Peer Review File

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Reviewer A

This article entitled "Intercept of Minute Ventilation versus Carbon Dioxide Output Relationship as anIndex of Ventilatory Inefficiency in Chronic Obstructive Pulmonary Disease"is an important issue in clinical assessment of patients with COPD with cardiopulmonary exercise testing. The article was well written by the author(s). The main findings of the study were that VE/VCO2 intercept (VEVCO2I) was more frequently and powerfully correlated with static and dynamic lung hyperinflation than VE/VCO2 nadir (VEVCO2N) and VE/VCO2 slope (VEVCO2S) across a spectrum of severity in COPD. The study successfully showed the possibility of clinical utility of VEVCO2I. Although it was well presented, some points are still needed to be clarified.

L107-108. Please consider isocapneic buffering occurred before the respiratory compensation point. Please see Wasserman's book.

R: Thank you for your comment. When the lactic acidosis first develops, both $\dot{V}_E$ and $\dot{V}CO_2$ increase curvilinearly, resulting in no further change in $\dot{V}_E/\dot{V}CO_2$ and $P_{ET}CO_2$. (isocapnic buffering), $\dot{V}_E$ retains a linear relation with $\dot{V}CO_2$ ($\dot{V}_E/\dot{V}CO_2$ is constant or decreases slightly). But after the isocapnic buffering period, $\dot{V}_E$ increases more rapidly than $\dot{V}CO_2$ (increase in $\dot{V}_E/\dot{V}CO_2$). This is the ventilatory compensation point (VCP). -- Wasserman's book (P.30-32, Fig.2.30). We have modified our text as you advised (see Page 5, Line 106-107).

L117. "The" should be "the".

R: Thank you. You are totally right. Please see Page 6, Line 117.

L125-130. It is not easy to read here. Perhaps, it is easier to read if the equation of VD/VT = 1 – (.863*VCO2/VE)/PaCO2 is provided. Herein, VD/VT is a ratio where VT is tidal volume but not total ventilation. Please clarify.

R: Thank you for your comment. $\dot{V}E = 863*\dot{V}CO2 / (PaCO2*(1- VD/VT))$. Not all respired air ventilates the lung effectively because some must ventilate the conducting airways, and some ventilation nonperfused or underperfused alveoli. Uneven ventilation will result in an increase in VD/VT and $\dot{V}E$ to clear a given volume of CO2 from lung. We have modified our text as you advised (see Page 6, Line 125-129).

L137-9. Dynamic LH has been reported by Tiopompi and others. (Respir Physiol Neurobiol. 2014;197:9–14). Static LH has not been reported.

R: Thank you for your comment. We have modified our text as you advised (see Page 7, Line 139-141).

L170. How many patients repeated the CPET because their peak work rate > = 200 watts? Please clarify. There were some reports on the selection of ramp slope in the literature. By these methods, the exercise duration was 10 +/- 2 min. Please report the exercise duration of your study. The duration of exercise is a key factor affecting VEVCO2I, S and N as it was mentioned in L110-114 and also reported in the literature.

R: Thank you for your comment. You are absolutely right. The duration of exercise is a key factor affecting the $\dot{V}E/\dot{V}CO2$ intercept, slope and nadir. We reported the data as you advised (see Page 11, Line 225-227).

L182. Please clarify that peak VE/VCO2 is not equal to VE/VCO2 @ peak exercise (the last 30 s).

R: Thank you for your comment. In this study, the peak $\dot{V}_E/\dot{V}CO_2$ by averaging the consecutive 30 s data points at the peak time. We have modified our text as you advised (see Page 9, Line 183,185-186).

L185. The data points for linear regression were those looked like linear by eyes. Were these data involved data at rest and during unloaded exercise? If yes, the results may be different. Some studies involved the data during loaded exercise (Tabet 2003). Will the different methods cause different results?

R: Thank you for your comment. These data point for linear regression were not involved in the date at rest and unloaded time. In this study, the data for linear regression was involved the beginning exercise data to the point just before ventilatory compensation point (VCP). We have modified our text as you advised (see Page 9, Line 189).

