

## **Relationship between maximal incremental and high-intensity interval exercise performance in elite athletes**

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## Abstract

It remains unclear whether the number of total bouts to limitation ( $B_{lim}$ ) in high-intensity interval testing (HIIT) differs among individuals, no matter if performed at the same relative intensity. This study aimed to explore the physiologic factors determining tolerance to effort during a HIIT. Forty-seven female participants (15-28 years old) were included: 23 athletes from Taiwan national or national reserve teams, and 24 moderately-active female. Each participant underwent maximal incremental (INC; modified-Bruce protocol) cardiopulmonary exercise testing and HIIT on treadmill, on separate days. HIIT protocol alternated a 1-min effort at 120% of the maximal speed and the same slope reached at the end of INC, with a 1-min rest, until volitional exhaustion. Gas-exchanges, and muscle oxygenation at right vastus lateralis by near-infrared spectroscopy, were continuously recorded. Additionally, bioelectrical impedance was utilized for body composition analysis. The result showed that  $B_{lim}$  differed greatly (range: 2.6 to 12) among participants. Stepwise regression revealed that  $B_{lim}$  was determined primarily by oxygen consumption ( $VO_2$ ) and heart rate (HR) at second-minute recovery ; and, muscle tissue saturation index at peak of INC ( $R=0.644$ ). Also, age and percent body fat were linearly correlated with  $B_{lim}$  (adjusted  $R=-0.475$ ,  $-0.371$ ,  $p<0.05$ ). Therefore, HIIT performance is determined by fast recovery of  $VO_2$  and HR, rather than maximal  $VO_2$  or muscle oxygenation recovery. Moreover, capacity to sustain a HIIT declines with age since as early as late adolescent. Further investigations on which factors should be manipulated to further improve athletes performance are warrant.

**Keywords** exercise testing; oxygen uptake; heart rate; near-infrared spectroscopy; HIIT

## Abbreviations

ATT adipose tissue thickness

$B_{lim}$ : number of total bouts to limitation (i.e. exhaustion) in high-intensity interval exercise testing

BCM: body cell mass

CWR: constant work rate testing

EqCO<sub>2</sub>: ventilatory equivalent for CO<sub>2</sub>

EqO<sub>2</sub>: ventilatory equivalent for O<sub>2</sub>

FFM: fat free mass

HHb: deoxy-(hemoglobin+myoglobin)

HR: heart rate

HR<sub>max</sub>: maximal HR

HR reserve: heart rate reserve

$\Delta HR_{0.5} = HR_{max} - HR$  at 0.5 min recovery

$\Delta HR_1 = HR_{max} - HR$  at 1 min recovery

$\Delta HR_2 = HR_{max} - HR$  at 2 min recovery

$\Delta HR_{0.5}/ = \Delta HR_{0.5} / HR_{max}$

$\Delta HR_1/ = \Delta HR_1 / HR_{max}$

$\Delta HR_2/ = \Delta HR_2 / HR_{max}$

HRt<sub>1/2</sub> = time span the heart rate recovers to half

HIIT: high-intensity interval testing

INC: maximal incremental exercise test

MA: moderately-active subjects

Max O<sub>2</sub> pulse= maximal VO<sub>2</sub>/HR

NIRS: near-infrared spectroscopy

O<sub>2</sub>Hb: oxy-(hemoglobin+myoglobin)

$\Delta O_2Hb_{0.5}$  = O<sub>2</sub>Hb at 0.5 min during recovery – peak O<sub>2</sub>Hb

$\Delta O_2Hb_1$  = O<sub>2</sub>Hb at 1 min during recovery – peak O<sub>2</sub>Hb

$\Delta O_2Hb_2$  = O<sub>2</sub>Hb at 2 min during recovery – peak O<sub>2</sub>Hb

$\Delta O_2Hb_{0.5}/$  =  $\Delta O_2Hb_{0.5}/$  (maximal O<sub>2</sub>Hb – minimal O<sub>2</sub>Hb) during recovery

$\Delta O_2Hb_1 /$  =  $\Delta O_2Hb_1/$  (maximal O<sub>2</sub>Hb – minimal O<sub>2</sub>Hb) during recovery

$\Delta O_2Hb_2/$  =  $\Delta O_2Hb_2/$  (maximal O<sub>2</sub>Hb – minimal O<sub>2</sub>Hb) during recovery

PBF: percent body fat

SLM: soft lean mass

SMM: skeletal muscle mass

SMLA: segmental muscle mass, left arm

SMLL: segmental muscle mass, left leg

SMRA: segmental muscle mass, right arm

SMRL: segmental muscle mass, right leg

SMTR: segmental muscle mass, trunk

TBM/FFM: total body mass/fat free mass

T<sub>1/2</sub>: the time span the variable changes in half

THb: total-(hemoglobin+myoglobin)

TSI: tissue saturation index

TSI<sub>peak</sub>: nadir of tissue saturation index during INC

$\Delta TSI_{0.5}$  = TSI at 0.5 min during INC recovery – peak TSI

$\Delta TSI_1$  = TSI at 1 min during INC recovery – peak TSI

$\Delta TSI_2$  = TSI at 2 min during INC recovery – peak TSI

$\Delta TSI_{0.5}/$  =  $\Delta TSI_{0.5}/$  (maximal TSI – minimal TSI) during recovery

$\Delta\text{TSI1} / = \Delta\text{TSI1} / (\text{maximal TSI} - \text{minimal TSI})$  during recovery

$\Delta\text{TSI2} / = \Delta\text{TSI2} / (\text{maximal TSI} - \text{minimal TSI})$  during recovery

$\text{VCO}_2$ : carbonic dioxide consumption

$\text{V}_E$ : minute ventilation

$\text{VO}_2$ : oxygen consumption

$\text{VO}_{2\text{max}}$ : maximal  $\text{VO}_2$

$\Delta\text{VO}_20.5 = \text{VO}_{2\text{max}} - \text{VO}_2$  at 0.5 min recovery

$\Delta\text{VO}_21 = \text{VO}_{2\text{max}} - \text{VO}_2$  at 1 min recovery

$\Delta\text{VO}_22 = \text{VO}_{2\text{max}} - \text{VO}_2$  at 2 min recovery

$\Delta\text{VO}_20.5 / = \Delta\text{VO}_20.5 / \text{maximal } \text{VO}_2$

$\Delta\text{VO}_21 / = \Delta\text{VO}_21 / \text{maximal } \text{VO}_2$

$\Delta\text{VO}_22 / = \Delta\text{VO}_22 / \text{maximal } \text{VO}_2$

$\text{VO}_2\text{rt}_{1/2}$  = time span the  $\text{VO}_2$  recovers to half

## INTRODUCTION

Incremental exercise testing (INC) is nowadays widely used to assess cardiopulmonary fitness among various populations, from elite athletes to semi-professional players, to chronic cardiovascular and lung disease patients [1, 2]. The INC provides quantification of whole-body all-out performance and, in the athletic world, it has become the gold-standard evaluation to identify exercise intensity zones upon which athletic training programs are designed.

In many competitive ball games, such soccer, basketball, rugby, badminton, a typical field performance is interspersed with multiple intervals (i.e. combination of succeeding effort and recovery phases in series). At high professional level, the intensity of this field performance can be compared to a high-intensity interval workout. This latter has been for decades a preferred athletic training method to increase not only whole-body aerobic capacity, but also to manipulate the response of the peripheral skeletal muscle system in reducing the time of activation and recovery kinetics of oxygen transport metabolism [3] as to, consequently, reduce the delay between mechanical request (i.e. exercise task) and muscle metabolic response (the so called metabolic phase or Phase II [4]). It is likely that the performance in high-intensity interval testing (HIIT) is more related to the court performance of athletes competing in the above-mentioned team sports. If that is true, the traditional INC should not be the first testing choice to evaluate ball games players performance and to base upon the seasonal training schedule. Nonetheless, HIIT has seldomly been used as testing protocol [5, 6] and INC results are still used to planning the training calendar, which is largely a combination of high-intensity exercise workloads and interval training phases.

In 2017, a group of reserve athletes from Taiwan national soccer, basketball and badminton teams came to our laboratory at the Chang Gung Memorial Hospital for a series of performance routine evaluations. In that occasion we administered a traditional INC protocol and a newly designed HIIT. Similar to the concept of “time to limitation” (i.e.  $T_{lim}$ ) in constant work rate (CWR) testing, we applied the same approach to HIIT test, using the number of bouts to limitation ( $B_{lim}$ ) and a supra-maximal intensity exercise (i.e. 120% INC maximal velocity) as indexes to assess the overall cardiopulmonary fitness in ball games sport players. Repeated transitions between supra-maximal intensity and recovery phase involve anaerobic and aerobic energy metabolisms both during and immediately after the exercise.

