Canadian Science Publishing | Applied Physiology, Nutrition, and Metabolism

Does blood lactate predict the chronic adaptive response to training: A comparison of traditional and talk test prescription methods

Journal:	*Applied Physiology, Nutrition, and Metabolism*
Manuscript ID	apnm-2018-0343.R1
Manuscript Type:	Article
Date Submitted by the Author:	29-Jun-2018
Complete List of Authors:	Preobrazenski, Nicholas; Queen's University School of Kinesiology and Health Studies, Bonafiglia, Jacob; Queen's University, School of Kinesiology and Health Studies Nelms, Matthew; Queen's University, School of Kinesiology and Health Studies Lu, Simo; Queen's University School of Kinesiology and Health Studies Robins, Lauren; Queen's University School of Kinesiology and Health Studies LeBlanc, Camille; Queen's University School of Kinesiology and Health Studies Gurd, Brendon; Queens University,
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)
Keyword:	blood lactate, talk test, VO2peak, metabolic stress, exercise training < exercise

Does blood lactate predict the chronic adaptive response to training: A comparison of traditional and talk test prescription methods

Nicholas Preobrazenski [1*], Jacob T. Bonafiglia [1*], Matthew W. Nelms [1], Simo Lu [1], Lauren Robins [1], Camille LeBlanc [1], and Brendon J. Gurd [1]

[1]School of Kinesiology and Health Studies, Queen's University, Kingston, Ontario, Canada, K7L 3N6

*Authors contributed equally to this work.

Corresponding Author:

Brendon J. Gurd, PhD

Telephone: 613-533-6000 ext.79023

Fax: 613-533-6000

E-mail: gurdb@queensu.ca

ORCIDs:

Nicholas Preobrazenski: 0000-0003-1222-221X

Jacob T. Bonafiglia: 0000-0003-4412-5481

Abstract

The purpose of this study was to test the hypotheses that: 1) inter-individual variability in acute blood lactate responses during exercise at 65% of peak work rate (WR_{PEAK}; REL) will predict variability in the chronic responses to exercise training, and 2) exercising at an intensity that causes uncomfortable speech production (negative [NEG] talk test [TT] stage) elicits high acute blood lactate responses and large adaptations to training. Twenty-eight participants competed four weeks of exercise training consisting of REL (n: 14) NEG (TT, n: 14). Fifteen additional participants were assigned to a no-exercise control group (CTL, n: 15). In REL, acute blood lactate responses during the first training session significantly predicted changes in VO_2peak (r=0.69) after training. TT resulted in consistently high acute blood lactate responses. REL and TT improved (p<0.05) maximal oxygen consumption (VO_2peak), WR_{PEAK}, and work rate at the onset of blood lactate accumulation (WR_{OBLA}). Despite non-significance, small to medium between-group effect sizes for changes in VO_2peak, WR_{PEAK}, and WR_{OBLA}, and a higher WR, heart rate, RPE, and blood lactate during training at NEG, support the potential superiority of TT over REL. When exercise is prescribed using a traditional method (%WR_{PEAK}; REL), acute metabolic stress may partly explain the variance in the adaptations to training. Additionally, TT elicited significant increases in VO_2peak, WR_{PEAK}, WR_{OBLA}, and although our small sample size limits the ability to confidently compare training adaptations between groups, our preliminary results suggest that future investigations with larger sample sizes should assess the potential superiority of TT over REL.

Key words: blood lactate, talk test, VO_2peak, metabolic stress, exercise training

Introduction

When endurance training is prescribed as a percentage of peak oxygen consumption (VO_2peak) or peak work rate (WR_{PEAK}), individual variability in the observed responses for VO_2peak (Bonafiglia et al. 2016, Gurd et al. 2016, Raleigh et al. 2016), WR_{PEAK} (Montero and Lundby 2017) and the work rate at the onset of blood lactate accumulation (WR_{OBLA}) are observed (Bonafiglia et al. 2016, Gurd et al. 2016). Although recent work has suggested that inter-individual variability in observed VO_2peak responses to standardized endurance training may be partially explained by genetic differences (Timmons et al. 2010, Ghosh et al. 2013), haematological adaptations (Montero et al. 2015), or exercise-induced oxidative stress (Margaritelis et al. 2017), the mechanism(s) underlying the variance in chronic adaptation to exercise training remain largely unexplored.

Acute exercise prescribed as a percentage of VO_2peak or WR_{PEAK} leads to considerable inter-individual variability in blood lactate responses (Coyle et al. 1988, Orok et al. 1989, Weltman et al. 1990, Meyer et al. 1999, Scharhag-Rosenberger et al. 2010, Egger et al. 2016, Bonafiglia et al. 2017). Because blood lactate reflects muscle lactate (Tesch et al. 1982, Jacobs and Kaiser 1982), intramuscular stress (Spriet et al. 2000), and potentially the induction of signaling pathways that trigger muscular remodeling (Hood 2001, Flück and Hoppeler 2003, Egan and Zierath 2013), the variability in metabolic stress associated with %WR_{PEAK} may contribute to inter-individual variability in the adaptive response to exercise. Although it has been speculated that individuals who experience large increases in metabolic stress (and blood lactate) during training will experience a larger adaptive response than individuals experiencing low metabolic stress (Mann et al. 2013, 2014), this speculation has yet to be tested. Accordingly, the first purpose of this study was to test the hypothesis that differences in blood lactate responses during acute exercise at 65% of WR_{PEAK} will predict, at least in part, the inter-individual variability in observed responses to exercise training.

If, as speculated above, in-training metabolic stress contributes to an individual's adaptive response, using an exercise prescription method that elicits consistently large increases in metabolic stress should also induce large adaptive responses. Although exercise can be prescribed to target OBLA and lactate threshold (Chwalbinska-Moneta et al. 1989, Coen et al. 1991, Philp et al. 2008), repeatedly collecting blood lactate samples can be invasive, costly, and inaccessible outside of a laboratory setting. The Talk Test (TT) is a non-invasive, free, accessible exercise prescription method that estimates exercise intensity through self-evaluation of the perceived difficulty of reciting a ~30-word phrase (Webster and Aznar-Lain 2008, Reed and Pipe 2014, 2016). Importantly, exercise

intensities characterized by uncomfortable speech (negative stage, NEG; Recalde et al. 2002) appear to produce consistently high blood lactate concentrations (Woltmann et al. 2015). It is tempting to speculate that exercising at NEG should induce consistently large increases in metabolic stress and thus subsequently large adaptive responses to training. However, the metabolic stress/exercise intensity induced by repeated exercise at NEG has not been characterized and the tolerability and efficacy of training at NEG is currently unknown. Thus, as a preliminary step towards developing an exercise prescription method that elicits consistently large increases in metabolic stress and large adaptive responses to training, the second purpose of this study was to characterize the in-training response and tolerability to exercise at NEG, and the efficacy of training at NEG for improving VO_2peak, WR_{PEAK} and WR_{OBLA}.

