Acute moderate-intensity exercise increases total antioxidant capacity and anti-inflammatory responses in competitive cyclists: The role of adiponectin

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Abstract
High-intensity exercise can elicit acute changes in the biochemical and physiological processes in the body of an athlete, including increased oxidative stress and inflammation. The purpose of this study was to explore the effect of acute moderate-intensity exercise on total antioxidant capacity (TAC) and serum levels of anti-inflammatory adiponectin (APN), and inflammatory markers in competitive cyclists. Ten male cyclists (age 15–26 years, body mass index 19.4–24.7 kg/m2) participated in this study. Each subject performed the maximal oxygen uptake test (VO2peak) and completed a 10-min cycling exercise at a workload of 50% of the peak of VO2peak. Blood samples were collected on three different occasions: after an overnight fasting and at the exercise workloads of 50% and 100% VO2peak. We measured APN, TAC, inflammatory markers as well as assessed nutrient and energy intake for each participant. Baseline concentration of serum APN (10.92 µg/mL) significantly increased at 50% and at 100% VO2peak. In addition, TAC also increased after acute exercise (0.079 vs 0.093 nmol/µL). The concentration of APN at 50% VO2peak positively correlated with the CRP ($r = 0.640$, $p = 0.046$) and negatively correlated with TNF-α ($r = -0.696$, $p = 0.025$). This test showed that short (10 min) and medium-intensity (50% VO2peak) exercise activity in trained athletes evoked beneficial antioxidant and anti-inflammatory responses. Importantly, this response correlates with the increase in APN levels thereby showing that highly trained individuals have beneficial responses originating from adipose tissue. Our observations show that a short training at moderate activity can be an important preservative strategy during the recovery training period.

Keywords
adiponectin, athlete, cycling, inflammation, oxidative stress, TAC

Introduction
The impact of physical activity on inflammation has received significant attention in recent years and has provided a mechanistic link between muscle contraction and its beneficial influence on the biomarkers of systemic inflammation.1 It is well documented that acute exercise increases expression and circulating levels of many inflammatory molecules such as cytokines and chemokines.2–8 Tumour necrosis factor α (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP) are cytokines that have pro-inflammatory effects; on the other hand, adiponectin (APN) and interleukin-10 (IL-10) are cytokines...
with anti-inflammatory effects. Acute training increases muscle production of IL-6 which is responsible for reducing pro-inflammatory TNF-α and increasing anti-inflammatory cytokines, such as APN. In addition, acute training may induce oxidative stress, but most studies have shown an increase in total antioxidant capacity (TAC) during medium or high intensity training. Little information is available on how low intensity anaerobic or aerobic exercise (≤50% VO\textsubscript{2max}) affects antioxidant levels. While many studies focused on skeletal muscles, only a few have investigated adipose tissue (AT) secretion following a vigorous workout in nonobese individuals. Recently, the training-induced secretion of cytokines from AT have been a new focus of research. A lower body fat percentage in master athletes (e.g. cyclists) is associated with higher overall antioxidant activity and less inflammation. Indeed, after repeated bouts of aerobic exercise or training AT demonstrates metabolic adaptation (fat mobilization through lipolysis). Acute exercise also affects the infiltration of immune cells into AT, thereby leading to increased expression of inflammatory response genes. AT is a source of pro- and anti-inflammatory cytokines, which are released both during and after vigorous exercise, such as competitive cycling exercise.

APN, a hormone abundantly secreted by adipocytes has a strong anti-inflammatory/oxidative and pro-myogenic effect on skeletal muscles exposed to acute or chronic inflammation and injury, mainly through the AdipoR1 (APN specific muscle receptor). It is important to notice that the published results investigating the serum levels of APN during acute exercise are contradictory. In highly trained individuals, several studies which involve trained adult male cyclists and male futsal players showed that acute exercise does not affect APN concentration and when nine well-trained cyclists were studied, similar results became apparent. On the other hand, a study involving trained male adult rowers showed that the APN concentration decreased immediately after the exercise. Furthermore, a study carried out on runners and weightlifters found that the concentration of APN increases after acute exercise. The study performed on nine professional male cyclists during the Giro d’Italia stage race showed an increase in APN throughout the race and similar was demonstrated by Voss et al. on the adult male competitive cyclists.

It is well established that acute or exhausting exercise induces oxidative stress. In fact, acute exercise increases inflammation, however, chronic adaptation to physical exercise also leads to a positive anti-inflammatory response. Acute training also induces antioxidant protection by increasing TAC, which was demonstrated in the studies at the end of short-acute exercise training in athletes. Although, intensive physical training can generate excessive reactive oxygen species, interestingly, regular training leads to an increase in TAC and antioxidant enzyme and their activity in skeletal muscles. Changes in enzyme activity and TAC under physical exhaustions in regularly trained versus untrained subjects are ambiguous. In many studies, the analysis of TAC level showed that its initial value is lower in untrained group than in the trained one. On the other hand, Park and Kwak showed that untrained and trained athletes had similar basal TAC. Others studies demonstrated that a change in the TAC levels depends on training intensity.