The recovery capacity is an important physiologic determinant affecting the performance in many types of ball games but is not well assessed with the traditional INC or CWR testing. In the same relative intensity during CWR, the time to limitation only slightly varies from person to person based on the concept of power duration hyperbolic curve (for example,  $T_{lim}$  is  $6 \pm 2$  minutes at 80% peak work rate in every subject) [7]. In contrast, in HIIT, whether  $B_{lim}$  varies considerably or not among individuals is still unclear.

Therefore, the main aim of this descriptive study was to determine which factors are most strongly associated with the bouts to limitation in HIIT. We hypothesized that  $B_{lim}$  in HIIT, unlike  $T_{lim}$  in CWR testing, has a wide discrepancy among individuals given the same relative intensity and exercise-recovery duration and pattern, which we suggest is primarily due to individual recovery capacities.

## MATERIALS AND METHODS

### *Participants*

This study enrolled 47 athletes and moderately active (MA) female participants. The athletes were reserves for the national Taiwan soccer, basketball and badminton teams. The participants assigned to the MA group were young women participating in moderate intensity exercise for at least 60 min in total weekly [8]. The experiment protocol was approved by the Chang Gung Memorial Hospital Institutional Review Board. All participants provided a written informed consent after receiving oral and written explanations of the experimental procedures and associated risks. This research was performed in accordance with the ethical standards of the Declaration of Helsinki.

### *Protocol*

Participants came twice at our laboratories at the Chang Gung Memorial Hospital to perform an INC maximal test and a HIIT. Each subject was instructed to refrain from vigorous exercise or caffeine intake for the 24 h prior testing, and to have at least 8-hour sleep the night before the test. All assessments took place approximately at the same time of the day under controlled environmental conditions (24°C, 63% humidity).

*Anthropometric and Body Composition Evaluation:* At the beginning of the visit, basic anthropometric characteristics (height and weight) were taken. Afterwards, whole-body composition was determined using the InBody s10 (Seoul, Korea) and by measuring the electrical resistance to four different frequencies (5, 50, 250, and 500 kHz) [9-11]. Each participant lied on a padded table for the entire duration of the testing, and sensors to measure electrical resistance were placed at the level of each body segment following the manufacturer instructions. To undergo this 20-min procedure, participants were instructed to fast for 2 hours prior to the test.

*Cardiopulmonary testing:* During the first visit a maximal INC test was carried out. The test started with 1 min of walking at 1 mile/h, followed by an incremental modified-Bruce protocol conducted until volitional exhaustion was reached. Immediately after exhaustion, an active recovery phase at individual walking pace was administered for 1 min, followed by 3 min of passive recovery. The INC was defined as maximal when the following criteria were present: (i) a plateau in  $\text{VO}_2$  between the final 2 stages, (ii) HR exceeded 85% of its predicted maximum, and (iii) respiratory exchange ratio exceeded 1.15 [2].

During the second visit, that took place at least 24 hours after the first one, a supra-maximal HIIT was performed. The speed was set at 120% of the maximal velocity reached during the INC, and the slope was the same as the INC final stage. The HIIT protocol consisted of intermittent 1-min sprinting interspersed with 1-min passive recovery, until exhaustion. Total number of bouts completed before exhaustion was recorded ( $B_{lim}$ ).

### **Measurements**

All exercise assessments were performed on an electromechanically braked treadmill (VIASYS™), while minute ventilation ( $V_E$ ),  $VO_2$ , and carbonic dioxide production ( $VCO_2$ ) were measured using a computer-based system (MasterScreen CPX, Cardinal-health, Germany). Before each test, the gas analyzers and the turbine flow meter of the system were calibrated following the manufacturer's instructions and by using a gas mixture of known concentration ( $FO_2$ : 0.16;  $FCO_2$ : 0.05;  $N_2$  as balance) and an automatically-pumping high and low flow system. Heart rate was determined from the R-R interval on a 12-lead electrocardiogram.

Muscle oxygenation was evaluated by means of a Bluetooth, portable, continuous-wave and spatially-resolved near-infrared spectroscopy (NIRS) (PortaMon, Artinis, The Netherlands). Relative concentrations of deoxy-hemoglobin+myoglobin (HHb), oxy-hemoglobin+myoglobin ( $O_2Hb$ ), and tissue saturation index (TSI, %) [12] were continuously recorded during the exercises at the level of the peripheral muscle tissue, ~1.5 cm beneath the probe (interoptode distance 3 cm). From these measurements, the relative changes in total hemoglobin and myoglobin ( $THb = HHb + O_2Hb$ ) were calculated. The NIRS probe was wrapped in a plastic foil and placed longitudinally to the muscle *vastus lateralis* belly on the right thigh, 15 cm above the upper margin of the patella, and secured with an elastic bandage to minimize the possibility that external light influenced the signal.

### **Data Analysis**

Maximal  $VO_2$  ( $VO_{2max}$ ) and maximal heart rate ( $HR_{max}$ ) were defined as the highest values obtained during the INC test. For the recovery phase, a three breaths moving average was applied to calculate  $VO_2$  and HR off-kinetics. Similarly, for the NIRS data (i.e. TSI and  $O_2Hb$ ), five 1-s data were used in the process of moving average.

$VO_2$ , HR,  $O_2Hb$ , and TSI changes at 0.5, 1, and 2 minutes recovery after INC were calculated as suggested by Turner et al.[13] (Figure 1):

$$\Delta VO_{20.5} = VO_{2max} - VO_2 \text{ at } 0.5 \text{ min recovery}; \Delta VO_{20.5}/ = \Delta VO_{20.5} / VO_{2max}.$$

Eq.

Above equation was applied to determine the 1<sup>st</sup> and 2<sup>nd</sup> minute change, and change ratio at the same three time points. The same approach was used for HR determination.

In addition, TSIRT<sub>1/2</sub>, O<sub>2</sub>HbRT<sub>1/2</sub>, VO<sub>2</sub>RT<sub>1/2</sub>, and HRRT<sub>1/2</sub> are the time span the value recovers to half.

### ***Statistical analysis***

Data are given as mean and standard deviation (SD). The criterion for significance was set at  $p < 0.05$ . Pearson correlation, correlation matrix and partial correlation were used to determine the degree of association between physiological variables measured during the cardiopulmonary testing and B<sub>lim</sub>. Since the population sample size allocate to final analyses was of 47, the first five parameters with the greatest correlation coefficient were included in the regression model. Forward stepwise linear regression was run to seek for predictors of B<sub>lim</sub>. Analyses were done using SPSS 22.0 (SPSS, Inc., Chicago, IL, USA).

## RESULTS

Forty-seven participants (n=23 athletes; n=24 MA) successfully completed the experimental protocol phases.  $B_{lim}$  differed greatly among subjects, ranging from 2.6 to 12. The representative results on the strongest physical parameters correlated to  $B_{lim}$  are shown in Figure 2. Among the anthropometric variables, age was significantly moderately correlated to  $B_{lim}$  ( $R=-0.475$ ;  $p=0.008$ ) after adjusting variables with co-linearity, including group, percent body fat (PBF),  $\Delta VO_2$ ,  $\Delta HR/$  and  $TSI_{peak}$  (Figure 2A). As to the variables derived from body composition analysis, PBF was significantly associated with  $B_{lim}$  (Figure 2B).

When the HIIT and INC were compared by univariate analyses, several physiological parameters during INC were significantly correlated with  $B_{lim}$ . Those parameters with a correlation coefficient  $>0.3$  and  $P<0.05$  are shown in S1. The five physical parameters with the highest correlation coefficients ( $\Delta VO_2$ ,  $VO_{2max}$ ,  $\Delta HR/$ ,  $TSI_{peak}$  and  $\Delta HR$ ) were put into the forward linear stepwise regression model (Table 1). The explanatory power in model 3 of the multiple linear regression model was 0.415. It revealed that  $B_{lim}$  were majorly determined by  $\Delta VO_2$ ,  $\Delta HR/$  and  $TSI_{peak}$ . Their scatter plots to  $B_{lim}$  are shown in Figure 3. Correlations were positive in  $\Delta VO_2$ ,  $\Delta HR/$ , and negative in  $TSI_{peak}$  (Figure 3A, 3B and 3C).

Figure 4 shows an example of a comparison between two subjects with similar  $VO_{2max}$  but significantly different  $B_{lim}$  (12 vs. 7.7). The subjects who tolerated more bouts in HIIT had a faster recovery in  $VO_2$  and HR and a lower  $TSI_{peak}$  value in INC.

The main findings for INC and HIIT are provided in Table 2. As expected, the athletes had a significantly higher  $VO_{2max}$ , maximal  $O_2$  pulse, peak  $V_E$  and lower  $TSI_{peak}$  in INC, and better performance in HIIT ( $B_{lim}$ :  $7.8 \pm 2.3$  vs.  $4.2 \pm 1.4$ ,  $p<0.05$ ) than MA.