It is important to recognize that the $\dot{V}_E/\dot{V}CO_2$ response contour is intrinsically linked to how $\dot{V}_E$ dynamically changes in relation to $\dot{V}CO_2$ taking into consideration its starting point (Forster HV, Adv Exp Med Biol 1988; 227:257–276; Poon C-S Respir Physiol Neurobiol. 2013; 186: 114–130; Ward SA. Respir Physiol Neurobiol 2013; 189: 203–210). The former is reflected by the slope and the latter by its intercept. It should be noted that considering all data points (i.e. including those after the VCP) will necessarily increase the computed slope and reduce the computed intercept. It

will not only underestimates ventilatory efficiency but it also does not
accurately describe the underlying response profile. As mentioned, however, most patients with moderate-to-severe COPD are unable to exercise beyond the RCP. In other words, there is no upward inflection in the V̇E versus V̇CO2 response in most of these patients. Thus, in practice, drawing a single line from unloaded to peak exercise fits well the overall $\dot{V}_E$ response in this particular sub-group of patients (Neder JA, Eur Respir J. 2015; 45: 377–387).

L187. "," is correct here? VT/TLC @ peak exercise … Please cite the original article.
R: Thank you for your comment. You are absolutely right. We have cited the original article (see Page 9, Line 191-194).

L195-197. What data were missed so that you used multivariate imputations? Please clarify.
R: Thank you for your comment. No data was missed in this study. We have modified our text as you advised (see Page 10, Line 200-202).
(This study was supported by the Capital's Funds for Health Improvement and Research. In this project, we used multivariate imputations for missed data.)

L203. Why peak VE/MVV and peak VO2/W represent ventilatory capacity?
R: Thank you for your comment. The breathing reserve is either expressed as the difference between the MVV and the exercise ventilation in absolute terms or this difference as a fraction of the MVV -- Wasserman's book (P.88, Fig.4.13). $\dot{V}O_2$-work rate relationship describes how much O_2 is utilized by exercise subject in relation to the quantity of external work performed. It gives important information concerning the external to internal respiration. We use those parameters represent exercise capacity. We have modified our text as you advised (see Page 10, Line 208).

L224. VE/MVV was not reduced but was increased. Please check up.
R: Thank you for your comment. You are absolutely right. We have modified our text as you advised (see Page 11, Line 230, 232).

L230-231. Suggest move to L219.
R: Thank you for your comment. We have modified our text as you advised (see Page 11, Line 224, Line 241-242).

L238-241. The data cannot be found in Table 2. It seems not easy to read.

R: Thank you for your comment. You are absolutely right. We have modified our text as you advised (see Table 2 Page 30).

L243-244. Suggest present the data in Table 3. Also, present the correlations of VEVCO2S and VEVCO2N with VO2/watt and VE/MVV at peak exercise. (Please note "peak" data sometimes do not occur "@ peak exercise"). Suggest move "Fig. 1……..." to where Figure legends (figure 1) were and move figure legends (figure 1) here. Suggest Delete Figure 2. Suggest put the data of figure 2 in Table 3.

R: Thank you for your comment. We have modified our text as you advised (see Table 2, 3. Page 30-32). Fig. 2 showed the intercept could correlate to exercise capacity. Would you please not to delete it?

L249. Suggest insert L260-261 in L249.

R: Thank you for your comment. We have modified our text as you advised (see Page 12, Line 260-261).

L251-254. Suggest insert Table 3 before r=0.574. Why RV% r=.571 and =.383 not found in Table 3?

R: Thank you for your comment. We have modified our text as you advised (see Page 12, Line 264. Table 3 Page 31).

L261. Suggest delete figure 3.

R: Thank you for your comment. We have modified our text as you advised (see Page 13, Line 274-276.Fig. 3. Page 27-28, Line 551-559.)

Discussion

General considerations:

It is a bit confusing when VEVCO2S, I, and N were presented in a mixed manner and even worse after they were discussed with previous reports. Suggest discuss your data about VEVCO2S, I, and N with those reported in the literature, separately, based on what the merits and demerits of these variables are.

L270-1. The statement here is not clear and not relevant to ref 23.

