Comparison of sprint interval and continuous endurance training on oxidative stress and antioxidant adaptations in young healthy adults

Gulbin Rudarli Nalcakan  
Ege University, Faculty of Sport Science, Department of Coaching Education, Izmir, Turkey, gulbinrn@gmail.com

Ece Onur  
Celal Bayar University, Faculty of Medicine, Department of Biochemistry, Manisa, Turkey, gulbinrn@gmail.com

Arzu Oran Oran  
Celal Bayar University, Faculty of Medicine, Department of Biochemistry, Manisa, Turkey, gulbinrn@gmail.com

S. Rana Varol  
Ege University, Faculty of Sport Science, Department of Coaching Education, Izmir, Turkey, rana.varol@ege.edu.tr

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Keywords
aerobic training, high-intensity interval, lipid peroxidation, total oxidant status, glutathione peroxidase

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Gulbin Rudarli Nalcakan1 ABCEF, Ece Onur2 ABCDE, Arzu Oran2 CDEF, S. Rana Varol1 ACDE
1 Ege University, Faculty of Sport Science, Department of Coaching Education, Izmir, Turkey
2 Celal Bayar University, Faculty of Medicine, Department of Biochemistry, Manisa, Turkey

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Conclusions: SIT was suggested as a safe exercise model to improve general health and the performance of traditional CET.

Key words: aerobic training, high-intensity interval, lipid peroxidation, total oxidant status, glutathione peroxidase.
INTRODUCTION

Aerobic exercise training induces numerous morphological and metabolic adaptations in skeletal muscle, including mitochondrial biogenesis and an enhanced capacity to oxidize fuels, such as glucose and fats [1]. These adaptations to continuous endurance training (CET) are linked with improved metabolic health and reduced risk for several metabolic diseases, such as obesity, insulin resistance, and type 2 diabetes [2–4].

Despite the proven positive effects of aerobic training on health, lack of time is the most common reason why many people fail to accomplish training programs [5, 6]. Low-volume, high-intensity interval training (HIT) has recently gained popularity as a time-efficient method of improving anaerobic and aerobic fitness [7] in only a few sessions in healthy people and also in patients with metabolic disorders [5, 8].

The most common model employed in low-volume HIIT studies has been the repeated Wingate test, which consists of a 30 s ‘all-out’ cycling effort against a supra-maximal workload. Wingate-based HIIT, which is known as sprint interval training (SIT), was typically performed for four to six work bouts separated by ∼4 min of recovery, for a total of 2–3 minutes of intense exercise during a training session that lasts ∼20 min with three sessions per week [5, 9, 10]. When compared on a matched-work basis or when estimated energy expenditure is equivalent, HIIT can serve as an effective alternative to traditional endurance training, inducing similar or even superior changes in a range of physiological, performance and health-related markers in both healthy individuals and diseased population [5, 9].

Exercise can increase the generation of reactive oxygen species (ROS), especially for single bouts of training, and elevated concentration of ROS causes oxidative damage of lipids, proteins, and DNA [11]. In fact, despite ROS having a fundamental role as signaling molecules in several determinant cellular pathways, redox changes induced by increased ROS production during exercise are negatively related to cellular homeostasis and might compromise the cellular function [12]. In skeletal muscle fibers, exercise-induced oxidative stress has been associated with fatigue, longer recovery time, and an increased injury rate [13].

The effectiveness of SIT training stems from high anaerobic demand, mainly in the first bouts, and increasing aerobic contribution with a higher number of sessions [14]. During repetitive high intensity effort, the oxidative metabolism causes an increase in the rate of oxygen transport due to workloads and fluctuations in oxygen uptake [15], while a decrease occurs in the ability to stimulate ATP production through the breakdown of phosphocreatine and glycogen [14]. Consequently, after repeated muscle contractions, oxidative stress can significantly reduce calcium intake by the sarcoplasmic reticulum and the ability to create action potentials that contribute to an acute decrease in performance during exercise [16].

Although during prolonged submaximal aerobic exercise, the increase in ROS production is mainly due to a disturbance in electron transport leading to increased leakage of superoxide radicals, oxidative stress specific to anaerobic exercise may be mediated through various other pathways, such as proton accumulation, due to lactic acidosis, auto-oxidation of catecholamine, catabolism of purines to xanthine and urate, and a transient and acute muscular deoxygenation, which resembles the ischemia-reperfusion syndrome [17].

