$, for breath-averaging strategies.

Equation 2,

$$Y_f = Y_i + 76.4 \times \text{LN}\left(\frac{X_i - 5}{X_f - 5}\right); \; (R^2_{GLMM} = 0.995, P < 0.001), \text{ for time-averaging strategies.}$$

Impact of the number of breaths or seconds included in the averaging block on the reproducibility of the $\dot{V}O_{2max}$ value

The CV of $\dot{V}O_{2max}$ was not influenced by the number of breaths or seconds included in the averaging block, regardless of exercise mode (running vs cycling), sex, RR_{max} and IE protocol (*P* > 0.05 for all comparisons in all averaging strategies) (Table 2 and Fig. 2).

The Vyntus Group had a lower CV (mean CV_f=2.2% for 30-b strategy) than Vmax (mean CV_f = 6.8% for 30-b strategy; $P < 0.05$ for all averaging block lengths). The CV_f for the breath and time-averaging strategies were almost identical when matched by the number of breaths or seconds (Table 2). Nonetheless, when the subjects were matched for fitness, no significant differences were observed in CV between Vyntus Group and Vmax Group (Table 4, Fig. 2d and Fig. 6).

In the Vmax Group, the CV for the F_IO_{2max} and HR_{max} tended to decrease with the reduction of the averaging block ($P < 0.001$). The remaining variables did not show significantly different CVs across strategies (Fig. 3). In the Vyntus Group, the CV of F_EO_{2max} tended to decrease with the reduction of the averaging block ($P = 0.006$).

DISCUSSION

This study demonstrates that the value imputed as $\dot{V}O_{2max}$ during an incremental test to exhaustion varies largely depending on the number of breaths or seconds included in the averaging strategy, yielding the strategy based on a fixed number of breaths slightly higher values compared to the strategy based on an equal number of seconds. To correct for the variability that this behaviour may introduce in the imputed $\dot{V}O_{2max}$, we have developed a mathematical model that allows standardising the $\dot{V}O_{2max}$ to a fixed number of breaths or seconds. We have also shown that the decay of $\dot{V}O_{2max}$ with the number of breaths or seconds included in the averaging strategy is highly influenced by fitness status and lower in bicycling than running, but is independent of sex, RR_{max} and incremental exercise protocol. This study provides with novel data showing that the variability of $\dot{V}O_{2max}$ is similar using breath- or time-averaging strategies and is maintained regardless of the number of breaths or seconds included in the averaging strategy, between 6 and 60. Besides, we have also demonstrated that the variability is lower in endurance-trained individuals than sedentary individuals, but is independent of sex, exercise mode, RR_{max} and incremental exercise protocol.

Our results agree with previous studies using time-based gas sampling intervals, showing a higher $\dot{V}O_{2max}$ the lower the averaging block, although rarely including more than five blocks, which precluded any precise mathematical modelling.[11,13,14,21,35] Nonetheless, application of the linear-log mathematical modelling for the decay in $\dot{V}O_{2max}$ to previous data in methodological articles shows good agreement (i.e. all $R^2 > 0.92$ when more than three data averaging strategies were reported in the respective article) as shown in Table 5.

The number of breaths in a given time-interval depends in part on the duration of the time interval. The problem arises when marked differences exist in breathing frequencies between the two tests. For example, if the RR_{max} in one test is 42 bpm, then a 20-s interval will include 14 breaths, while if the same subject repeats the test and reaches an RR_{max} of 60 bpm, then 20 breaths would be included in the averaging interval. Given the high impact that the number of breaths included in the averaging interval has on the $\dot{V}O_{2max}$ value, this should be corrected. This investigation provides a tool to standardise the test to a fixed breath- or time-averaging strategy, which can be settled to any value between 6 and 60.

Averaging strategy and reproducibility

This investigation shows that the reproducibility of the $\dot{V}O_{2max}$ is similar for averaging strategies between 6 and 60 breaths or seconds using two widely commercialised metabolic carts (Vmax N29, Sensormedics and Vyntus CPX, Jaeger-CareFusion). To the best of our knowledge, this is the first study reporting better reproducibility of $\dot{V}O_{2max}$ in subjects with a higher $\dot{V}O_{2max}$ value, which may be due to the lack of experience of untrained subjects in performing strenuous exercise.

It has been reported that $\dot{V}O_{2max}$ can be determined using automated metabolic carts with CVs between 4% and 9%.[37] No previous study has reported the reproducibility of $\dot{V}O_{2max}$ assessment with the Vyntus CPX Jaeger-CareFusion metabolic cart. Here we have observed CVs just above 2%, which are remarkably low for a breath-by-breath metabolic cart, and close to the best achievable with the Douglas bag method.[38] Moreover, this high reproducibility is also achieved by endurance-trained individuals tested both on cycle-ergometers and treadmills, despite the inherent error due to the multiple assessments needed to carry out breath-by-breath assessments at high respiratory rates.[39] In addition, our data demonstrate a high level of agreement (CV_f lower than 4.7% and CCC > 0.97) between Vmax N29 SensorMedics and the Vyntus CPX Jaeger-CareFusion metabolic carts.

Physiological implications

Since different values of $\dot{V}O_{2max}$ can be obtained depending on the averaging strategy, achieving accurate and reliable measures of $\dot{V}O_{2max}$ has important implications for integrative physiology. From a physiological perspective is not the same to compute the $\dot{V}O_{2max}$ value with 6-s than 60-s averaging strategy, since the latest may be 6-10% lower, depending on the fitness status. The relevance of this finding is further highlighted by the fact that the $\dot{V}O_{2max}$ has low plasticity, improving 10-30% with endurance training. [4] For example, the assessment of effect sizes from training studies or calculations used to obtain cardiac output by the direct Fick method would be highly affected by an over- or underestimated $\dot{V}O_{2max}$.

In the equations proposed, the intercept of the linear-log model is a random value that depends on the $\dot{V}O_{2max}$ of each subject. The slope is a common slope representing the drop in $\dot{V}O_{2max}$ with time. This slope would have been equal to 0, had the $\dot{V}O_{2max}$ been steady from 6 to 60 s. The existence of this slope indicates that the $\dot{V}O_{2max}$ cannot be maintained more than a few seconds. This is expected since neither the cardiac output nor the pulmonary ventilation reaches a plateau during IE. The same applies to muscular O_2 extraction which does not seem to plateau during incremental exercise.[40] A lower slope could indicate a higher capacity to maintain $\dot{V}O_2$ values close to $\dot{V}O_{2max}$ near exhaustion. The fact that the slope was lower during treadmill than cycle ergometer exercise could indicate that when a smaller muscle mass is used the time during which the plateau can be maintained is shorter. The latter agrees with the fact that a plateau is more often seen for treadmill than cycle ergometer tests.[41]

Limitations

Although part of the $\dot{V}O_2$ data included in the current study originates from previous research, the presence of a newly recruited group tested in the two metabolic carts evidenced a high agreement between carts. It remains unknown whether similar results will be obtained with other metabolic carts. Nevertheless, our log-linear model predicts with high accuracy the $\dot{V}O_{2max}$ when applied to previous studies using different metabolic carts (Table 5). The equations have been specifically developed for mean group values, and therefore are very precise when applied to groups or mean data. The individual slope of the $\dot{V}O_{2max}$ decay may differ markedly in some subjects. Although the equations were developed using a population with a broad range of $\dot{V}O_{2max}$ values (20-60 $mL \cdot kg^{-1} \cdot min^{-1}$), the accuracy of the prediction when applied to other populations with $\dot{V}O_{2max}$ outside this range (e.g. patients with cardiorespiratory diseases or elite athletes), which may have different $\dot{V}O_{2max}$ decay slopes, should be specifically determined in future studies. Nonetheless, the excellent fit of the log-linear model in cardiac patients [8,11,14] suggests robust applicability of the model to other populations.

FUTURE PERSPECTIVES

Our results imply that caution should be taken when comparing $\dot{V}O_{2max}$ across studies with different averaging strategies. New studies with physiological manipulations (i.e., haemodilution, hypoxia, hyperoxia, etc.) will be needed to ascertain whether the slope of the prediction model has a specific physiological meaning beyond what we have intuitively deducted. From the outcomes of this investigation, no particular sampling strategy can be suggested as optimal for $\dot{V}O_{2max}$ assessment based on our reproducibility approach. On the other hand, given the limited time at which $\dot{V}O_{2max}$ can be sustained, a shorter averaging strategy has a higher probability for capturing the real $\dot{V}O_{2max}$, while facilitating the identification of the plateauing criteria. Thus, we think that averaging intervals including between 15 and 20 breaths or seconds are likely preferable.

ACKNOWLEDGEMENTS

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APPENDIX

Application of the correction equation

To compare two $\dot{V}O_{2max}$ values obtained with different sampling strategies, a specific breath- or time-averaging strategy between 6 and 60 breaths or seconds should be first chosen for standardisation of the two values. In the following example, the given rolling breath-average $\dot{V}O_{2max}$ values were aimed to be standardised to a 10-b rolling strategy.

Study 1 presented a mean $\dot{V}O_{2max}$ of 2430 mL·min^{-1} with a 10-b rolling strategy.

Study 2 had a mean $\dot{V}O_{2max}$ of 2320 mL·min^{-1} with a 50-b rolling strategy.

The equation for breath-averaging strategies (see Results section) should be applied as follows:

$$Y_f = Y_i + A \times LN\left(\frac{X_i - 5}{X_f - 5}\right);\ \dot{V}O_{2max}\ 10\text{-b} = 2320 + 68.8 \times LN\left(\frac{50 - 5}{10 - 5}\right) = 2471\ \text{mL}\cdot\text{min}^{-1}$$

Where A is the fixed slope for breath-averaging strategies; Y_f, represents the corrected final $\dot{V}O_{2max}$ value for the aimed 10-b averaging strategy (in mL·min^{-1}); Y_i, the known initial $\dot{V}O_{2max}$ value (random intercept); X_i, the number of breaths for the given initial 50-b averaging strategy; X_f, the number of breaths for the aimed final 10-b averaging strategy and LN the natural logarithm.

Therefore, the corrected 10-b $\dot{V}O_{2max}$ value for study 2 is 2471 mL·min^{-1}. Thus, this allows to correct for the wrong initial conclusion that $\dot{V}O_{2max}$ was higher in study 1 than 2, since when both studies are compared using a standardised averaging strategy (10-b in this example), it turns out that $\dot{V}O_{2max}$ was greater in study 2 (2471 $mL \cdot min^{-1}$) than 1 (2430 $mL \cdot min^{-1}$). This should be considered in meta-analysis examining $\dot{V}O_{2max}$ changes.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

AUTHOR CONTRIBUTIONS

All authors read and approved the final manuscript. All co-authors read, contributed with comments and approved the final version of the manuscript.

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FIGURE LEGENDS

Figure 1. Flow chart of participants in the different groups of the study.

Figure 2. Variation of $\dot{V}O_{2max}$ determination and reproducibility using two different automated metabolic carts operated in breath-by-breath mode with different rolling breath-averages. (a) $\dot{V}O_{2max}$ response in a heterogeneous group of subjects assessed with Vmax N29 Sensormedics (Vmax Group) (n=62). (b) $\dot{V}O_{2max}$ response in a group of endurance-trained subjects assessed with Vyntus CPX (Vyntus Group) (n=11). (c) $\dot{V}O_{2max}$ response in a group of recreationally active and endurance-trained subjects performing one test with Vmax N29 and a duplicate test with Vyntus CPX in random order (Combined group) (n=11). (d) $\dot{V}O_{2max}$ response for a Vmax subgroup with matched fitness level (relative-to-weight $\dot{V}O_{2max}$) to Vyntus group (n=9).

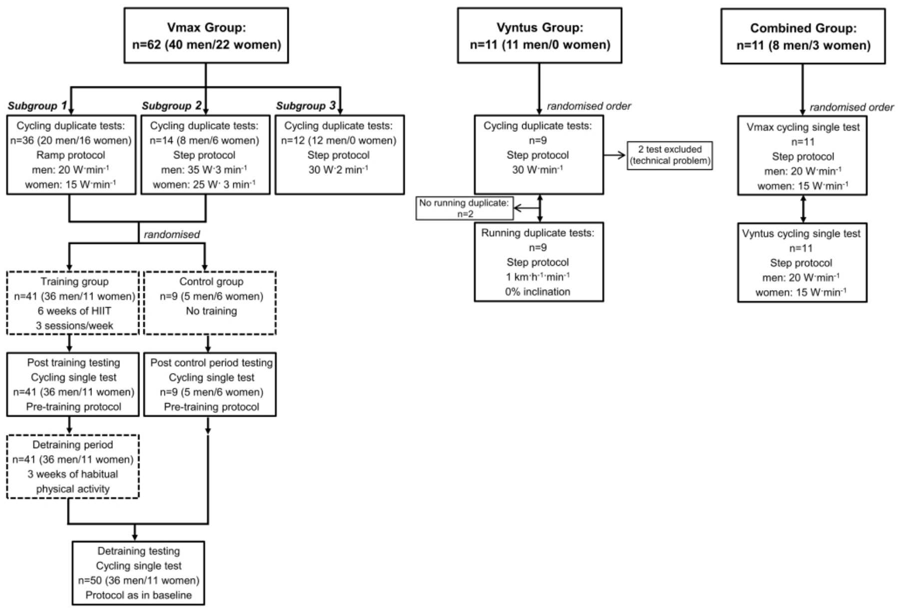
Figure 3. Variation and reproducibility of ergospirometric variables assessed with the Vmax N29 Sensormedics metabolic cart operated in breath-by-breath mode, applying different rolling breath-averages (n=62). (a) Carbon dioxide production ($\dot{V}CO_{2max}$), respiratory exchange ratio (RER_{max}), pulmonary ventilation ($\dot{V}_{Emax}$), alveolar ventilation ($\dot{V}_{Amax}$), end-tidal O_2 pressure ($P_{ET}O_{2max}$), end-tidal CO_2 pressure ($P_{ET}CO_{2max}$), fraction of expired O_2 (F_EO_{2max}), fraction of expired CO_2 (F_ECO_{2max}), fraction of inspired O_2 (F_IO_{2max}). (b) Tidal volume (VT_{max}), respiratory rate (RR_{max}) and heart rate (HR_{max}). Coefficient of variation (CV%).

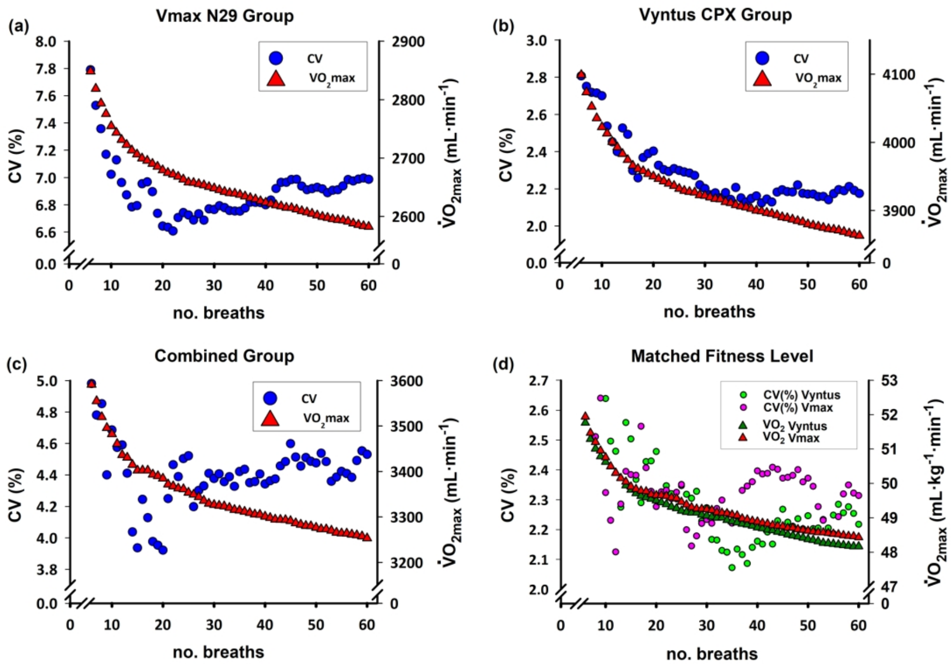
Figure 4. $\dot{V}O_2$ data for the response to an incremental exercise to exhaustion in a representative subject from Vyntus Group using different averaging blocks (breaths and seconds). Data are presented as (a) raw breath-by-breath, (b) 15 breaths and 15-s, (c) 30 breaths and 30-s, (d) 60 breaths and 60-s.