Main results in the recovery phase of INC are presented in Table 3. HR recovery at the 2<sup>nd</sup> minute and  $VO_2$  recovery are faster in Ath than MA. Muscle oxygenation recovery showed no difference between Ath and MA. It is noteworthy that  $\Delta HR/$  and  $\Delta VO_2$  that were the major determinants of  $B_{lim}$  picked up by the stepwise regression are significantly higher in Ath than MA.

Anthropometric data showed no difference in body weight, body height and BMI except age ( $19 \pm 3$  vs.  $24 \pm 2$  years) between Ath and MA. Table 4 shows the body composition data. Ath has higher SMM, SLM, FFM, SMRA, SMTR, SMRL, protein, BCM and lower PBF. The P-value of SMLA and SMLL are 0.051 and 0.055.

## DISCUSSION

Traditionally, an INC protocol is administered to obtain a physiological quantification (e.g.  $\dot{V}O_{2\max}$ ) reflecting the cardiopulmonary fitness of an individual. Previous research showed that  $\dot{V}O_{2\max}$  is poorly correlated with the athletic performance on the court [14]. One reason seems to be the different exercise patterns between the laboratory testing procedure (i.e. INC) and the field performance of several sportive ball games (e.g. badminton, basketball, soccer). This descriptive study aimed to investigate the HIIT performance under the same relative intensity and duty cycle (120% speed and the same incline as one's INC in the final stage alternating with a 1-min rest) on a group of 47 participants in order to explore the physiologic factors determining tolerance to effort during a HIIT. Our results showed that total number of bouts to limitation ( $B_{\text{lim}}$ ) was widely distributed among study population, ranging from 2.6 to 12 bouts (mean:  $6.0 \pm 2.6$ ). Further regression analyses, seeking to reveal which physiological parameters determine this large distribution, suggests that HR and  $\dot{V}O_2$  recovery are the key variables to reach a longer HIIT duration (i.e. higher number of bouts completed).

### *Parameters derived from INC influencing $B_{\text{lim}}$*

We were interested in determining what variables extracted from a comprehensive analyses of the traditional INC test, influence  $B_{\text{lim}}$  and therefore the individual tolerance of high-intensity intermittent exercise. Results from linear stepwise regression analysis revealed that  $B_{\text{lim}}$  was more strongly associated with three physiological parameters:  $\Delta\dot{V}O_2$ ,  $\Delta\text{HR}_2$ , and  $\text{TSI}_{\text{peak}}$ .

$\dot{V}O_2$  recovery ( $\Delta\dot{V}O_2$ ), rather than  $\dot{V}O_{2\max}$ , was found the strongest determinant of  $B_{\text{lim}}$  in the linear stepwise regression analysis. This result is in agreement with a previous study by Harris and colleagues [15] that investigated the time course of phosphocreatine (PCr) resynthesis in a group of adults undergoing a maximal test performed on cycle-ergometer. These authors observed that muscle recovery kinetics after exhaustive maximal exercise is biphasic, with alactacid component (from 10 s to a few minutes) and lactacid component (a few minutes to hours) [16, 17]. The alactacid component consists of oxygen-dependent adenosine triphosphate (ATP) and PCr replenish, which is the primary energy replenish pathway during HIIT. Participants with faster ATP/PCr replenish have more rapid  $\dot{V}O_2$  recovery within the first minutes following the dynamic exhaustive exercise and thereby, a

broader  $\text{VO}_2$  reserve in the next HIIT bout. Thus, as evidenced by our statistical model, these individuals are characterized by having a greater  $B_{\text{lim}}$ .

The second parameter that determines performance capacity during an HIIT is the HR recovery ( $\Delta\text{HR}2/$ ). HR recovery consists of rapid (parasympathetic reactivation) and subsequent phase (sympathetic withdrawal and humoral factors) [18]. It reflects the regulatory capability of cardiac autonomic nervous system. Previous studies showed that HR recovery reflects the  $\text{VO}_{2\text{max}}$  [19-21] and training status [22, 23]. The present study further found that it is related to performance in HIIT. During HIIT, faster HR recovery suggests a broader HR reserve for the next bout. This highlighted the importance of cardiac autonomic nervous system regulation in determining HIIT performance.

Together with the recovery rate for  $\text{V}'\text{O}_2$  and HR,  $\text{TSI}_{\text{peak}}$  was found a significant parameter in explaining individual  $B_{\text{lim}}$  and performance in HIIT among the participants enrolled in the current study. TSI represents a balance between muscular oxygen delivery and consumption [24, 25]. Change in TSI has been shown to increase after HIIT training, which is accompanied by increase in mitochondrial biogenesis, capillarization, and mitochondrial enzyme activity [26-28]. It is likely that participants with lower  $\text{TSI}_{\text{peak}}$  (larger TSI change) tolerate regional metabolic acidosis better and are, thereby, prone to have greater  $B_{\text{lim}}$ .

In addition,  $B_{\text{lim}}$  and age showed strong relationship in our cohort (age range 15 to 28 years) suggesting that the capacity of sustaining the HIIT for long duration is dependent by the maturity (age) of the individual undergoing the test. In a study by Ratel et al. [29], authors compared the recovery capacity among prepubescent boys ( $n=11$ , age  $9.6 \pm 0.7$  years), pubescent boys ( $n=9$ , age  $15 \pm 0.7$  years), and men ( $n=10$ , age  $20.4 \pm 0.8$  years) undergoing a ten-bout intermittent sprinting cycling test (friction load = 50% optimal force), separated by 30 s, 1 and 5 min passive recovery. The capacity of maintaining peak cycling power from the first to tenth set decreased ( $p<0.01$ ) by 11.3% in men and 15.3% in pubescent boy, with no changes in the prepubescent group, when the recovery interval was of 1 min, i.e. same duration we applied in the current study HIIT test. Ratel study suggested that for pubescent and men categories a longer time of recovery is needed to the higher muscle glycolytic activity and slower PCr resynthesis. Similarly, in a study by Zafeiridis et al. [30]. the effect of age was investigated respect the recovery capacity after high-intensity intermittent isokinetic strength exercise. A group of boys (age,  $11.4 \pm 0.5$  years), teens (age,  $14.7 \pm 0.4$  years), and

men (age,  $24.1 \pm 2$  years) were enrolled and performed two sets of exercise of 30 and 60 s bout duration, intermitted by a 1 or 2 min rest, respectively. Results showed that the teens tended to recover faster than men, suggesting that the rate of recovery for both type of task was age-dependent. Accordingly, recovery capacity from an anaerobic performance decreases with age, starting as early as 9 to 11 years. Age-related exercise capacity decline is multi-factorial, such as decrease of intramuscular PCr concentration, intramuscular creatine kinase concentration, rate of PCr hydrolysis, and glycolytic enzyme activity, as well as change in muscle architecture and speed of neural activation [16, 31-35]. However, due to the wide age range and exercise type (aerobic or anaerobic) [36-38], the detailed mechanism of age-related decline of anaerobic performance from teens to young-adult remains uncertain.

Finally, we found also that the percentage of body fat is inversely associated with  $B_{lim}$  in our cohort ( $r = -0.371$ ). Higher body fat proportion means less muscle mass that can produce power. Body fat, an excessive loading to carry during any type of physical effort, has been proven to decrease the performance of anaerobic exercise [39].

## Limitations

This study has few limitations. First, the explanatory power in model 3 of the multiple linear regression model was 0.415. As consequence, other factors should be considered to explain the 1–2 bout deviation of participants' maximal  $B_{lim}$  we seen in our cohort. A plausible factor to consider in describing which are the determinants of HIIT performance could be found on the role played by motivation and compliance of participants [14]. To minimize the variability of this latter, before data collection, each participant was informed on the relevance of the results to planning the training season. We are quite confident that view the fact that the athletes were all nationals, our motivation point was effective in obtaining reliable effort to intolerance.

In addition, it could seem contradictory that from our statistical model analyses that the recovery of HR and  $VO_2$  at the second minute ( $\Delta VO_{22}$ ,  $\Delta HR_{2/}$ ), rather than those at 30 s or 1 min or  $t_{1/2}$ , were more related to  $B_{lim}$ . It can be explained by the difference between active and passive recovery. During the recovery phase, all participants were instructed to walk at their casual speed for 1 min (active recovery) and then stand still for another 2 min (passive recovery). However, HR decreased less in active recovery compared with passive recovery, in that the latter has a lower central command from the motor cortex and muscle mechano-metabo receptor activity from skeletal muscle contraction than the former [40]. Oxygen

consumption is also higher in active recovery [41]. The competition of oxygen between PCr replenish and muscle activity during active recovery produces higher  $VO_2$  [42, 43]. Due to variable casual speed during active recovery among every subject, the recovery kinetics is not comparable.

Furthermore, limitations exist in the use of NIRS methodology in estimating muscle metabolism, i.e. high melanin and large adipose tissue thickness (ATT) cause a signal attenuation, reducing the amount of light reaching the muscle tissue under investigation. Nevertheless, the low content of melanin (participants were all Asians) and the ATT below 1.8 mm confirm the reliability of our NIRS data [44]. Finally, the subjects in our study were all female. As consequence, whether sex might influence the results of our analyses deserve future investigation.

