European Journal of Applied Physiology (2019) 119:1725–1733
https://doi.org/10.1007/s00421-019-04162-1

ORIGINAL ARTICLE

# Acute effects of very low-volume high-intensity interval training on muscular fatigue and serum testosterone level vary according to age and training status

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Received: 21 January 2019 / Accepted: 11 May 2019 / Published online: 4 June 2019


## Abstract

**Purpose** To compare the acute physiological responses of three different very low-volume cycling sessions (6 × 5 s, 3 × 30 s, and 3 × 60 s) and their dependence on age and training status.

**Methods** Subjects were untrained young men (mean ± SD; age 22.3 ± 4.6 years, $VO_2$peak 42.4 ± 5.5 ml/kg/min, $n = 10$), older untrained men (69.9 ± 6.3 years, 26.5 ± 7.6 ml/kg/min, $n = 11$), and endurance-trained cyclists (26.4 ± 9.4 years, 55.4 ± 6.6 ml/kg/min, $n = 10$). Maximal voluntary contraction (MVC) and electrically stimulated knee extension torque, and low-frequency fatigue, as ratio of stimulation torques at 20–100 Hz (P20/100), were measured only 24 h after exercise. Serum testosterone (Te) and blood lactate concentrations were measured only 1 h after exercise.

**Results** All protocols increased the blood lactate concentration and decreased MVC and P20/100 in young men, but especially young untrained men. In old untrained men, 6 × 5 s decreased P20/100 but not MVC. Te increased after 3 × 30 s and 3 × 60 s in young untrained men and after 3 × 60 s in older untrained men. The increase in Te correlated with responses of blood lactate concentration, MVC, and P20/100 only in old untrained men.

**Conclusions** As little as 6 × 5 s all-out cycling induced fatigue in young and old untrained and endurance-trained cyclists. Slightly higher-volume sessions with longer intervals, however, suppressed contractile function more markedly and also transiently increased serum testosterone concentration in untrained men.

**Keywords** Anabolic response · Low-frequency fatigue · High-intensity interval training · Sprint interval training

### Abbreviations

| | |
|---|---|
| HIIT | High-intensity interval training |
| HR | Heart rate |
| LFF | Low-frequency fatigue |
| MVC | Maximal voluntary contraction |
| SD | Standard deviation |
| SIT | Sprint interval training |
| Te | Serum testosterone concentration |
| $VO_2$peak | Peak oxygen uptake |

Communicated by Philip D. Chilibeck.

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## Introduction

The number of people aged over 60 years is expected to triple worldwide by 2050, with the "oldest old" group (≥ 85 years) being the most rapidly expanding segment (Garatachea et al. 2015). Many in the elderly population become dependent on others, which increases the health care costs. Cardiorespiratory fitness decreases with age and this decrease is associated with increased risk of morbidity and mortality (Lee et al. 2010). Given the wide range of benefits of exercise and physical activity (Garatachea et al. 2015), exercise has been proposed for the prevention or attenuation of the decrease in quality of life with ageing. However, the lack of transfer of scientifically based physical activity recommendations into practice creates a need for attractive yet effective training regimens that better suit the needs of society.

High-intensity interval training (HIIT), which comprises short high-intensity bursts of exercise interspaced with periods of rest, is one of the most efficient training modes to improve cardiovascular fitness and aerobic capacity (Buchheit and Laursen 2013a; Laursen and Jenkins 2002; Gibala 2007; Sökmen et al. 2018). Recent studies have shown that as little as 6 × 30 s all-out cycling is sufficient to trigger changes in the muscle tissue expression of genes involved in adaptations related to the endurance phenotype (Place et al. 2015; Little et al. 2010). This finding suggests that HIIT may be an attractive exercise training mode in terms of time economy.

However, standard HIIT requires repeated efforts of intense exertion, which can cause unpleasant sensations (Gibala et al. 2012). Recent attempts have focused on determining whether a small volume of HIIT in each session is effective. For instance, Verbickas et al. (2018) reported that 12 sets of 5 s all-out cycling (that is sprint interval training, SIT, session) induced a long-lasting decrease in the plasma concentration of brain-derived neurotrophic factor in young men; the authors suggested that this decrease may promote cognitive functions. Krusnauskas et al. (2018) reported that all-out cycling of even lower volume (6 sets of 5 s) in young untrained women created significant physiological stress and therefore may be an effective mode of training. These strategies look promising because they can be enjoyable, which may enhance compliance with training. However, the effects may depend on many factors such as the participant's gender, physical capacity, and age. These protocols of very low-volume HIIT, including SIT (Bagley et al. 2016), need further scrutiny to determine their efficacy and mechanisms of action.