R: Thank you for your comment. You are absolutely right. We have modified our text as you advised (see Page 13, Line 305-306).

L273-275. Please briefly explain or comment or discuss why VEVCO2N was better than VEVCO2S in a repeated test study in ref 24. In L110-4, it seems not in line with this notion.

R: Thank you for your comment. As the lactate threshold may not always be identified, particularly in clinical populations with low exercise capacity (Zacarias EC, J Cardpulm Rehabil. 2000; 20: 369–376. Agostoni P. Circ Heart Fail. 2013; 6: 977–987), the V̇E/V̇CO2 nadir seems a more accurate indication of ventilatory efficiency than the V̇E/V̇CO2 at the lactate threshold. The V̇E/V̇CO2 nadir has been found to be highly reproducible in patients with COPD (ref. 24 Barron A. European J Prev.Cardio. 2014; 21(4):445-53). In this study, each patient underwent two bicycle ergometer tests; the first, a familiarization test, with a 10 W/min ramp, and the second a personalized ramp based on the results of the familiarization test to elicit maximal effort within 8–10 min. However, the V̇E/V̇CO2 nadir, might underestimate ventilatory efficiency if the V̇E/V̇CO2 descending curve is prematurely interrupted by lactic acidosis or an excessively short test duration (Neder JA. Am J Respir Crit Care Med 2001; 164: 1481–1486). We have modified our text as you advised (see Page 13, Line 305-306).

L275-276.The notion does not just come from ref 25 and 26 only. Also, reported in Neder 2015, Teopompi 2014, 2014, Chuang 2020.

R: Thank you for your comment. You are absolutely right. We have modified our text as you advised (see Page 13, Line 306-308).

L276-9. Need ref. Suggest review thoroughly and cite correctly. Indeed, there are some reports on this notion. Also, you mentioned it in L272-273. Suggest assort your statement.

R: Thank you for your comment. You are absolutely right. We have modified our text as you advised (see Page 14, Line 311-321).

L283-4. Not easy to understand.

R: Thank you for your comment. We have modified our text as you advised (see Page 15, Line 331-334).

L297. You mentioned emphysema here. In Teopompi's study, VTpeak/FEV1 was considered as emphysema factor. Please discuss about the variable. Suggest add on the variable.

R: Thank you for your comment. We added the peak V_T/FEV_1 in present study. We have modified our text as you advised (see Page 16, Line 354-358).

L318-322. IC/TLC is a reverse variable of FRC/TLC (i.e. IC/TLC + FRC/TLC =100%). FRC/TLC, RV/TLC, RV%, and FRV% are highly related. Thus, VEVCO2I vs. IC/TLC (r=-0.574) is close to VEVCO2I vs. RV/TLC (r=0.588) and VEVCO2I vs. RV% (r=0.571).

Lastly, please discuss peak VO2/W and peak VE/MVV as you also showed these data.

R: Thank you for your comment. We have modified our text as you advised (see Page 15, Line 346-351; Page 17, Line 378-381).

Study limitation

L342. How many female subjects in each group? Were the proportions of female subjects similar across the three groups?

R: Thank you for your comment. There were 2 female subjects in control, 5 female in $COPD_{1\text{-}2stages}$ and 3 female patients in $COPD_{3\text{-}4stages}$. The three groups were well matched in proportions of female. We have modified our text as you advised (see Page 18, Line 402-404).

L347. Please show the duration of CPET in your study.

R: Thank you for your comment. Ranges of exercise duration in current study were found from 6 to 10 min (see Page 11, Line 248-249).

1. Why not correlated VEVCO2S, I, and N with dyspnea?

R: Thank you for your comment. We did not correlated the $\dot{V}_E/\dot{V}CO_2$ slope, nadir and intercept with dyspnea for some subjects in control stopping exercise for leg fatigue (see Page 18, Line 404-405).