Methods

Forty-seven, recreationally active (self-reported < three hours of physical activity per week), and healthy young males volunteered to participate in the current study. We selected recreationally active participants because we have previously shown that prescribing exercise at 65% WR_{PEAK} in this population results in variability in acute and chronic responses (Bonafiglia et al. 2016, 2017). Participants were only enrolled in the study if they were between 18 and 30 years of age, nonsmokers, non-obese (BMI < 30 kg/m^2), were not taking any medication, and free of cardiovascular and metabolic disease. Each participant attended a preliminary screening session where they were briefed on the study, provided informed consent, and had their height and weight recorded. Participants were not previously trained in cycling and were not involved in a training program at the start of the study. Participants were informed to maintain their regular physical activity and nutritional habits throughout the duration of the study. All experimental procedures performed on human participants were approved by the Health Sciences Human Research Ethics Board at Queen's University. Verbal and written explanation of the experimental protocol and associated risks were provided to all participants prior to obtaining written informed consent.

Experimental Design

Participants completing four weeks of training using the relative WR_{PEAK} protocol (REL: n = 14; see details below) were part of a randomized control trial where participants were assigned to either REL or a no-exercise control group (CTL: n = 15) via minimization. Briefly, the first participant was randomly allocated to REL or CTL, and every subsequent participant was allocated to REL or CTL in a manner that minimized the imbalance of baseline VO_2peak between groups (Treasure and MacRae 1998). The REL and CTL groups were part of a larger

data collection including muscle biopsies (biopsy data not included). Participants that completed four weeks of training using the talk test training protocol (TT: see details below; n=14) were recruited separately but concurrently. All participants were recruited from the same undergraduate population and met the same inclusion/exclusion criteria. All participants completed physiological testing in the week preceding the first week of training and 72 hours following their last training session (or at the equivalent time for CTL). All physiological testing and training was performed on a Monark Ergomedic 874 E stationary ergometer (Vansbro, Sweden). All participants were asked to refrain from ingesting nutritional supplements and exercising 24 hours before, and alcohol and caffeine 12 hours before all physiological testing. The study protocol is depicted in Figure 1.

Physiological Testing

In the week preceding (PRE) and the week following (POST) the four-week interventions, participants reported to the laboratory on two separate occasions separated by at least 24 hours. During the first visit to the laboratory at both PRE and POST, participants completed a VO_2peak incremental step test to volitional exhaustion on a cycle ergometer in the fed state, as previously described (Edgett et al. 2013). Briefly, the VO_2peak test consisted of a five-minute warm-up at 80RPM, followed by a step increase to 80 watts for one minute, and then a progressive 24 watts per minute increase until volitional fatigue (1-minute test). Gas exchange and heart rate (HR) were collected throughout the test using a metabolic cart (Moxus, AEI Technologies, Pittsburgh, PA) and Polar HR monitors (Polar Team2 Pro, Kempele, Finland), respectively. VO_2peak and peak HR (HR_{PEAK}) were calculated as the highest 30-second average VO_2 and HR observed during the 1-minute test, respectively. RPM was continuously collected throughout the step test and WR_{PEAK} was calculated as the highest 30-second average WR from the 1-minute test. Participants in the REL and CTL group had a resting muscle biopsy taken ~30 minutes prior to their VO_2peak test; however, biopsy data is not included in the present manuscript.

During the second PRE and POST visit to the laboratory, participants completed an additional incremental step test following an identical protocol as the test described above, with the exception that work rate was increased by 24W every three minutes (3-minute test). Fingertip capillary blood (~20 uL) was collected at rest and within the last 30 seconds of each successive three minute stage using a Lactate Scout + (EEK Diagnostics, Madgeberg, Germany); a device with acceptable accuracy and reliability (Bonaventura et al. 2014). Because blood lactate is unstable during incremental step tests with short stage durations (Bentley 2007), fingertip capillary blood was only collected during the three-minute test. The WR at the onset of blood lactate accumulation during the 3-minute test

(WR_{OBLA}), a submaximal measure where a blood lactate concentration of at least 4.0 mmol•L^{-1} is reached (Sjödin and Jacobs 1981), was determined as the average WR in the final 30 seconds of the stage where OBLA was reached. Gas exchange was only collected for participants in the REL and CTL groups, as described for the 1-minute test above (data not presented). Gas exchange was not collected for participants in the TT group during the 3-minute test so that the talk test could be administered during the final 30 seconds of each stage. The talk test required participants to count aloud from one to thirty at a regular conversational pace and volume. Subsequently, participants were asked whether they could speak comfortably and chose one of the following three options: "Yes, I could speak comfortably" (positive stage; POS), "Yes, I could speak, but not entirely comfortably" (equivocal stage; EQ), "No, I could not speak comfortably" (NEG; Recalde et al. 2002, Reed and Pipe 2014). The first stage where participants in the TT group reported to be exercising at NEG was used to select the initial WR for the first training session.

Training Protocols

All groups were asked to maintain habitual physical activity levels throughout the duration of the trial. Training for both exercise groups consisted of 15 training sessions over a four-week period. During each training session the REL group participants cycled for 30 minutes at 65% of $WR_{PEAK,}$ whereas the TT group performed the talk test every two-and-a-half to five minutes to ensure that the intensity was sufficient to elicit a NEG response. As mentioned above, the TT involved participants counting to 30 before choosing one of three options: POS, EQ, or NEG. Based on participant responses, WR was either increased, decreased, or remained the same, to target a sustainable intensity within the NEG stage (see Figure 2 for details on TT training session protocol).

All training sessions for both groups were fully supervised and were preceded by a one-minute loadless warm-up. During each 30-minute training protocol participants were instructed to maintain a cadence of 80RPM and received verbal encouragement. Blood lactate concentrations were measured at the 10 and 30-minute point of the first training session of each week from fingertip capillary blood (~20uL). HR, rating of perceived exertion (RPE; 6-20 Borg Scale; Borg 1982), and WR were measured every five minutes during each training session.

Statistical Analysis

A one-way ANOVA was used to compare baseline characteristics across groups. A two-way mixed ANOVA (group x time) was used to compare mean training blood lactate, HR, RPE and WR between REL and TT. Additional two-way mixed ANOVAs were used to compare changes in VO_2peak, WR_{PEAK}, WR_{OBLA}, and body mass

following four weeks of training among groups. Any significant interaction or main effects were subsequently analyzed using Bonferroni post-hoc analyses. A linear regression was used to determine whether blood lactate concentrations during the first training session predicted changes in VO_2peak, WR_{PEAK}, and WR_{OBLA} following training in the REL group. Statistical analysis was performed using SPSS (version 20; IBM Corp., Armonk, NY, USA).

A priori sample size calculations were calculated for the primary outcome, VO_2peak. Given that we have previously found a 1.69 ± 3.19 mL•kg^{-1}•min^{-1} increase in VO_2peak after three weeks of training at 65% WR_{PEAK} (Bonafiglia et al. 2016), we expected a 2 mL•kg^{-1}•min^{-1} increase in VO_2peak after four weeks of training at 65% WR_{PEAK} (REL). Using the sample size formula (Overall and Doyle 1994; Eq. 13) relevant to repeated measures ANOVA, we determined that a sample size of 13 was needed in each group ($Z\alpha$ = 1.96, $Z\beta$ = 0.85, r = .85, $\bar{X}_1 - \bar{X}_2$ = 2, SD = 3.19 mL•kg^{-1}•min^{-1}) to give 80% power to detect a difference in VO_2peak change scores (POST-PRE) of 2 mL•kg^{-1}•min^{-1} between REL/TT and CTL.