## **CONCLUSION**

Bouts to limitation (i.e.  $B_{lim}$ ) in HIIT differ greatly among individuals, provided the same relative intensity and duty-recovery cycle.  $VO_2$  recovery, HR recovery, and  $TSI_{peak}$  are the major determining physical factors to  $B_{lim}$ . In addition, our results showed that age seems to represent a strong influencing factor.  $B_{lim}$  declines remarkably since as early as late adolescent in the study participants. The study adds interpretational value in the recovery phase of maximal incremental cardiopulmonary test and further improves our understanding of the factors that predict a good performance in HIIT.

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## **Author contribution statement**

HSC, AA, and LYC conceived and designed the experiments. CSC, LHC, LYC, CCPC, and HSC performed the experiments. AA, LHC, LYC, CCPC, HCC and HSC analyzed the data.

CSC, LHC, HCC and HSC contributed analysis tools. AA, CSC, LHC, and HSC wrote the paper. All authors revised, proof-read and approved the final version of the manuscript. HSC, CCPC, HCC and LYC obtained funding.

### **Disclosures**

No conflicts of interest, financial or otherwise are declared by the authors.

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## FIGURE LEGENDS

### Figure 1

The data of TSI and O<sub>2</sub>Hb after moving average in the recovery phase of INC are graphed. TSI is labeled as black line and O<sub>2</sub>Hb is labeled as grey. Maximal and minimal value of TSI and O<sub>2</sub>Hb are labeled as Max, and Min, respectively. Peak value is the value at peak during INC. T<sub>1/2</sub> is the time span the variable changes in half. For simplicity, data during exercise is not shown. The calculation formula were as follows.  $\Delta\text{TSI}_{0.5} = \text{TSI at 0.5 min during INC recovery} - \text{peak TSI}$ ;  $\Delta\text{TSI}_{0.5}/ = \Delta\text{TSI}_{0.5}/ (\text{maximal TSI} - \text{minimal TSI})$  during recovery. The same formula applies to the 1st and 2nd minute, and also O<sub>2</sub>Hb. O<sub>2</sub>Hb: oxy-(hemoglobin+myoglobin), TSI: tissue saturation index, INC: Incremental exercise testing,

### Figure 2

Scatter plots of age and body composition against total number of bouts in high-intensity interval test. In Figure 2A, partial correlation is utilized to adjust the variables with co-linearity, including group, PBF,  $\Delta\text{VO}_{22}$ ,  $\Delta\text{HR}_{2/}$  and TSI<sub>peak</sub>. PBF, percent body fat;  $\Delta\text{VO}_{22} = \text{VO}_{2\text{max}} - \text{VO}_2$  at 2 min during recovery;  $\Delta\text{HR}_{2/} = (\text{HR}_{\text{max}} - \text{HR at 2 min during recovery}) / \text{maximal HR}$ ; TSI<sub>peak</sub>, nadir of tissue saturation index during INC

### Figure 3

Scatter plots of physical parameters derived from the incremental exercise testing against total number of bouts to limitation in the HIIT.  $\Delta\text{VO}_{22} = \text{VO}_{2\text{max}} - \text{VO}_2$  at 2 min during recovery;  $\Delta\text{HR}_{2/} = (\text{HR}_{\text{max}} - \text{HR at 2 min during recovery}) / \text{maximal HR}$ ; TSI<sub>peak</sub>, nadir of tissue saturation index during INC.

### Figure 4

The two subjects had very close maximal VO<sub>2</sub> in INC but differed greatly in B<sub>lim</sub>(12 vs. 7.7 bouts) in HIIT. The dark and light horizontal lines denote the peak values in their INCs. The subject who tolerated more bouts in HIIT had a faster recovery in VO<sub>2</sub>, HR, and lower TSI<sub>peak</sub>. HIIT, high-intensity interval testing; INC, maximal incremental exercise test; VO<sub>2</sub>, oxygen consumption; TSI<sub>peak</sub>, nadir of tissue saturation index during INC.

**Table 1. Stepwise linear regression of  $B_{lim}$  based on physiologic parameters**

	$\beta$	t	P( $\beta$ )	R	$\Delta R^2$	F
Model 1				0.471	0.222	9.396*
$\Delta VO_2$	0.471	3.065	0.04			
Model 2				0.572	0.106	7.798*
$\Delta VO_2$	0.368	2.419	0.021			
$\Delta HR$	0.342	2.247	0.032			
Model 3				0.644	0.086	7.304*
$\Delta VO_2$	0.282	1.884	0.069			
$\Delta HR$	0.317	2.192	0.036			
$TSI_{peak}$	-0.309	-2.139	0.040			

\*p < 0.05; the p-value indicates the overall significance of the linear regression model

P( $\beta$ ): p-value for  $\beta$

**Table 2.** Physical parameters during INC and HIIT

	<b>Ath</b>	<b>MA</b>
<b>INC</b>		
VO <sub>2</sub> max (L.min <sup>-1</sup> .Kg <sup>-1</sup> )	45.7 ± 6.0*	37.8 ± 7.6
peak HR (min <sup>-1</sup> )	185.0 ± 8.4	187.4 ± 10.0
peak O <sub>2</sub> pulse (ml)	12.8 ± 1.8*	10.4 ± 1.9
EqCO <sub>2</sub> nadir	24.3 ± 2.2	24.1 ± 2.2
EqO <sub>2</sub> nadir	20.4 ± 2.1	21.1 ± 2.7
peak V <sub>E</sub> (L.min <sup>-1</sup> )	83.0 ± 13.5*	76.3 ± 14.0
V <sub>E</sub> /VCO <sub>2</sub> slope	26.8 ± 3.3	25.4 ± 6.9
TSI <sub>peak</sub> (%)	56.4 ± 6.6*	61.0 ± 5.1
HHb <sub>peak</sub> (μM)	0.9 ± 5.0	2.1 ± 5.0
O <sub>2</sub> Hb <sub>peak</sub> (μM)	-4.1 ± 4.7	-6.6 ± 4.5
THb <sub>peak</sub> (μM)	-3.3 ± 6.5	-4.5 ± 7.9
<b>HIIT</b>		
B <sub>lim</sub> (times)	7.8 ± 2.3*	4.2 ± 1.4

Value are shown as mean ± SD, \*  $p < 0.05$  in Mann-Whitney U test; Ath: athletes; MA: moderately-active subjects; HR: heart rate; V<sub>E</sub>: ventilation; TSI: tissue saturation index; HHb: deoxyhemoglobin; O<sub>2</sub>Hb: oxyhemoglobin; THb: total hemoglobin; TSI<sub>peak</sub>: nadir of tissue saturation index during INC; EqCO<sub>2</sub>: ventilator equivalent for CO<sub>2</sub>; EqO<sub>2</sub>: ventilatory equivalent for O<sub>2</sub>

**Table 3** Physiologic response during the recovery phase at the end of the incremental exercise test

	Ath	MA	Ath	MA	Ath	MA	Ath	MA			
$\Delta HR_{0.5}$	9.4 ± 6.3	8.8 ± 3.5	$\Delta VO_2_{0.5}$	14.1 ± 5.9*	8.8 ± 3.5	$\Delta TSI_{0.5}$	3.52 ± 3.42	3.01 ± 2.50	$\Delta O_2Hb_{0.5}$	3.73 ± 2.52	3.05 ± 2.33
$\Delta HR_1$	24.2 ± 9.0	20.1 ± 6.3	$\Delta VO_2_1$	23.8 ± 4.3*	20.1 ± 6.3	$\Delta TSI_{1.0}$	8.19 ± 4.42	7.35 ± 3.55	$\Delta O_2Hb_{1.0}$	8.24 ± 3.05	7.72 ± 4.00
$\Delta HR_2$	56.5 ± 11.8*	45.5 ± 10.1	$\Delta VO_2_2$	34.6 ± 5.5*	27.6 ± 6.8	$\Delta TSI_{2.0}$	12.73 ± 6.47	11.23 ± 2.93	$\Delta O_2Hb_{2.0}$	11.82 ± 3.37	10.94 ± 4.53
$\Delta HR_{0.5}/$	0.05 ± 0.03	0.05 ± 0.02	$\Delta VO_2_{0.5}/$	0.3 ± 0.1	0.05 ± 0.02	$\Delta TSI_{0.5}/$	0.23 ± 0.21	0.21 ± 1.16	$\Delta O_2Hb_{0.5}/$	0.27 ± 0.17	0.20 ± 0.13
$\Delta HR_1/$	0.13 ± 0.05	0.11 ± 0.03	$\Delta VO_2_1/$	0.5 ± 0.1	0.11 ± 0.03	$\Delta TSI_{1.0}/$	0.57 ± 0.23	0.51 ± 0.21	$\Delta O_2Hb_{1.0}/$	0.59 ± 0.16	0.50 ± 0.19
$\Delta HR_2/$	0.31 ± 0.06*	0.24 ± 0.06	$\Delta VO_2_2/$	0.7 ± 0.0	0.24 ± 0.06	$\Delta TSI_{2.0}/$	0.84 ± 0.16	0.77 ± 0.15	$\Delta O_2Hb_{2.0}/$	0.83 ± 0.12	0.70 ± 0.19
$HR_{t_{1/2}}$ (s)	84.9 ± 18.6	92.4 ± 12.2	$VO_{2rt_{1/2}}$ (s)	53.5 ± 8.6	92.4 ± 12.2	$TSI_{t_{1/2}}$ (s)	53.1 ± 14.1	29.3 ± 14.1	$O_2Hb_{t_{1/2}}$ (s)	59.5 ± 23.7	49.0 ± 15.6