2. Does VD/VT (and PETCO2peak-rest) affect VEVCO2S, I, and N?

R: Thank you for your comment. $\dot{V}_E/\dot{V}CO_2 = 1/\{P_aCO_2*(1-V_D/V_T)\}$. V_D/V_T decrease in a curvilinear manner as exercise progresses. $\dot{V}E/\dot{V}CO2$ decreases in direct proportion to V_D/V_T. Thus, PaCO2 is kept constant (↔) during mild-to-moderate exercise in healthy humans. (Forster HV Compr Physiol 2012; 2:743–777. Whipp BJ. Am Rev Respir Dis 1984; 129: S17–S20). In the healthy, P_aCO_2 is approximately 2mm Hg greater than $P_{ET}CO_2$. However, $P_{ET}CO_2$ increases relative to P_aCO_2 during exercise. See Wasserman's book (P.91-92, Fig.4.19). $\dot{V}_E/\dot{V}CO_2$ intercept were also directly related to the $P_{ET}CO_2$peak-rest values, which may be considered as an estimate of ventilatory limitation to exercise (Stickland et al., 2012; Thirapatarapong et al., 2013).

3. What is the mechanism that VEVCO2S is lower in the COPD patients with more advanced stages compared to the earlier stages? Are there any reasons other than flow limitation? Can the VD

combined dLH affect VEVCO2S, I, and N?

R: Thank you for your comment. Lower $\dot{V}_E/\dot{V}CO_2$ slopes in more advanced disease, were explained by worsening mechanical constraints (Paoletti P. Respir Physiol Neurobiol 2011; 179: 167–173. Teopompi E. Respir Physiol Neurobiol 2014; 197: 9–14). and, another explaination probably, an increase in carbon dioxide set-point (Oren A. J Appl Physiol Respir Environ Exerc Physiol 1981; 51: 185–189). Paolotti et al. [12] proposed another two hypotheses: (1) an improvement in ventilatory efficiency during exercise due to reduced physiological dead space; (2) a higher arterial CO2

(PaCO2) set-point, as they found that the hypercapnia was related to emphysema. Chuang ML reported the relationship between peak V_D/V_T and $\dot{V}_E/\dot{V}CO_2$ slope and intercept (Chuang ML. Respir Res 2020) (see Page 18, Line 406-407, Line 413-415).

4. Why the sum of VEVCO2S and VEVCO2I equals or mimics VEVCO2N?

R: Thank you for your comment. The relationship between the sum of $\dot{V}_E/\dot{V}CO_2$ slope and $\dot{V}_E/\dot{V}CO_2$ intercept and $\dot{V}_E/\dot{V}CO_2$ nadir has been reported to be mathematical--See Wasserman's book (P.93, Fig.4.21). Neder reported the nadir–slope differences not equal to the intercept, particularly in more advanced COPD. These data indicate that too short a test resulted in $\dot{V}_E/\dot{V}CO_2$nadir overestimating the slope in these patients (Neder JA.et al. Eur Respir J 2015; 45: 377–387).

Conclusions

L351-352. How to draw the conclusion here by the data reported?

R: Thank you for your comment. You are absolutely right. We have modified our text as you advised (see Page 18-19, Line 418-421).

Figure legends

L517. C. ……. decreased in $COPD_{stages3-4}$ or looks similar compared to control?

Suggest delete figures 2 and 3 and move the data to Table 3.

R: Thank you for your comment. Compared to control (24.75±3.07), the slope was increased in $COPD_{stages1-2}$ (30.58±3.62) and decreased in $COPD_{stages3-4}$ (26.84±4.96) (see Page 24, Line 543-544).

Fig. 2 showed the intercept could correlate to exercise capacity. Would you please not to delete it?

We have deleted figures 3 and move the data to Table 3 (see Page 25-26, Line 553-560).

L548-9. In Table 1, you may delete FEV1 (L), IC (L) lines as lung volumes are closely related to body size. Usually, % predicted is a sufficient way to present. Please keep either one of IC/TLC and FRC/TLC lines as IC/TLC + FRC/TLC = 100%

R: Thank you for your comment. You are absolutely right. We have modified our text as you advised (see Page27, Table 1).

L557-558. Table 2. For a better comparison to VEVCO2 at peak exercise, please insert the data of VEVCO2S, I, and N beneath VEVCO2 and give a footnote to describe S, I, and N.

R: Thank you for your comment. We have modified our text as you advised (see Page28-29, Table 2).