One of the newly proposed mechanisms by which HIIT/SIT increases physical fitness via temporal free radical production in the exercising muscles is specific modifications induced to structural protein ryanodine receptor channels making them "leaky" (Place et al. 2015), which in turn increases the free cytosol calcium concentration and activates genes that promote mitochondrial biogenesis (Gehlert et al. 2015). On the other hand, it has been suggested that elevation of circulating testosterone and other anabolic agents also increase intracellular calcium, which serves as mediator of a diverse array of cell responses including myocyte hypertrophy (Vicencio et al. 2011). Old age is associated with impaired production of anabolic hormones as well as endurance, strength, and power (Metter et al. 1997; Aguirre et al. 2014; Grey et al. 2015; Martin et al. 2015). It is unclear which exercise interventions are best able to stimulate muscle adaptations and ameliorate the ageing-induced impairment in motor functions.

The increase in blood testosterone level depends on the volume of resistance exercise (Gotshalk et al. 1997), but further study is needed to understand the responses of testosterone to HIIT/SIT sessions of different types and volumes in people of different training status and age (Kilian et al. 2016). The adaptations to HIIT may depend on intramuscular antioxidant capacity, which is associated with age (Andersson and Hellstrand 2012) and fitness level (Place et al. 2015). Therefore, the primary aim of the current study was to compare the acute effects of three different types of very low-volume interval exercise on neuromuscular and serum testosterone responses in untrained young and older men, and in endurance-trained young male cyclists. We hypothesized that neuromuscular fatigue, the testosterone response, and psychological tolerance to exercise (enjoyability) would depend on the volume of interval training session, and that both training status and age would influence the ability to produce muscle power and induce metabolic stress. We presumed that older people require a larger volume of intense exercise to induce the same response as in young untrained and especially young endurance-trained athletes because of the reduced ability to produce power with ageing. We also presumed that well-trained cyclists would exhibit a greater increase in circulating testosterone level than untrained men because of their ability to perform more work during a short exercise bout.

## Materials and methods

### Participants and familiarization

In total, 31 men were enrolled in the study and were classified into three groups: young untrained, old untrained, and young endurance-trained cyclists. The participants' characteristics are included in Table 1. The health status of all participants was checked by a medical doctor before the study. The exclusion criteria were cardiovascular problems, metabolic disease, or chronic joint pain. Untrained participants did not perform any regular exercises for at least 6 months before the study began. The participants read and voluntarily signed an informed consent form, which was consistent with the principles outlined in the Declaration of Helsinki. All procedures were approved by the Kaunas Region Biomedical Research Ethics Committee.

Each participant performed a familiarization session 7 days before the first cycling exercise session that comprised a brief warm up followed by a few attempts at maximal knee extension while seated in the dynamometer chair (System 3; Biodex Medical Systems, Shirley, NY, USA). This was followed by several submaximal cycling sprints on a stationary ergometer (Monark 824E; Monark, Vansbro, Sweden) with a brake weight of 3.75% of body weight.

### Measurement of peak oxygen consumption ($VO_2$peak)

$VO_2$peak was measured 7 days before the first cycling exercise session. Expired gases were collected and analysed using a breath-by-breath analyser (Oxygen Mobile; Jaeger/

**Table 1** Participants' characteristics and peak performance in the progressive cycling test (mean ± SD)

| | Young ($n$ = 10) | Old ($n$ = 11) | Cyclists ($n$ = 10) |
|---|---|---|---|
| Age (years) | 22.3 ± 4.6[a] | 69.9 ± 6.3 | 26.4 ± 9.4[a] |
| Height (cm) | 183.3 ± 4.7 | 176.8 ± 6.9 | 181.5 ± 6.6 |
| Body mass (kg) | 88.1 ± 13.6 | 78.5 ± 15.3 | 78.0 ± 9.0 |
| Body mass index (kg/m$^2$) | 26.4 ± 5.2 | 25.0 ± 3.9 | 24.0 ± 2.9 |
| Fat-free mass (%) | 71.8 ± 7.6[a] | 59.4 ± 7.4 | 70.6 ± 5.7[a] |
| $VO_2$ peak (ml/kg/min) | 42.4 ± 5.5[a,b] | 26.5 ± 7.6[b] | 55.4 ± 6.6 |
| Peak heart rate (bpm) | 188.8 ± 5.9[a] | 150.3 ± 18.3[b] | 185.1 ± 13.6 |
| Peak power (W) | 344.4 ± 50.7[a,b] | 215.0 ± 49.3[b] | 441.4 ± 68.5 |

[a]Different from old at $p < 0.05$

[b]Different from cyclists at $p < 0.05$

VIASYS Healthcare, Hoechberg, Germany), and heart rate (HR) was measured with a HR metre (S-625X; Polar Electro, Kempele, Finland). Testing was performed on a stationary cycle ergometer (Ergometrics 800S; ErgoLine, Medical Measurement Systems; Binz, Germany). Before the test, participants performed 4–5 min of warm up on the cycle ergometer against a load in watts corresponding to the body weight in kilograms. The protocol started with 3 min of cycling at 50 W, after which the load was increased by 5 W every 10 s. Trained cyclists maintained the pedalling cadence at 80 rpm and untrained participants aimed at 60 rpm. The test was continued until volitional fatigue, which was defined as an inability to maintain the required pedalling cadence.