Although not a primary aim of this study, we completed a secondary exploratory analysis comparing changes in VO_2peak, WR_{PEAK}, and WR_{OBLA} following REL and TT, despite not powering a priori to detect differences in change scores between training groups. Differences in change scores between REL and TT were compared using t-tests, and the corresponding effect sizes were calculated using Cohen's *d* (small = 0.2; medium = 0.5; large = 0.8; Cohen 1992). Pooled (REL and TT) SD of change scores were used for VO_2peak, WR_{PEAK}, and WR_{OBLA} Cohen's *d* calculations.

Results

A total of 75 individuals expressed interest in participating in the current study with 50 and 25 being screened for the REL/CTL and TT arm, respectively (Fig 3). 30 and 17 participants completed baseline testing for the REL/CTL and TT arms, respectively. 1 participant dropped out of the REL group (due to discomfort caused during the baseline muscle biopsy), while two participants dropped out of the TT group citing a lack of time and an unrelated sickness. Importantly, an additional TT participant dropped during their second week of training because they were unable to complete the TT protocol.

The remaining 43 participants completed the study, including all PRE and POST testing. 28 participants completed all 15 training sessions (REL: n = 14, TT: n = 14). Data from 4 participants (CTL: n = 2, REL: n = 1, TT: n = 1) was excluded from the WR_{OBLA} analysis as blood lactate was unable to be collected during their POST tests.

Additionally, data from 3 participants were excluded from the WR_{PEAK} analysis as the RPM recording software failed during data collection (CTL: n = 2, TT: n = 1). Sample size and baseline participant characteristics for the REL and TT groups are presented in Table 1. Only age differed among groups, where the mean age was significantly ($p < 0.05$) higher in the REL group. Weight did not significantly change from baseline in any group (CTL: +0.02 ± 0.88 kg, REL: +0.35 ± 1.07 kg, TT: +0.30 ± 1.34kg).

Figure 4 illustrates the mean weekly WR (Fig 4A), HR (Fig 4C), and RPE (Fig 4D), and blood lactate (Fig 4B) from the first training session of each week for both REL and TT groups. Significant interactions (group x time) were observed for each measure ($p < 0.05$ for all). Bonferroni post-hoc analyses are presented within Figures 4A-D.

The mean blood lactate concentration of the first training session positively predicted changes in VO_2peak in the REL group (Fig 5; $r^2 = 0.5$, r = 0.7, $p < 0.01$). No other significant linear regressions were observed between mean blood lactate responses and chronic responses to training for both REL and TT.

A significant ($p < 0.001$) main effect of time (POST – PRE) and a significant ($p < 0.001$) interaction effect (group x time) were observed for VO_2peak (CTL: +0.17 ± 2.8 $mL•kg^{-1}•min^{-1}$, REL: +3.67 ± 3.1 $mL•kg^{-1}•min^{-1}$, TT: +5.43 ± 3.0 $mL•kg^{-1}•min^{-1}$), WR_{PEAK} (CTL: +5.4 ± 19.6 W, REL: +33.1 ± 20.0 W, TT: 46.5 ± 20.8 W), WR_{OBLA} (CTL: +0.21 ± 17.2 W, REL: +34.2 ± 31.3 W, TT: +42.4 ± 33.1 W). Bonferroni post-hoc analyses are presented in Figure 6. Although secondary analysis using independent samples t-tests of the change score differences between REL and TT for VO_2peak ($p = 0.14$), WR_{PEAK} ($p = 0.10$), and WR_{OBLA} ($p = 0.52$) revealed no significant differences between groups, effect size calculations indicate a medium, medium, and small effect of TT increasing VO_2peak ($d = 0.58$), WR_{PEAK} ($d = 0.66$), and WR_{OBLA} ($d = 0.25$) more than REL, respectively.

Discussion

The current study examined the adaptive responses to training when exercise was prescribed as a fixed percentage of WR_{PEAK} (REL) or using the NEG TT stage. Our study was designed to 1) test the hypothesis that variability in acute blood lactate responses predicts the variability in the adaptive responses to training at REL, and 2) to characterize the in-training response and tolerability of repeated exercise at NEG and to examine the efficacy of training at NEG for improving VO_2peak, WR_{PEAK} and WR_{OBLA}. The major novel findings of the current study are 1) variability in blood lactate responses in the first training session predicted changes in VO_2peak following training in the REL group, 2) compared to REL, training at NEG resulted in higher in-training WR, HR, RPE and blood lactate

as training progressed, 3) training at NEG was well tolerated with only one participant unable to complete the training protocol (all other participants completed all 15 training sessions without incident), and 4) training at NEG elicited significant improvements in VO_2peak, WR_{PEAK}, and WR_{OBLA}, demonstrating the efficacy of the TT as a non-invasive and accessible exercise training prescription tool.

Acute blood lactate responses predict changes in VO_2peak following training at 65% of WR_{PEAK}

Exercise prescribed as a percentage of VO_2peak/WR_{PEAK} results in considerable variability in acute blood lactate responses (Coyle et al. 1988, Orok et al. 1989, Weltman et al. 1990, Meyer et al. 1999, Scharhag-Rosenberger et al. 2010, Egger et al. 2016, Bonafiglia et al. 2017) and changes in VO_2peak (Bouchard and Rankinen 2001, Vollaard et al. 2009, Zelt et al. 2014, Bonafiglia et al. 2016), WR_{PEAK} (Montero and Lundby 2017), and WR_{OBLA} (Bonafiglia et al. 2016, Gurd et al. 2016) following training. Consistent with the hypothesis that heterogeneity in acute exercise-induced metabolic stress may contribute to the variability in the adaptive responses to training (Mann et al. 2013, 2014), we observed a positive relationship between acute blood lactate responses during the first training session and changes in VO_2peak following training in the REL group. Only the acute blood lactate responses in the first REL training session significantly correlated with changes in VO_2peak. Unsurprisingly, there was no relationship between first session blood lactate and any adaptive response in the TT group. It is possible that some participants in the TT group may have had a lower blood lactate concentration during week 1 compared to the remaining 3 weeks because as training progressed their WRs were increased based on their TT responses (see Fig 2). Thus, there would be no expectation of a relationship between first session blood lactate and adaptations to training in TT, whereas the opposite would be true in REL. Further, because the mean blood lactate responses in REL decreased over the training period (Fig 4B), our data suggest that the ability to predict changes in VO_2peak disappears as participants in the REL group adapted to training.