Since excessive generation of ROS in unaccustomed muscles is harmful while the modest generation by regular exercise is beneficial to upregulating defense mechanisms against oxidative stress. It appears that chronic exercise training has a protective effect through
the improvement in antioxidant capacity [12], thus forming a basis of hormetic effects of exercise [11]. Furthermore, anaerobic exercise training can induce adaptations that act to attenuate the exercise-induced oxidative stress. These may be specific to increased antioxidant defenses and may reduce the generation of pro-oxidants during and after exercise [18]. Although SIT has been shown to produce several positive health benefits, such as improved glycemic control and insulin sensitivity [19], cardiac function [20], blood lipid profile [21] and fat oxidation [22], scholarly data have been scarce regarding the effects of oxidative stress on short-term training [23]. Therefore, this study aimed to compare the effects of SIT and CET on oxidative stress and antioxidant indices in healthy young males. We hypothesized that similar oxidative stress and antioxidant adaptation would be observed in SIT and CET.

**MATERIAL AND METHODS**

**Participants**

Following medical history inquiries, physical examinations, and blood testing, 15 healthy young recreationally active university students who met the inclusion criteria (age: 21.7±2.2 years, body mass index (BMI): 25.0±2.1 kg.m⁻², percentage of body fat (BF%): 16.2±3.2%, \( VO_{2}\max \): 40.3±5 ml.min⁻¹.kg⁻¹) voluntarily participated in the study. The inclusion criteria were to be 20–26 years old; not being obese (BMI < 30), anemic, and actively infected; not having any health problems and injuries; not using alcohol, tobacco products, and drugs that affect lipid, lipoprotein and antioxidant metabolism; being habitually active but not engaged in any sort of structured training program for at least five months before the study. Moreover, participants were instructed not to perform any additional exercise, modify their regular daily diet regimen, and take any medications and supplements during the study period.

The study protocol was approved by the Local Scientific Research Ethics Committee (Approval No: 20.478.486-159). Participants were informed about the study procedures and associated risks and were asked to sign a written informed consent form.

**Experimental design**

A repeated measures study design was employed for this prospective laboratory experiment. All experimental procedures were performed in standard 20–22°C temperature and 50–55% relative humidity condition. Before the training sessions, participants visited the laboratory to collect fasting blood samples and record anthropometric measurements. The following day, participants joined in a familiarization session to adapt to the testing/training procedures, the laboratory environment, and to meet with the study group. Following a submaximal test, a maximal graded exercise test and a verification phase were performed to determine the subjects’ \( VO_{2}\max \) levels. After the baseline measurements, subjects were matched according to their initial \( VO_{2}\max \) levels and divided into two groups as follows (numbers indicate ranking of \( VO_{2}\max \) values; 1 = the highest, 15 = the lowest) [24]:

**CET:** 1 4 5 8 9 12 13

**SIT:** 2 3 6 7 10 11 14 15

Sprint intervals and endurance training periods were continued for 7 weeks. At the end of the training period, the baseline tests were repeated in the same order. All procedures were performed on a mechanically braked cycle ergometer (894E, Monark, Sweden), which was set up to replicate the participants’ normal riding position for all tests.
**Procedures**

*Anthropometric measurements*
Height and body weight were measured with minimal clothing (Seca 767, Hamburg, Germany) using standard methods. The body fat ratio was measured on the body composition analysis device (Tanita MC 780MA, USA), which operates with the bioelectric impedance method.

*Determination of VO₂max*
Familiarization sessions consisted of a 20-min submaximal cycling and a 30-sec all-out sprint cycling bout that used the load of 10% of the body mass.

(1) A submaximal graded exercise test that consisted of four 5-min stages were performed to find subjects’ respiratory anaerobic thresholds because maximal graded exercise tests were adjusted from the respiratory anaerobic threshold to exhaustion. (2) We focused on terminating the graded exercise tests approximately 10±2 minutes. Test velocities were fixed at 60 rpm. Test loads were increased by ~20 watts at the 4th, 6th, 8th, 10th, 11th, and continued with one-minute intervals till volunteer exhaustion. Strong verbal encouragement, yet avoiding to give progress feedback, was given to the participants to reveal volitional exhaustion. Peak VO₂ uptake was calculated as the highest value of VO₂ observed over 30 seconds. (3) Following the maximal graded exercise test, a constant load verification phase was performed corresponding to the workload of peak VO₂ revealed.