## Interval cycling exercises

Participants performed cycling exercise sessions on a mechanically braked stationary cycling ergometer (Monark 824E; Monark, Vansbro, Sweden). Sessions were interspaced by a 5-week rest period and performed in random order. Exercise sessions were 6 × 5 s all-out with a 90 s rest interval between each; 3 × 30 s all-out with a 4 min rest interval between each; and 3 × 60 s submaximal efforts with a 4 min rest interval between each. All-out exercise sessions (SIT) were performed using a brake weight of 7.5% of the participant's body weight, and submaximal exercise session was performed using a brake weight of 3.75% of body weight (Krusnauskas et al. 2018) and required the subjects to select the highest possible pedalling cadence which would still allow them to complete the training session without a significant drop in the pedalling cadence. HR was recorded during all interval cycling sessions, and 5-s averaged peak values during each bout then used to calculate the average peak HR of the session. During rest intervals, subjects stayed on the ergometer and were light pedalling with zero brake weight. Each bout was always started from a standstill position, and after a verbal signal from the investigator the subject started pedalling as fast as possible in 5-s and 30-s bouts and up to the required efforts during the 60-s bouts. Each time the brake weight was automatically applied upon reaching the 100 rpm pedalling rate. After completion of all three interval training sessions, each participant was asked to list the exercise modalities from most to least preferable as a mode of training.

## Maximal voluntary contraction (MVC) and electrically evoked torque

MVC peak torque was measured for the knee extensor muscles on the dominant leg using an isokinetic dynamometer (System 3; Biodex Medical Systems). The participant sat in the dynamometer chair with the knee joint positioned at a 60° angle (0° = full knee extension). MVC was reached and maintained for ~ 2 s before relaxation and was measured twice; the larger value was used in the analysis. Direct muscle stimulation to the same leg was applied using two carbonized rubber surface electrodes covered with a layer of electrode gel (Medigel, Modi'in, Israel). Electrodes were positioned over both the proximal and distal ends of the quadriceps femoris. A standard electrical stimulator (MG 440; Medicor, Budapest, Hungary) was used to deliver supramaximal 0.5 ms square-wave pulses for 1 s trains of stimuli at frequencies of 20 (P20) and 100 (P100) Hz. Low-frequency fatigue (LFF) was derived from the P20/P100 ratio (Jones 1996; Skurvydas et al. 2016; Kamandulis et al. 2017).

## Blood analyses

Venous blood samples (5 ml) were collected at the baseline and then 5 min, 1 h, and 24 h after exercise. The blood was allowed to clot at room temperature and was centrifuged for 15 min at 1200$g$. The serum concentration of total testosterone was measured using an enzyme-linked immunoassay system (IBL; International GMBH, Hamburg, Germany). Whole-blood lactate concentration was measured at the baseline, and 5 min, and 1 h after cycling exercise using a portable lactate analyser (Pro™ LT-1730; Arkray Inc., Kyoto, Japan) in the same fresh samples of venous blood.

### Statistics

Data are presented as mean ± standard deviation (SD). The normality of the data distribution was evaluated using the Kolmogorov–Smirnov test; all data were found to be normally distributed except for lactate and testosterone concentrations. For normally distributed data, repeated-measures analysis of variance (general linear model) and Tukey's post hoc test were used to identify the within-factor effects (time: before, immediately after, 60 min, and 24 h after exercise) for outcome measures (MVC, P20/P100 ratio). The non-parametric Friedman's test with Dunn's post hoc test were used to identify the within-factor effects of testosterone and lactate (time: before, immediately after, 60 min after exercise). Correlational analysis was performed to assess the relationships between the changes in testosterone concentration and other outcome measures. Comparisons between groups at a single time point were analysed using the non-parametric Kruskal–Wallis test with Dunn's post hoc test. Significance was set at $p < 0.05$.

## Results

Peak and average powers were the highest during 5 s sprints, followed by 30 s sets, and smallest during the 60 s bouts in all three groups (Fig. 1). Cyclists developed the highest peak power and average power during all-out (6 × 5 s and 3 × 30 s) sessions, and the old untrained had the lowest peak and average powers ($p < 0.05$, Fig. 1). Peak cadence was attained during 6 × 5 s sprints (156.9 ± 12.8, 155.1 ± 9.7 and 115.8 ± 9.4 rpm in young untrained, old untrained and cyclists, respectively; $p < 0.001$ between old vs. Any of the young groups), and it was similar in the first but declined during the second and the third 30 s intervals. Power output did not change across sets of 5-s sprints in either group and no major differences in the fatigue index (the power drop) during the 30 s all-out sprint was evident either between the sets or between the groups. However, both peak power and average power during the 3 × 30 s session dropped the fastest in young untrained compared to the other two groups which maintained the peak power and average power much better across the sets.