Although the mechanisms underlying the relationship between acute blood lactate and VO_2peak responses are unclear, it is possible that differences in acute blood lactate responses reflect the magnitude of exercise-induced perturbations and an associated induction of signaling pathways that ultimately underlie chronic improvements in VO_2peak. For example, the concentration of blood lactate rises proportionately with plasma epinephrine levels (Lehmann et al. 1985), and epinephrine has been implicated as an important signaling molecule that initiates the induction of chronic cardiac and skeletal muscle adaptations (Williams and Barnes 1989). In addition to

epinephrine-mediated signalling, blood lactate concentrations throughout training may reflect disturbances in intramuscular energetics (Spriet et al. 2000) and subsequent activation of the AMPK-PGC-1α pathway in exercising muscle (Jäger et al. 2007), which may affect VO_2peak through the induction of mitochondrial biogenesis and angiogenesis (Ingjer 1979, Calvo et al. 2008, Hawley et al. 2014). It is also possible that variability in acute blood lactate responses reflects inter-individual differences in other determinants of VO_2peak responses to endurance training, including (but potentially not limited to) acute oxidative stress (Margaritelis et al. 2017) and haematological adaptations (Montero et al. 2015). Furthermore, it is possible that genetic factors may explain, at least in part, the significant relationship between acute blood lactate and VO_2peak responses in REL. Specifically, the predictor genes that explain a portion of the variance in VO_2peak response to exercise training (e.g. ACSL1, PRDM1, GRIN3A) may also partially explain the variability in acute blood lactate responses (Bouchard et al. 2011). Although future work is needed to elucidate the mechanisms linking acute blood lactate responses and changes in VO_2peak, our findings suggest that acute blood lactate responses to the first training session may partially explain the variability in VO_2peak responses when training is prescribed as a fixed percentage of WR_{PEAK}.

The TT can be used as an exercise training prescription tool

To our knowledge, this is the first study to demonstrate that training at the NEG TT stage improves VO_2peak, WR_{PEAK}, WR_{OBLA} in young and healthy males. Recently, Porcari et al. (2018) demonstrated that 10 weeks of cycling at the last-positive TT stage (i.e. the highest intensity that allows for comfortable speech) improves VO_2max, peak power output, and VO_2 at the ventilatory threshold. Taken together, these results suggest that the TT can be used to prescribe exercise when access to the facilities and resources required to prescribe exercise as a percentage of a given physiological variable (e.g. WR_{PEAK}, VO_2peak, lactate threshold, etc.) is limited.

The difference in change scores between REL and TT for VO_2peak (TT – REL = 1.76 $mL \cdot kg^{-1} \cdot min^{-1}$), WR_{PEAK} (TT – REL = 13.42 W), and WR_{OBLA} (TT – REL = 8.13 W) may suggest that TT elicits superior adaptations to training than REL. The larger adaptations in TT are likely attributable to the higher in-training metabolic stress (Fig 4), which is likely a result of consistently adjusting exercise intensity during each exercise session (Fig 2). Conversely, because exercise intensity remained unchanged during REL, the in-training metabolic stress decreased throughout training (Fig 4), which may have contributed to the relatively smaller adaptive responses (Fig 6). Unlike TT, adjustments in the prescribed exercise intensity in REL require reassessment of WR_{PEAK}. Therefore, it remains possible that reassessing WR_{PEAK} midway through training in REL would have increased the prescribed exercise

intensity, elevated the in-training metabolic stress, and perhaps reduced the difference in the adaptive responses between TT and REL.

Although it appears that TT resulted in larger training responses than REL, our study design limits our confidence in this conclusion. Specifically, we justified our sample size based on our primary analysis, which required 13 participants in each group to detect an expected difference in VO_2peak change scores of 2 $mL \cdot kg^{-1} \cdot min^{-1}$ between REL/TT and CTL. However, because no other study has used the NEG stage to prescribe exercise training, we were unable to estimate an expected difference in VO_2peak change scores between REL and TT. Accordingly, we were unable to perform an a priori sample size calculation for our secondary analysis (i.e. comparing VO_2peak change scores between REL and TT). Given our small sample size, the observed power for our secondary analysis was only ~30%, suggesting that the non-significant difference in VO_2peak change scores between REL and TT may reflect a type II error. Using equation 8 from Overall and Doyle (1994) and a statistical power of 80%, we calculate that future studies would need a total sample size of 96 (TT = 48, REL = 48) to detect our observed effect size (d = 0.58) for the difference in VO_2peak change scores between REL and TT as significant. Additionally, REL and CTL participants received their group assignment following the completion of baseline testing, whereas participants were recruited separately for TT. Therefore, we were unable to blind TT participants to their group assignment during baseline testing. Considering the above limitations, there is a need for future work that is designed to test the hypothesis that exercising at the NEG TT stage improves training adaptations more than REL.

Conclusion

Acute blood lactate responses partially explain the variability in chronic responses when training is prescribed as a percentage of WR_{PEAK}. These results suggest that acute metabolic stress may underlie inter-individual differences in observed changes in VO_2peak. We demonstrated for the first time that exercising at the NEG TT stage is a simple and accessible exercise prescription tool that leads to consistently high in-training responses and improves VO_2peak, WR_{PEAK}, and WR_{OBLA} in recreationally active young men. Thus, studies in other populations, including sedentary, old, and obese individuals, are needed before generalizing these findings. Additionally, although not a primary aim of the present study, our findings may suggest that the TT may be potentially more effective at improving VO_2peak, WR_{PEAK}, and WR_{OBLA} compared to prescribing exercise intensity at 65% of WR_{PEAK}; however, future work is needed to confirm these preliminary results.

Acknowledgements

The authors thank the volunteers and students who participated in this study.

Conflict of Interest

The authors have no conflicts of interest to report.

References

Bentley, D.J. 2007. Incremental exercise test design and analysis. Sport. Med. **37**(7): 575–586. doi:10.2165/00007256-200737070-00002.

Bonafiglia, J.T., Rotundo, M.P., Whittall, J.P., Scribbans, T.D., Graham, R.B., and Gurd, B.J. 2016. Inter-individual variability in the adaptive responses to endurance and sprint interval training: A randomized crossover study. PLoS One. **11**(12): e0167790. doi:10.1371/journal.pone.0167790.

Bonafiglia, J.T., Sawula, L.J., and Gurd, B.J. 2017. An examination of the Counting Talk Test's ability to discern individual variability in exercise-induced metabolic stress. Meas. Phys. Educ. Exerc. Sci. **22**(1): 38–47. doi:10.1080/1091367X.2017.1373650.

Bonaventura, J.M., Sharpe, K., Knight, E., Fuller, K.L., Tanner, R.K., and Gore, C.J. 2014. Reliability and accuracy of six hand-held blood lactate analysers. J. Sport. Sci. Med. **14**(1): 203–214.

Borg, G.A.V. 1982. Psychophysical bases of percieved exertion. Med. Sci. Sports Exerc. **14**(5): 377–381.

Bouchard, C., and Rankinen, T. 2001. Individual differences in response to regular physical activity. Med. Sci. Sports Exerc. **33**(6 Suppl): S446–S451. doi:10.1097/00005768-200106001-00013.