Most of the studies on elite athletes analyzed serum levels of cytokines before and after the maximum exercise load (100% VO\textsubscript{2max}) or endurance training lasting longer than several hours, such as marathon and triathlon. Several other studies showed that responses of cytokines on the short-duration exercise are not dependent on exercise-induced muscle damage but are instead related to the exercise intensity (physiological load/stress). Therefore, the aim of this study was to confirm the hypothesis that even short-term exercise (10 min) at low intensity (50% VO\textsubscript{2peak}) in trained athletes can affect the release of anti-inflammatory APN, as well as the level of TAC.

**Materials and methods**

This cross-sectional study was performed in the early phase of the cycling season (in November 2018). The study was approved by the Slovenian National Medical Ethics Committee (No. 93/07/13), and all participants provided written informed consent before the study.

**Subjects**

Ten healthy competitive Slovenian cyclists participated in the study. The key inclusion criteria were as follows: male, aged 15–30 years, active competitive
cyclist within the active race season, BMI 18.5–27.0 kg/m², free from any musculoskeletal injury and cardiovascular disease, non-smokers, and not taking any medications. All participants competed either at the national level or on European races and have VO₂\text{max} rates higher than 50 mL/kg BW/min.

**Measurements**

All measurements were carried out at the University of Primorska, Faculty of Health Sciences and taken between 7.00 a.m. and 10.00 a.m. after a night of fasting in a thermoneutral environment (20–22°C).

**Anthropometric measurements**

Anthropometric measurements were performed between 7.00 a.m. and 9.00 a.m. in standardized conditions by the same examiner and were obtained using standard protocols and techniques.

Subject’s body mass (kg), body composition (total percentage body fat (% BF)), percentage of fat free mass (% FFM), percentage of muscle mass (% MM), percentage of water (% water) and height (cm) were determined in standing position, with subjects wearing light clothes and no shoes.

Body composition was determined by multiple-frequency bioelectrical impedance analysis using a Tanita BC 418 MA (Tanita Corporation, Arlington Heights, IL). Prior to the measurements, subjects were informed about the nature of the tests and asked to follow a pre-test protocol that included a 12-h fasting cure, 24-h abstinence from alcohol and caffeine, 24-h abstinence from physical activity and adequate fluid intake. Before the measurements, the subjects rested on a chair in a thermoneutral environment (20–22°C) for 10 min. All participants were asked to empty their bladders before the measurement.

Body height was measured with a 0.1 cm precision using a stadiometer (Invicta Plastic, Oadby, England). Body mass index (BMI) was calculated by dividing body mass (kg) by square of height (m).

**Resting metabolic rate**

Resting metabolic rate (RMR) was measured using the hand-held indirect calorimeter (MedGem Microlife, Golden, CO). RMR measurements were performed under standard conditions: at least 24 h without exercise, in fasting state in the last 12 h, in thermo neutral environment (20–22°C) and after the device was auto-calibrated. RMR measurement lasted approximately 10 min and after completing the measurements, the RMR value in kcal/day was displayed on a computer screen. CVs for RMR were <8%.

**Dietary assessment**

Macronutrients, vitamins and mineral intakes were recorded using the 3-day food record method. The subjects were instructed to complete 3-day dietary records: the subjects recorded their food intake for 2 weekdays and 1 weekend day during the week prior to the exercise trial. Nutrient composition energy content and dietary total antioxidant capacity were analyzed using the web tool Open platform for clinical nutrition (OPEN) http://opkp.si/.

OPEN food composition data was taken from the Slovenian food composition database (FCDB); or if not available there, from the Souci-Fachmann-Kraut FCDB and/or from the USDA National Nutrient Database for Standard Reference.\textsuperscript{36,37} Food composition data applied by the OPEN met the European standard for food data CEN/TC 387.

**Assessment of physical activity**

Information on physical activity was estimated using a physical activity diary which gathered information on the frequency, type, duration and intensity of every day physical activity. Metabolic equivalents (METs) for each activity were assigned according to a standardized protocol, published by Ainsworth et al.\textsuperscript{38} Total amount of physical activity per day was calculated by multiplying the time spent on the activities with the intensity index expressed in MET, where 1 MET equals a resting oxygen consumption of 3.5 mL/kg/min.

**Experimental design**

During the 1-week study period, subjects were admitted to the biochemical laboratory of the Faculty of Health Sciences. On their first visit, after an overnight fast, resting metabolic rate measurements and anthropometric measurements were performed between 7.00 a.m. and 9.00 a.m. To maintain optimal hydration, each subject drank 500 mL of water 30 min before exercise.