Oxygen uptake was measured breath-by-breath using a Cosmed Quark b2 with expired gas concentrations (Cosmed Srl, Rome, Italy). The device was calibrated according to the manufacturer’s instructions. The turbine flow meter was calibrated using a 3-L syringe (Quinton Instruments, USA). HR data were collected with a telemetric system belonging to the same gas analyzer (Polar Electro OY, Kempele, Finland).

*Biochemical Measurements*
Fasting blood samples were drawn from an antecubital forearm vein between 08.00 and 10.00 am by a nurse, and then centrifuged at 4000 rpm for 10 min. Plasma/sera were removed and kept at -80°C until analysis. To minimize the influence of analytical variation, all samples were analyzed on the same day for each measurement. Within-batch CV’s and internal controls of the lab for all the analyzed parameters were at an acceptable level.

*The plasma malondialdehyde* (MDA) level was determined via the spectrophotometrical method (Shimadzu UV-1201 V, Japan) described by Ohkawa et al. [25]. An external standard curve was prepared using 1,1,3,3-tetraethoxypropane. Results are given in the µmol/l unit.

*Plasma glutathione peroxidase* (GSH-Px) activities were determined by the method of Paglia and Valentine [26]. Cumene hydroperoxide was added as a substrate. Oxidized glutathione produced by the action of GSH-Px present in the sample was then reduced by glutathione reductase in the presence of NADPH. The decrease in NADPH was recorded at 340 nm spectrophotometrically. Results are given in the µmol/l unit.

*Total antioxidant status* (TAS) and *total oxidant status* (TOS) were determined with a commercial kit (Rel Assay Diagnostics, Turkey) spectrophotometrically by an autoanalyzer (Siemens Advia 1800, USA) using an automated colorimetric method developed by Erel [27, 28]. The coefficient of variation (CV) of the kit was calculated as 10%. The measuring range for serum TAS level was 0–2.75 mmol Trolox Eq/L, and for serum TOS the level was 0–33.5 µmol H₂O₂ Eq/L.
Training intervention
The SIT regimen consisted of 4–6 Wingate all-out sprints with 4.5 min recoveries, while CET consisted of 30–50 min of cycling at a workload corresponding to 60% of VO$_2$max. SIT repetitions and CET durations were gradually increased every seven sessions throughout the training periods. Training sessions were performed three times a week for seven consecutive weeks. A rest of one to two days between training sessions was provided to allow enough recovery.

Statistical methods
Descriptive statistics were reported as the mean ± standard deviations. The Shapiro–Wilk W test showed that the data obtained met the assumptions of normality. Group (CET and SIT) was the between-subjects factor, and Time (0. Week and 7. Week) was the withinsubjects factor in the present study. The main effects and the interaction effect of these factors on the dependent variables were assessed using a 2 × 2 (Group × Time) two-factor mixed-design analysis of variance (ANOVA). These tests were also performed in case of insignificant overall mixed-design ANOVA results to identify the effect size (d) of the difference, which is an essential determinant of practical significance. Baseline values of dependent variables were compared between CET and SIT using an Independent-Samples T-Test. The Mann-Whitney U test was used to compare deltas (W1–W0) between the groups. The level of statistical significance was set at p ≤ 0.05.

Results
Similar mean age, height, mass, body fat percentage, and VO$_2$max level of the SIT and CET groups are displayed in Table 1 (p > 0.05).

Table 1. Physical and physiological characteristics of CET (n = 7) and SIT (n = 8) groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CET</th>
<th>SIT</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>21.3 ± 2.36</td>
<td>22.0 ± 2.14</td>
<td>0.549</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.80 ± 0.06</td>
<td>1.84 ± 0.01</td>
<td>0.149</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>79.3 ± 6.69</td>
<td>86.2 ± 8.00</td>
<td>0.098</td>
</tr>
<tr>
<td>Body fat ratio (%)</td>
<td>15.8 ± 2.62</td>
<td>16.5 ± 3.72</td>
<td>0.668</td>
</tr>
<tr>
<td>VO$_2$max (ml.min$^{-1}$.kg$^{-1}$)</td>
<td>40.46 ± 5.99</td>
<td>40.16 ± 4.26</td>
<td>0.912</td>
</tr>
</tbody>
</table>

Note: CET – continuous endurance training; SIT – sprint interval training; VO$_2$max – maximal oxygen consumption.