Figure 5. The goodness of fit. The goodness of fit of the linear-log model was checked in a random sample of 50 participants. Down-sampling was performed to enhance the clarity of the graph. (a) Raw $\dot{V}O_{2max}$ decay for breath-averages, (b) $\dot{V}O_{2max}$ decay after logarithmic

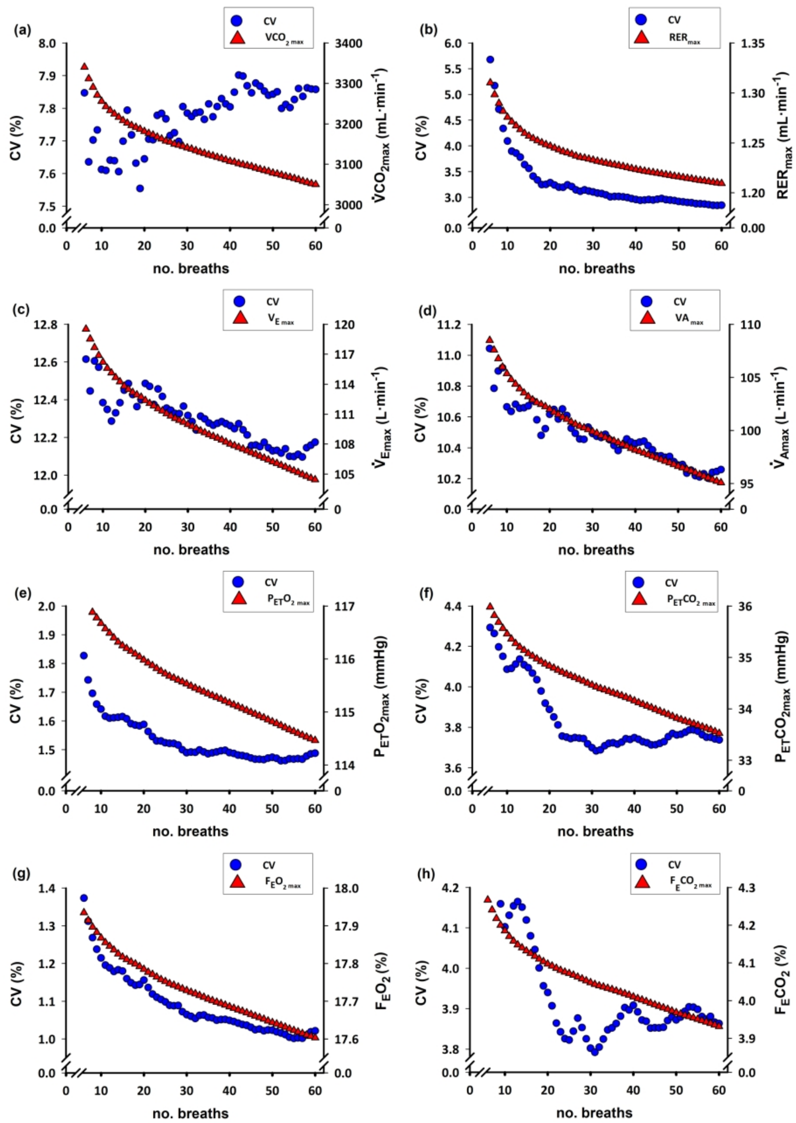
transformation for breath-averages (c) Raw $\dot{V}O_{2max}$ decay for time-averages, (d) $\dot{V}O_{2max}$ decay after logarithmic transformation for time-averages.

Figure 6. Bland and Altman plots with 95% limits of agreement for duplicate $\dot{V}O_{2max}$ tests in subjects belonging to Vmax and Vyntus groups expressed in relative-to-weight values ($mL \cdot kg^{-1} \cdot min^{-1}$) in a representative breath- and time-averaging strategy. Dotted lines represent the threshold for significant differences in reproducibility found in the study between untrained and moderately trained subjects (n=73, 80 duplicates, Combined Group not included).

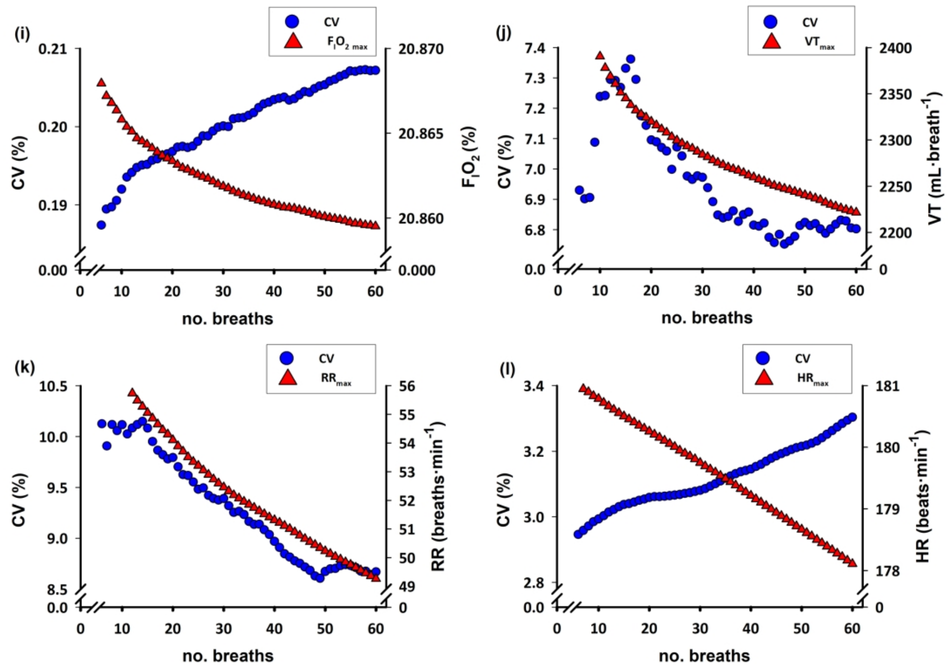

Vmax Group:
n=62 (40 men/22 women)
Vyntus Group:
n=11 (11 men/0 women)
Combined Group:
n=11 (8 men/3 women)
Subgroup 1
Subgroup 2
Subgroup 3
Cycling duplicate tests:
n=36 (20 men/16 women)
Ramp protocol
men: 20 W·min⁻¹
women: 15 W·min⁻¹
Cycling duplicate tests:
n=14 (8 men/6 women)
Step protocol
men: 35 W·3 min⁻¹
women: 25 W· 3 min⁻¹
Cycling duplicate tests:
n=12 (12 men/0 women)
Step protocol
30 W·2 min⁻¹
randomised order
Cycling duplicate tests:
n=9
Step protocol
30 W·min⁻¹
2 test excluded
(technical problem)
No running duplicate:
n=2
Running duplicate tests:
n=9
Step protocol
1 km·h⁻¹·min⁻¹
0% inclination
randomised order
Vmax cycling single test
n=11
Step protocol
men: 20 W·min⁻¹
women: 15 W·min⁻¹
Vyntus cycling single test
n=11
Step protocol
men: 20 W·min⁻¹
women: 15 W·min⁻¹
randomised
Training group
n=41 (36 men/11 women)
6 weeks of HIIT
3 sessions/week
Control group
n=9 (5 men/6 women)
No training
Post training testing
Cycling single test
n=41 (36 men/11 women)
Pre-training protocol
Post control period testing
Cycling single test
n=9 (5 men/6 women)
Pre-training protocol
Detraining period
n=41 (36 men/11 women)
3 weeks of habitual
physical activity
Detraining testing
Cycling single test
n=50 (36 men/11 women)
Protocol as in baseline



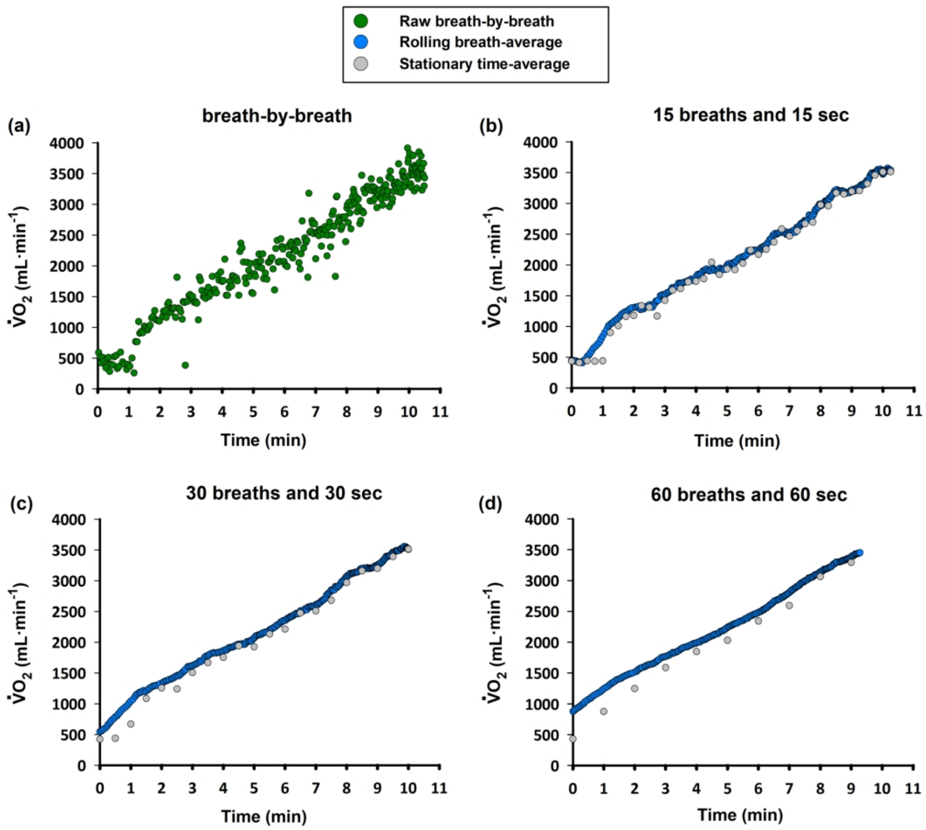
(a)
Vmax N29 Group
CV
VO2max
CV (%)
V̇O2max (mL·min-1)
no. breaths
(b)
Vyntus CPX Group
CV
VO2max
CV (%)
V̇O2max (mL·min-1)
no. breaths
(c)
Combined Group
CV
VO2max
CV (%)
V̇O2max (mL·min-1)
no. breaths
(d)
Matched Fitness Level
CV(%) Vyntus
CV(%) Vmax
VO2 Vyntus
VO2 Vmax
CV (%)
V̇O2max (mL·kg-1·min-1)
no. breaths



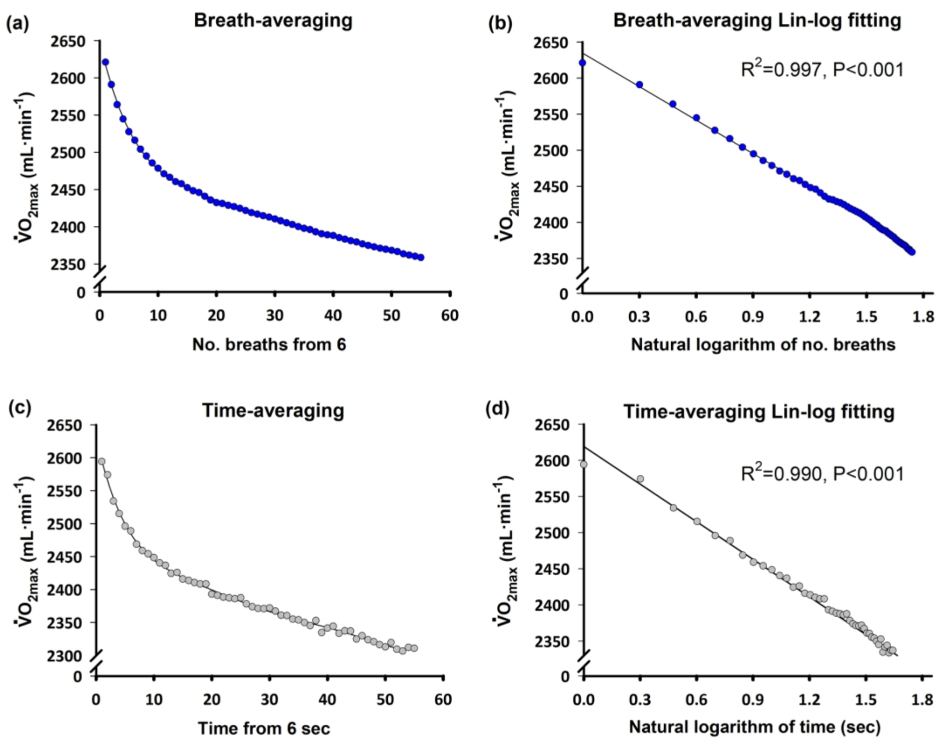
(a)
CV
VCO2 max
CV (%)
V̇CO2max (mL·min-1)
no. breaths
(b)
CV
RERmax
CV (%)
RERmax (mL·min-1)
no. breaths
(c)
CV
VE max
CV (%)
V̇Emax (L·min-1)
no. breaths
(d)
CV
VA max
CV (%)
V̇Amax (L·min-1)
no. breaths
(e)
CV
PETO2 max
CV (%)
PETO2max (mmHg)
no. breaths
(f)
CV
PETCO2 max
CV (%)
PETCO2max (mmHg)
no. breaths
(g)
CV
FEO2 max
CV (%)
FEO2 (%)
no. breaths
(h)
CV
FECO2 max
CV (%)
FECO2 (%)
no. breaths



(i)
CV
$F_IO_{2\ max}$
CV (%)
no. breaths
(j)
CV
VT_{max}
F_IO_2 (%)
CV (%)
VT (mL·breath^{-1})
no. breaths
(k)
CV
RR_{max}
CV (%)
RR (breaths·min^{-1})
no. breaths
(l)
CV
HR_{max}
CV (%)
HR (beats·min^{-1})
no. breaths

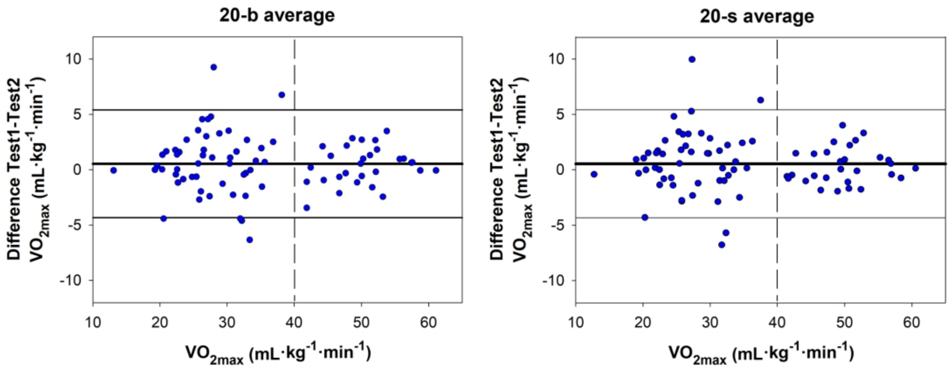


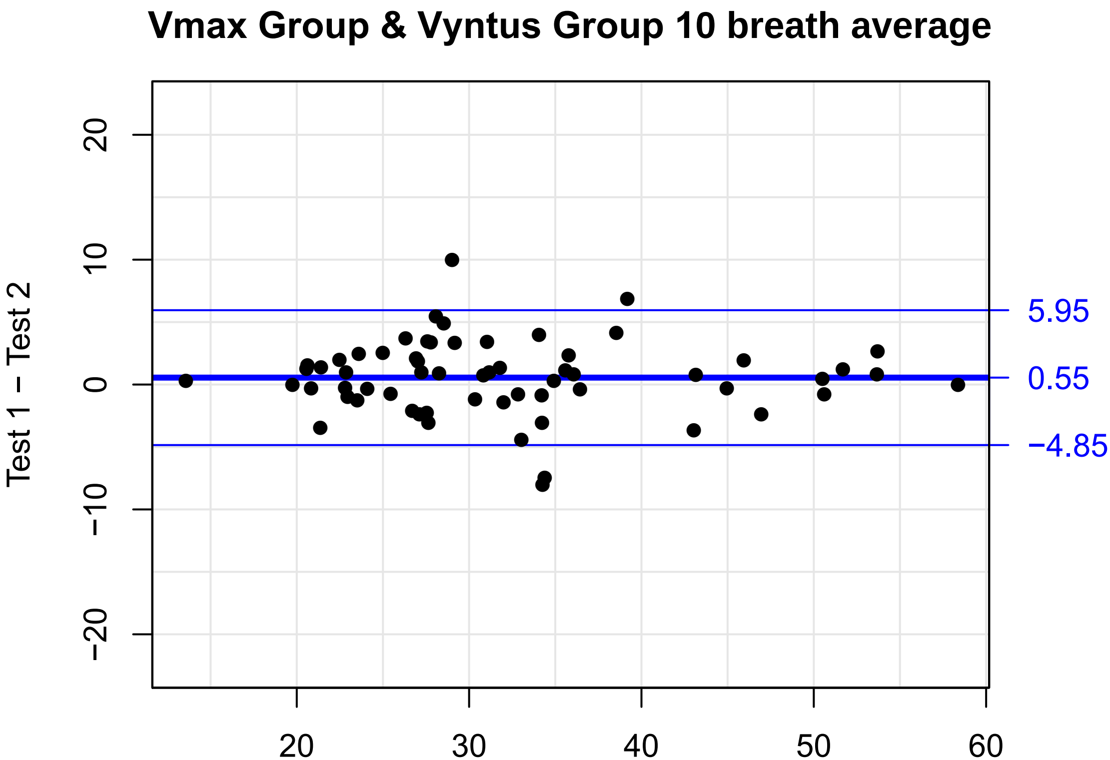
186x160mm (300 x 300 DPI)