Data are mean ± SD, \* Ath vs. MA,  $p < 0.05$  in Mann-Whitney U test

$\Delta HR_{0.5}$ ' ( $\text{min}^{-1}$ )= peak HR – HR at 0.5 min recovery;  $\Delta HR_1$  ( $\text{min}^{-1}$ )= peak HR – HR at 1 min recovery;  $\Delta HR_2$ ( $\text{min}^{-1}$ ) = peak HR – HR at 2 min recovery;  $\Delta HR_{0.5}/$  =  $\Delta HR_{0.5}/$  maximal HR;  $\Delta HR_1/$  =  $\Delta HR_1/$  maximal HR;  $\Delta HR_2/$  =  $\Delta HR_2/$ maximal

The above equations also applied to  $VO_2$ ( $\text{ml}\cdot\text{min}^{-1}\cdot\text{Kg}^{-1}$ )

$\Delta TSI_{0.5}$  ( $\mu\text{M}$ ) = TSI at 0.5 min recovery - peak TSI;  $\Delta TSI_1$  ( $\mu\text{M}$ ) = TSI at 1 min recovery - peak TSI;  $\Delta TSI_2$  ( $\mu\text{M}$ ) = TSI at 2 min recovery - peak TSI;  $\Delta TSI_{0.5}/$  =  $\Delta TSI_{0.5}/$  (maximal TSI – minimal TSI) recovery;  $\Delta TSI_1/$  =  $\Delta TSI_1/$  (maximal TSI – minimal TSI) recovery;  $\Delta TSI_2/$  =  $\Delta TSI_2/$  (maximal TSI – minimal TSI) recovery;

The above equations also applied to  $O_2Hb$  ( $\mu\text{M}$ )

**Table 4.** Body composition data

	<b>Ath</b>	<b>MA</b>
SMM (Kg)	23.6 ± 2.3*	22.2 ± 2.3
Fat (Kg)	11.8 ± 2.7	13.7 ± 3.8
PBF (%)	21.5 ± 4.1*	24.9 ± 4.5
SLM (Kg)	40.4 ± 3.7*	38.3 ± 3.7
FFM (Kg)	42.9 ± 3.9*	40.6 ± 3.9
SMRA (Kg)	2.1 ± 0.3*	1.9 ± 0.3
SMLA (Kg)	2.0 ± 0.3	1.9 ± 0.3
SMTR (Kg)	18.8 ± 1.8*	17.9 ± 1.7
SMRL (Kg)	6.9 ± 0.8*	6.5 ± 0.8
SMLL (Kg)	6.9 ± 0.7	6.6 ± 0.8
Protein (Kg)	8.5 ± 0.8*	8.0 ± 0.8
BCM (Kg)	28.0 ± 2.6*	26.6 ± 2.6
TBM/FFM (%)	73.3 ± 0.2	73.3 ± 0.2

Value are shown as Mean ± SD, \*  $p < 0.05$  in Mann-Whitney U test; Ath: athletes; MA: moderately-active subjects; SMM: Skeletal Muscle Mass; PBF: percent body fat; SLM: Soft Lean Mass; FFM: Fat Free Mass; SMRA: segmental muscle right arm; SMLA: segmental muscle left arm; SMTR: segmental muscle trunk; SMRL: segmental muscle right leg; SMLL: segmental muscle left leg; BCM: body cell mass; TBM/FFM: total body mass/fat free mass

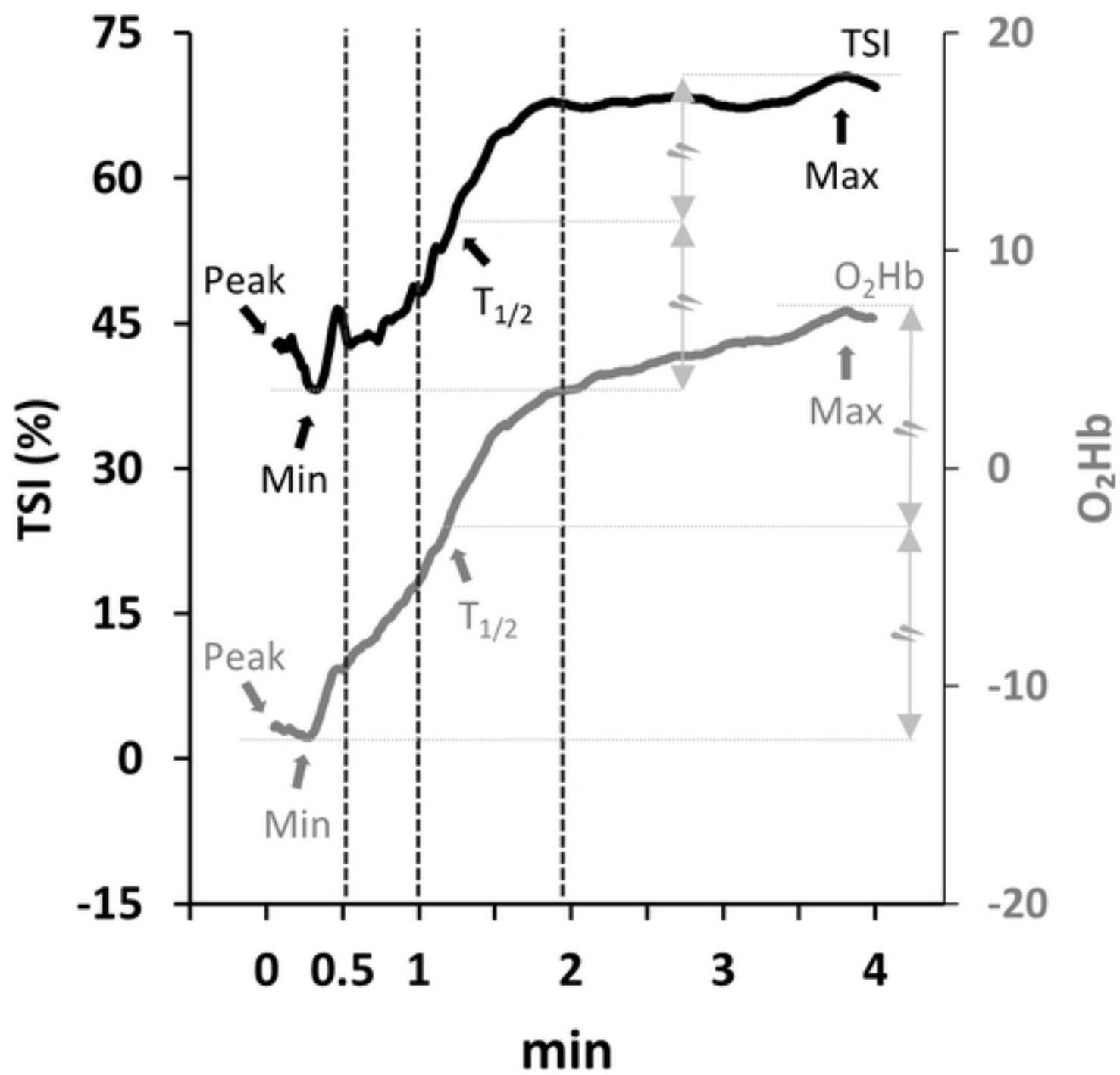
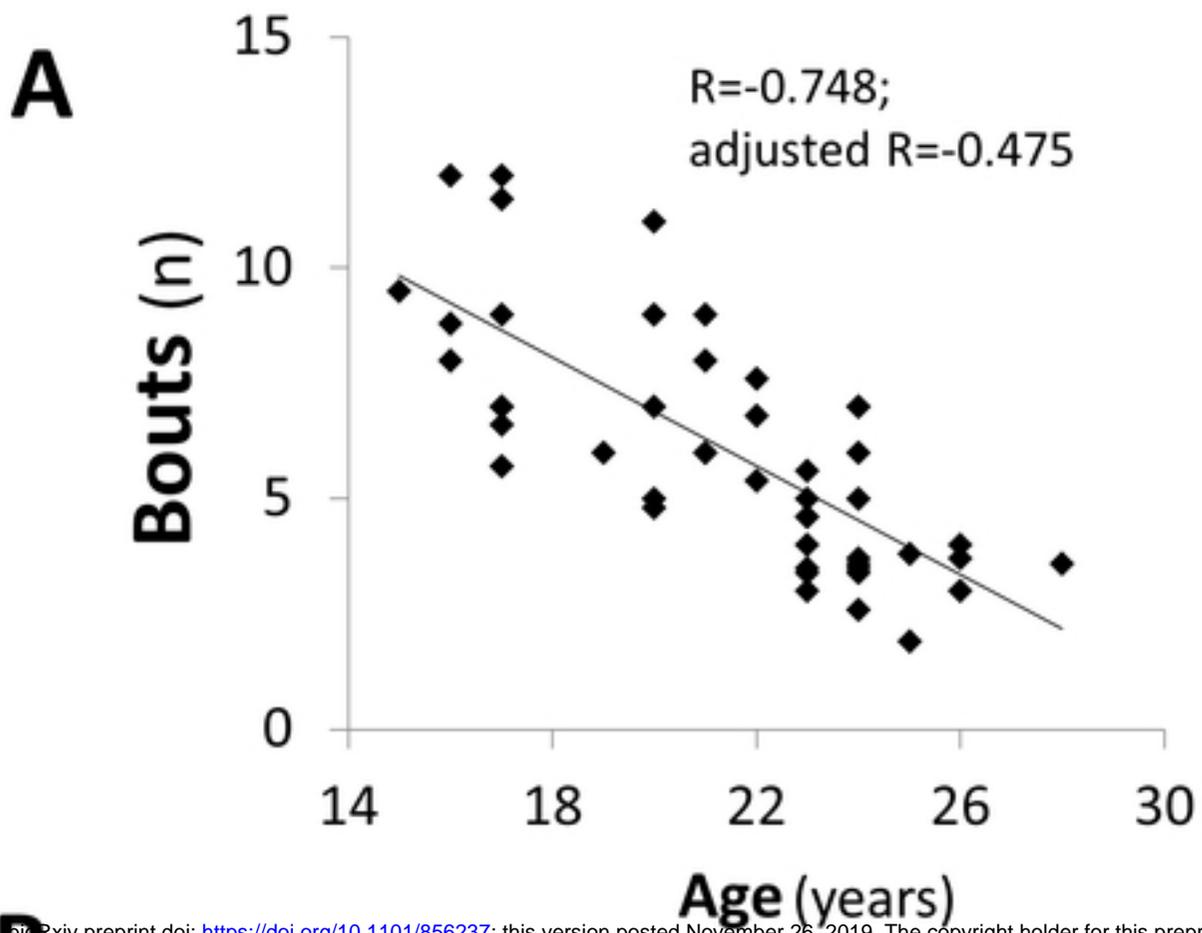