After that, between 9.00 a.m. and 10.00 a.m., the measurement of maximal oxygen uptake (VO₂\text{peak}) was assessed using a cycle ergometer.
During the second visit (1 week later), blood samples were taken before workload began (0%), immediately after 10 min of the workload at 50% and immediately after 1 min following the workload at 100% VO$_{2\text{peak}}$. The workload test was performed between 9.00 a.m. and 10.00 a.m.

Exercise protocol

Heart rate, volume of oxygen and carbon dioxide consumption (VO$_2$, VCO$_2$), ventilation (VE), respiratory quotient (QR) and power (W) were monitored during the exercise.

VO$_{2\text{peak}}$ protocol. The peak oxygen uptake was determined using a progressive continuous test to exhaustion on a cycle ergometer (Monark, Model Ergomedic 894E, Varberg, Sweden). Before the test, each subject had to complete a 15-min warm-up at 75 watts (W) power output maintaining 70 revolutions per minute (rpm). The subjects wore a Polar heart rate monitor and a device for measuring oxygen consumption with the accompanying face mask. VO$_2$ was measured via a portable open-air spirometer system (Cosmed K4b$^2$, Rome, Italy). The device was heated prior to use for approximately 30 min and then the calibration of gas and volume was carried out. The protocol began at 75 W (70 rpm) and was increased by 25 W every minute until exhaustion. Oxygen consumption and other parameters were measured continuously during the exercise test. Maximal workload (watt) was considered to represent the power output during the last completed stage of the incremental exercise test, while VO$_{2\text{peak}}$ represents the highest 10-s rolling average during the test and is expressed relative to body mass. The criteria for attaining VO$_{2\text{peak}}$ included any two of the following: heart rate within 10 beats/min of age-predicted maximum, respiratory-exchange ratio $\geq$1.1, and rating of perceived exertion $\geq$17.

On the second visit, each subject after the 15-min warm-up completed a 10-min cycling exercise at a workload of 50% of the peak VO$_{2\text{peak}}$ followed by 1-min of 100% (VO$_{2\text{peak}}$). Exercise loads were calculated from the previously obtained results of subject’s maximal oxygen uptake on a cycle ergometer (from 1 week before).

Serum analysis

Standard laboratory tests including C-reactive protein (CRP), IL-6, APN, bilirubin, glucose, lipids, ferritin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), haemoglobin (Hb), haematocrit (Hct), red (RBC) and white (WBC) blood cell and platelet count (Plt), were performed after overnight fasting and before and after each workload. All of the biochemical measures were done in duplicate.

Blood samples were collected by venipuncture on three different occasions: after an overnight fasting (0%), and immediately at the exercise workloads of 50% and 100% VO$_{2\text{peak}}$. Blood samples (4 mL) were collected in EDTA – containing vacuum tubes and centrifuged. Plasma was separated from the cells and stored at −20°C until analyzed.

The measurements of serum concentration of APN (BioVendor, Lab.Med.Inc., Brno, Czech Republic), IL-6, TNF-α (both Thermo Fischer Scientific Inc., Rockford, USA) were performed in duplicate on a microplate reader (Tecan, Männedorf, Switzerland) by commercially available enzymatic reagents. Using Olympus reagents and AU 680 analyzer (Beckman Coulter) we analyzed serum concentrations of bilirubin, glucose, triglycerides (TG), total cholesterol, low-density lipoprotein, (LDL cholesterol), high-density lipoprotein (HDL cholesterol), CRP and ferritin. The MCV, RBC, WBC, Hct, Plt, Hb and haematocrit variables were analysed by haematological analyser LH780 (Beckman Coulter, Sweden) and using Beckman Coulter reagents. The antioxidant capacity (TAC) of serum samples was measured by Photochem® instrument (Analytic Jena, Jena, Germany) using ACW kits (Water Antioxidant Capacity) to detect the activity of hydrophilic compounds. Measured serum TAC levels in our study, expressed as nanomoles of ascorbic acids per µL of serum. Assays interassay and intraassay CVs were typically <10% (for TNF-α 5.2%, for APN 6.3%, for IL-6 7.1%). CVs for TAC were <8%. CVs for all other biochemical parameters were <2.5%.

Statistical analysis

Statistical analyses were performed using SPSS (version 19.0, IBM, Armonk, New York). Prior to the analysis, all the data that were not normally distributed was log base 10-transformed to approximate a normal distribution (in our experiment: data for the concentration of IL-6). The data are presented as the mean ± SD. Pearson Product Moment correlations were calculated to evaluate associations.
among different biochemical variables. In order to determine changes in inflammatory and anti-inflammatory markers during the exercise, each marker was analyzed by the paired-sample t-test. The sample size of our study population was calculated considering the expected mean of the paired differences in the APN level $5 \mu g/mL$ and expected standard deviation of the change in the APN level $3.0 \mu g/mL$.

To achieve a power of 80% and a level of significance of 5% (two sided), we estimated we would need a minimum sample size of six participants. Considering potential drop-outs, we aimed to enroll 10 participants.