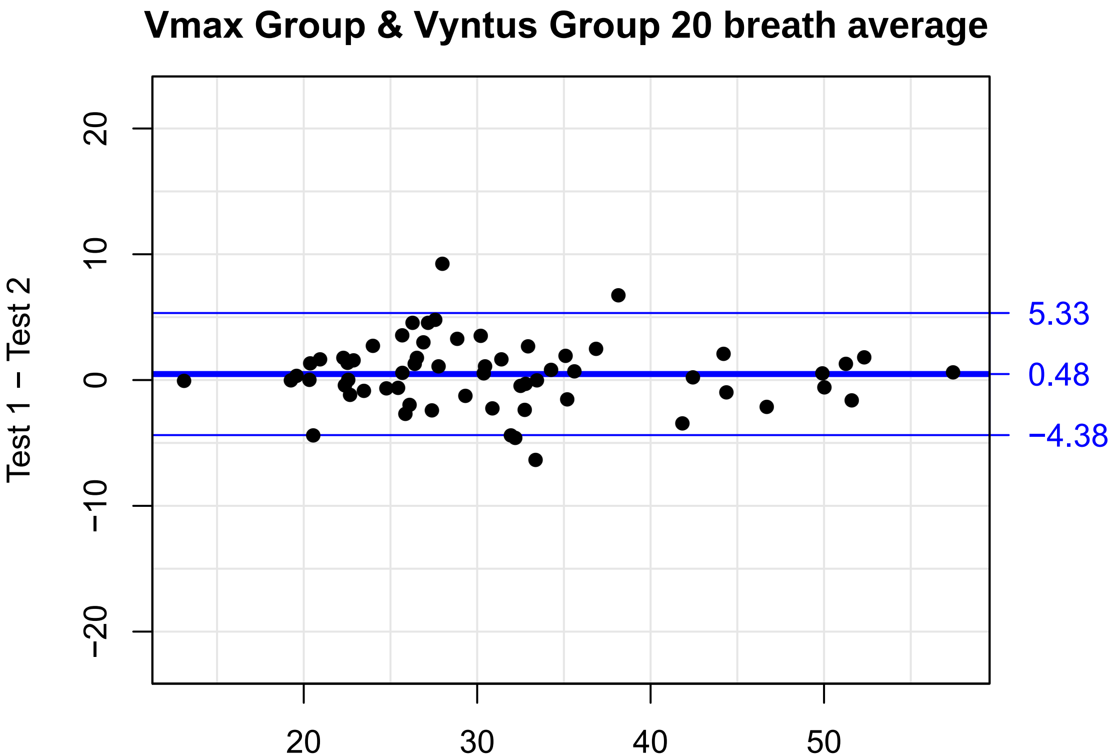
(a)
Breath-averaging
VO2max (mL·min-1)
No. breaths from 6
(b)
Breath-averaging Lin-log fitting
R2=0.997, P<0.001
Natural logarithm of no. breaths
(c)
Time-averaging
Time from 6 sec
(d)
Time-averaging Lin-log fitting
R2=0.990, P<0.001
Natural logarithm of time (sec)

Duplicate testing for Vmax and Vyntus groups

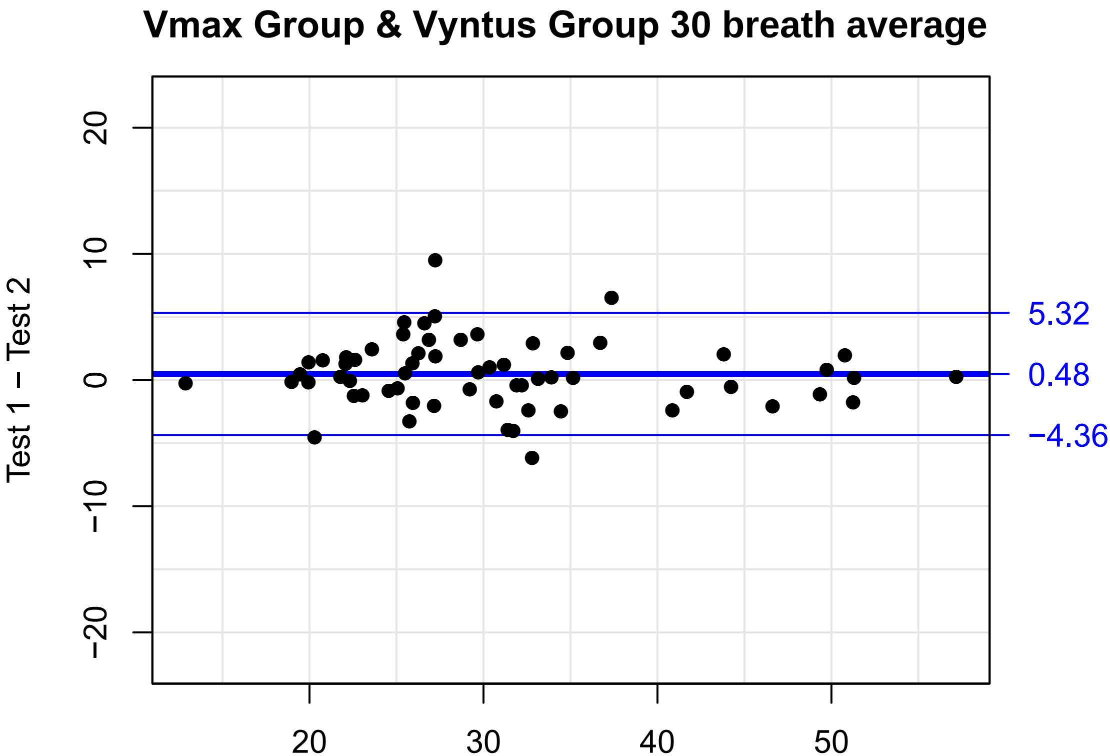




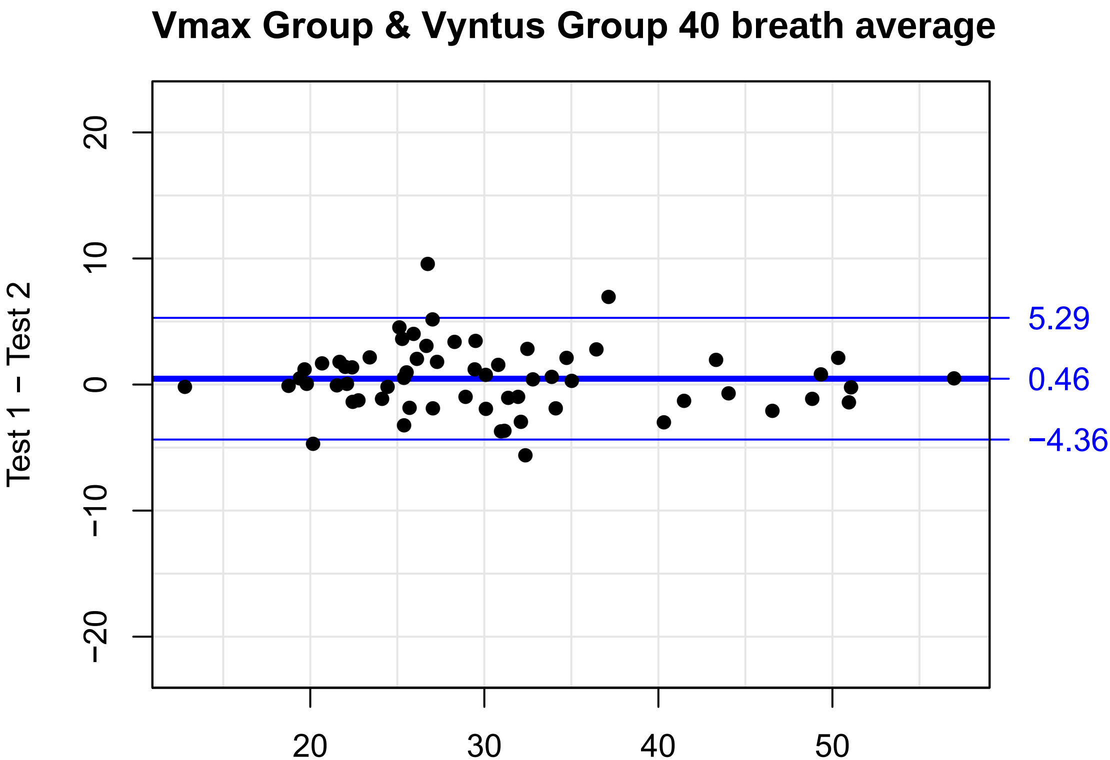
Vmax Group & Vyntus Group 10 breath average
Test 1 − Test 2
20
10
0
−10
−20
20
30
40
50
60
5.95
0.55
−4.85

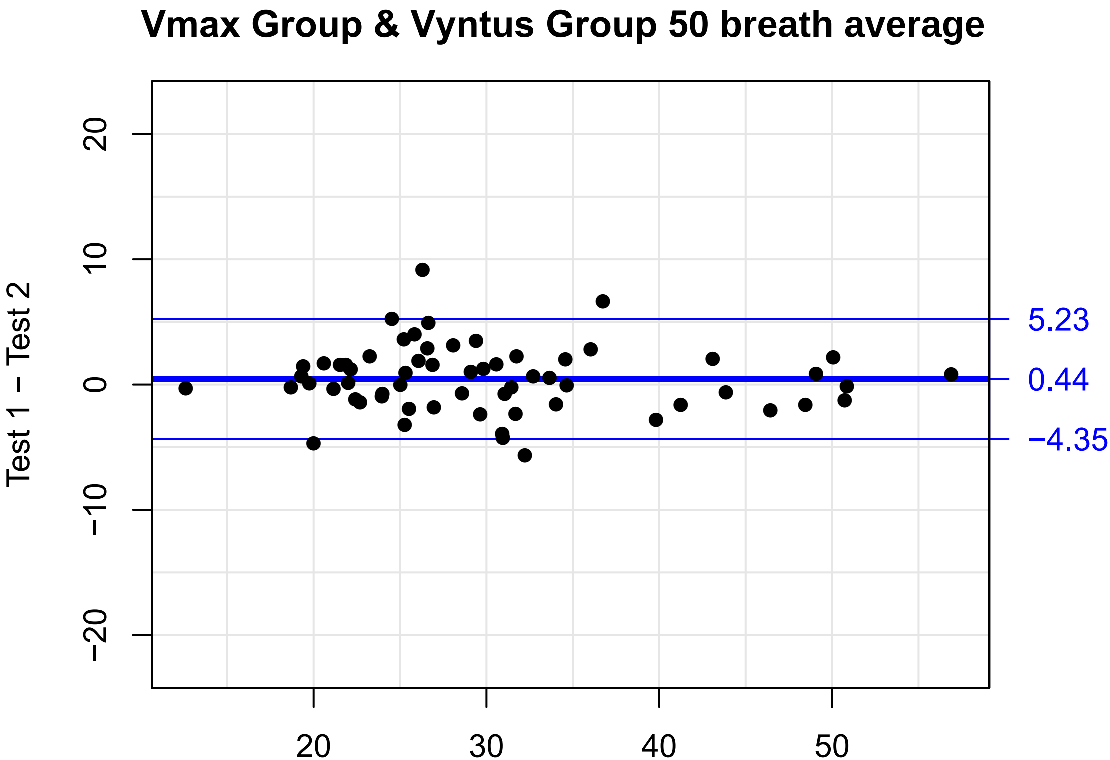


Vmax Group & Vyntus Group 20 breath average
Test 1 − Test 2
20
10
0
−10
−20
20
30
40
50
5.33
0.48
−4.38



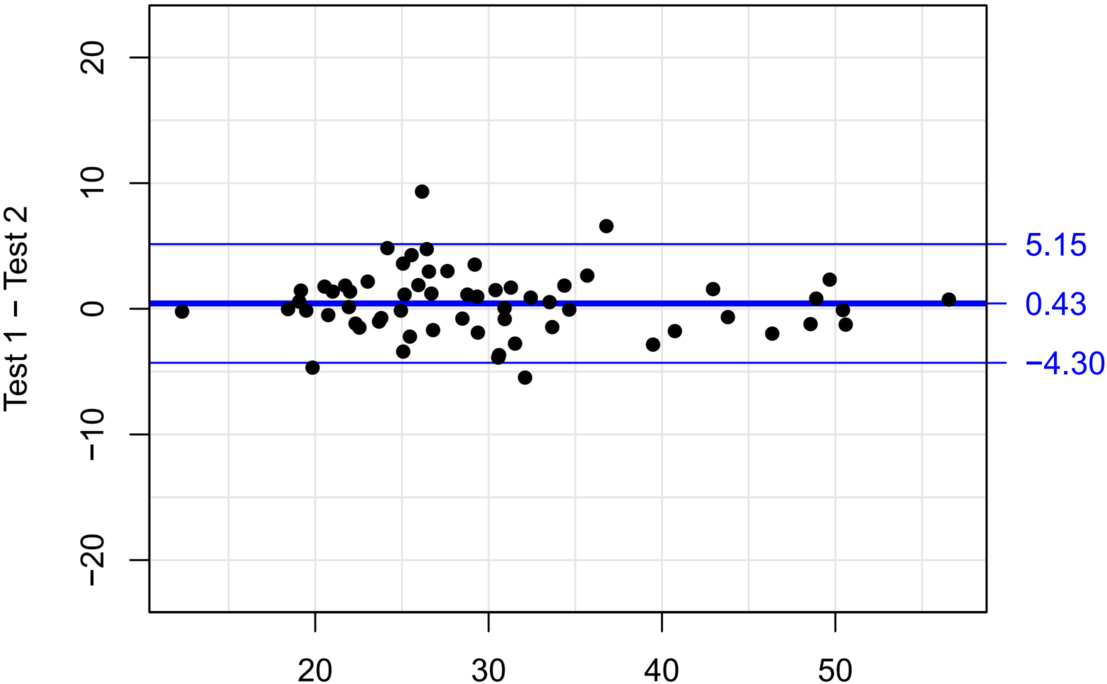
Vmax Group & Vyntus Group 30 breath average
Test 1 − Test 2
20
10
0
−10
−20
20
30
40
50
5.32
0.48
−4.36

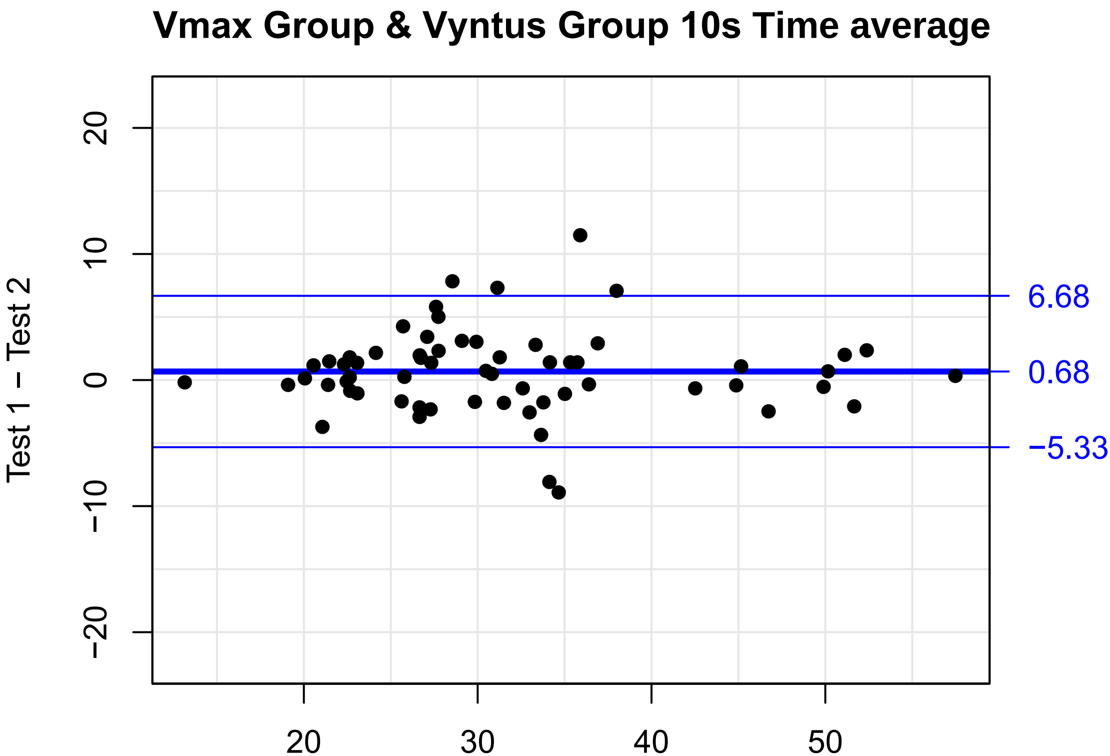

Vmax Group & Vyntus Group 40 breath average
Test 1 − Test 2
20
10
0
−10
−20
20
30
40
50
5.29
0.46
−4.36