Figure 1



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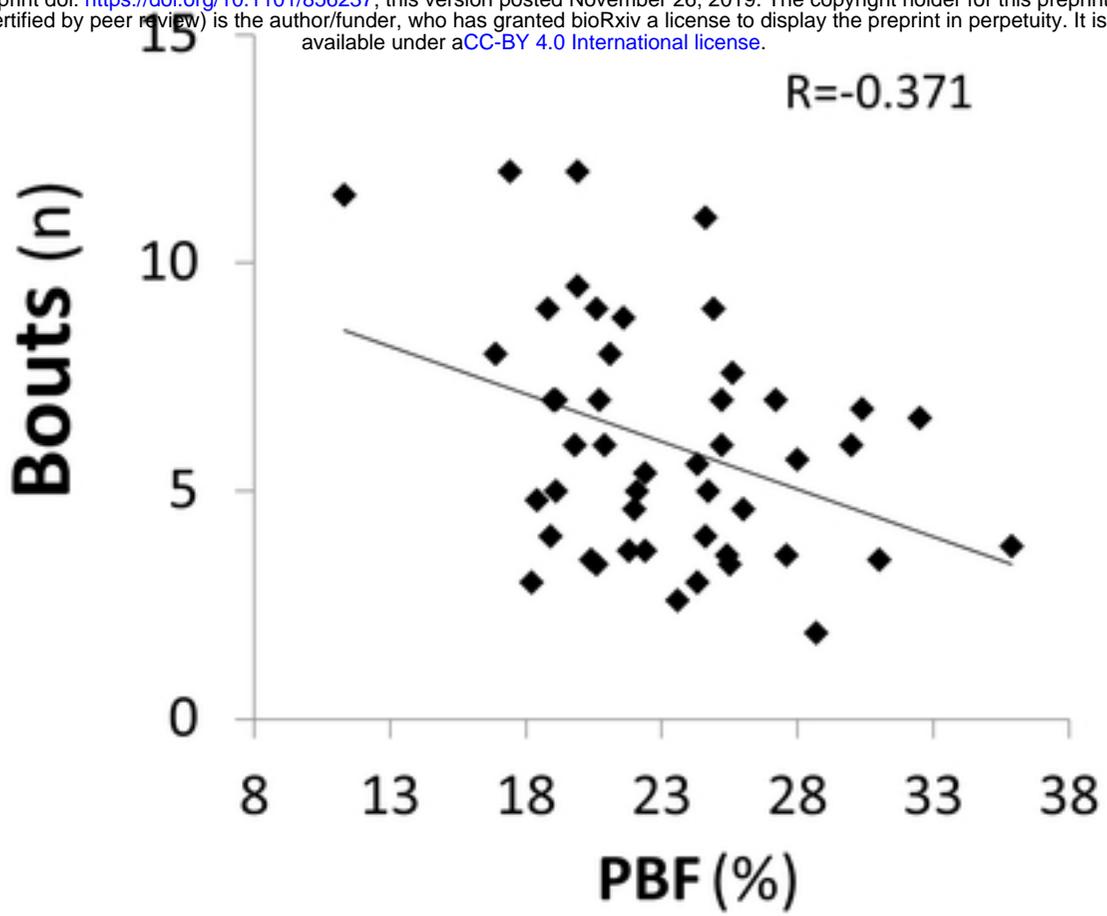
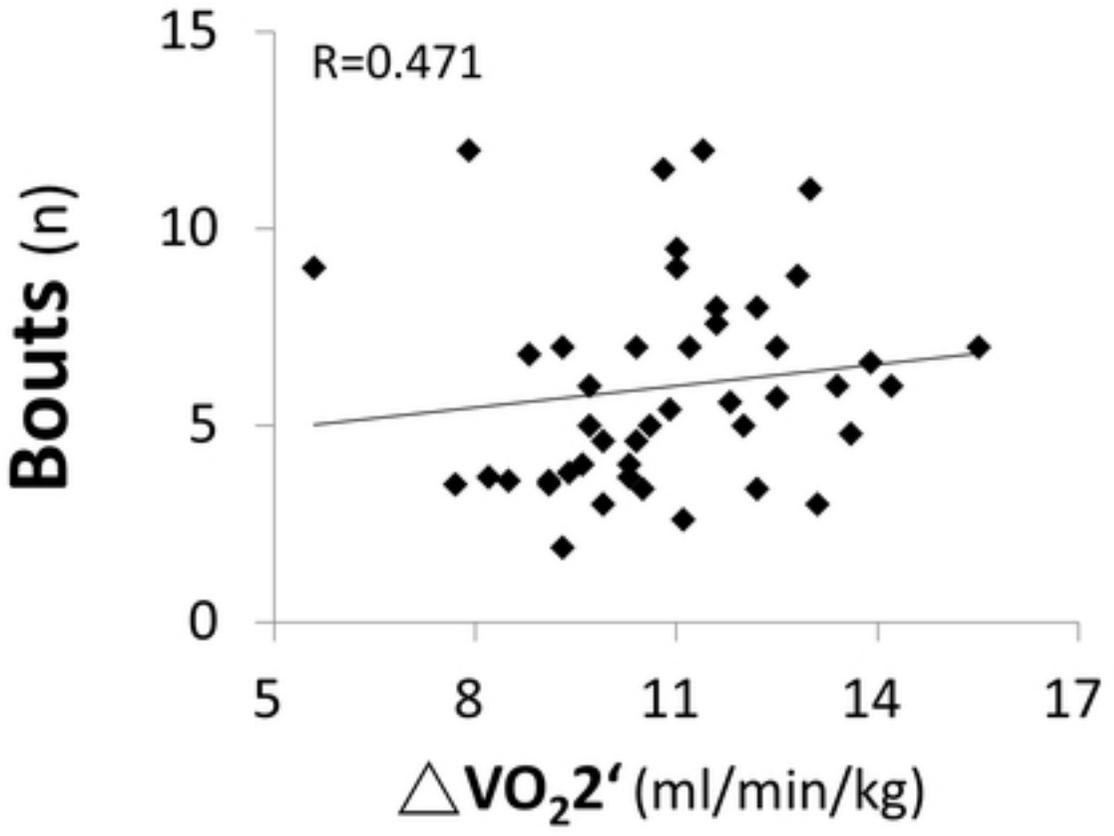