L565-568.Table 3. As aforementioned.

R: Thank you for your comment. We have modified our text as you advised (see Page29-30, Table 3).

Although the authors revised the manuscript according to the reviewer's comments, there are some points not clear which needs to be clarified.

General comments:

The line numbers along the text were not provided and thus it is very difficult to review and may cause confusion when communications between the reviewer and the authors.

Specific comments:

L108 isocapnic buffering. It is clear in the authors' response whereas not clear in the revised text. Please clarify.

R: Thank you for your comment. When the lactic acidosis first develops, at the isocapnic buffering period, $\dot{V}_E$ retains a linear relation with $\dot{V}CO_2$ ($\dot{V}_E/\dot{V}CO_2$ is constant or decreases slightly). But after that, $\dot{V}_E$ increases more rapidly than $\dot{V}CO_2$ to compensate for lactic acidosis (increase in $\dot{V}_E/\dot{V}CO_2$). We have modified our text as you advised (see Page 5, Line 106-108).

L129. Two periods after "…..- VD/VT))"

R: Thank you for your comment. We have modified our text as you advised (see Page 6, Line 130).

L206. Peak VE/MVV is not appropriate for representing exercise capacity; however, it may be suitable for dyspnea index or use "Peak VE/MVV" directly. Like in lines 224-225, "Peak exercise capacity"…… that the authors used peak exercise capacity. It is clear to represent VO2/watt but should not be peak VE/MVV. Please clarify.

Peak VO2/watt is ok for exercise capacity (like in Line 259) although exercise capacity is usually used as VO2% predicted.

R: Thank you for your comment. You are absolutely right. The peak $\dot{V}_E$/MVV expressed as the difference between the MVV and the exercise ventilation as a fraction at peak. It may be suitable for dyspnea index. $\dot{V}O_2$-work rate relationship describes how much O_2 is utilized by exercise subject in relation to the quantity of external work performed. Peak VO2/watt is an index for exercise capacity. We have modified our text as you advised (see Page 10, Line 211-212).

L216-7. IC/TLC + FRC/TLC = 100%. Please use one of them and that will be OK.

R: Thank you for your comment. You are absolutely right. We have modified our text as you advised (see Page 11, Line 223).

L295. "()." before "A lower slope, stable……."

R: Thank you for your comment. I am a little bit confused. It doesn't seem to need "()." before "A lower slope, stable …" from context. (see Page 15, Line 316).

L297 or L316. Please discuss the differences between linear regression for the data involving and not involving after RCP (respiratory compensation point). In the current study, the correlations of VE/VCO2 slope with the variables in Table 3 were poor except peak VT/FEV1 whereas Neder et al reported that VE/VCO2S was correlated with EELVpeak/TLC in patients with GOLD stage 2-4 (r= 0.48-0.60, Neder, 2015).

R: Thank you for your comment. It should be recognized that including the data points after the RCP will necessarily increase the computed slope and decrease the computed intercept. Furthermore, including the data points after the RCP does not accurately reflect $\dot{V}_E$ vs. $\dot{V}CO_2$ plot profile. In present study, we excluded the data above the RCP for linear regression to calculate the slope and intercept. We have modified our text as you advised (see Page 14, Line 299-304).

Neder et al reported that $\dot{V}_E/\dot{V}CO_2$ slope increased in $COPD_{stage\ 1}$ and declined as the disease progresses (please see Fig.4, Neder JA, Arbex FF, Alencar MC, et al. Exercise ventilatory inefficiency in mild to end-stage COPD. Eur Respir J. 2015; 45(2):377-87). This was consistent with our results. Neder et al reported that $\dot{V}_E/\dot{V}CO_2$ slope was correlated with mechanical

ventilatory contrains in patients with GOLD stage 2-4, (r from 0.48 to 0.60). But in current study, we investigated the correlations of $\dot{V}_E/\dot{V}CO_2$ slope with ventilatory contrains in entire group.

L316. Consistent with Neder et al's reports that VEVCO2I was correlated with peak (r= -0.58- -0.72, Neder et al, 2015).

R: Thank you for your comment. We have modified our text as you advised (see Page 16, Line 336-338).