Bouchard, C., Sarzynski, M.A., Rice, T.K., Kraus, W.E., Church, T.S., Sung, Y.J., et al. 2011. Genomic predictors of the maximal O_2 uptake response to standardized exercise training programs. J. Appl. Physiol. **110**(5): 1160–1170. doi:10.1152/japplphysiol.00973.2010.

Calvo, J.A., Daniels, T.G., Wang, X., Paul, A., Lin, J., Spiegelman, B.M., et al. 2008. Muscle-specific expression of PPARγ coactivator-1α improves exercise performance and increases peak oxygen uptake. J. Appl. Physiol. **104**(5): 1304–1312. doi:10.1152/japplphysiol.01231.2007.

Chwalbinska-Moneta, J., Robergs, R. A, Costill, D.L., and Fink, W.J. 1989. Threshold for muscle lactate accumulation during progressive exercise. J. Appl. Physiol. **66**(6): 2710–2716. doi:10.1152/jappl.1989.66.6.2710.

Coen, B., Schwarz, L., Urhausen, A., and Kindermann, W. 1991. Control of training in middle and long distance running by means of the individual anaerobc threshold. Int. J. Sports Med. **12**(6): 519–524. doi:10.1055/s-2007-1024727.

Cohen, J. 1992. A power primer. Psychol. Bull. **112**(1): 155-159. doi:10.1037/0033-2909.112.1.155.

Coyle, E.F., Coggan, A.R., Hopper, M.K., and Walters, T.J. 1988. Determinants of endurance in well-trained cyclists. J. Appl. Physiol. **64**(6): 2622–30. doi:10.1152/jappl.1988.64.6.2622.

Edgett, B.A., Foster, W.S., Hankinson, P.B., Simpson, C.A., Little, J.P., Graham, R.B., et al. 2013. Dissociation of increases in PGC-1α and its regulators from exercise intensity and muscle activation following acute exercise. PLoS One. **8**(8): e71623. doi:10.1371/journal.pone.0071623.

Egan, B., and Zierath, J.R. 2013. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab. **17**(2): 162-184. doi:10.1016/j.cmet.2012.12.012.

Egger, F., Meyer, T., and Hecksteden, A. 2016. Interindividual variation in the relationship of different intensity markers - A challenge for targeted training prescriptions. PLoS One. **11**(10): 1–11. doi:10.1371/journal.pone.0165010.

Flück, M., and Hoppeler, H. 2003. Molecular basis of skeletal muscle plasticity-from gene to form and function. *In* Reviews of Physiology, Biochemistry and Pharmacology. pp. 159–216. doi:10.1007/s10254-002-0004-7.

Ghosh, S., Vivar, J.C., Sarzynski, M.A., Sung, Y.J., Timmons, J.A., Bouchard, C., et al. 2013. Integrative pathway analysis of a genome-wide association study of VO_2max response to exercise training. J. Appl. Physiol. **115**(9): 1343–1359. doi:10.1152/japplphysiol.01487.2012.

Gurd, B.J., Giles, M.D., Bonafiglia, J.T., Raleigh, J.P., Boyd, J.C., Ma, J.K., et al. 2016. Incidence of nonresponse and individual patterns of response following sprint interval training. Appl. Physiol. Nutr. Metab. **41**(3): 229–234. doi:10.1139/apnm-2015-0449.

Hawley, J.A., Hargreaves, M., Joyner, M.J., and Zierath, J.R. 2014. Integrative biology of exercise. Cell. **159**(4): 738–749. doi:10.1016/j.cell.2014.10.029.

Hood, D.A. 2001. Invited review: Contractile activity-induced mitochondrial biogenesis in skeletal muscle. J. Appl. Physiol. **90**(3): 1137-1157. doi:10.1152/japplphysiol.00017.2004.

Ingjer, F. 1979. Effects of endurance training on muscle fibre ATP-ase activity, capillary supply and mitochondrial content in man. J. Physiol. **294**(5): 419–32. doi:10.1113/jphysiol.1979.sp012938.

Jacobs, I., and Kaiser, P. 1982. Lactate in blood, mixed skeletal muscle, and FT or ST fibres during cycle exercise in man. Acta Physiol. Scand. **114**(3): 461–6. doi:10.1111/j.1748-1716.1982.tb07010.x.

Jäger, S.S., Handschin, C.C., St-Pierre, J.J., and Spiegelman, B.M. 2007. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1α. Proc. Natl. Acad. Sci. U.S.A. **104**(29): 12017–12022. doi:10.1073/pnas.0705070104.

Lehmann, M., Schmid, P., and Keul, J. 1985. Plasma catecholamine and blood lactate cumulation during incremental exhaustive exercise. Int. J. Sports Med. **6**(2): 78–81. doi:10.1055/s-2008-1025817.

Mann, T., Lamberts, R.P., and Lambert, M.I. 2013. Methods of prescribing relative exercise intensity: Physiological and practical considerations. Sport. Med. **43**(7): 613–625. doi:10.1007/s40279-013-0045-x.

Mann, T.N., Lamberts, R.P., and Lambert, M.I. 2014. High responders and low responders: Factors associated with individual variation in response to standardized training. Sport. Med. **44**(8): 1113–1124. doi:10.1007/s40279-014-0197-3.

Margaritelis, N. V., Theodorou, A.A., Paschalis, V., Veskoukis, A.S., Dipla, K., Zafeiridis, A., et al. 2017. Adaptations to endurance training depend on exercise-induced oxidative stress: Exploiting redox interindividual variability. Acta Physiol. **222**(2): e12972. doi:10.1111/apha.12898.

Meyer, T., Gabriel, H.H., and Kindermann, W. 1999. Is determination of exercise intensities as percentages of VO_2max or HRmax adequate? Med. Sci. Sports Exerc. **31**(9): 1342–1345. doi:10.1097/00005768-199909000-00017.

Montero, D., Cathomen, A., Jacobs, R.A., Flück, D., de Leur, J., Keiser, S., et al. 2015. Haematological rather than skeletal muscle adaptations contribute to the increase in peak oxygen uptake induced by moderate endurance training. J. Physiol. **593**(20): 4677–4688. doi:10.1113/JP270250.

Montero, D., and Lundby, C. 2017. Refuting the myth of non-response to exercise training: 'Non-responders' do respond to higher dose of training. J. Physiol. **11**: 3377–3387. doi:10.1113/JP273480.

Orok, C.J., Hughson, R.L., Green, H.J., and Thomson, J.A. 1989. Blood lactate responses in incremental exercise as predictors of constant load performance. Eur. J. Appl. Physiol. **59**: 262–267. doi:10.1007/BF02388326.

Overall, J.E., and Doyle, S.R. 1994. Estimating sample sizes for repeated measurement designs. Control. Clin. Trials. **15**(2): 100–123. doi:10.1016/0197-2456(94)90015-9.

Philp, A., Macdonald, A.L., Carter, H., Watt, P.W., and Pringle, J.S. 2008. Maximal lactate steady state as a training stimulus. Int. J. Sports Med. **29**(6): 475–479. doi:10.1055/s-2007-965320.