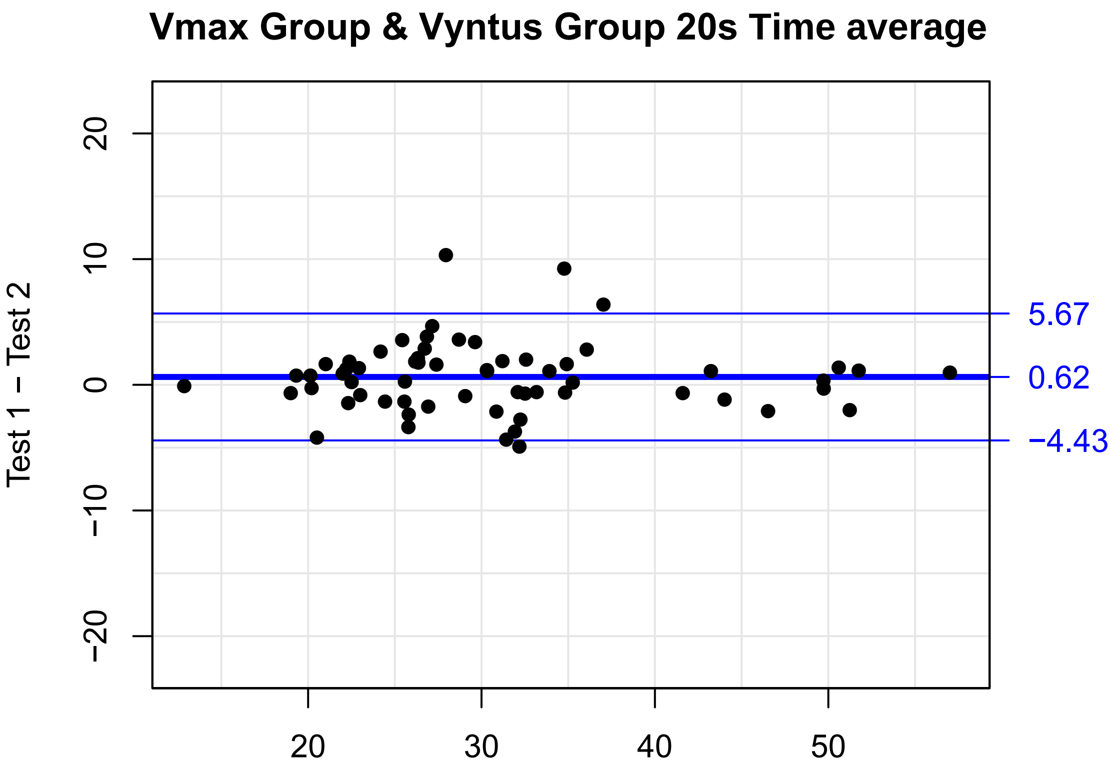


Vmax Group & Vyntus Group 50 breath average
Test 1 − Test 2
20
10
0
−10
−20
20
30
40
50
5.23
0.44
−4.35

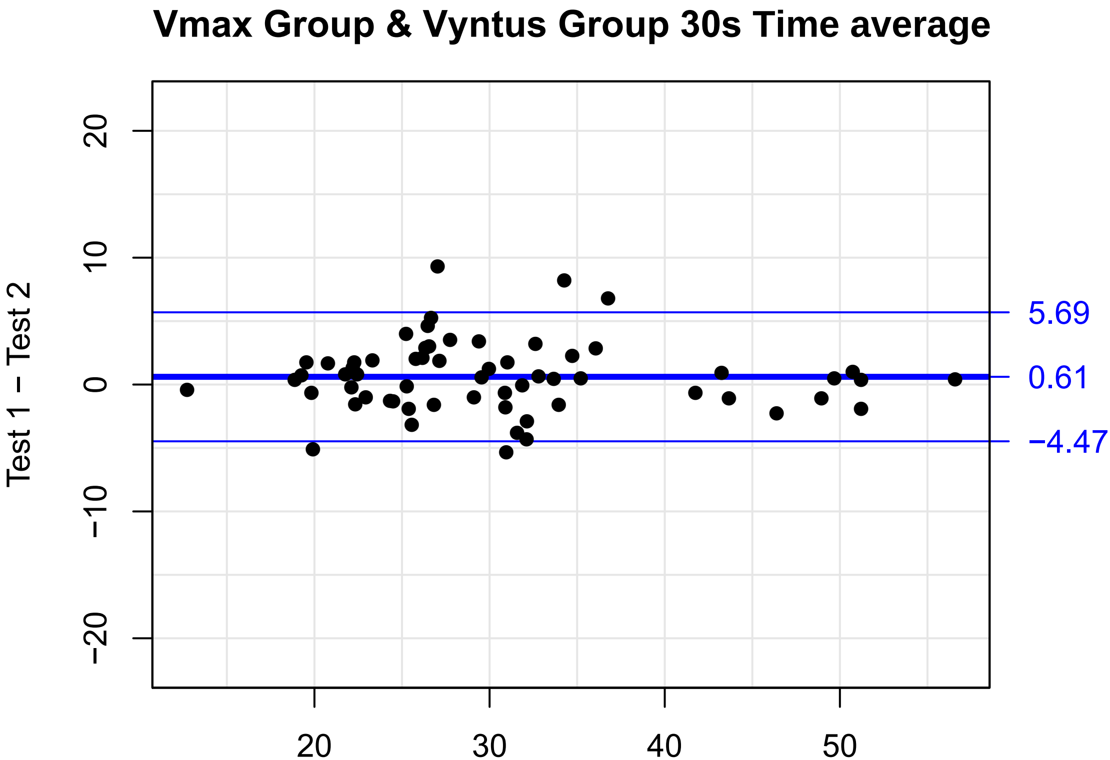
Vmax Group & Vyntus Group 60 breath average



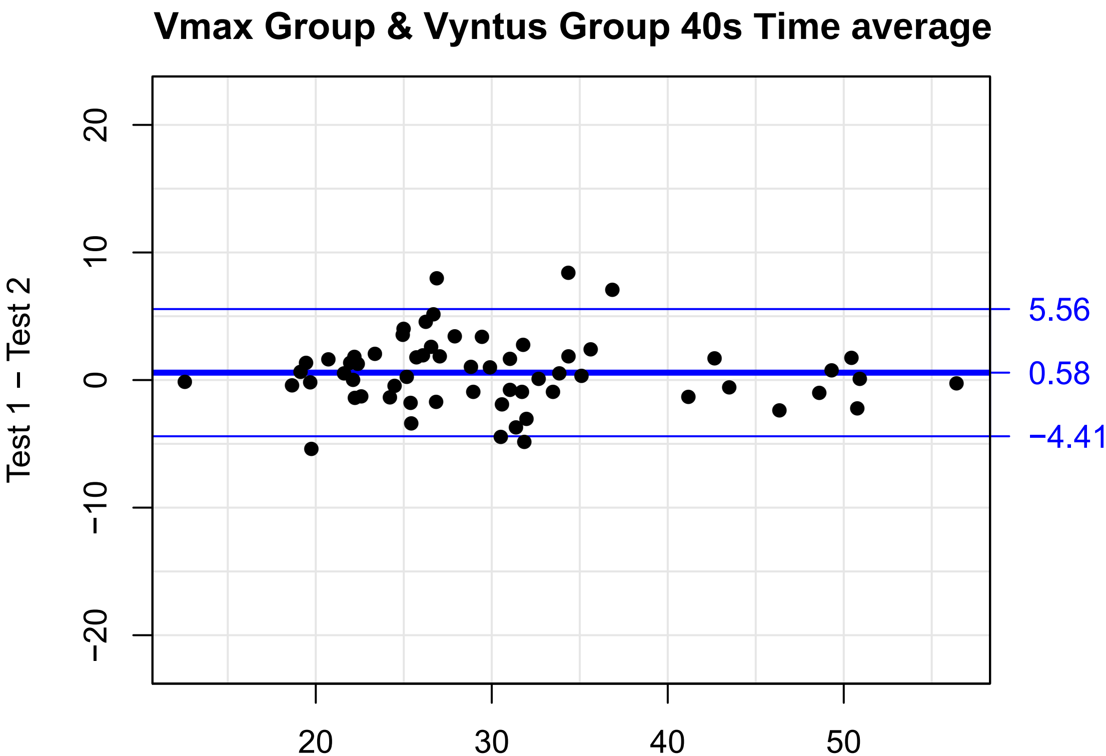

Vmax Group & Vyntus Group 10s Time average
Test 1 − Test 2
20
10
0
−10
−20
20
30
40
50
6.68
0.68
−5.33



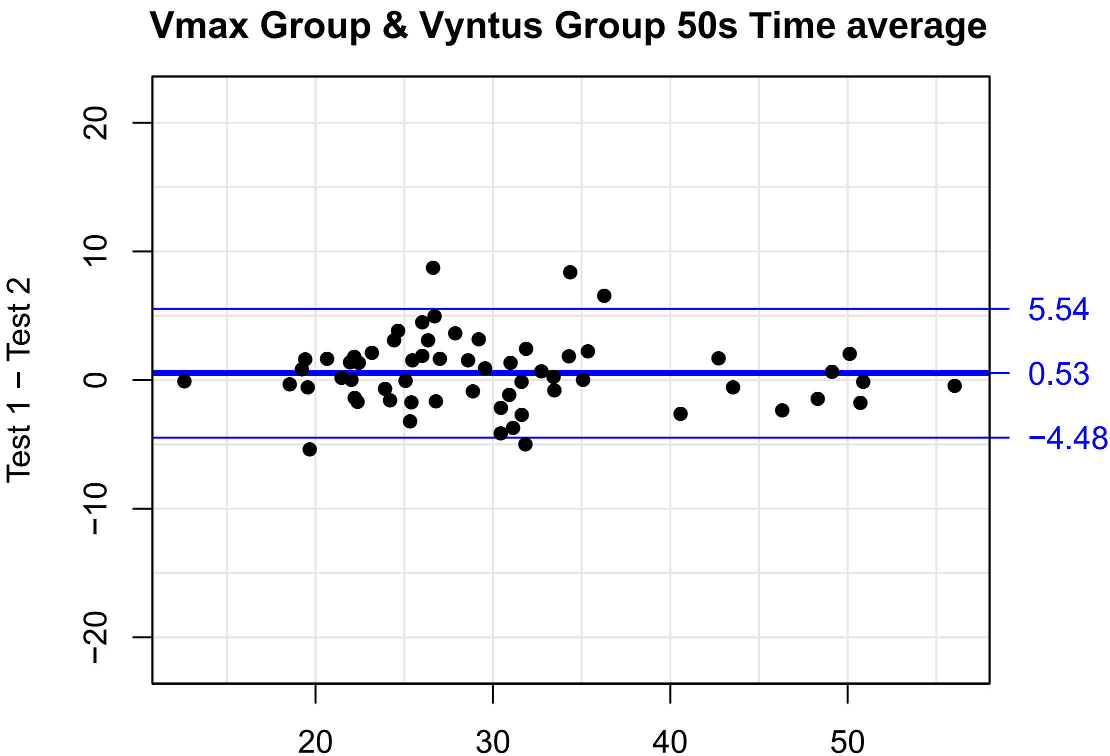
Vmax Group & Vyntus Group 20s Time average
Test 1 − Test 2
20
10
0
−10
−20
20
30
40
50
5.67
0.62
−4.43



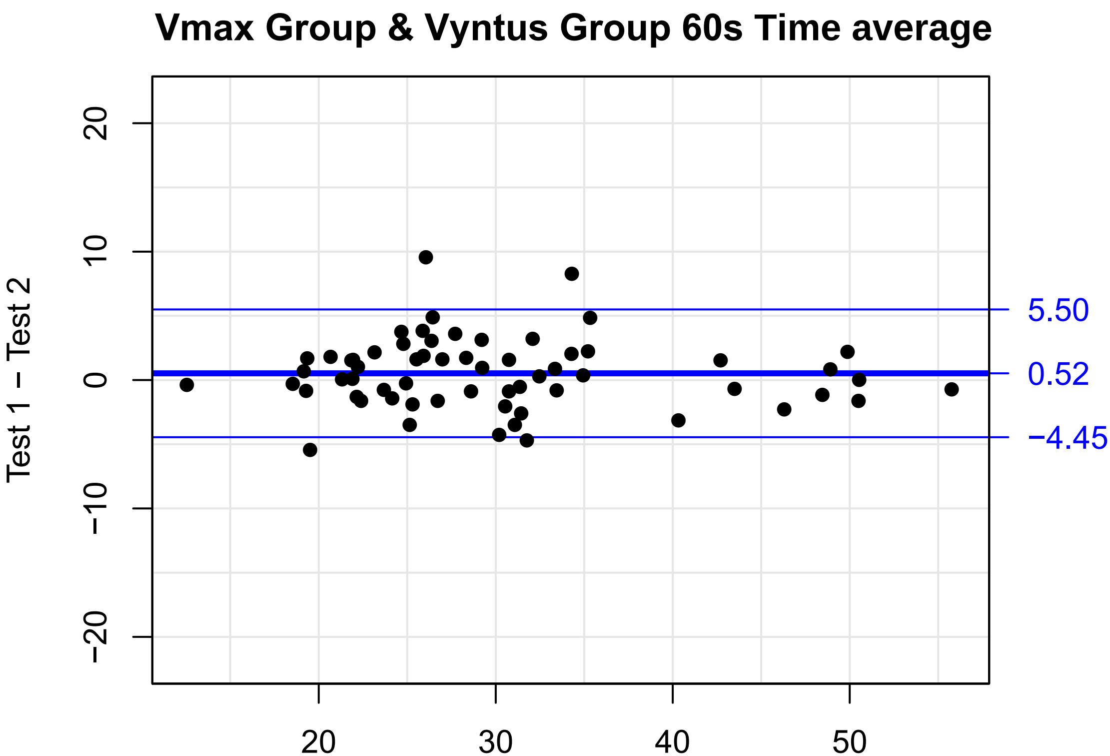
Vmax Group & Vyntus Group 30s Time average
Test 1 − Test 2
20
10
0
−10
−20
20
30
40
50
5.69
0.61
−4.47



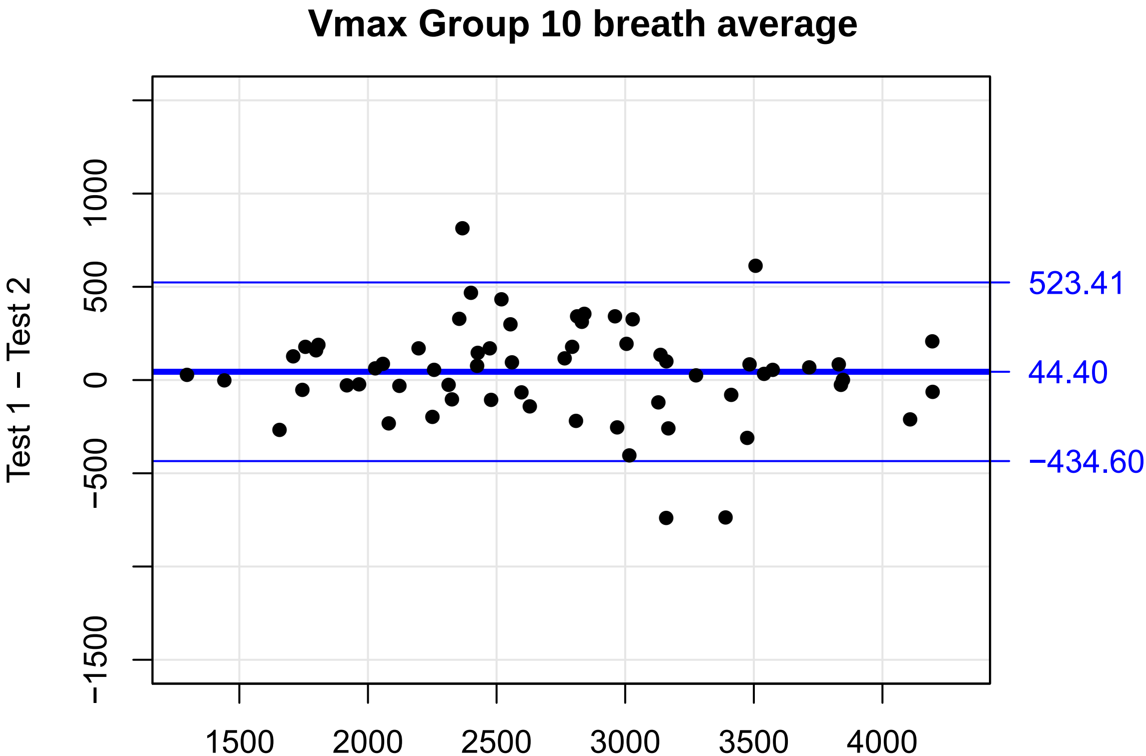
Vmax Group & Vyntus Group 40s Time average
Test 1 − Test 2
20
10
0
−10
−20
20
30
40
50
5.56
0.58
−4.41