L368. Consistent with Teopompi et al's reports (r= -0.38- -0.48, 2013, 2014).

R: Thank you for your comment. We have modified our text as you advised (see Page 18, Line 388-390).

L542. "Figure 3" Please delete it.

R: Thank you for your comment. We have deleted Fig.3 (see Page 25-26, Line 569-577).

L574. Table 3. P value for 0.000 is OK but for 0.00? Is it correct?

R: Thank you for your comment. You are absolutely right. We have modified our text as you advised (see Page 29-30, Table 3).

Reviewer B

In the study, the conclusion obtained that the VE/VCO2 intercept is significantly correlated with static and dynamic hyperinflation, has been well demonstrated with the measured parameters, and the statistical analysis performed. The paper has been neatly written in an understandable sequence and language. As a weakness, it is a retrospective study of 53 patients with a variability in BMI from 18 to 35 Kg/m2. Another weakness, (common in retrospective studies), is the involuntary highlight of statistically significant parameters, with the expected influence in the hypothesis design.

Nevertheless, the conclusion of this investigation is interesting, since it adds information to recent reports that have shown that the VE/VCO2 ratio and hyperinflation at rest, are associated with the intensity of dyspnea during exercise. As a suggestion, it would be interesting to design a prospective study with more selected individuals. In this way, the authors could certify their hypothesis, and advance in the application of these concepts as a routine practice in pulmonary function laboratories.

In relation with the analysis of the VE/VCO2 ratio at nadir, is cumbersome the demonstration that patients with different degrees of COPD, have a VE/VCO2 ratio without statistical difference. This observation previously reported, open a series of new questions. It occurs, that for more than a decade the determinations of the ventilatory threshold are carried out at this level, and important conclusions related to severity and prognosis have been obtained. As example, if the VE/VCO2 value exceeds a cut-off of 34 prior to lung resection surgery, (considered a risk factor), might be assumed that different severity groups of COPD, share the same surgical risk.

On the other hand, it is also noteworthy, (as the authors describe), to mention that the determinations of dynamic hyperinflation in an exercise test, ends with an inspiratory capacity maneuver. In this condition, GOLD patients 3 and 4 presents an important polypnea and the flow/volume curve present multiple artifacts, with serious limitations in the measurements. This aspect, also complicate the conclusions of multiple authors during the last years. In the previous scenario, the Vt/TLC ratio seems a convenient parameter.

These observations remark the necessity to re-evaluate some accepted physio-pathological concepts of the usual practice, as the behavior of the PCO2 set point, and the assumption that physiological dead space decreases at the nadir, supposing an optimization of the ventilation/perfusion ratio.

Two comments are requested to the authors:

1. In respect to conclusions of different studies, about the relevance of the VE/VCO2 ratio at the nadir, in severity and prognosis of COPD. Their opinion will be insightful, if there is no statistic difference in the VE/VCO2 ratio between the different groups.

R: Thank you for your comment. The $\dot{V}_E/\dot{V}CO_2$ nadir has been found to be highly reproducible in patients with COPD exercising to elicit maximal effort within 8–10 min (Barron A et al. European J Prev Cardio.2014,21(4)：445-53. However, the $\dot{V}_E/\dot{V}CO_2$ nadir, might underestimate ventilatory efficiency if the $\dot{V}_E/\dot{V}CO_2$ descending curve is prematurely interrupted by lactic acidosis or an excessively short test duration (Neder JA. Am J Respir Crit Care Med 2001; 164: 1481–1486). Too short a test duration resulted in $\dot{V}_E/\dot{V}CO_2$ nadir overestimating the slope in these patients. Nadir–slope difference may decrease after beneficial interventions.

2. Also, would be interesting their opinion, regarding the conclusions obtained in multiple studies of dynamic hyperinflation, considering the countless artifacts of the flow/volume curve at the end of the exercise.

R: Thank you for your comment. Dynamic measurements were averaging the consecutive 30 s data points to alleviate the artifacts of the flow/volume curve at the peak of the exercise.

Authors performed the commentaries indicated in the revision.

R: Thank you for your comment.