Porcari, J.P., Foster, C., Falck, K., Suckow, S., Turek, J., Wargowsky, A., et al. 2018. Comparison of the Talk Test and percent heart rate reserve for exercise prescription. Kinesiology. **50**(1): 3-10. doi:10.1249/01.mss.0000519000.47583.0a.

Raleigh, J.P., Giles, M.D., Scribbans, T.D., Edgett, B. A, Suwula, L.J., Bonafiglia, J.T., et al. 2016. The impact of work-mathced interval training on VO_2peak and VO_2 kinetics: Diminishing returns with increasing intensity. Appl. Physiol. Nutr. Metab. **713**(February): 706–713. doi:10.1139/apnm-2015-0614.

Recalde, P.T., Foster, C., Skemp-Arlt, K.M., Fater, D.C.W., Neese, C.A., Dodge, C., et al. 2002. The Talk Test as a simple marker of ventilatory threshold. South African J. Sport. Med. **8**(January): 5–8.

Reed, J.L., and Pipe, A.L. 2014. The Talk Test. Curr. Opin. Cardiol. **29**(5): 475–480. doi:10.1097/HCO.0000000000000097.

Reed, J.L., and Pipe, A.L. 2016. Practical approaches to prescribing physical activity and monitoring exercise intensity. Can. J. Cardiol. **32**(4): 514–522. doi:10.1016/j.cjca.2015.12.024.

Scharhag-Rosenberger, F., Meyer, T., Gäßler, N., Faude, O., and Kindermann, W. 2010. Exercise at given percentages of VO_2max: Heterogeneous metabolic responses between individuals. J. Sci. Med. Sport. **13**(1): 74–79. doi:10.1016/j.jsams.2008.12.626.

Sjödin, B., and Jacobs, I. 1981. Onset of blood lactate accumulation and marathon running performance. Int. J. Sports Med. **2**(1): 23–26. doi:10.1055/s-2008-1034579.

Spriet, L.L., Howlett, R.A., and Heigenhauser, G.J. 2000. An enzymatic approach to lactate production in human skeletal muscle during exercise. Med. Sci. Sports Exerc. **32**(4): 756–763. doi:10.1097/00005768-200004000-00007.

Tesch, P.A., Daniels, W.L., and Sharp, D.S. 1982. Lactate accumulation in muscle and blood during submaximal exercise. Acta Physiol. Scand. **114**(3): 441–446. doi:10.1111/j.1748-1716.1982.tb07007.x.

Timmons, J.A., Knudsen, S., Rankinen, T., Koch, L.G., Jensen, T., Keller, P., et al. 2010. Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. J. Appl. Physiol. **108**(6): 1487–1496. doi:10.1152/japplphysiol.01295.2009.

Treasure, T., and MacRae, K.D. 1998. Minimisation: The platinum standard for trials? BMJ. **317**(7155): 362–3. doi:10.1136/bmj.317.7155.362.

Vollaard, N.B.J., Constantin-Teodosiu, D., Fredriksson, K., Rooyackers, O., Jansson, E., Greenhaff, P.L., et al. 2009. Systematic analysis of adaptations in aerobic capacity and submaximal energy metabolism provides a unique insight into determinants of human aerobic performance. J. Appl. Physiol. **106**(5): 1479–1486. doi:10.1152/japplphysiol.91453.2008.

Webster, A.L., and Aznar-Lain, S. 2008. Intensity of physical activity and the “Talk Test ." ACSMs. Health Fit. J. **12**(3): 13–17. doi: 10.1249/FIT.0b013e31817047b4.

Weltman, A., Snead, D., Seip, R., Schurrer, R., Weltman, J., Rutt, R., et al. 1990. Percentages of maximal heart rate, heart rate reserve and VO_2max for determining endurance training intensity in male runners. Int. J. Sports Med. **11**(3): 218–222. doi:10.1055/s-2007-1024795.

Williams, J.H., and Barnes, W.S. 1989. The positive inotropic effect of epinephrine on skeletal muscle: A brief review. Muscle Nerve. **12**(12): 968–975. doi: 10.1002/mus.880121204.

Woltmann, M.L., Foster, C., Porcari, J.P., Camic, C.L., Dodge, C., Haible, S., et al. 2015. Evidence that the talk test can be used to regulate exercise intensity. J. Strength Cond. Res. **29**(5): 1248–1254. doi:10.1519/JSC.0000000000000811.

Zelt, J.G.E., Hankinson, P.B., Foster, W.S., Williams, C.B., Reynolds, J., Garneys, E., et al. 2014. Reducing the volume of sprint interval training does not diminish maximal and submaximal performance gains in healthy men. Eur. J. Appl. Physiol. **114**(11): 2427–2436. doi:10.1007/s00421-014-2960-4.

Table

Table 1. Participant baseline characteristics.

	REL (n = 14)	TT (n = 14)	CTL (n = 15)
Age*	21.9 ± 2.0	19.8 ± 1.1	20.5 ± 1.4
Height (cm)	180.5 ± 7.3	184.4 ± 14.6	176.0 ± 7.6
Weight (kg)	79.7 ± 12.0	80.0 ± 13.0	76.1 ± 11.7
VO_2peak (mL•kg^{-1}•min^{-1})	46.0 ± 6.6	45.8 ± 5.9	45.1 ± 8.8
VO_2peak (mL•min^{-1})	3659 ± 738	3640 ± 629	3406 ± 709.8
WR_{OBLA} (W)	155.4 ± 54.3[a]	163.2 ± 52.1[a]	161.0 ± 41.3[a]
WR_{PEAK} (W)	281.8 ± 55.5	286.1 ± 61.6[a]	259.1 ± 50.1[b]

Values are means ± standard deviation. VO_2peak, peak oxygen uptake; WR, work rate; OBLA, onset of blood lactate accumulation.

* Significantly higher in REL ($p < 0.05$).

[a] n = 13.

[b] n = 14.

Figure Captions

Fig. 1. Study protocol.

Fig. 2. Flow chart used to guide exercise intensity to the NEG TT stage.

Fig. 3. Participant flow diagram.

Fig. 4. Mean weekly work rate (WR), heart rate (HR), and RPE, and blood lactate of the first training session of each week in CTL, REL, and TT.

Significant interaction effect. (4A; $p < 0.05$, 4B; $p < 0.01$, 4C; $p < 0.01$, 4D; $p < 0.01$).

a Significantly different from week 1 ($p < 0.05$).

b Significantly different from week 2 ($p < 0.05$).

c Significantly different from week 3 ($p < 0.05$).

* Significant between-group difference ($p < 0.05$).

Fig. 5. Linear regression between mean blood lactate responses in the first training session and changes in VO_2peak following training.

Fig. 6. VO_2peak, WR_{PEAK}, WR_{OBLA} before (PRE) and after (POST) four weeks of CTL, REL, and TT.

Main effect of time ($p < .001$) for all. Interaction effect ($p < .001$) for all.

* Significantly different from PRE ($p < 0.001$).