Blood lactate concentration increased significantly in response to all three sessions in all three groups. The magnitude of the increase was similar in the young untrained men and trained cyclists but was blunted in old untrained men (Fig. 2). After 1 h of passive rest, blood lactate concentration returned to baseline in all groups and conditions except it remained slightly above the baseline after the 3 × 30 s session ($p < 0.05$) in young untrained men (Fig. 2a). HR response, calculated as percentage of HRpeak attained during progressive $VO_2$peak test to exhaustion, did not differ between the groups during all three types of sessions and was ~ 82, ~ 95, and ~ 97% in 5-s, 30-s and 60-s bout sessions, respectively). Old untrained men and cyclists ranked the 6 × 5 s session as the preferred mode for further training (56% of all participants), and the 3 × 30 s session was most frequently preferred by young untrained men (50% of all participants).

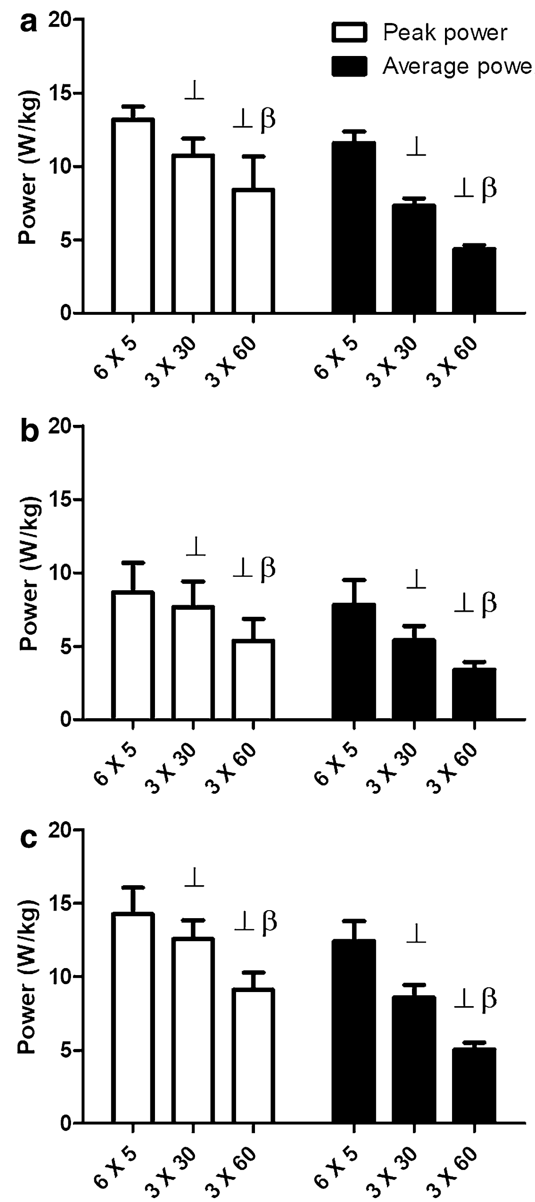


**Fig. 1** Wingate power (mean and SD) in young (**a**) and old (**b**) untrained men and endurance-trained cyclists (**c**). [⊥]Lower than in the 6 × 5 s session ($p < 0.05$). [β]Lower than in the 3 × 30 s session ($p < 0.05$)