## Results

### Baseline characteristics of studied subjects

Table 1 shows the physiological characteristics of the 10 competitive cyclists studied, including their body mass, height, percentage of the body fat, percentage of the fat free mass and muscle mass, BMI, VO$_{2peak}$ values, resting heart rate and maximal heart rate. All data are expressed as mean ± SD. The average (SD) body mass of subjects was 69.8 (8.7) kg. The average BMI value was 21.1 (1.8) kg/m$^2$. Subjects had a high proportion of lean muscle mass 89.8 (3.3) %, and a low percentage of fat mass 9.9 (3.6) %, which is also reflected in the high resting metabolic rate (1905 (189) kcal/day). Competitive athletes in general are known to have a low heart rate at rest, which was also confirmed in our case since our subjects had a heart rate of 49 (10) beats/min. All subjects completed maximal oxygen test with their average VO$_{2peak}$ of 64.2 (3.8) mL/kg BW/min and maximal heart rate of 191 (5) beat/min.

The dietary habits of the subjects are presented in Table 1. Their average energy intake was amounted 13021 (3010) kJ per day. The mean daily intake of carbohydrates was 54 (7) %, while recommendations for endurance athletes recommend the intake of 55–65%. Of total ingested calories, 29 (5) % were from fat and 15 (5) % from protein, which is in accordance with the recommendations. The average intake of vitamin C was 132 (126) mg/day and total

### Table 1. The characteristics, metabolic parameters, nutrient and energy intake of the subjects involved in the study.

| Variable                      | Mean     | Minimum value | Maximum value |
|-------------------------------|----------|---------------|---------------|
| Age (years)                   | 19.6 (4.0) | 15            | 26            |
| Height (cm)                   | 181.8 (4.2) | 176.3         | 182.0         |
| Body mass (kg)                | 69.8 (8.7)  | 59.8          | 85.5          |
| Fat mass (%)                  | 9.9 (3.6)   | 5.7           | 14.9          |
| Fat free mass (%)             | 89.8 (3.3)  | 85.1          | 94.3          |
| Muscle mass (%)               | 85.8 (3.2)  | 81.4          | 90.1          |
| Water (%)                     | 65.8 (2.5)   | 62.4          | 69.1          |
| BMI (kg/m$^2$)                | 21.1 (1.8)   | 19.4          | 24.7          |
| RMR (kcal/day)                | 1905.9 (188.9) | 1460.0       | 2070.0        |
| MET (h/day)                   | 14.0 (3.4)   | 10.6          | 20.4          |
| VO$_{2peak}$ (mL/kg BW/min)   | 64.2 (3.8)   | 57.6          | 71.4          |
| VO$_2$ at 75 W (mL/kg BW/min) | 22.2 (1.6)   | 19.2          | 24.0          |
| HR$_{rest}$ (beat/min)        | 49 (10)      | 40            | 69            |
| HR$_{max}$ (beat/min)         | 191 (5)       | 184           | 200           |
| Nutrient and energy intake    |           |               |               |
| Energy intake (kJ/day)        | 13021 (3010) | 8910         | 18328         |
| Carbohydrates (% of energy)   | 54 (7)       | 40            | 63            |
| Proteins (% of energy)        | 15 (5)       | 11            | 28            |
| Fats (% of energy)            | 29 (5)       | 22            | 38            |
| Saturated fatty acids (% of energy) | 10 (4)    | 6             | 16            |
| Monounsaturated fatty acids (% of energy) | 6 (2)    | 3             | 9             |
| Iron (mg/day)                 | 20 (6)       | 10            | 27            |
| ORAC (µmol Trolox equivalents/100 g) | 8153 (6382) | 705          | 16344         |
| Vitamin C (mg/day)            | 132 (126)    | 17            | 380           |

Values are means (SD). BMI: body mass index; RMR: resting metabolic rate; MET: metabolic equivalent; VO$_{2peak}$: peak of oxygen uptake; HR$_{rest}$: heart rate at rest; HR$_{max}$: maximum heart rate at exercise; ORAC: total oxygen radical absorbance capacity.
Oxygen radical absorbance capacity (ORAC) was 8153 (6382) µmol Trolox equivalents/100 g.

Table 2 shows the overall data for the studied serum biochemical and haematological variables. All parameters are within reference values.

### Relationships between adipokines, cytokines and other variables

Simple linear regression analysis was used to determine associations between serum levels of APN and BW, BMI, FFM, RMR, MCV and MCH. Basal serum levels of APN were in positive correlation with the BW \((r=0.700, p=0.024)\), BMI \((r=0.800, p=0.005)\), FFM \((r=0.641, p=0.046)\) and RMR \((r=0.615, p=0.005)\). Interestingly, APN levels were in negative correlation with red blood cells indices, that is, MCV \((r=-0.654, p=0.040)\) and MCH \((r=-0.786, p=0.007)\).