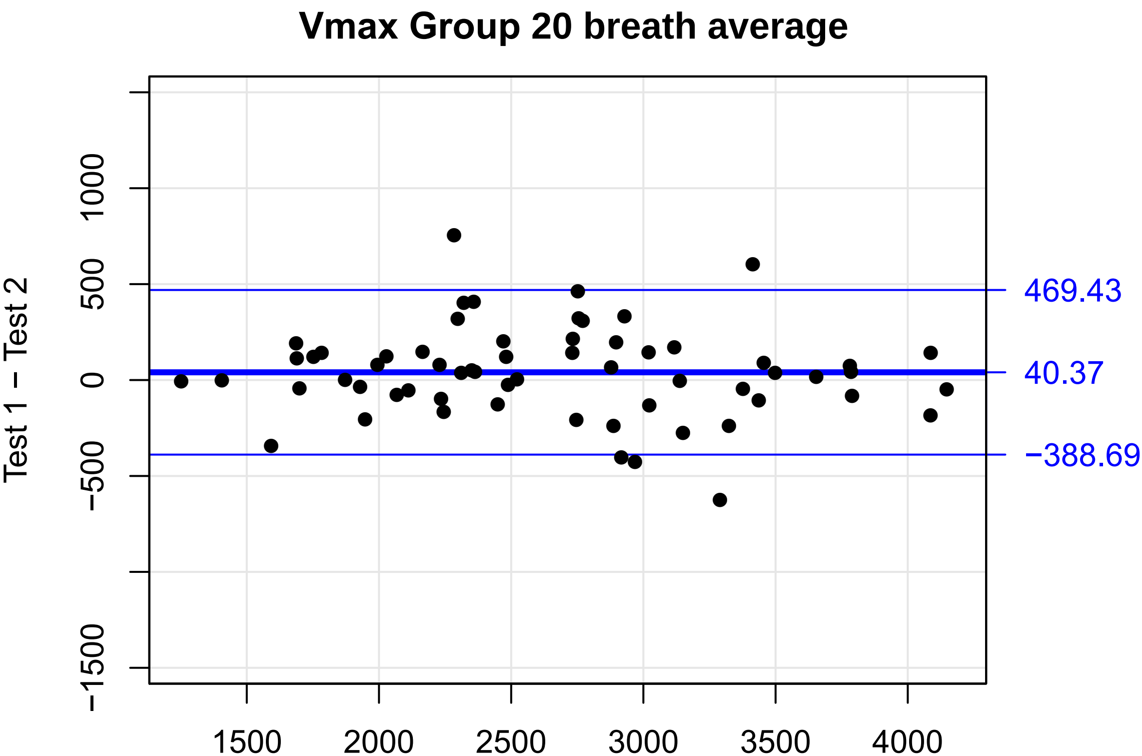
Vmax Group & Vyntus Group 50s Time average
Test 1 − Test 2
20
10
0
−10
−20
20
30
40
50
5.54
0.53
−4.48



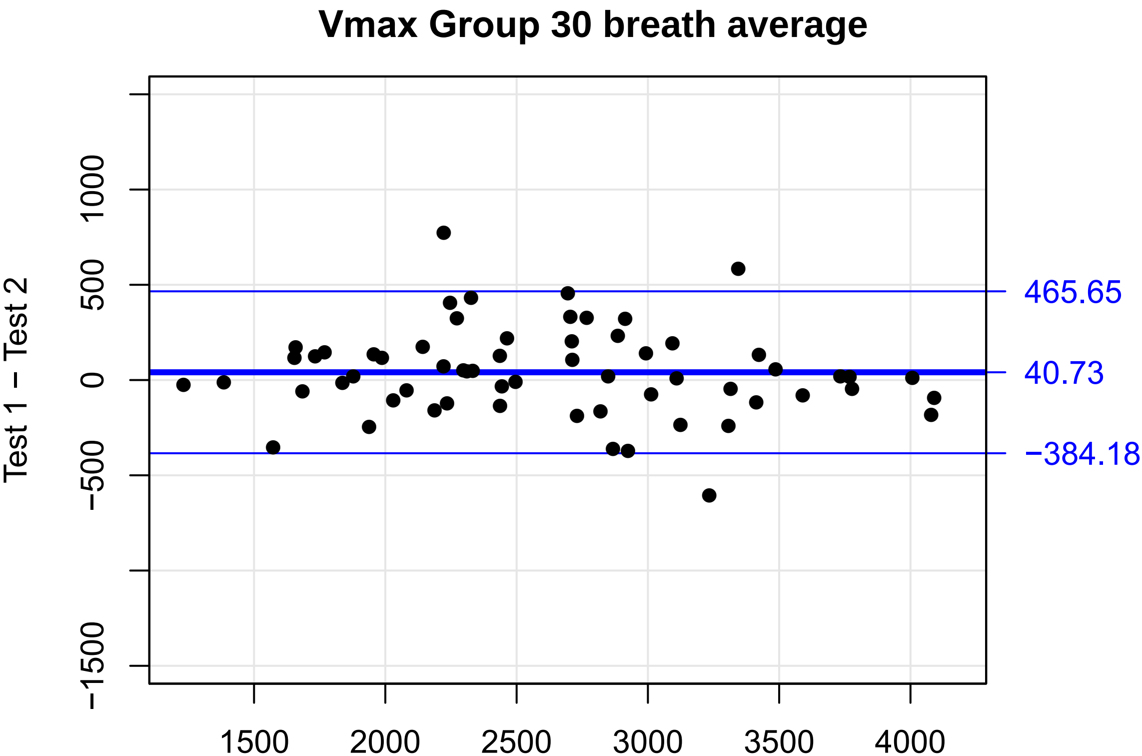
Vmax Group & Vyntus Group 60s Time average
Test 1 − Test 2
20
10
0
−10
−20
20
30
40
50
5.50
0.52
−4.45

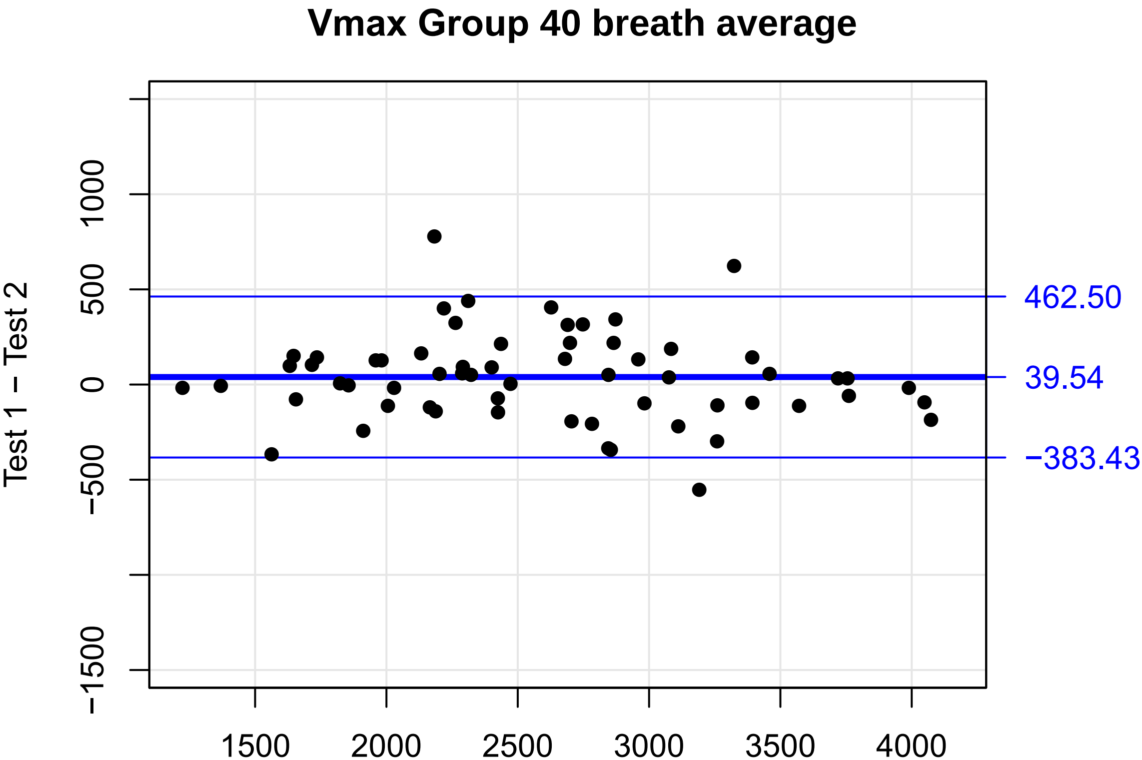


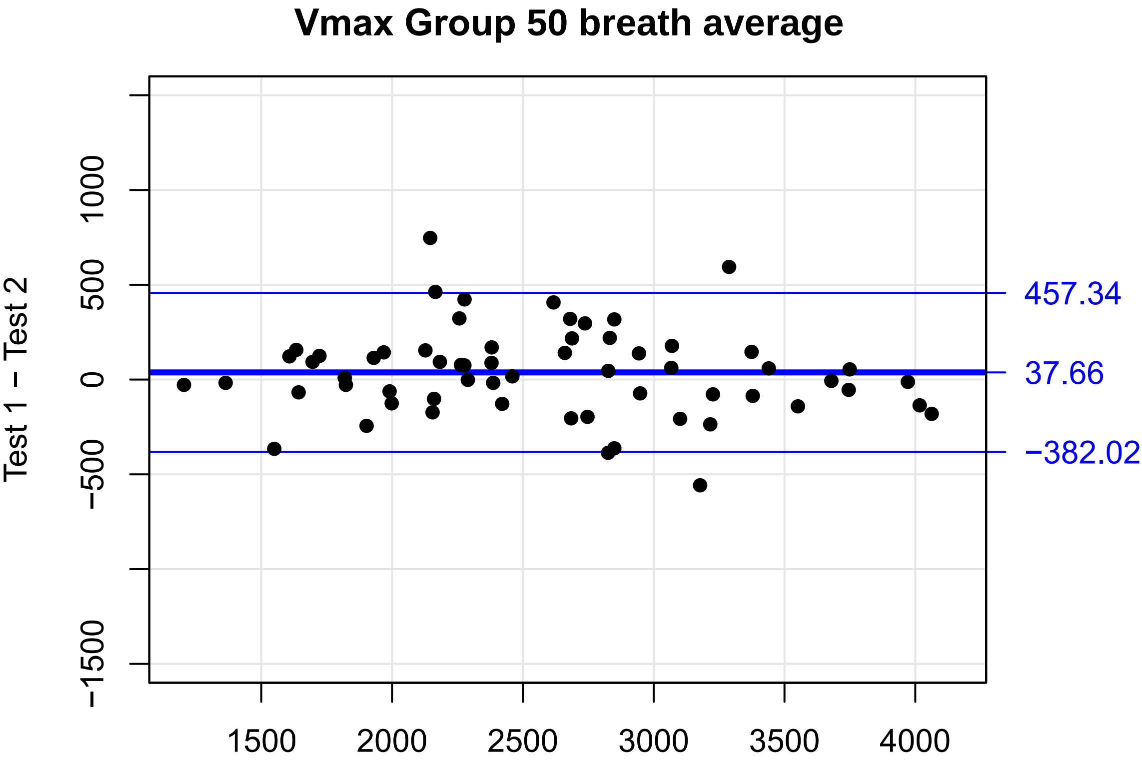
Vmax Group 10 breath average
Test 1 − Test 2
1000
500
0
−500
−1500
1500
2000
2500
3000
3500
4000
523.41
44.40
−434.60

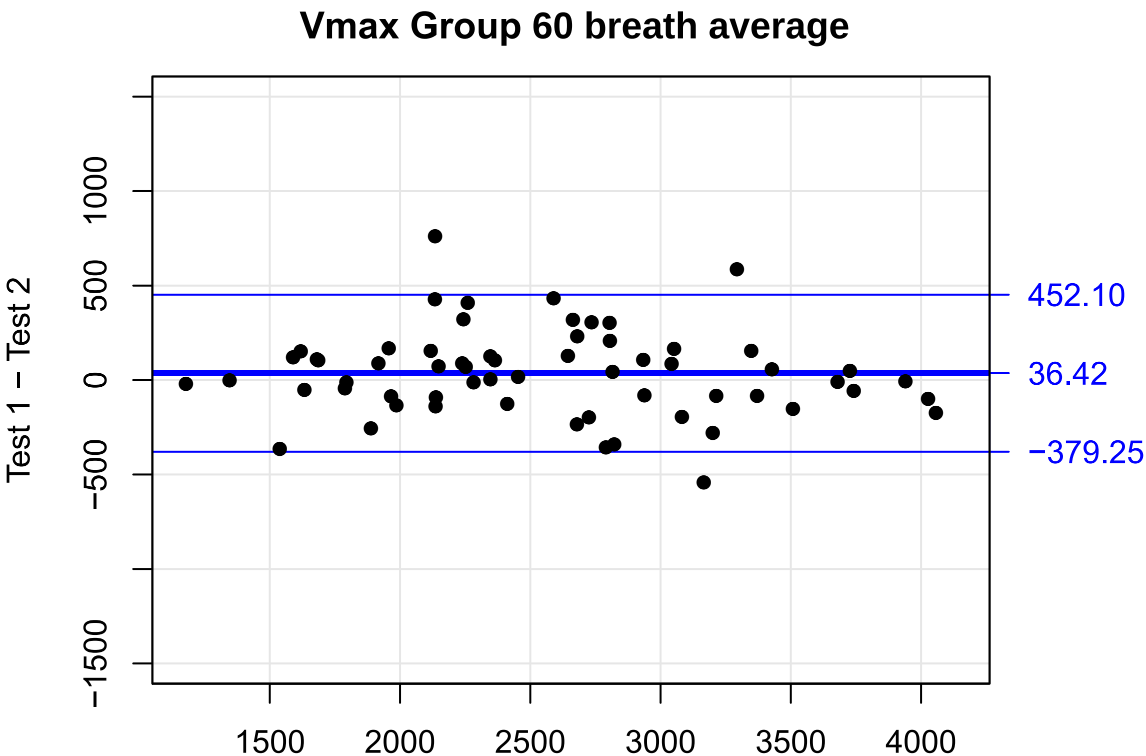


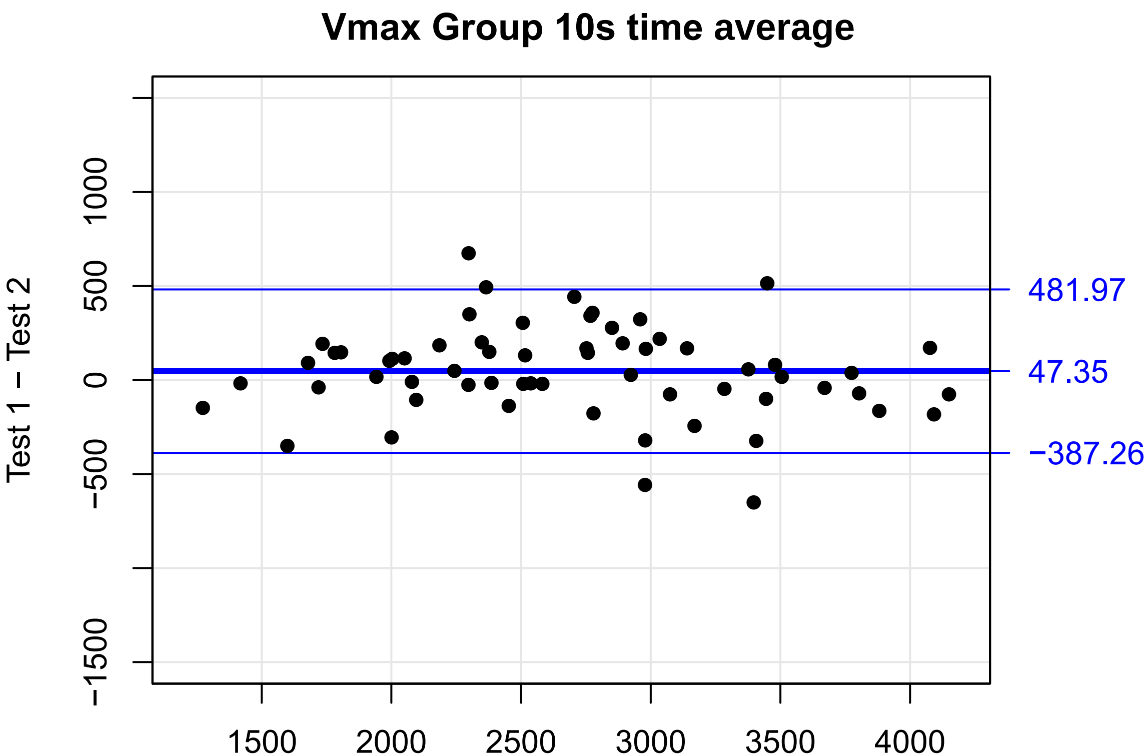
Vmax Group 20 breath average
Test 1 − Test 2
1000
500
0
−500
−1500
1500
2000
2500
3000
3500
4000
469.43
40.37
−388.69