No significant Group × Time interaction effect was found for any of the dependent variables, indicating that changes in these variables over the course of the study showed similar patterns in the CET and SIT groups: GPx (F[1,13] = 0.064, p=0.804, n$^2_p = 0.005$); MDA (F[1,13] = 0.468, p = 0.506, n$^2_p = 0.035$); TAS (F[1,13] = 1.099, p=313, n$^2_p = 0.078$); TOS (F[1,13] = 0.299, p = 0.594, n$^2_p = 0.022$).

After the training period, GSH-Px (30%) and TOS (33%) levels were increased while MDA (8.6%, p < 0.003) and TAS (62%) levels were decreased for CET. On the other hand, GSH-Px (55%), TOS (18%), and TAS (17%) levels were increased, while MDA (6.8%, p < 0.016) was significantly decreased for SIT. There were no significant differences between CET and SIT in average values and percentage change in means (Δ%) of oxidative stress and antioxidant results (Table 2).
Table 2. Influence of CET and SIT on oxidative stress and antioxidant indices

<table>
<thead>
<tr>
<th></th>
<th>CET (n=7)</th>
<th>SIT (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week</td>
<td>M ± SD</td>
</tr>
<tr>
<td>SH-Px (µmol/l)</td>
<td>W0</td>
<td>687.9 ± 195.0</td>
</tr>
<tr>
<td></td>
<td>W7</td>
<td>832.3 ± 191.9</td>
</tr>
<tr>
<td>MDA (µmol/l)</td>
<td>W0</td>
<td>1.01 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>W7</td>
<td>0.92 ± 0.05</td>
</tr>
<tr>
<td>TAS (Trolox Eq/L)</td>
<td>W0</td>
<td>1.62 ± 1.12</td>
</tr>
<tr>
<td></td>
<td>W7</td>
<td>0.92 ± 1.15</td>
</tr>
<tr>
<td>TOS (µmol H2O2 Eq/L)</td>
<td>W0</td>
<td>4.96 ± 0.59</td>
</tr>
<tr>
<td></td>
<td>W7</td>
<td>6.52 ± 2.13</td>
</tr>
</tbody>
</table>

Note: *p≤0.05, #p=0.037 (statistically significantly higher compared with data of CET); CET – continuous endurance training; SIT – sprint interval training; GSH-Px, glutathione peroxidase; MDA – malondialdehyde; TAS – total antioxidant status; TOS – total oxidant status; M – mean; SD – standard deviation; Δ% – percentage change in means (−: decrease); d, Cohen’s d (<0.2 trivial; 0.2 ≤ d <0.5 small; 0.5 ≤ d <0.8 moderate; d ≥ 0.8 large effect size); W – week.

DISCUSSION

The main contribution of the study is the result of a 7-week SIT in a marked reduction of MDA and TOS levels as oxidative stress markers and the elevated antioxidant status indices (TAS and GSH-Px level) with similar results that was noted for the CET group. Most importantly, these adaptations were achieved in only 21 exercise sessions with 52.30 min of pure exercise time and a total of 8.45-hour time commitment in the whole 7-week period, even though total active time spent for CET (14 hours) was fifteen times more than SIT. Therefore, the SIT modality may be preferred due to its greater time efficiency compared to CET. As a result, SIT may be accepted as a time-efficient strategy for achieving favorable oxidative stress and antioxidant adaptation.

Several studies in the literature have investigated the effects of exercise models of different intensities on oxidative stress and antioxidant status. However, while there is research on the acute effect of HIIT in healthy individuals [17, 29–31], studies showing the regular training effects of HIIT on oxidative stress and antioxidant status are scarce. Moreover, the use of different exercise models, a single sampling period of the studies, and the different measured oxidative stress and antioxidant status markers makes it difficult to compare the results of the previous investigations.