### Discussion

Our main finding is that short (10 min) and medium-intensity (50% VO\(_{2}\)peak) exercise activity in highly trained athletes can evoke beneficial antioxidant and anti-inflammatory responses. Thus, a short training at moderate activity can be an important preservative strategy during the recovery training period.
This is strongly correlated with the increase in APN levels, thereby suggesting that these beneficial responses originate from adipose tissue.

Physical exercise induces metabolic changes in the organism, leading to the activation of adaptive mechanisms aimed at establishing a new dynamic equilibrium. An AT adaptation leads to the secretion of various inflammatory and anti-inflammatory markers. People with a high percentage of fat mass have more adipocytes, which release many cytokines that can cause metabolic disorders.41

Vilarrasa et al.42 showed that subjects with low APN levels have higher BMI than subjects with high APN levels. Kappes and Löffler43 assume that the difference in concentration of APN is probably due to the increase in the expression of TNF-α in people with a high percentage of fat mass.
subjects with higher BMI, which reduces the expression of APN. Today, it is known that regular exercise in obese subjects leads to higher serum levels of APN and lower levels of several pro-inflammatory adipokines.\textsuperscript{44–46} On the other hand, the effect of exercise on APN concentration in normally fed patients are contradictory.\textsuperscript{25,47} Importantly, in our study we showed that the body mass and BMI of competitive cyclists are positively correlated to the concentration of the APN, which is in contrast to the active males with elevated BMI indexes.\textsuperscript{24} Also, there is a gap of knowledge in understanding the secretion of APN during different intensity levels and durations of the exercise in the elite athletes; indeed, several published results are contradictory.\textsuperscript{20,22,23}

While exercise-induced oxidative stress and inflammation has received significant attention, the relationship between exercise intensity and the resultant increase of antioxidant capacity and anti-inflammatory markers remains unclear.\textsuperscript{34} The athletes are inherently prone to higher baseline APN and TAC, which can be attributed to the fact that they are exposed to constant high loads resulting in a higher susceptibility for inflammatory mediators compared to other individuals.\textsuperscript{28,31} This is also supported by the average measured baseline APN (10.9 µg/mL) which is in the middle of the baseline APN of the healthy active individuals (5–20 µg/mL).\textsuperscript{4} Changes in TAC and APN, however, are not only detected at the basal level. Although most studies show that low-intensity exercise does not change APN concentrations\textsuperscript{20,48} and that TAC increases especially with high-intensity exercise,\textsuperscript{34} we have proven that changes occur even during short-term, low-intensity exercise. It was previously suggested that exercise must be exhaustive to overwhelm endogenous antioxidant defenses.\textsuperscript{49}

However, in our study we demonstrated that even short-term low-intensity exercise can trigger a significant increase in TAC and APN, and revealed positive association between them. In accordance with the results of Lombardi et al.\textsuperscript{8} and Voss et al.\textsuperscript{26} in the elite cyclists, we observed an increase in the serum levels of APN at 50% \( \text{VO}_{2\text{peak}} \) and further increase at 100% \( \text{VO}_{2\text{peak}} \). A similar trend was shown for TAC plasma concentration. These findings contradict the results of Parker et al.,\textsuperscript{34} which showed no changes in TAC in low intensity (50%) and short time exercise (5 min). The mentioned study and other studies\textsuperscript{50,51} showed that the TAC increases at a higher intensity. However, these studies investigated physically active and untrained individuals, while we showed responses in highly-trained (competitive) individuals at lower intensity (50%) and in a short time period (10 min). In our study, we also noticed that the early increase of the APN and TAC concentrations coincides with the increasing concentration of IL-6, CRP and TNF-\( \alpha \). This can be attributed to the fact that APN has anti-inflammatory activity.\textsuperscript{4}

Also, this type of acute exercise can be the contributing factor to the observed changes in TAC and APN. Oxidative stress is increased in exercises with concentric muscle contractions at an early phase, whereas in eccentric contraction exercise (e.g. cycling) exercise after a certain delay.\textsuperscript{52}

We also found a positive relationship between APN and CRP at intensity of 50% \( \text{VO}_{2\text{peak}} \), which is in accordance with similar studies to date.\textsuperscript{4} The increase in serum levels of CRP by two to three folds, known as a low systemic inflammation, may also occur during acute exercise.\textsuperscript{4} Some of the existing studies detected the increase of CRP after 24 h of exercise.\textsuperscript{4} Similarly, we detected the increase in the concentration of CRP during the exercise load. In addition, many other factors have the potential to influence the serum levels of CRP.\textsuperscript{4}