Vmax Group 30 breath average
Test 1 − Test 2
1000
500
0
−500
−1500
1500
2000
2500
3000
3500
4000
465.65
40.73
−384.18

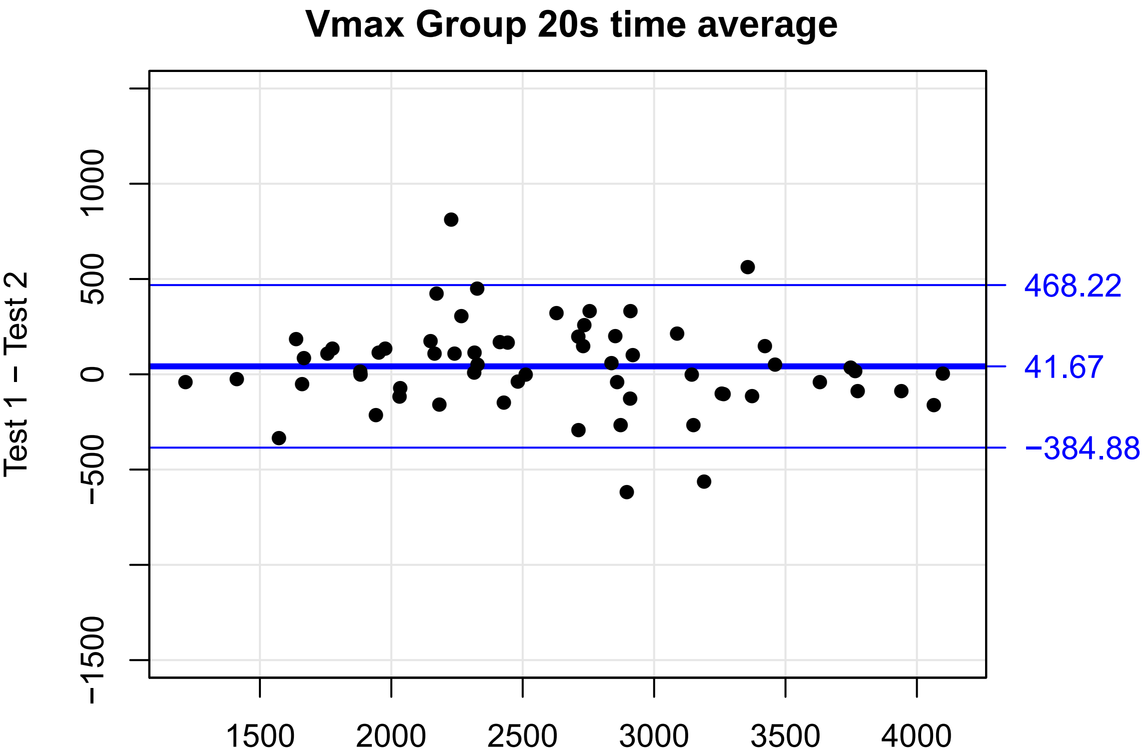

Vmax Group 40 breath average
Test 1 − Test 2
1000
500
0
−500
−1500
1500
2000
2500
3000
3500
4000
462.50
39.54
−383.43

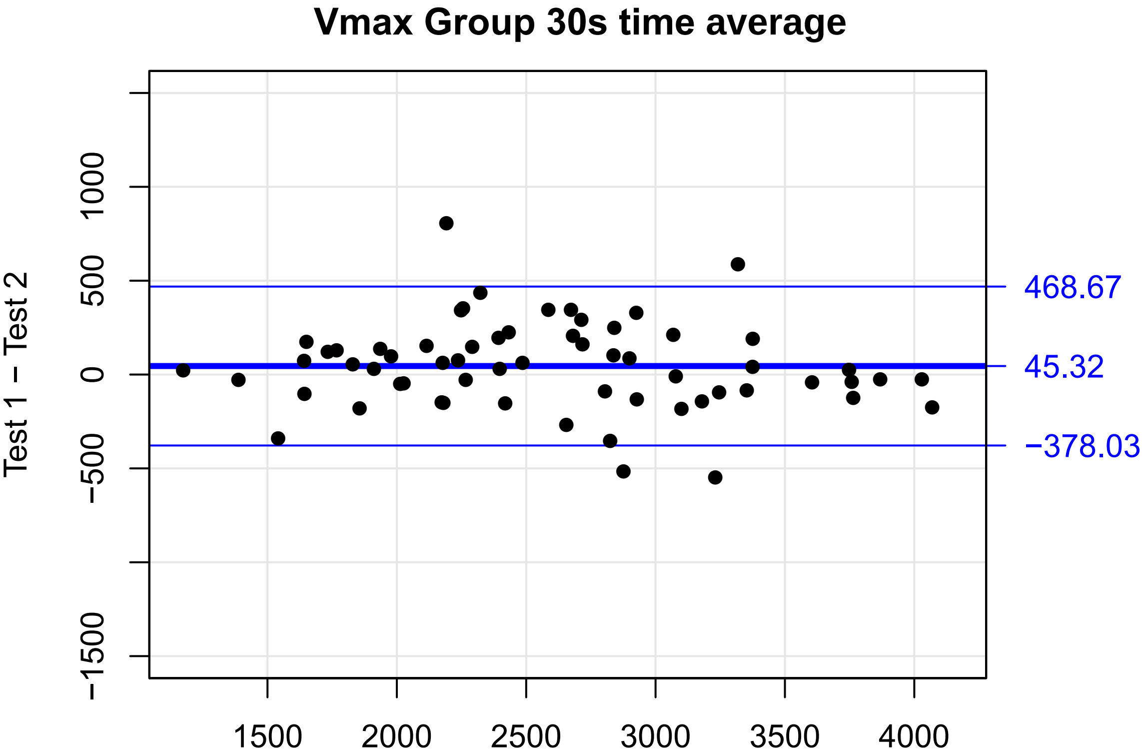

Vmax Group 50 breath average
Test 1 − Test 2
1000
500
0
−500
−1500
1500
2000
2500
3000
3500
4000
457.34
37.66
−382.02

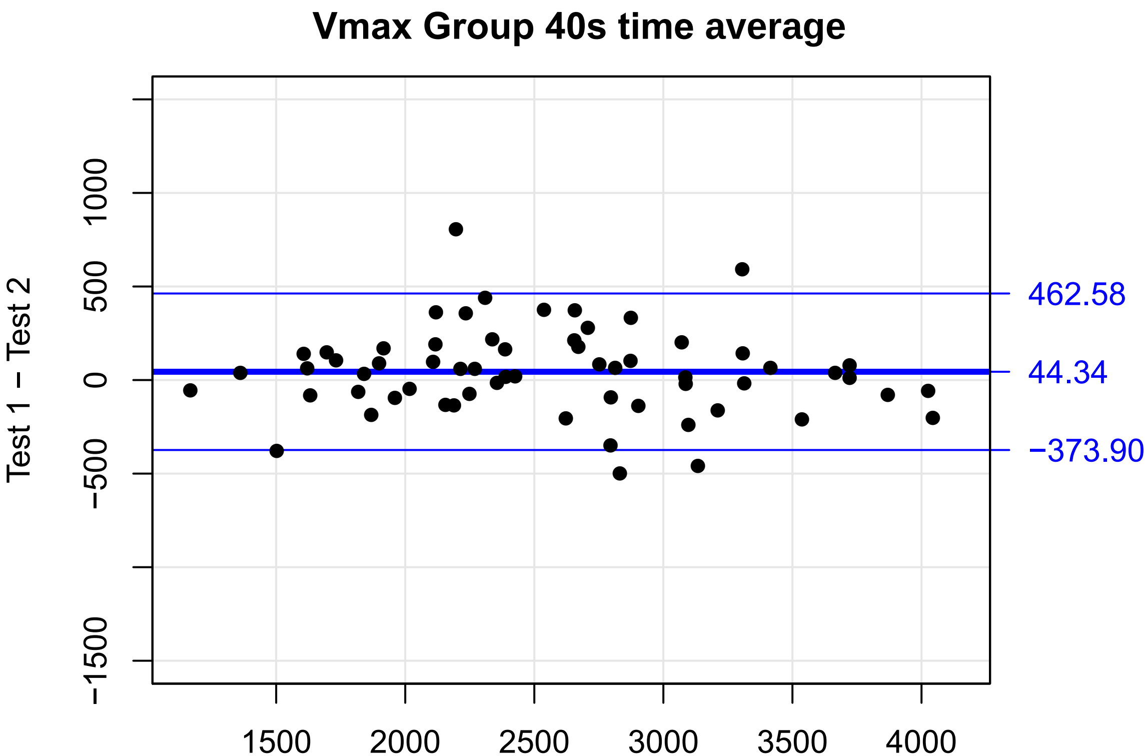

Vmax Group 60 breath average
Test 1 − Test 2
1000
500
0
−500
−1500
1500
2000
2500
3000
3500
4000
452.10
36.42
−379.25

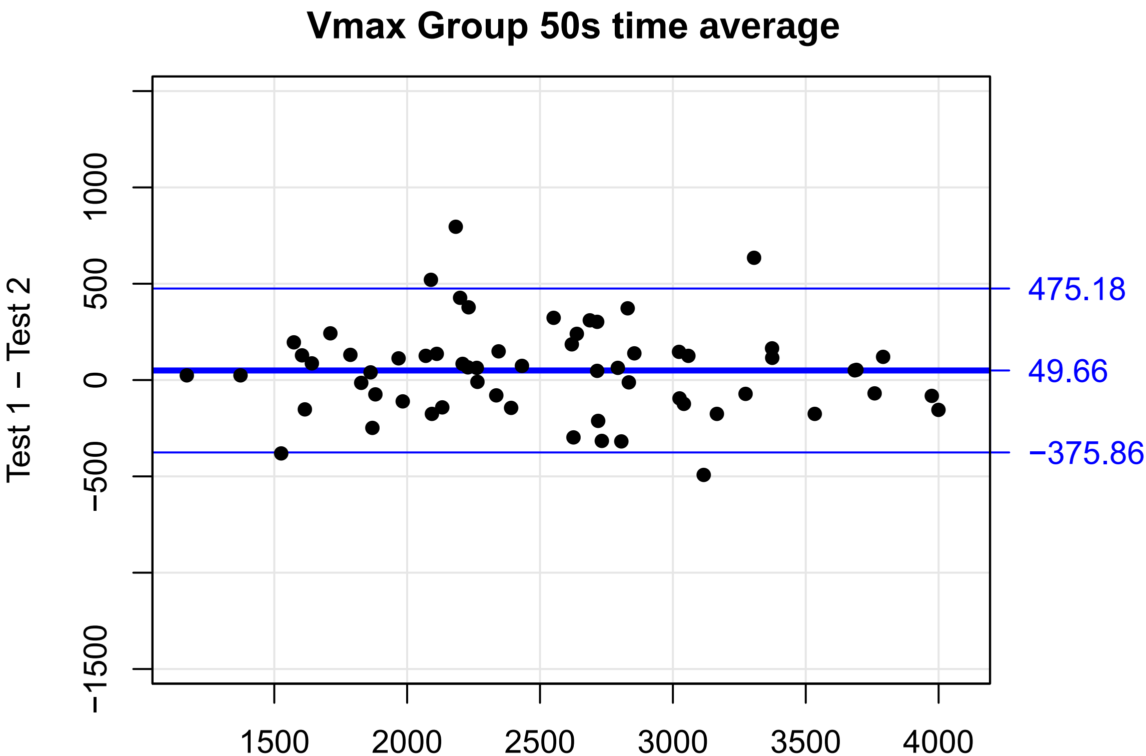


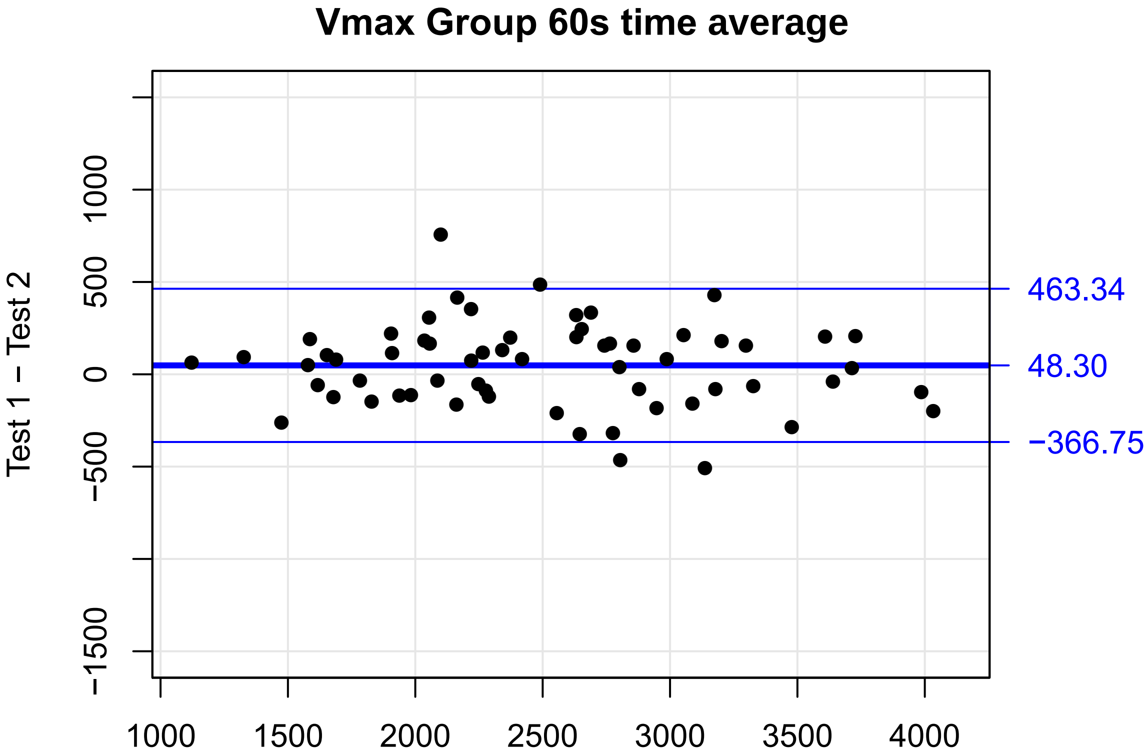
Vmax Group 10s time average
Test 1 − Test 2
1000
500
0
−500
−1500
1500
2000
2500
3000
3500
4000
481.97
47.35
−387.26

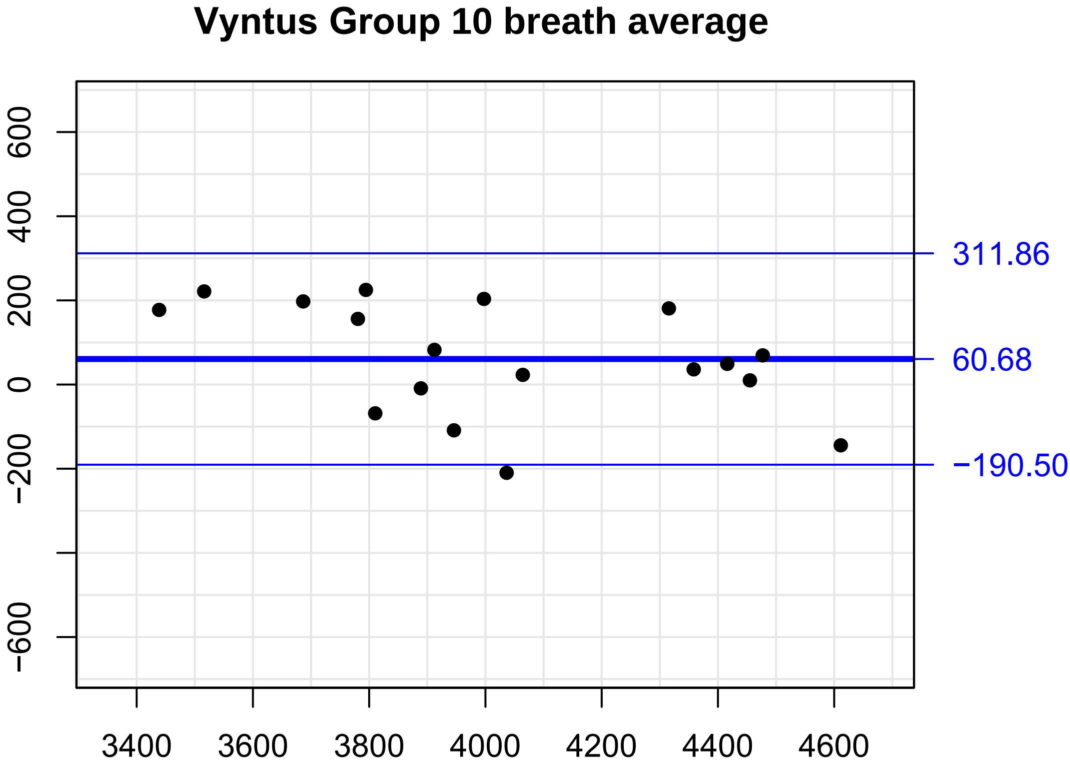

Vmax Group 20s time average
Test 1 − Test 2
1000
500
0
−500
−1500
1500
2000
2500
3000
3500
4000
468.22
41.67
−384.88