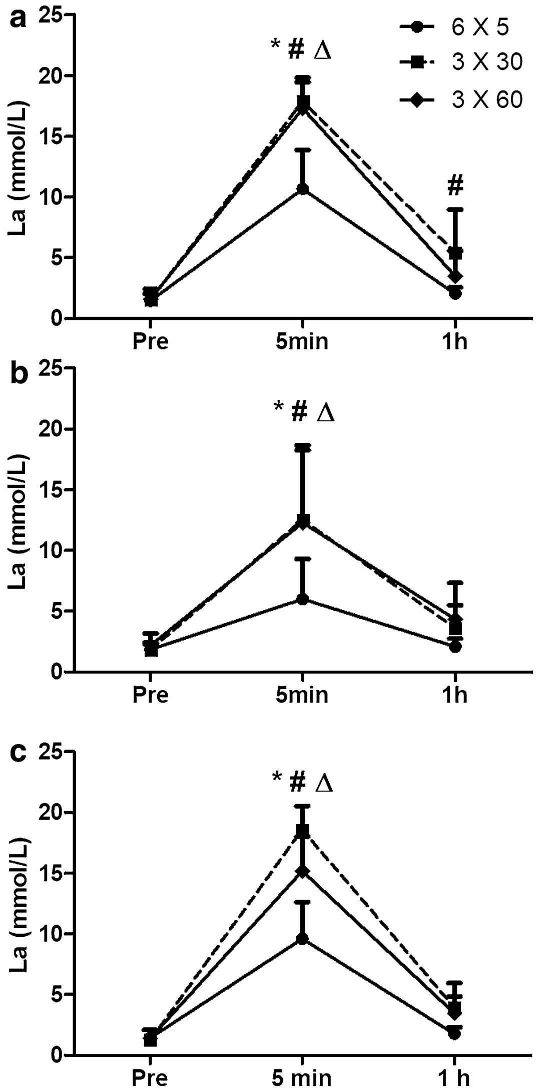


**Fig. 2** Blood lactate concentration (mean and SD) after exercise in young (**a**) and old (**b**) untrained men and endurance-trained cyclists (**c**). *Higher ($p<0.05$) than before exercise (pre) in the 6×5 s session. [#]Higher ($p<0.05$) than before exercise (pre) in the 3×30 s session. [Δ]Higher ($p<0.05$) than before exercise (pre) in the 3×60 s session

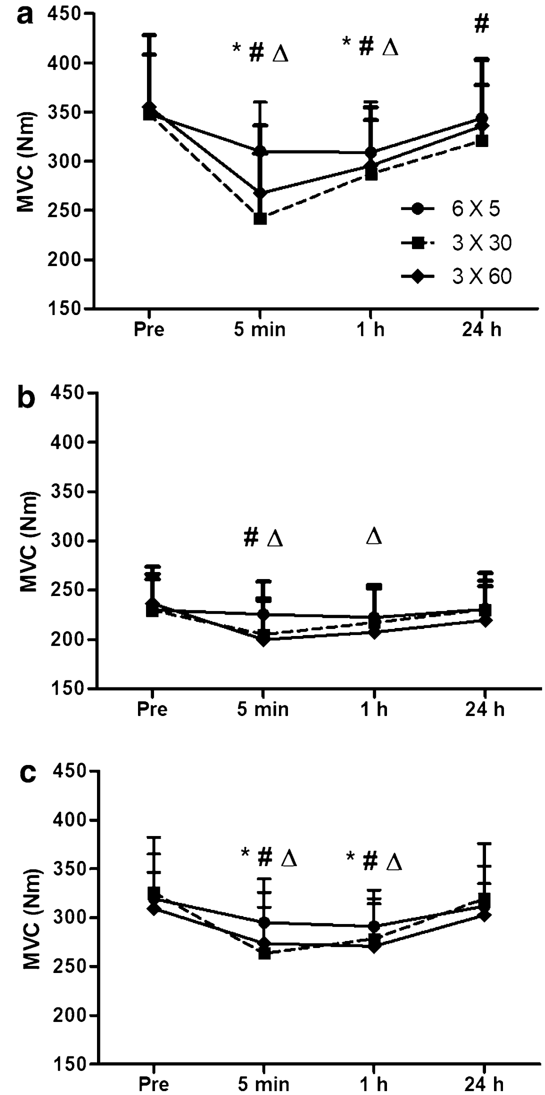


**Fig. 3** Maximal voluntary contraction (MVC, mean and SD) in young (**a**) and old (**b**) untrained men and endurance-trained cyclists (**c**). *Lower ($p<0.05$) than before exercise (pre) in 6×5 s session. [#]Lower ($p<0.05$) than before exercise (pre) in 3×30 s session. [Δ]Lower ($p<0.05$) than before exercise (pre) in 3×60 s session

MVC was reduced in all groups immediately after all three types of sessions (Fig. 3) except in old untrained men after the 6×5 s all-out cycling task (Fig. 3b). After 1 h of passive rest, MVC remained lower in young untrained and endurance-trained cyclists (Fig. 3a, c) after all three sessions and did not recover in old untrained men after the 3×60 s bout (Fig. 3b). In old untrained men and endurance-trained cyclists, MVC returned to baseline within 24 h after all sessions (Figs. 3b, c) but remained lower 24 h after the 3×30 s session in young untrained men (Fig. 3a). The baseline MVC was lowest in old untrained men ($p<0.05$). The reduction in MVC was greatest in young untrained men and smallest in old untrained men ($p<0.05$).