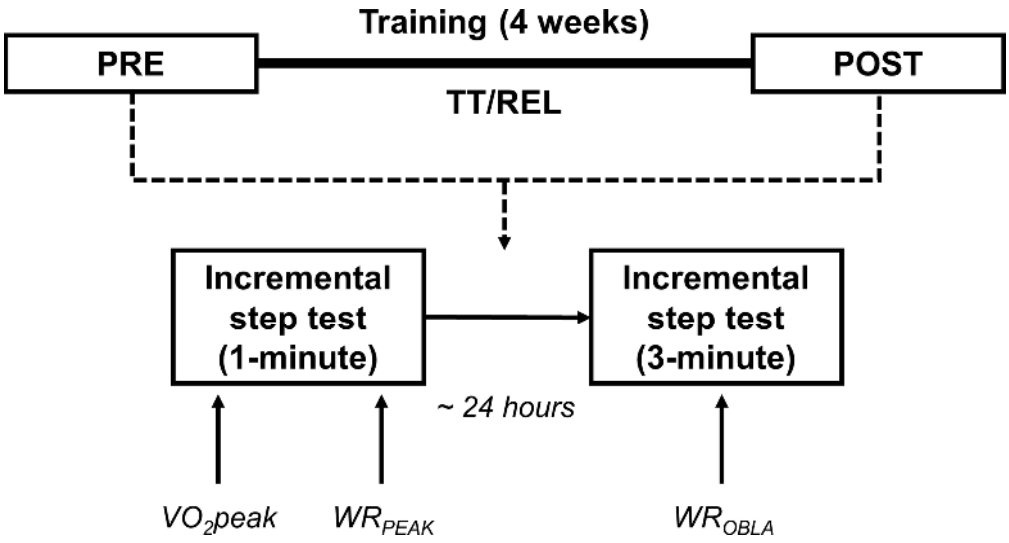

Fig. 1. Study protocol.

180x94mm (300 x 300 DPI)

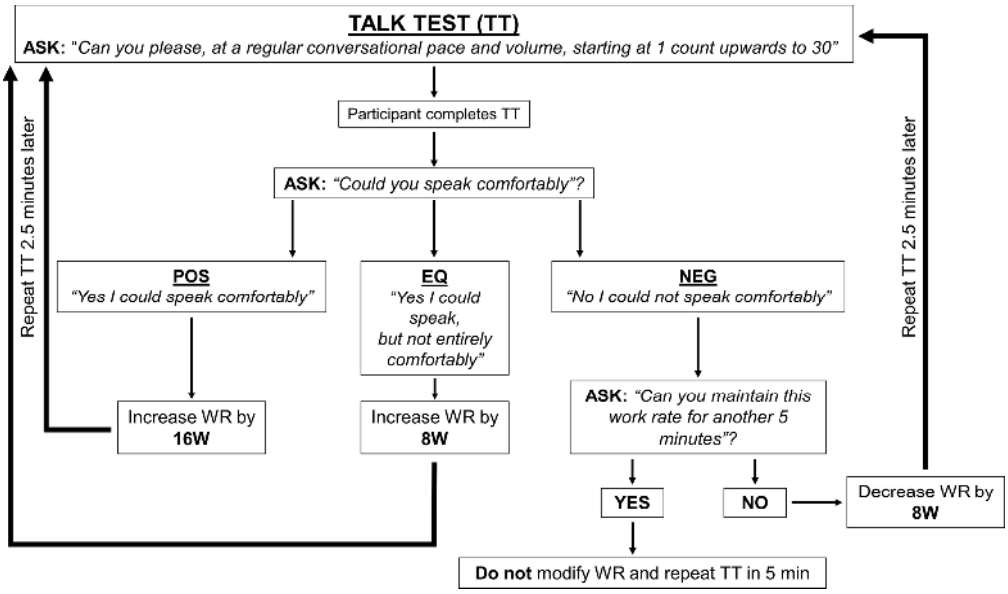

Fig. 2. Flow chart used to guide exercise intensity to the NEG TT stage.

180x104mm (300 x 300 DPI)

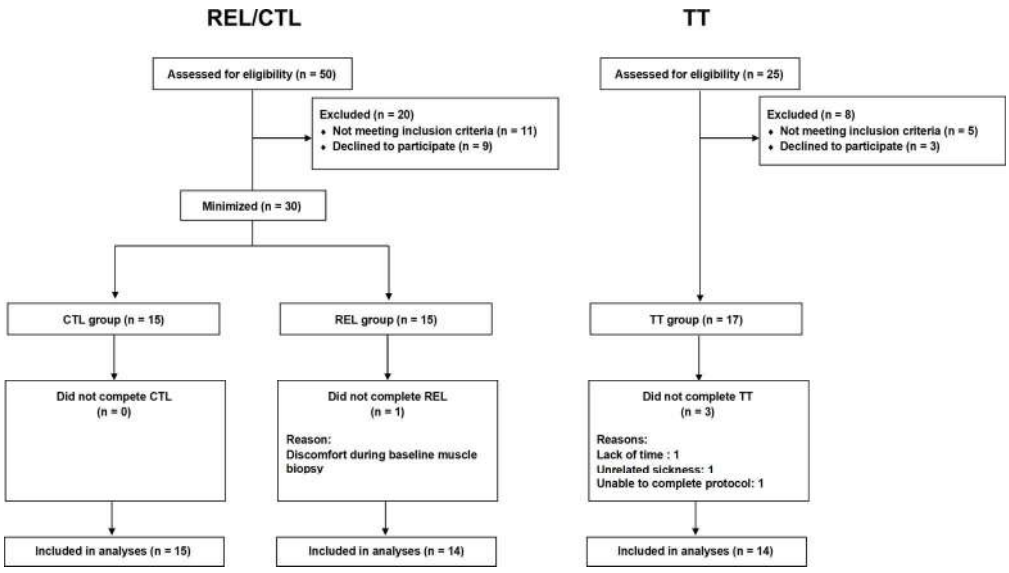

Fig. 3. Participant flow diagram.

180x100mm (300 x 300 DPI)