Vmax Group 30s time average
Test 1 − Test 2
1000
500
0
−500
−1500
1500
2000
2500
3000
3500
4000
468.67
45.32
−378.03

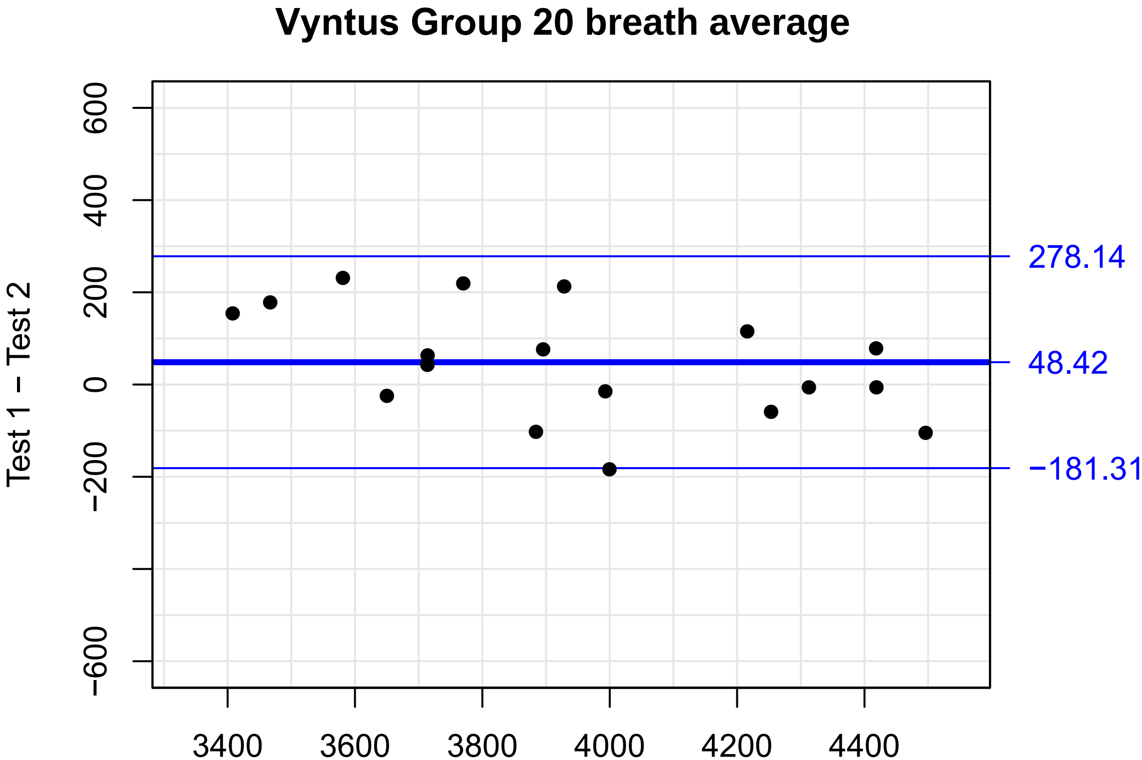

Vmax Group 40s time average
Test 1 − Test 2
1000
500
0
−500
−1500
1500
2000
2500
3000
3500
4000
462.58
44.34
−373.90


Vmax Group 50s time average
Test 1 − Test 2
1000
500
0
−500
−1500
1500
2000
2500
3000
3500
4000
475.18
49.66
−375.86

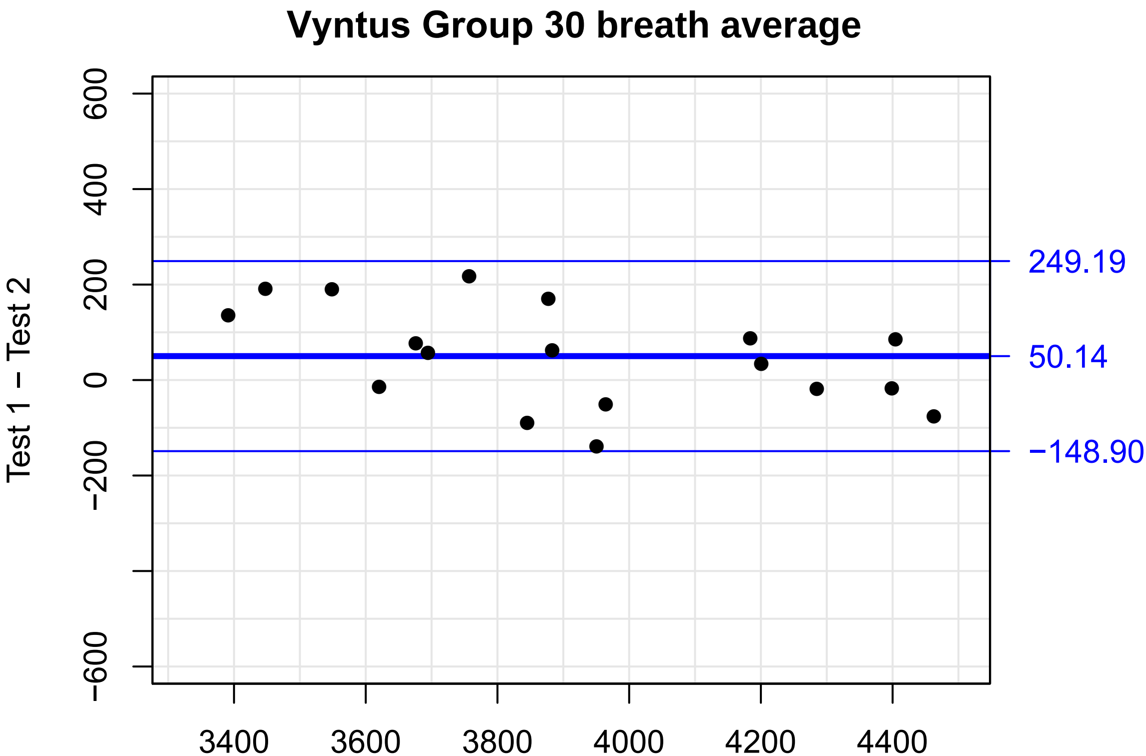

Vmax Group 60s time average
Test 1 − Test 2
1000
500
0
−500
−1500
1000
1500
2000
2500
3000
3500
4000
463.34
48.30
−366.75



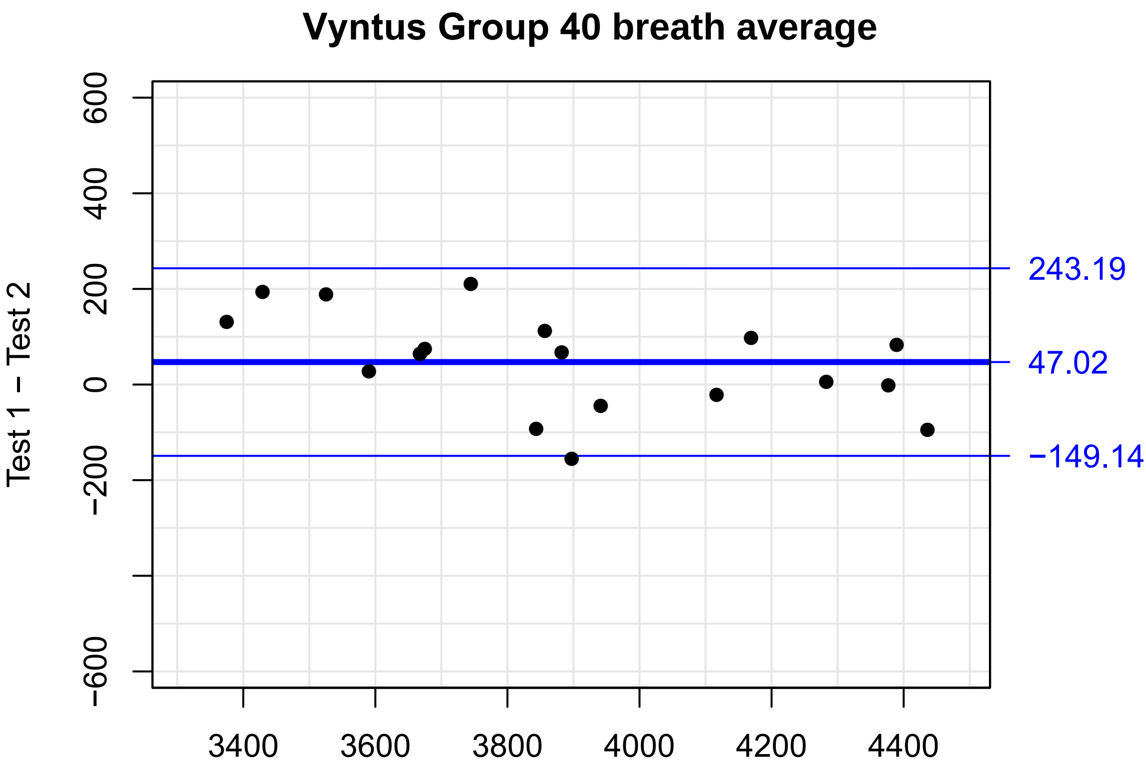
Vyntus Group 10 breath average
600
400
200
0
−200
−600
3400
3600
3800
4000
4200
4400
4600
311.86
60.68
−190.50

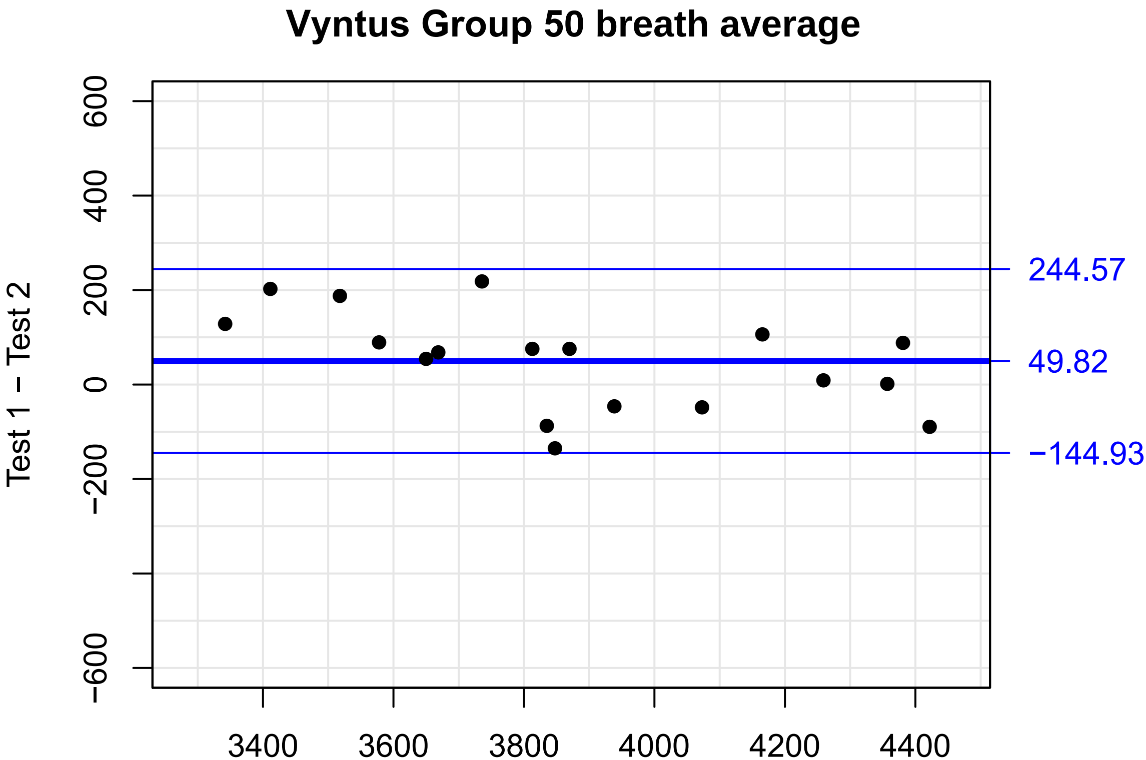


Vyntus Group 20 breath average
Test 1 − Test 2
600
400
200
0
−200
−600
278.14
48.42
−181.31
3400
3600
3800
4000
4200
4400

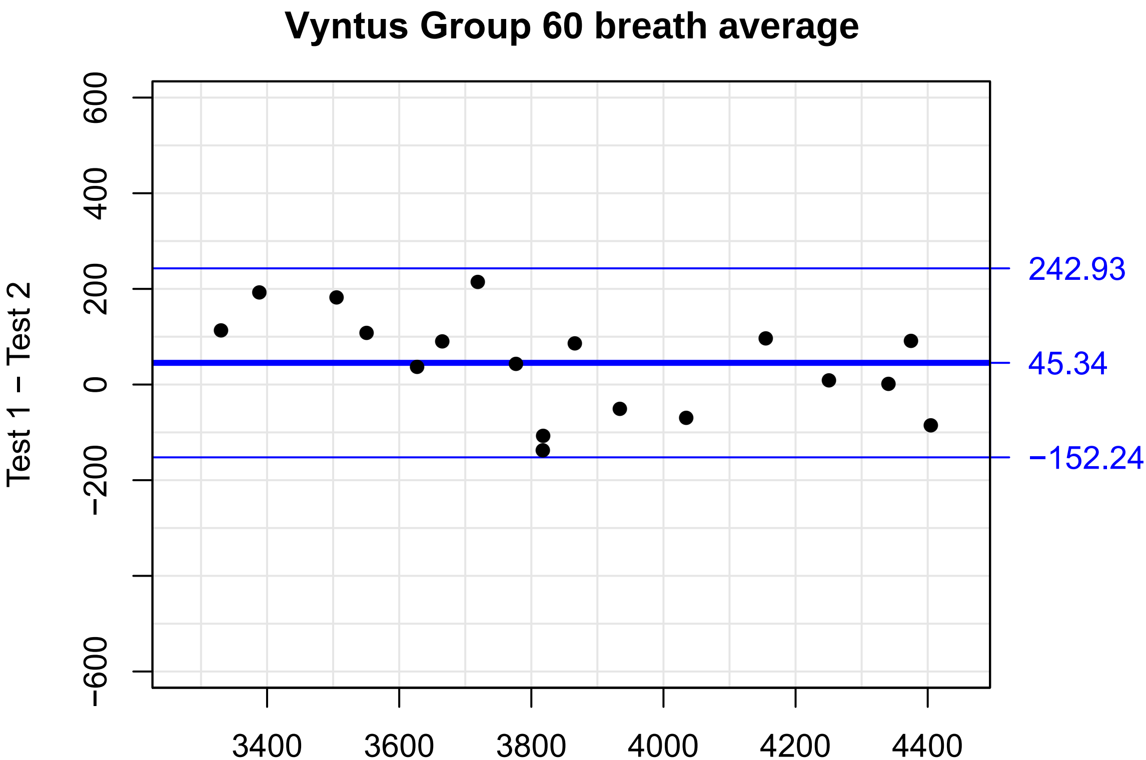


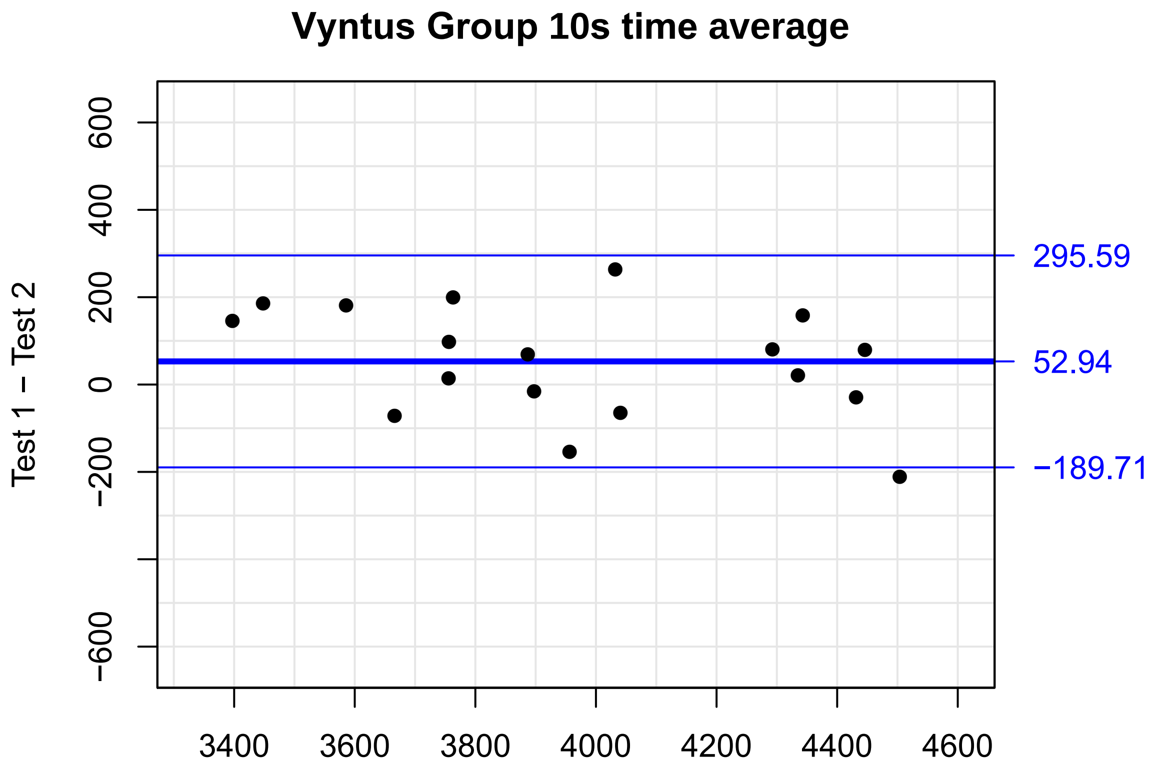
Vyntus Group 30 breath average
Test 1 − Test 2
600
400
200
0
−200
−600
3400
3600
3800
4000
4200
4400
249.19
50.14
−148.90