LFF (expressed as a ratio of 20 Hz to 100 Hz electrically stimulated torques) was induced by all three types of interval training sessions in all three groups immediately (5 min) and 1 h after the exercise (Fig. 4) except for 1 h after 3×30 s all-out cycling in the old untrained men (Fig. 4b). After 24 h of recovery, LFF was still present after the 3×30 s and 3×60 s sessions in young untrained men (Fig. 4a), 3×60 s session in old untrained men (Fig. 4b), and 6×5 s and 3×60 s sessions in endurance-trained cyclists (Fig. 4c).

Young untrained men experienced the most pronounced LFF compared with the two other groups ($p<0.05$).

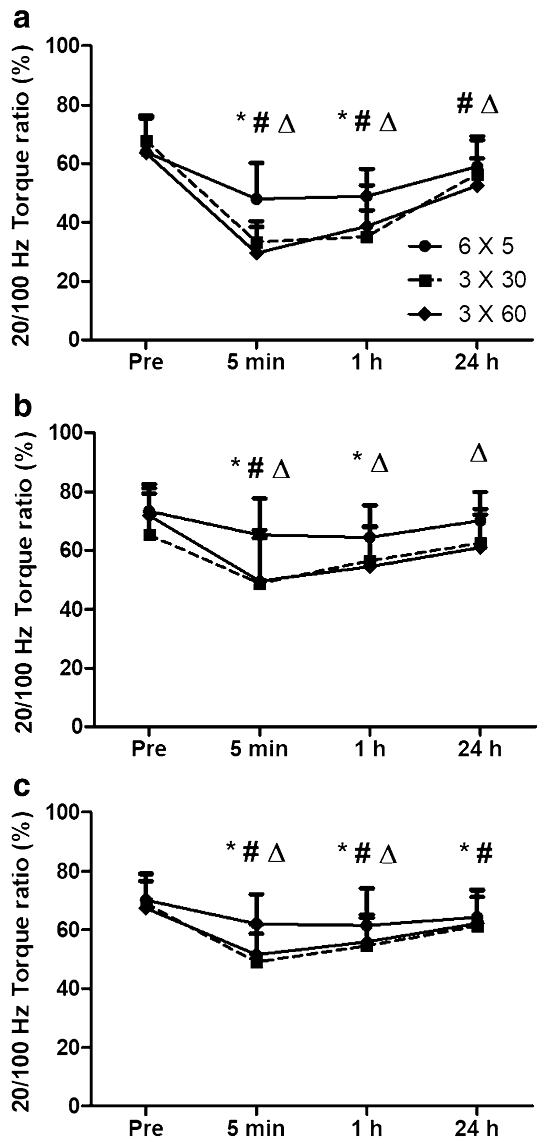


**Fig. 4** Low-frequency fatigue (ratio of 20 Hz–100 Hz electrical stimulated torques, mean and SD) in young (**a**) and old (**b**) untrained men and endurance-trained cyclists (**c**). *Smaller ($p<0.05$) than before exercise (Pre) in 6×5 s session. #Smaller ($p<0.05$) than before exercise (pre) in 3×30 s session. ΔSmaller ($p<0.05$) than before exercise (pre) in 3×60 s session

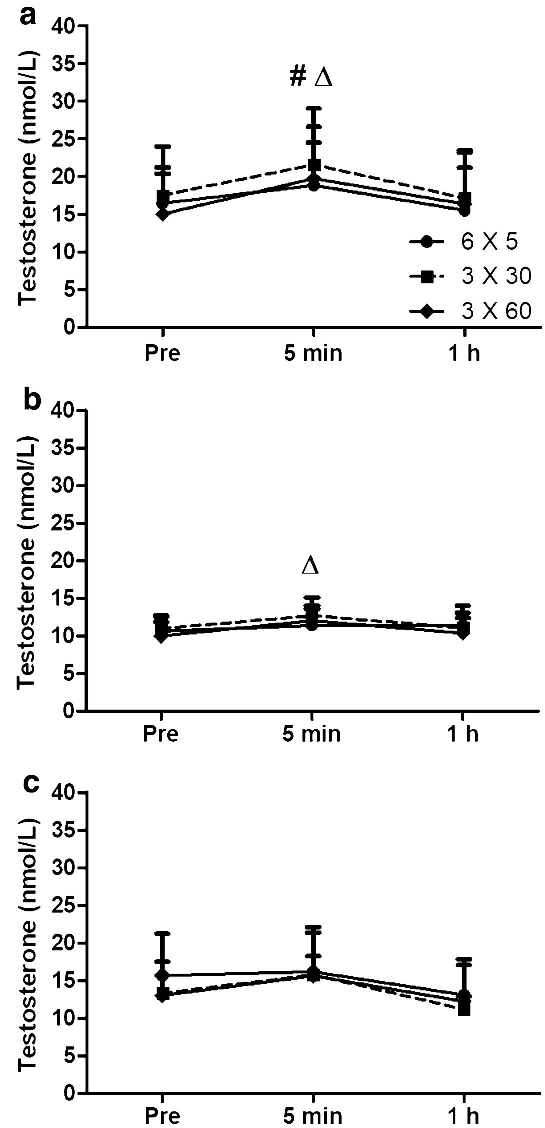


**Fig. 5** Serum testosterone concentration (mean and SD) response to exercise in young (**a**) and old (**b**) untrained men and endurance-trained cyclists (**c**). #Higher ($p<0.05$) than before exercise (pre) in 3×30 s session. ΔHigher ($p<0.05$) than before exercise (pre) in 3×60 s session