Previous research showed that the adaptation seen with increased activity of antioxidant enzymes is one of the fundamental changes in aerobic or anaerobic skeletal muscle. Thus, oxidative stress decreases, and lipid peroxidation levels attenuate [12, 32, 33]. Although the influence of oxidative stress on exercise performance is not apparent, inhibition of lipid peroxidation has been shown to be associated with reducing muscular fatigue and alleviating inflammation [34].

The elevated ROS levels produced during the high energy turnover rate required in the active muscle leads to increased lipid peroxidation, which is measured by the formation of MDA after a SIT session [12, 34], thus reducing membrane fluidity, permeability, and
excitability, as well as altering membrane-bound enzyme functions [18]. With compelling evidence that exercise-induced increases in oxidative stress are adaptive, lipid peroxidation may stimulate adaptations such as structural remodeling of external cellular membranes and lipoproteins [35]. Training has been suggested to upregulate antioxidant enzyme levels in tissues that perform systematic exercise, and reduce resting lipid peroxidation [36]. It has been reported that eight weeks of continuous moderate-intensity training and high-intensity training in master runners may be sufficient for antioxidant systems to reduce the acute damage of each high-intensity training session [37]. A decrease in MDA was observed with the 12-week SIT training performed on rats. Although the mechanism behind the peroxidation reduction is unclear, it is suggested that an upregulation of GSH-Px activity may be responsible. Due to its sensitivity to intracellular levels of reactive oxygen and its role in the destruction of the end products of oxidation (including lipid peroxides), GSH-Px may be the most important antioxidant enzyme for cell survival. An increase in GSH-Px may also contribute to the training adaptation during the study [38]. This interpretation may explain the results of the seven-week SIT and CET observations that revealed a similar decrease in MDA and a similar increase in TOS levels, reflecting the total amount of oxidant in the body.

To prevent exercise-induced oxidative stress, antioxidant defense systems including superoxide dismutase (SOD), catalase (CAT) and GPx enzymes, and non-enzymatic substances such as reduced glutathione (GSH), and vitamins A, C, and E, and selenium act in synergy. GSH, an important intracellular antioxidant, plays a prominent role in cellular defense against oxidative stress by directly removing reactive oxygen species and as a substrate for GSH-Px [32, 38]. The TAS level can be considered as a reliable biomarker reflecting the body’s total antioxidant defense. Our study showed that SIT caused a more positive change in TAS and GSH-Px levels compared to CET. This result suggests that the CET group has low basal values, and intensity is more effective than the duration of the exercises. The increase in antioxidant system indicators has been interpreted that higher intensity exercise can promote more redox health benefits and help protect against chronic oxidative stress-related diseases [39].

In addition, the results of two other studies with healthy participants are in line with our results. Bogdanis et al. [40] employed a 3-week classic SIT protocol (4–6 × 30 sec, 4 min rest) with eight healthy active men to investigate the oxidative stress markers. At the end of nine sessions of the SIT period, they observed that oxidative stress markers decreased (protein carbonyl and thiobarbituric acid reactive substances (TBARS)) while antioxidant capacity and CAT activity increased. Fisher et al. [23] implemented 90% of the maximum anaerobic power (HIIT protocol), 4 × 30-sec loadings, and 4-min active rest with 15% of the maximum anaerobic power for a week to a group of eight healthy active men. Participants completed a total of 3 sessions at 48-hour intervals. Results demonstrated an increase in oxidative stress (plasma TBARS levels). Also, there were significant increases in SOD, CAT, and GPx activities in lymphocytes after HIIT. Although the antioxidant system improved as a result of repetition of similar high intensities in these studies, the reduction in oxidative stress markers observed at the end of 9 sessions did not occur at the end of the 3-session period.

The major limitation of the current study is the absence of control of the antioxidant content in the diet regimen of the participants. Therefore, the study results assume that each participant followed his regular daily diet regimen and did not take any supplements following the researcher’s instructions.
CONCLUSIONS

Besides its popularity among athletes and the general population, a study investigating the effects of regular SIT training on oxidative stress and antioxidant status with a comparison to CET could not be found in the scholarly literature. In this study, it is assumed that oxidant and antioxidant balance increases the effectiveness of the SIT exercise model for healthy and patient individuals with similar adaptations provided by short term SIT and CET.

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