Figure 2

**A****B**

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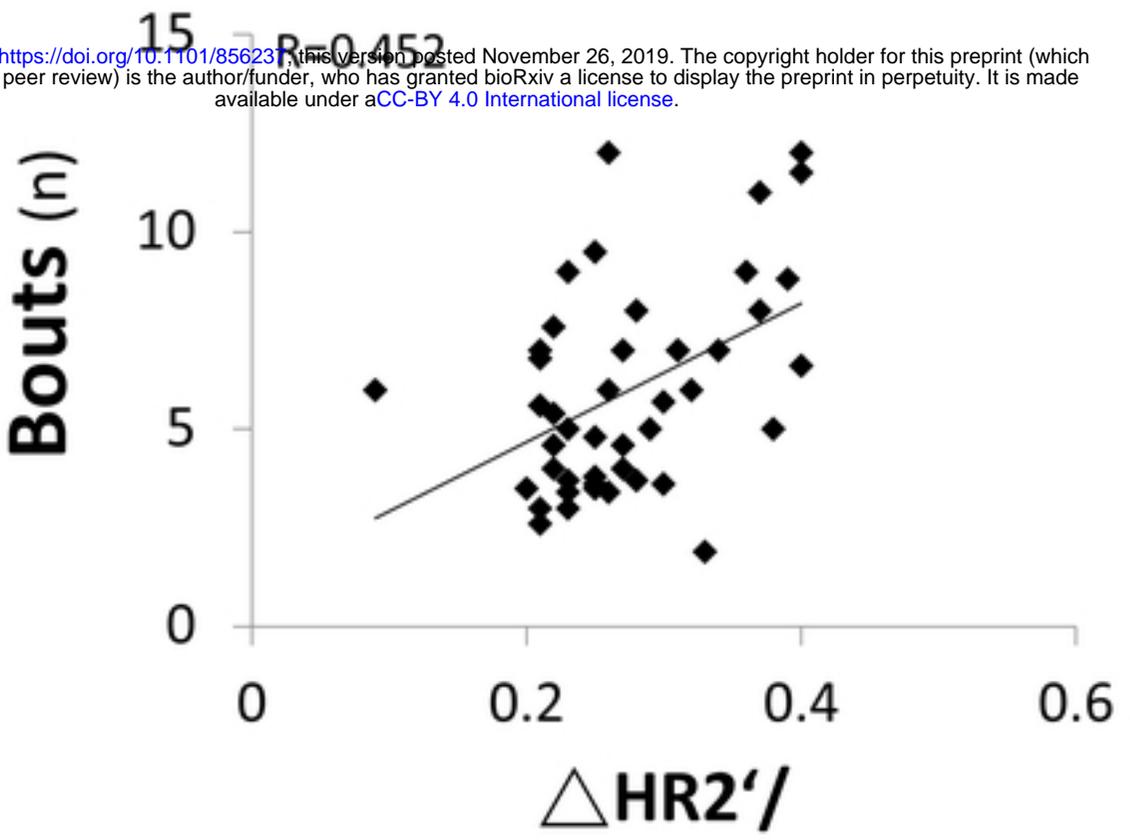
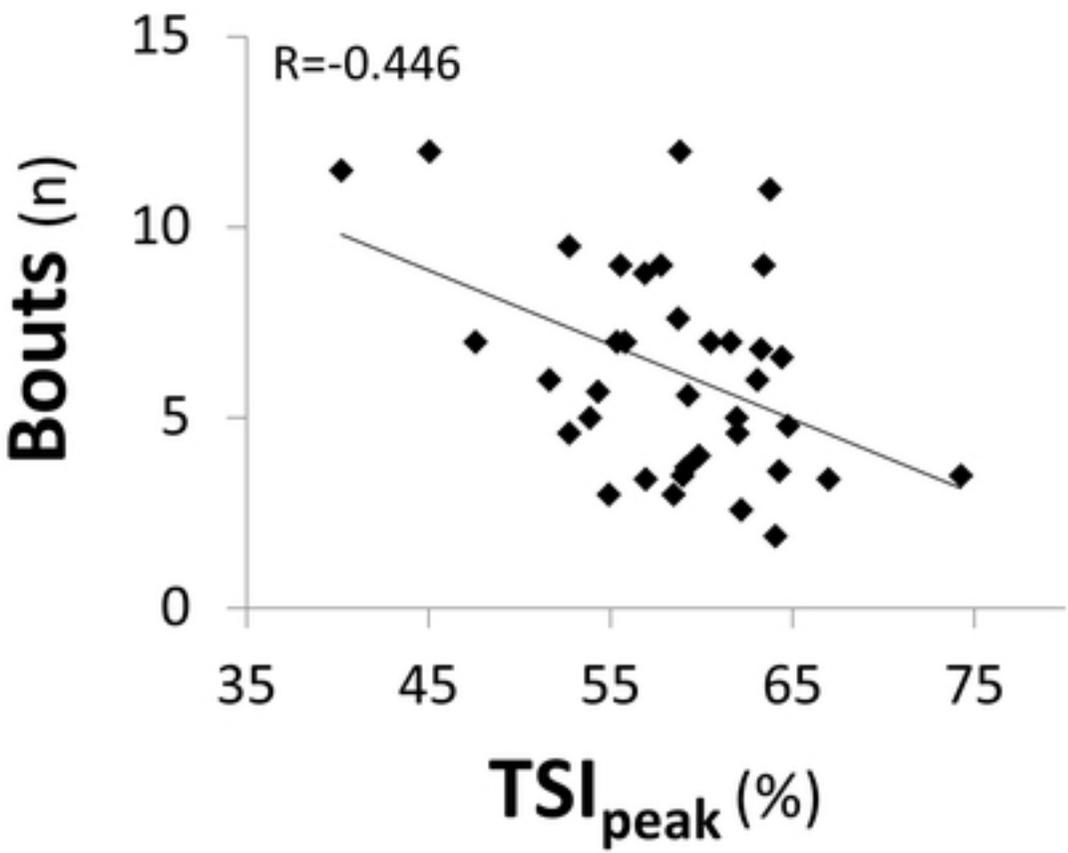
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Figure 3