In our study, changes in TNF-\( \alpha \) concentration during exercise were not significant. This finding was expected since literature indicates that TNF-\( \alpha \) levels increase after 30 min of exercise.\textsuperscript{1} Nevertheless, our study showed that the concentration of TNF-\( \alpha \) was slightly increased at 50% \( \text{VO}_{2\text{peak}} \), while it decreased at higher loads. The same was demonstrated on the elite cyclist in the study of Luk et al.\textsuperscript{7} The decrease of TNF-\( \alpha \) concentration at higher load (100% \( \text{VO}_{2\text{peak}} \) vs 50% \( \text{VO}_{2\text{peak}} \)) can be attributed to the marked increase in skeletal muscle IL-6, which subsequently had an anti-inflammatory effect and inhibited TNF-\( \alpha \) release. This may explain why TNF-\( \alpha \) values are lower at higher loads.\textsuperscript{53} In addition to the decrease in TNF-\( \alpha \), IL-6 is also responsible for the production of APN.\textsuperscript{54}

Therefore, the observed upregulation of APN and TAC serum levels in response to the acute low-intensive exercise is probably a compensatory action to the induced increase in pro-inflammatory mediators. These findings provide novel support for the use of low-intensity and short duration (\( \leqslant 10 \text{min} \)) exercise in prescribing exercise for the improvement and/or maintenance in health.
In contrast to low-intensity exercise, high-intensity exercise is known to cause an increase of muscle metabolism markers. Recent evidence suggests that high intensity exercise can induce oxidative stress and inflammation after exercise. Our study, also showed that during a short exercise of high intensity (100%) the inflammatory factors CRP, IL-6, TNF-α increase, as well as APN and TAC, which is in accordance with other studies.

The inflammatory and anti-inflammatory response of the body to exercise is also influenced by the athlete’s VO$_{2\text{max}}$ capacity and diet. Therefore, in our study we included elite athletes and controlled their performance and nutrition.

The present study has several limitations that should be mentioned. This study focuses exclusively on professional cyclists. The analysis focuses only on APN without considering certain hormones that can modulate APN concentration. In addition, this study did not evaluate the influence of professional training on serum oxidant status. The study was conducted in the laboratory and not in a real outdoor environment where the athlete is influenced by many other factors such as temperature, wind and humidity.

Conclusions

In conclusion, the novel findings presented in this paper suggest that a single bout of short-term exercise (10 min) at low intensity (50% VO$_{2\text{peak}}$) triggers a transient stimulation of pro- and anti-inflammatory cytokine production and endogenous antioxidants. The cytokine response induced by short-term training at low intensity leads to an increased production of pro-inflammatory cytokines. The pro-inflammatory response is followed by increase in anti-inflammatory markers and antioxidant potential. Both, anti-inflammatory markers and antioxidant potential play a crucial role in the containment and resolution of the inflammation processes. Our results show that highly trained individuals can efficiently counteract the negative effects of oxidative stress and inflammation.

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Declaration of conflicting interests

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Ethics approval

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Informed consent

Written informed consent was obtained from all subjects before the study. Written informed consent was obtained from legally authorized representatives before the study.

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References

1. Pedersen BK and Febbraio MA (2012) Muscles, exercise and obesity: Skeletal muscle as a secretory organ. *Nature Reviews Endocrinology* 8(8): 457–465.
2. Pedersen BK and Hoffmann-Goetz L (2000) Exercise and the immune system: Regulation, integration, and adaptation. *Physiological Reviews* 80(3): 1055–1079.
3. Panagiotakos DB, Pitsavos C, Chrysohoou C et al. (2005) The associations between leisure-time physical activity and inflammatory and coagulation markers related to cardiovascular disease: The ATTICA study. *Preventive Medicine* 40(4): 432–437.
4. Mansouri M, Keshtkar A, Hasani-Ranjbar S et al. (2011) The impact of one session resistance exercise on plasma APN and RBP4 concentration in trained and untrained healthy young men. *Endocrine Journal* 58(10): 861–868.
5. Varady KA, Bhutani S, Church EC et al. (2010) Adipokine responses to acute resistance exercise in trained and untrained men. *Medicine and Science in Sports and Exercise* 42(3): 456–462.
6. Cosio-Lima ML, Desai VB, Schuler BP et al. (2011) A comparison of cytokine responses during prolonged cycling in normal and hot environmental conditions. *Open Access Journal of Sports Medicine* 2: 7–11.
7. Luk HY, Levitt DE, Lee EC et al. (2016) Pro and antiinflammatory cytokine responses to a 164km road cycle ride in a hot environment. *European Journal of Applied Physiology* 16(10): 2007–2015.
8. Lombardi G, Lanteri P, Graziani R et al. (2012) Bone and energy metabolism parameters in professional cyclists during the Giro d’Italia 3-weeks stage race. *PLoS One* 7(7): 42077.
9. Starkie RL, Rolland J, Angus DJ et al. (2001) Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-α levels after prolonged running. *The American Journal of Physiology-Cell Physiology* 280(4): C769–C774.