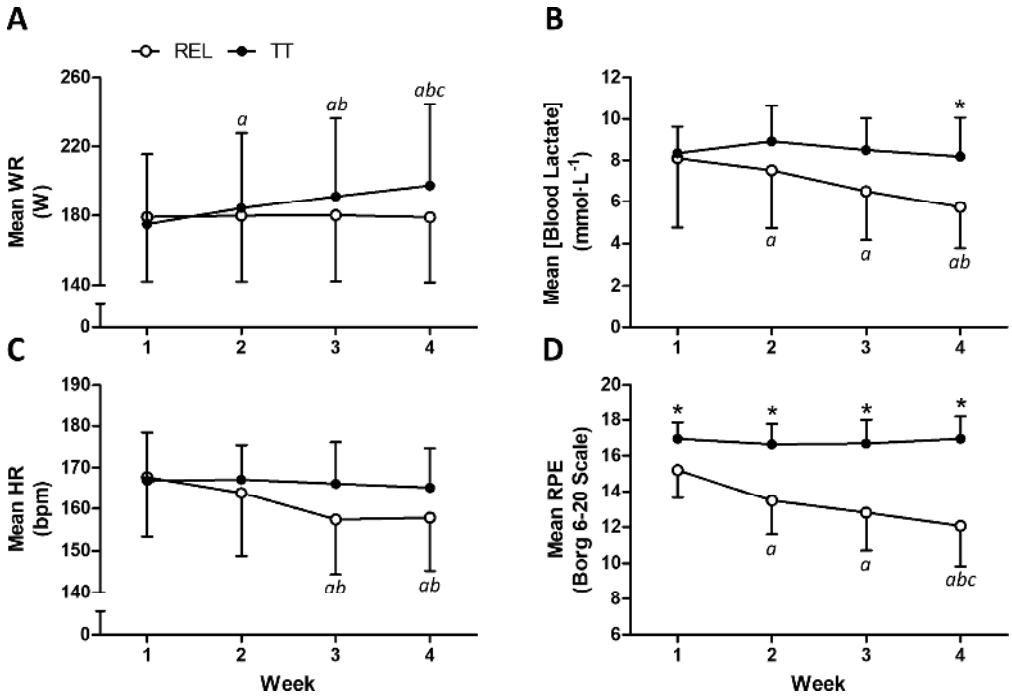

Fig. 4. Mean weekly work rate (WR), heart rate (HR), and RPE, and blood lactate of the first training session of each week in CTL, REL, and TT.
Significant interaction effect. (4A; $p < 0.05$, 4B; $p < 0.01$, 4C; $p < 0.01$, 4D; $p < 0.01$).
a Significantly different from week 1 ($p < 0.05$).
b Significantly different from week 2 ($p < 0.05$).
c Significantly different from week 3 ($p < 0.05$).
* Significant between-group difference ($p < 0.05$).

180x122mm (300 x 300 DPI)

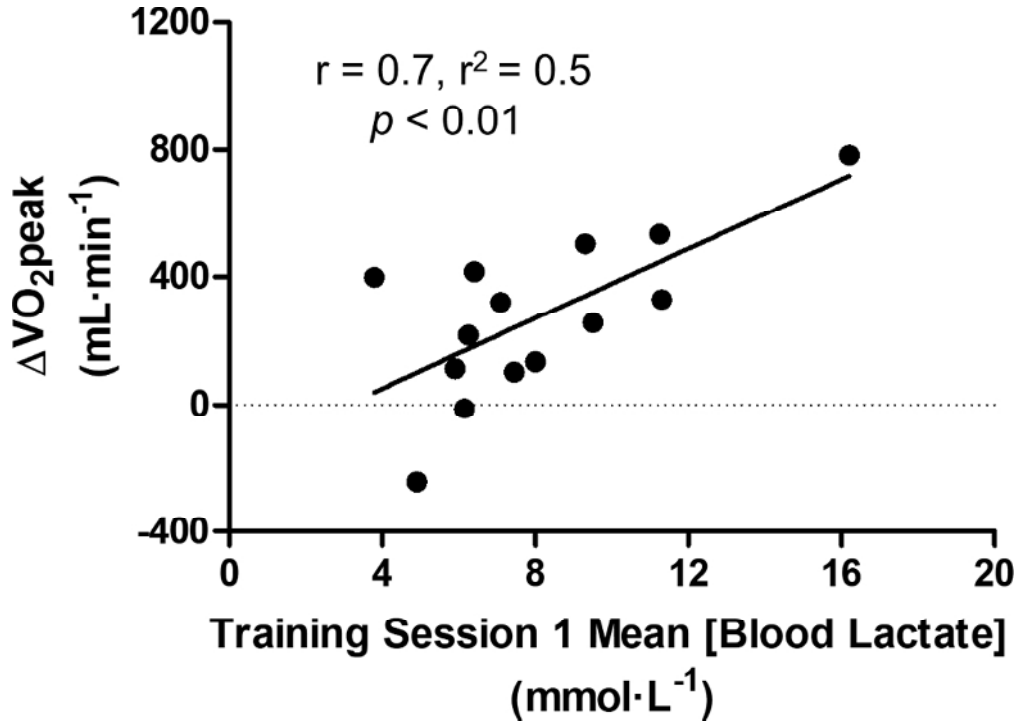

Fig. 5. Linear regression between mean blood lactate responses in the first training session and changes in VO2peak following training.

85x60mm (300 x 300 DPI)

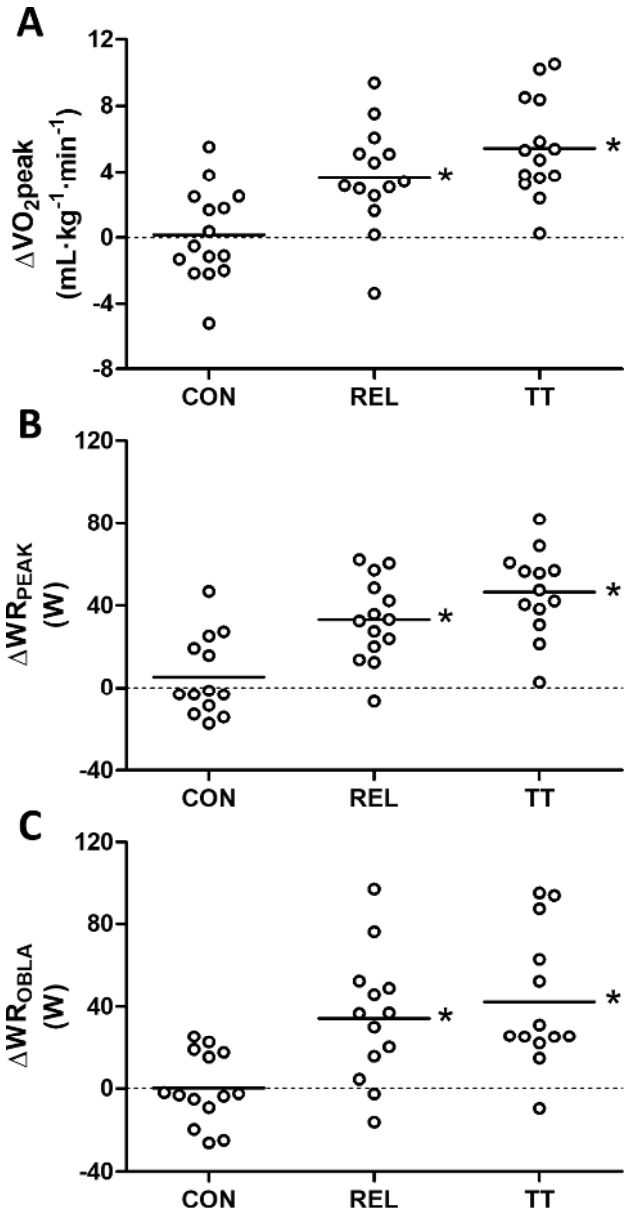

Fig. 6. VO2peak, WRPEAK, WROBLA before (PRE) and after (POST) four weeks of CTL, REL, and TT.‼ ┼ Main effect of time ($p < .001$) for all. Interaction effect ($p < .001$) for all. ‼ ┼ * Significantly different from PRE ($p < 0.001$).

85x165mm (300 x 300 DPI)