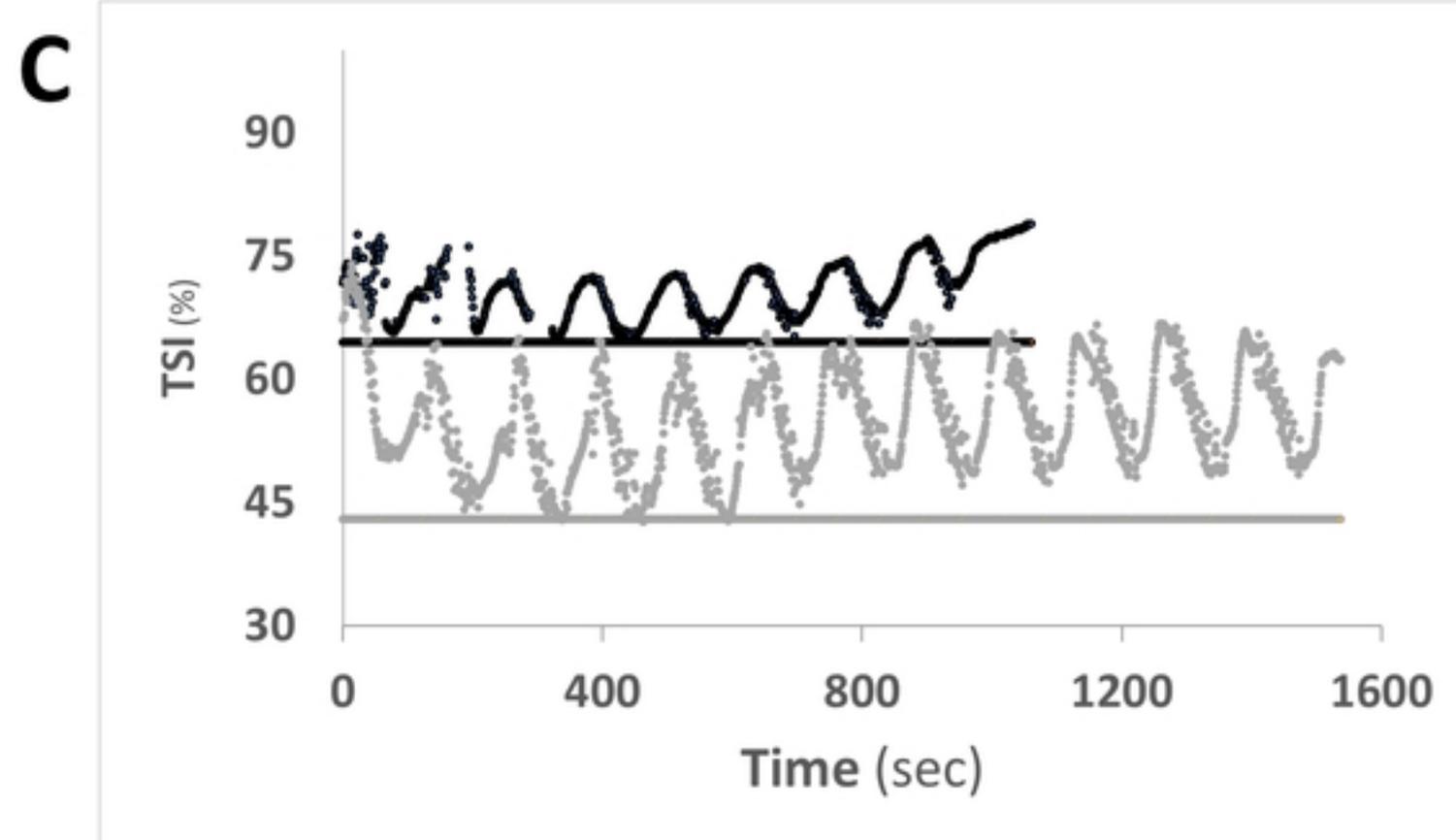
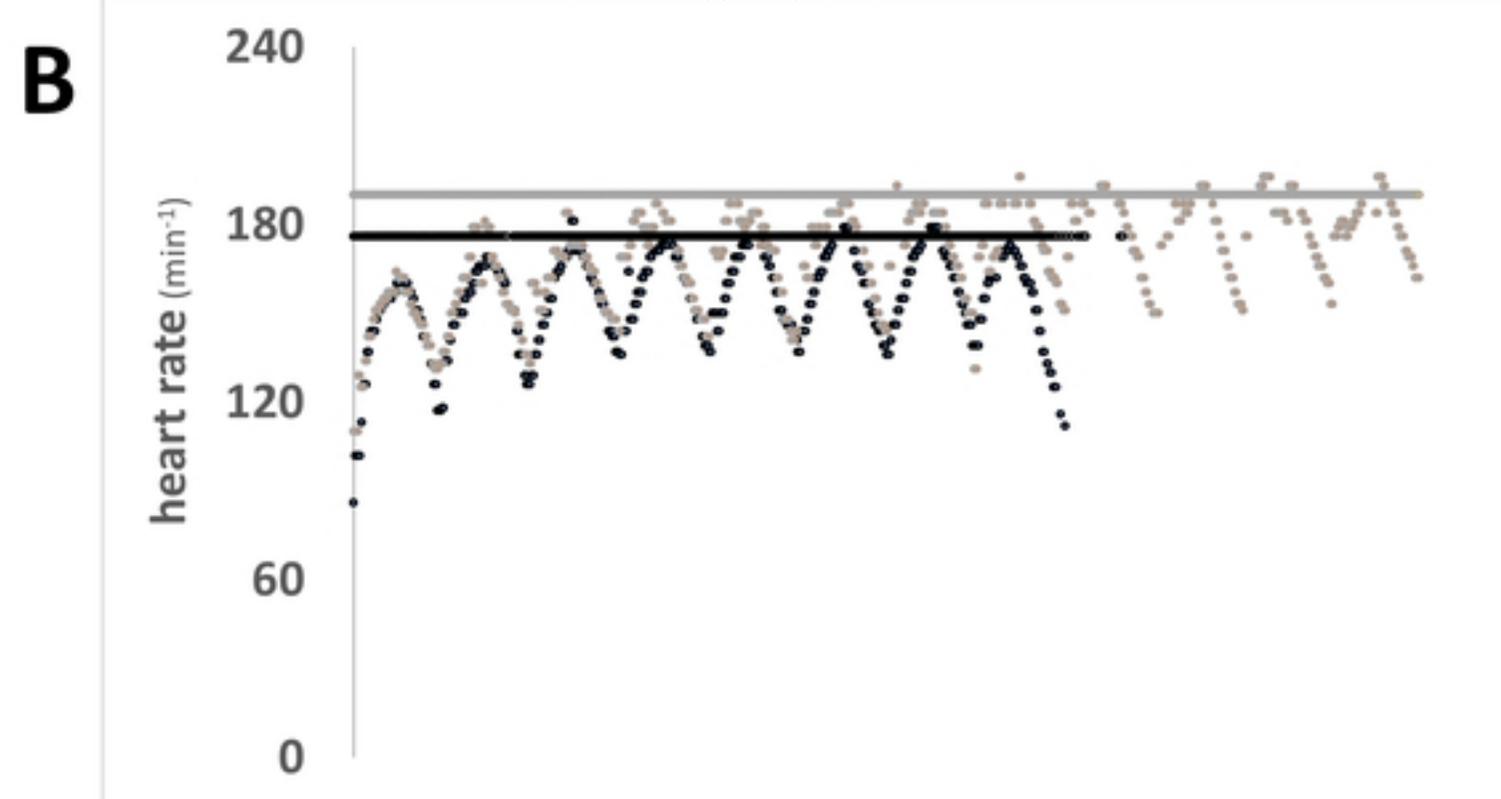
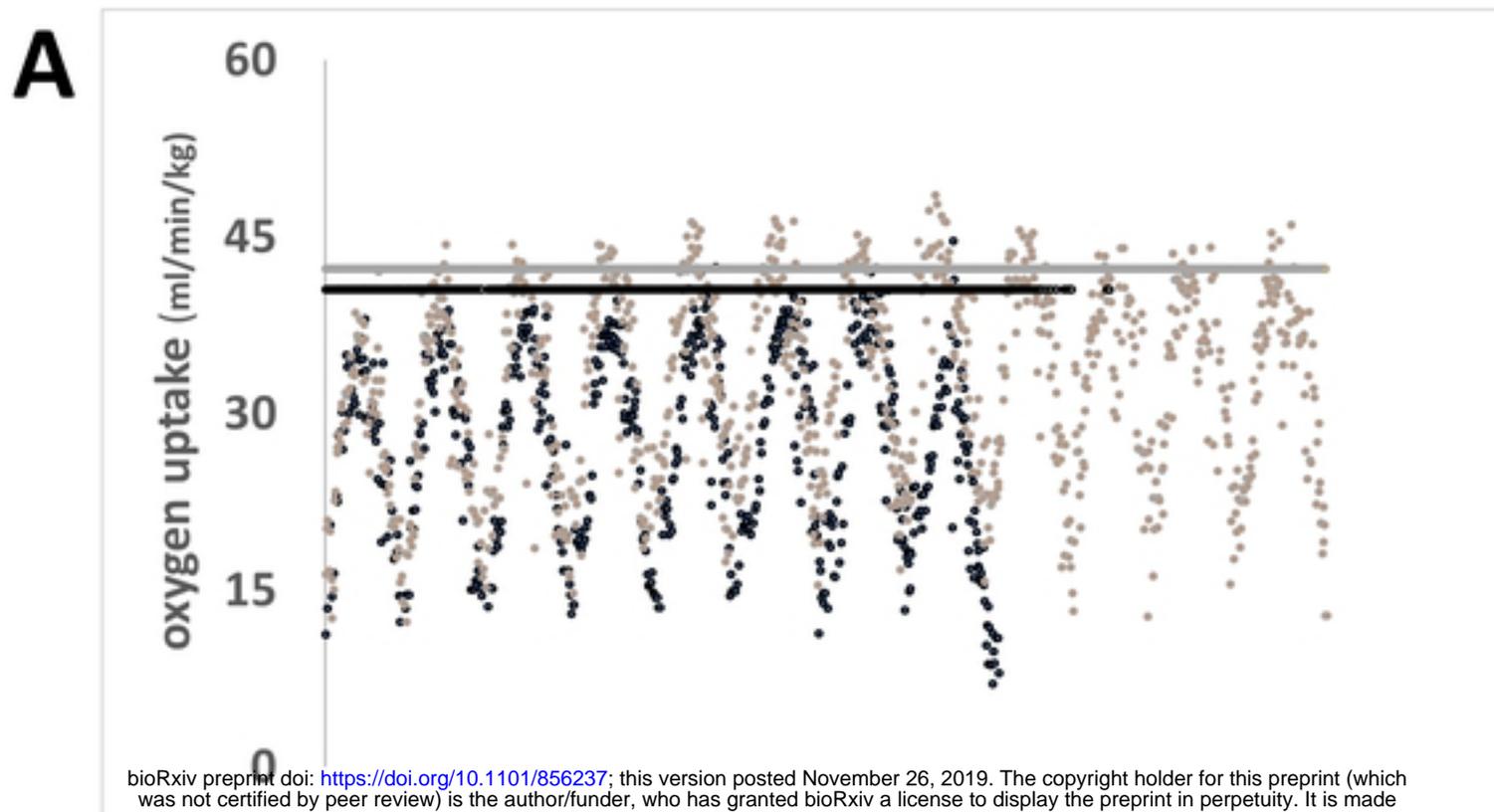


Figure 4