10. Varamenti E, Tod D and Pullinger SA (2020) Redox homeostasis and inflammation responses to training in adolescent athletes: A systematic review and meta-analysis. *Sports Medicine-Open* 6(1): 1–17.

11. Lewis NA, Howatson G, Morton K et al. (2015) Alterations in redox homeostasis in the elite endurance athlete. *Sports Medicine* 45(3): 379–409.

12. Frydelund-Larsen L, Akerstrom T, Nielsen S et al. (2007) Visfatin mRNA expression in human subcutaneous adipose tissue is regulated by exercise. *American Journal of Physiology-Endocrinology and Metabolism* 292(1): E24–E31.

13. Hojbjerre L, Rosenzweig M, Dela F et al. (2007) Acute exercise increases adipose tissue interstitial adiponectin concentration in healthy overweight and lean subjects. *European Journal of Endocrinology* 157(5): 613–623.

14. Fabre O, Ingerslev LR, Garde C et al. (2018) Exercise training alters the genomic response to acute exercise in human adipose tissue. *Epigenomics* 10(8): 1033–1050.

15. Bas S, Finckh A, Puskas GJ et al. (2014) Adipokines correlate with pain in lower limb osteoarthritis: Different associations in hip and knee. *International Orthopaedics* 38(12): 2577–2583.

16. Aguilar SS, Sousa CV, Deus LA et al. (2020) Oxidative stress, inflammatory cytokines and body composition of master athletes: The interplay. *Experimental Gerontology* 130: 110806.

17. Pedersen BK (2000) Exercise and cytokines. *Immunology and Cell Biology* 78(5): 532–535.

18. Jortay J, Senou M, Abou-Samra M et al. (2012) Adiponectin and skeletal muscle: Pathophysiological implications in metabolic stress. *The American Journal of Pathology* 181(1): 245–256.

19. Abou-Samra M, Selvaïs CM, Dubuisson N et al. (2020) Adiponectin and its mimics on skeletal muscle: Insulin sensitizers, fat burners, exercise mimickers, muscling pills... or everything together? *International Journal of Molecular Sciences* 21(7): 2620.

20. Lakhdar N, Saad HB, Denguezi M et al. (2013) Effects of intense cycling training on plasma leptin and adiponectin and its relation to insulin resistance. *Neuroendocrinology Letters* 34(3): 229–235.

21. Zar A, Abedi H, Ahmadi F et al. (2017) Effect of training intensity on serum leptin and adiponectin in male and female futsal players. *Report of Health Care* 3(4): 43–50.

22. Suzuki K, Takahashi M, Li CY et al. (2015) The acute effects of green tea and carbohydrate congestion on systemic inflammation and oxidative stress during sprint cycling. *Applied Physiology Nutrition and Metabolism* 40(10): 997–1003.

23. Jürimäe J, Purge P and Jürimäe T (2005) Adiponectin is altered after maximal exercise in highly trained male rowers. *European Journal of Applied Physiology* 93(4): 502–505.

24. Schön M, Kovaničová Z, Košútka Z et al. (2019) Effects of running on adiponectin, insulin and cytokines in cerebrospinal fluid in healthy young individuals. *Scientific Reports* 9(1): 1959.

25. Martínez EC, Fortes MS and Anjos LA (2011) Influence of nutritional status and VO2max on adiponectin levels in men older than 35 years. *Arquivos Brasileiros de Cardiologia* 96(6): 471–476.

26. Voss SC, Nikolauski Z, Bourdon PC et al. (2016) The effect of cumulative endurance exercise on leptin and adiponectin and their role as markers to monitor training load. *Biology Sport* 33(1): 23–28.

27. Cunniffe B, Hore AJ, Whitcombe DM et al. (2010) Time course of changes in immuneendoctrine markers following an international rugby game. *European Journal of Applied Physiology* 108(1): 113–122.

28. Carlsson A, Rohn S, Bittmann F et al. (2008) Exercise increases the plasma antioxidant capacity of adolescent athletes. *Annals of Nutrition and Metabolism* 53(2): 96–103.

29. Mukherjee S and Chia M (2009) Urinary total antioxidant capacity in soccer players. *Acta Kinesiologica* 3(1): 26–33.

30. de Souza TP Jr, de Oliveira PR and Pereira B (2005) Efeitos do exercicio fisico intenso sobre a quimioluminescencia urinaria e malondialdeido plasmatico. *Revista Brasileira de Medicina do Esporte* 11(1): 91–96.

31. Marin DP, dos Santos M, de Cassia R et al. (2011) Cytokines and oxidative stress status following a handball game in elite male players. *Oxidative Medicine and Cellular Longevity* 2011: 804873.

32. Park SY and Kwak YS (2016) Impact of aerobic and anaerobic exercise training on oxidative stress and antioxidant defense in athletes. *Journal of Exercise Rehabilitation* 12(2): 113.

33. Wadley AJ, Chen YW, Lip GY et al. (2016) Low volume–high intensity interval exercise elicits anti-oxidant and anti-inflammatory effects in humans. *Journal of Sports Sciences* 34(1): 1–9.