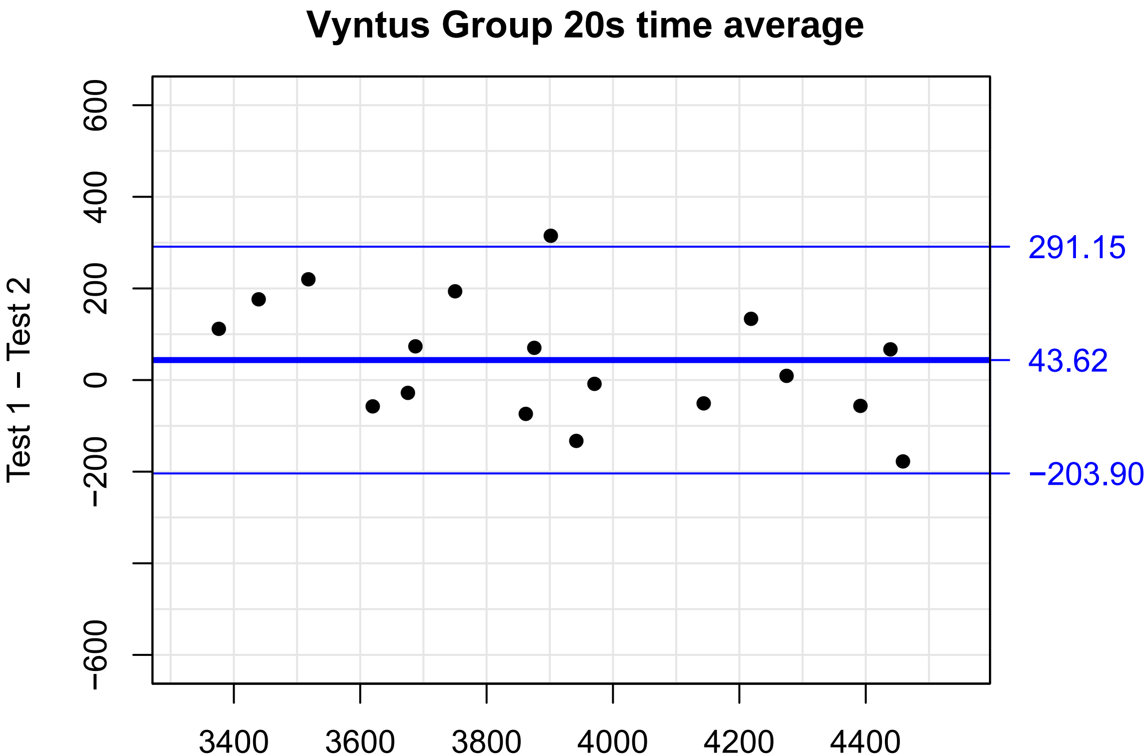

Vyntus Group 40 breath average
Test 1 − Test 2
600
400
200
0
−200
−600
3400
3600
3800
4000
4200
4400
243.19
47.02
−149.14

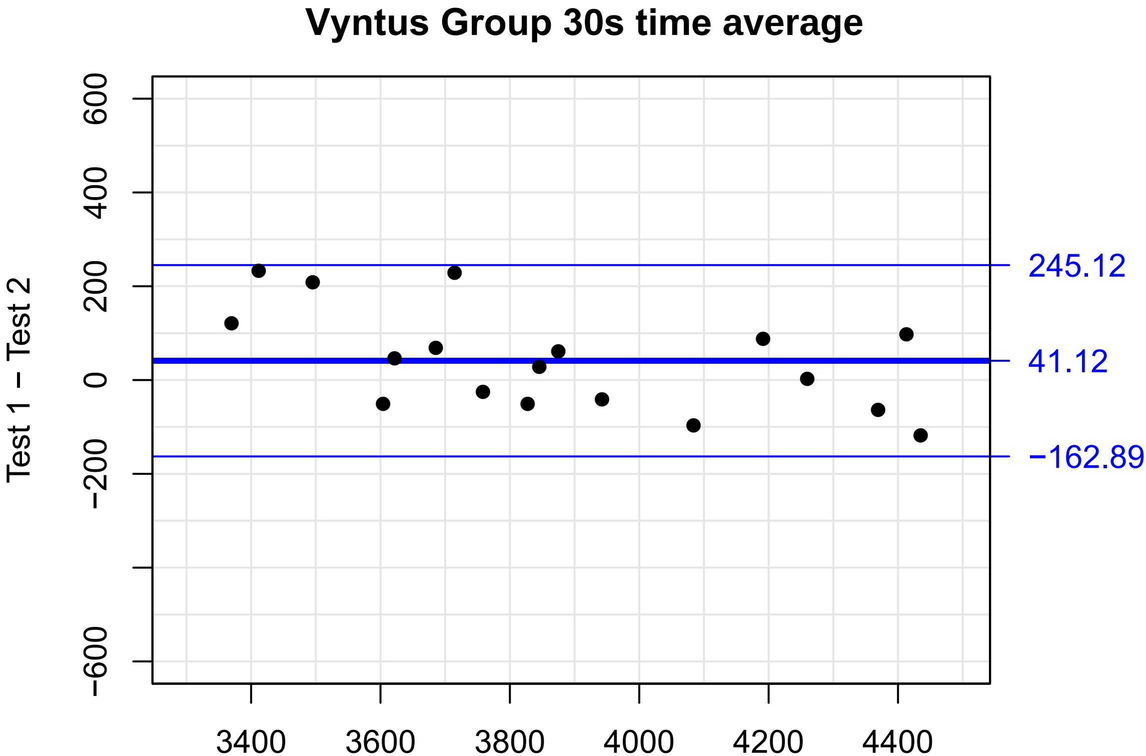


Vyntus Group 50 breath average
Test 1 − Test 2
600
400
200
0
−200
−600
3400
3600
3800
4000
4200
4400
244.57
49.82
−144.93

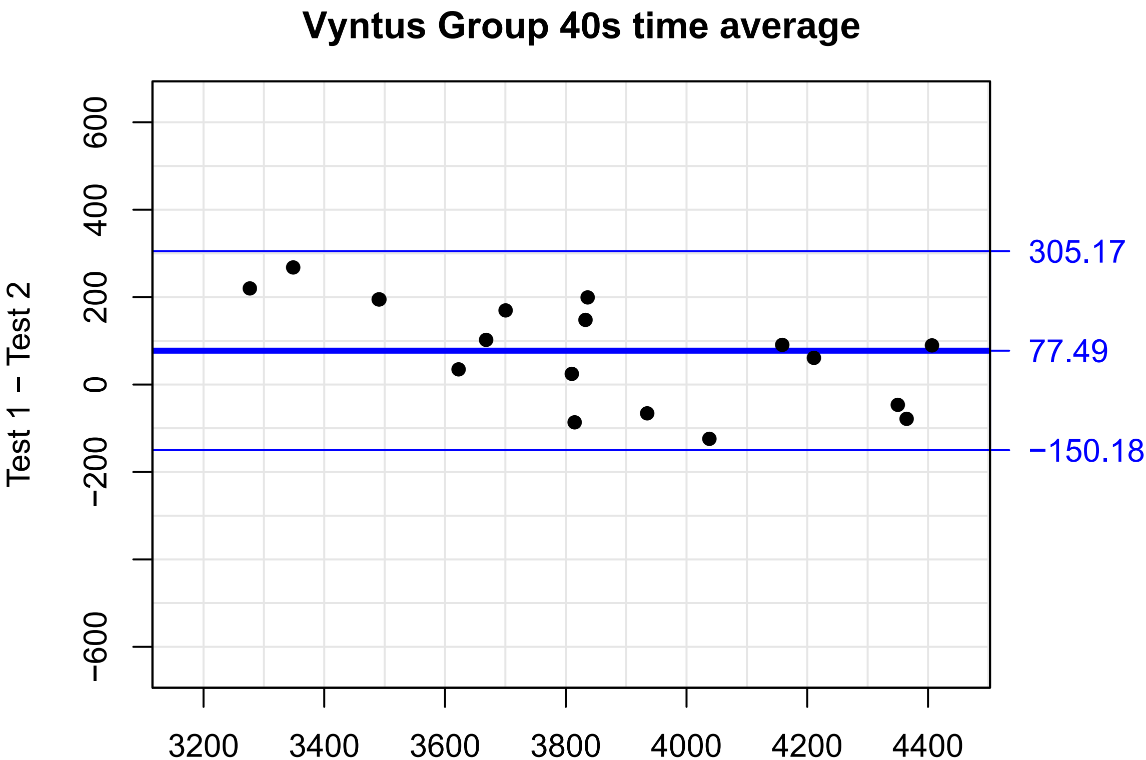

Vyntus Group 60 breath average
Test 1 − Test 2
600
400
200
0
−200
−600
3400
3600
3800
4000
4200
4400
242.93
45.34
−152.24


Vyntus Group 10s time average
Test 1 − Test 2
600
400
200
0
−200
−600
3400
3600
3800
4000
4200
4400
4600
295.59
52.94
−189.71

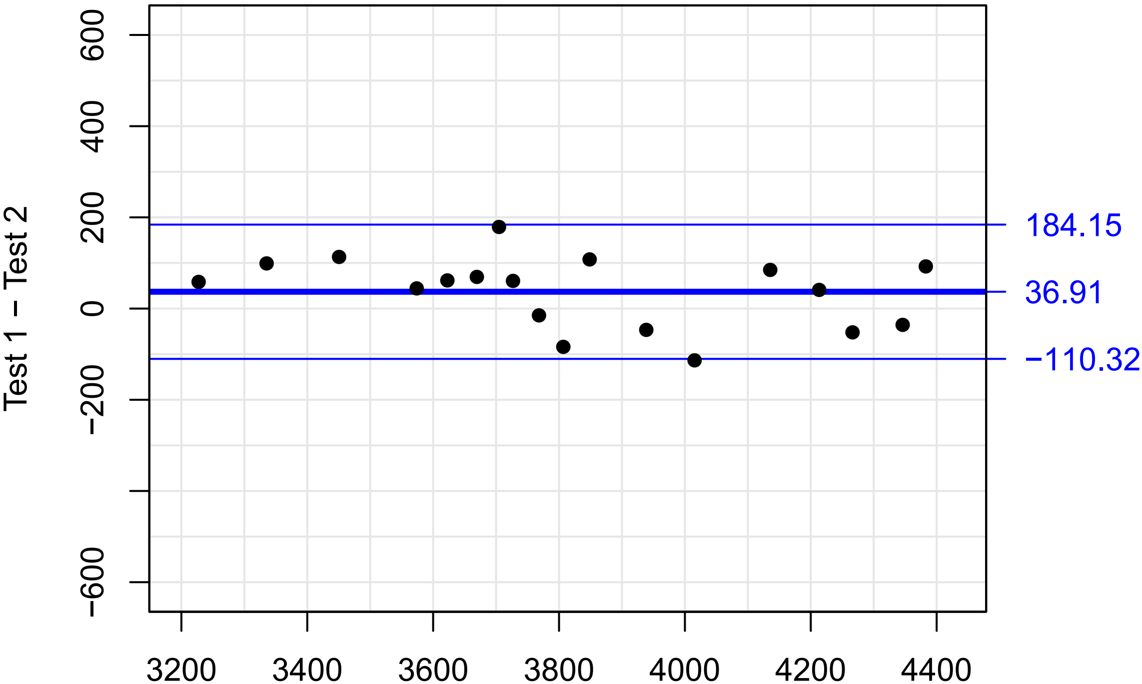

Vyntus Group 20s time average
Test 1 − Test 2
600
400
200
0
−200
−600
3400
3600
3800
4000
4200
4400
291.15
43.62
−203.90

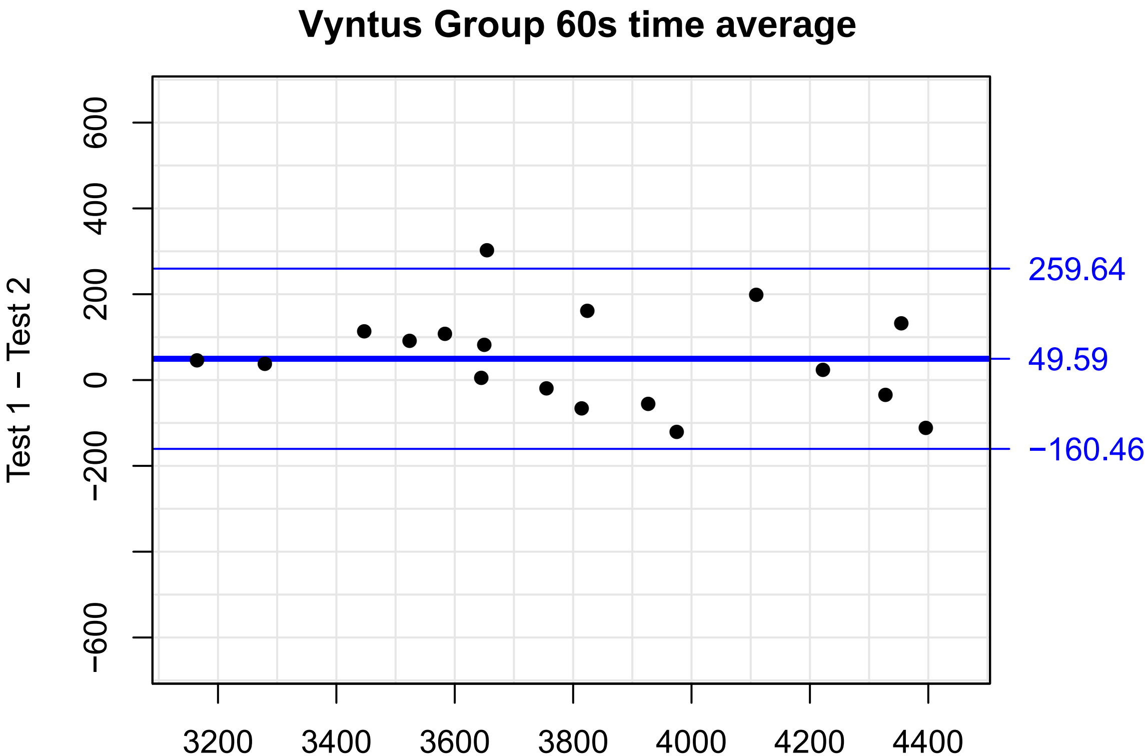


Vyntus Group 30s time average
Test 1 − Test 2
600
400
200
0
−200
−600
3400
3600
3800
4000
4200
4400
245.12
41.12
−162.89


Vyntus Group 40s time average
Test 1 − Test 2
600
400
200
0
−200
−600
3200
3400
3600
3800
4000
4200
4400
305.17
77.49
−150.18

Vyntus Group 50s time average




Vyntus Group 60s time average
Test 1 − Test 2
600
400
200
0
−200
−600
259.64
49.59
−160.46
3200
3400
3600
3800
4000
4200
4400