Sessions of 3×30 s and 3×60 s evoked a similarly larger degree of LFF and MVC depression in all three groups compared with the 6×5 s session (Figs. 3 and 4).

Total testosterone concentration was lower in old men than in the other two groups ($p<0.05$, Fig. 5). An increase in testosterone concentration was evident immediately (5 min) after the 3×60 s session in both untrained groups (Fig. 5a, b) and after the 3×30 s session in young untrained men (Fig. 5a). Testosterone concentration did not change significantly in endurance-trained cyclists (Fig. 5c).

In old untrained men at 5 min after exercise (data pooled for the three types of sessions), the increase in testosterone concentration correlated significantly with the decrease in MVC ($r=-0.59$, $p<0.05$), decrease in P20/P100 ratio ($r=-0.43$, $p<0.05$), and increase in blood lactate concentration ($r=0.70$, $p<0.05$). There were no significant correlations between the measured parameters in the other two groups.

## Discussion

The results of the current study show that as little as 6 × 5 s all-out cycling induces significant metabolic perturbations and prolonged depression of skeletal muscle contractile function in both trained and untrained young as well as in untrained elderly men. However, longer bouts (3 × 30 s of all-out and 3 × 60 s of submaximal cycling) were more effective in all groups in inducing prolonged neuromuscular fatigue, and they also transiently elevated the total serum testosterone level in untrained young and old men.

It is well established that HIIT induces multiple adaptations in the exercising skeletal muscles including augmented glycolytic capacity, increased glycogen and triglyceride stores, expanded capillary density, and upregulated mitochondrial biogenesis (Gibala et al. 2006; Burgomaster et al. 2008; Little et al. 2010; Buchheit and Laursen 2013b; Scribbans et al. 2014). One of the mechanisms underlying the adaptation of skeletal muscle aerobic capacity is the exercise-induced increase of free radicals which serve as signalling for a cascade of modifications of intracellular macromolecules, finally leading to upregulation of genes required for enhanced performance (Powers et al. 2011). One of the targets via which free radicals seem to exert their training effect has recently been shown to be ryanodine receptor protein, which, when affected, leads to calcium leakage and prolonged LFF and alleged increase in mitochondrial biogenesis (Place et al. 2015). Indeed, as Little et al. (2010) have shown, as short as 2 weeks of HIIT increased resting levels of SIRT1, Tfam and PGC-1α as well as mitochondrial proteins in the muscle along with enhanced endurance cycling performance. Intriguingly, scavenging free radical with supplemented antioxidants hampered the training-induced mitochondrial biogenesis (Paulsen et al. 2014).

Intriguingly, intense HIIT may well be a sufficient stimulus to induce muscle hypertrophy in previously untrained subjects (Bagley et al. 2018). It is tempting to ascribe part of the effect of HIIT on increased lean body mass in previously untrained people to elevation in testosterone levels. In lifelong sedentary ageing men, exercise training, including HIIT, has been shown to increase circulating testosterone concentration (Hayes et al. 2017). Mechanistically, testosterone improves muscle protein accretion by activating satellite cells and thus promoting muscle hypertrophy (Kadi 2008). It has also been hypothesized that increase in circulating levels of testosterone and other anabolic androgenic steroids may lead to increased intracellular calcium concentration which, among other effects, may also mediate myocyte hypertrophy (Vicencio et al. 2011). However, the factors responsible for the increase in serum testosterone concentration in response to exercise and its effects on muscle mass and strength remain far from clear (O'Leary and Hackney 2014; Peltonen et al. 2018; Vaara et al. 2015; Morton et al. 2018). As shown by the results of the current study, elevation in serum testosterone and therefore its effects in untrained men may be expected when some threshold volume of high to all-out intensity exercise session is passed. In this respect, an increase in circulating testosterone has also been linked to the volume of resistance training (Gotshalk et al. 1997). In the older men in this study, the significant correlations between the increase in testosterone and lactate concentration response and muscle force decrement imply that triggering the adaptations is dependent on the effort and/or metabolic stimulus of the training session. We have to admit that the kinetics of lactate and testosterone in response to exercise were not precisely followed in our study and peak value or area under the curve thus were not available for the analysis. It was rather a temporal correlation between the lactate (‘metabolic stimulus‘) and testosterone (‘anabolic effect‘) responses tested, which may not give the full picture of the relationship between the two blood markers and may depend on the way these metabolites change in different groups.