34. Parker L, McGuckin TA and Leicht AS (2014) Influence of exercise intensity on systemic oxidative stress and antioxidant capacity. *Clinical Physiology and Functional Imaging* 34(5): 377–383.

35. Suzuki K (2018) Involvement of neutrophils in exercise-induced muscle damage. *General Internal Medicine and Clinical Innovations* 3: 1–8.

36. Golob T, Stibilj V, Žlender B et al. (2006) *Slovenian food composition tables – meat and meat products.*
Ljubljana: University of Ljubljana, Biotechnical Faculty (in Slovene).
37. Souci SW, Fachmann W and Kraut H (2008) Food composition and nutrition tables. 7th ed. Germany: MedPharm Scientific Publishers.
38. Ainsworth BE, Haskell WL, Herrmann SD et al. (2011) Compendium of physical activities: A Second Update of Codes and MET Values. Medicine and Science in Sports and Exercise 43(8): 1575–1581.
39. American College of Sports Medicine (2006) ACSM’s guidelines for exercise testing and prescription. Philadelphia, PA: Lippincott, Williams & Wilkins, pp.99–102.
40. Kraemer RR, Aboudehen KS, Carruth AK et al. (2003) Adiponectin responses to continuous and progressively intense intermittent exercise. Medicine and Science in Sports and Exercise 35(8): 1320–1325.
41. Stehno-Bittel L (2008) Intricacies of fat. Journal of Physical Therapy Science 88(11): 1265–1278.
42. Vilarrasa N, Vendrell J, Maravall J et al. (2005) Distribution and determinants of adiponectin, resistin and ghrelin in a randomly selected healthy population. Clinical Endocrinology 63(3): 329–335.
43. Kappes A and Löffler G (2000) Influences of ionomycin, dibutyryl-cycloAMP and tumour necrosis factor-α on intracellular amount and secretion of apM1 in differentiating primary human preadipocytes. Hormone and Metabolic Research 32(11–12): 548–554.
44. Eriksson M, Johnson O, Boman K et al. (2008) Improved fibrinolytic activity during exercise may be an effect of the adipocyte-derived hormone leptin and adiponectin. Thrombosis Research 122(5): 701–708.
45. Torres-Leal F, Fonseca-Alaniz HM, Teodoror FRG et al. (2011) Leucine supplementation improves adiponectin and total cholesterol concentrations despite the lack of changes in adiposity or glucose homeostasis in rats previously exposed to a high-fat diet. Nutrition and Metabolism 8(1): 62.
46. Zemel BM and Bruckbazer A (2012) Effects of a leucine and pyridoxine-containing nutraceutical on fat oxidation, and oxidative and inflammatory stress in overweight and obese subjects. Nutrients 4(6): 529–541.
47. Oberbach A, Tönjes A, Klöting N et al. (2006) Effect of a 4 week physical training program on plasma concentrations of inflammatory markers in patients with abnormal glucose tolerance. European Journal of Endocrinology 154(4): 577–585.
48. Magkos FB, Mohammed S and Mittendorfer B (2010) Enhanced insulin sensitivity after acute exercise is not associated with changes in high-molecular weight adiponectin concentration in plasma. European Journal of Endocrinology 162(1): 61–66.
49. Vina J, Sanchis-Gomar F, Martinez-Bello V et al. (2012) Exercise acts as a drug; the pharmacological benefits of exercise. British Journal of Pharmacology 167(1): 1–12.
50. Tyldum GA, Schjerve IE, Tjonna AE et al. (2009) Endothelial dysfunction induced by post-prandial lipemia: Complete protection afforded by high-intensity aerobic interval exercise. Journal of the American College of Cardiology 53(2): 200–206.
51. Gabriel B, Ratkevičius A, Gray P et al. (2012) High-intensity exercise attenuates postprandial lipaemia and markers of oxidative stress. Clinical Science (London) 123(5): 313–321.
52. Kawamura T and Muraoka I (2018) Exercise-induced oxidative stress and the effects of anti-oxidant intake from a physiological viewpoint. Antioxidants 7(9): 119.
53. Chevrel G, Granet C and Miossec P (2005) Contribution of tumour necrosis factor α and interleukin (IL) β to IL6 production, NF-κB nuclear translocation, and class I MHC expression in muscle cells: In vitro regulation with specific cytokine inhibitors. Annals of the Rheumatic Diseases 64(9): 1257–1262.
54. Starkie RL, Rolland J, Angus DJ et al. (2001) Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-α levels after prolonged running. American Journal of Physiology-Cell Physiology 280(4): C769–C774.
55. Hood MS, Little JP, Tarnopolsky MA et al. (2011) Low-volume interval training improves muscle oxidative capacity in sedentary adults. Medicine and Science in Sports and Exercise 43(10): 1849–1856.