From the practical perspective, given the low daily physical activity levels among the general population, the short duration of interval training used here makes it attractive in terms of both efficacy and time saving. However, for training to remain effective, the volume of the sessions should probably increase progressively with increasing fitness, and commitment may then become an issue. Indeed, long-term compliance with HIIT programmes seems to be complicated (Bagley et al. 2016) thus more attractive training modes can probably be achieved with mixing training sessions between high-volume workouts with longer exercise bouts and those of sprint-like bouts during the week, or by combining different durations and intensities of the bouts in one session (Venckunas et al. 2016), which may reduce monotony and improve adherence. Of the interval training protocols used in the current study, the shortest intervals (6 × 5 s) were considered to be the most comfortable/preferred by most of the old untrained men and young cyclists. This has also been previously reported in old and young untrained women (Krusnauskas et al. 2018). Unexpectedly, the 3 × 30 s all-out sprints, which were physiologically more demanding (as shown by the higher lactate concentration compared with that for the 6 × 5 s session) was considered to be most preferred by most young untrained men in this study.

A myriad of combinations of the number of bouts, duration, intensity, and rest periods are possible. The factors that influence the effectiveness and enjoyability of HIIT/SIT sessions are complex (Buchheit and Laursen 2013b) and depend much on the physical fitness of the participants. Age and athletic status were related to the working capacity during HIIT/SIT in our study: cyclists reached the highest and old men the lowest average and peak power, and while fatigue index within the 30-s sets was similar among the groups, cyclists showed a superior recovery as reflected by better maintained average power across the sets. Thus, for the trained people it would probably be required to perform more sets of 30-s cycling and/or diminish the recovery period to create the same degree of training stimulus as in untrained ones. Interestingly enough, the power output during all types of intervals used in the current study was much lower in cyclists as compared to other two groups when calculated in relation to peak power attained during the incremental $VO_2$peak test. However, it has just been reported (Degens et al. 2019, in press) that endurance athletes have much higher aerobic to anaerobic power/capacity compared to other athletes or untrained persons, thus it could not just be extrapolated directly that their efforts and physiological demands during the HIIT/SIT sessions were lower. On the contrary, the lactate values after the workouts and HR response (as %HRpeak, ~82, ~95, and ~97% in 5-s, 30-s and 60-s bouts, respectively) between the groups during all three types of interval sessions were similar.

Even the session of the lowest exercise volume (i.e., 6×5 s all-out cycling interspaced with 90 s of recovery) elicited substantial and prolonged muscle fatigue, which confirms that as little as 30 s (and perhaps even less) of exercise per session and a total session duration of < 12 min including the rest periods may be effective in inducing endurance-type adaptations during the initial phases of training. It is indeed highly possible that the use of other interval training protocols (e.g., more sets and/or shorter rest intervals in between) might have produced different responses of muscle function and testosterone. Therefore, even though we did not observe any increase in circulating testosterone in any of the groups of young or older men in response to 6×5 s all-out cycling session, we cannot definitely exclude that workouts of very short (up to 5 s) all-out bursts of muscular activity provide an insufficient stimulus to induce anabolic responses. It could well be that larger overall volume of all-out exertion performed with shorter rest periods between bout would lead to different outcomes. A good analogy on aerobic capacity adaptation aspect is a recent study on well-trained boxers which reported that voluminous sprint interval training encompassing ~2 min of all-out punching per session performed every other day was sufficient to induce substantial improvement in upper body $VO_2$peak (Kamandulis et al. 2018).

In conclusion, the results of the study show that as little as 6×5 s of all-out interval cycling is effective in inducing prolonged neuromuscular fatigue in old and especially young untrained men, as well as in young endurance-trained cyclists. The results also suggest that this form of SIT is both an attractive and effective means of training, at least in the initial phases. However, slightly higher volumes of interval training (either 3×30 s all-out or 3×60 s submaximal cycling) were even more effective in inducing metabolic fatigue and prolonged contractile deficit as well as transiently elevating the serum testosterone level in young and old untrained men.

**Acknowledgements** This work was supported by the Research Council of Lithuania [Grant no. SEN-08/2016].

**Author contributions** Venckunas T, Krusnauskas R, Snieckus A, Eimantas N, Baranauskiene N, Skurvydas A, Brazaitis M, Kamandulis S. TV, RK, AS, NE, NB, AS, MB, and SK designed the study; RK, AS, NE, NB and MB conducted the study; TV, RK, AS, NE, NB, AS, MB, and SK analysed the data; TV, RK, AS, AS and SK wrote the paper. All authors approved the final version of the manuscript.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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