High intensity interval training and molecular adaptive response of skeletal muscle

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ABSTRACT

Increased cardiovascular fitness, VO2max, is associated with enhanced endurance capacity and a decreased rate of mortality. High intensity interval training (HIIT) is one of the best methods to increase VO2max and endurance capacity for top athletes and for the general public as well. Because of the high intensity of this type of training, the adaptive response is not restricted to Type I fibers, as found for moderate intensity exercise of long duration. Even with a short exercise duration, HIIT can induce activation of AMPK, PGC-1α, SIRT1 and ROS pathway as well as by the modulation of Ca2+ homeostasis, leading to enhanced mitochondrial biogenesis, and angiogenesis. The present review summarizes the current knowledge of the adaptive response of HIIT.

Introduction

High intensity interval training (HIIT) emerged several decades ago in response to the need for new training techniques for athletic events of high intensity and often also of long duration. In the 1920s the legendary runner “Flying Finn” Paavo Nurmi started to introduce “interval training” sessions to his annual training cycle.1 In the Nordic athletic community “natural” interval training gained popularity, was named Fartlek and was formalized by Gösta Holmér in 1937. The Fartlek method originally was developed for cross country, multi-terrain runners and consisted of a continuous distance run in which very high (sprints, or uphill runs) and low velocity running periods were integrated. In the 1950s interval training methods started to infiltrate several European athletic training programs. It became prominent probably because the four-time Olympic gold medalist Emil Zatopek (Czechoslovakian) and other eminent runners like Vladimir Kuts (Russian), Gordon Pirie (British) or Sigfried Hermann (German) used this training method effectively during their preparation.2 Some of the first scientific papers which described HIIT in detail were published in the late 50s–early 60s by Roskamm and Reindell3 as well as Per Olaf Astrand.4 However, HIIT research gained full attention in the 1970s.5 Early protocols used the average velocity or the velocity corresponding to the personal best time of the distance of interest as reference instead of the maximal aerobic capacity. The use of maximal oxygen uptake (VO2max), introduced by the Nobel laureate Archibald Vivian Hill, become widespread only after the Second World War.

Depending on the applied protocol, the exercise resembled endurance moderate intensity continuous training (MICIT) or a strength-speed training adaptation. For an in depth historical review the reader is directed to the review of Véronique Billat.2

It became clear that high intensity exercise is a fundamental tool for improving peak athletic performance. It is impossible to reach the required physiological limit of athletic performance during competitions without challenging the musculoskeletal and cardiovascular systems by vigorous exercise sessions. As early as 1986 Cox and colleagues showed that after seven weeks working at 90% or higher intensity exercise induced left ventricular morphology changes in previously sedentary individuals.6 HIIT is shown to increase aerobic capacity even more efficiently than volume-based endurance training protocols.7 The time spent with doing physical exercise is also an important factor, not only for nonprofessional athletes but for professionals, as well. Recently time-efficiency became a pinnacle aspect for athletes to improve diverse skill sets. HIIT induces comparable chronic physiological adaptation with less expenditure of time than classical endurance programs,8 and the recovery time after HIIT appears to be shorter than after moderate intensity exercise of long duration.9 Moreover, some authors claim that HIIT is a superior method compared to aerobic training, in order to
improve diverse physiological functions. The question that needs to be answered is “what are the differences in the adaptive response to HIIT and MICT.”

In this review we focus on investigations that applied 85–90% [taking peak power output (PPO) or PVO_{2max} \times VVO_{2max}] or higher loads during HIIT. This range of intensity is suggested by Wisloff et al. who reviewed the literature on interval training and its cardiac benefits. The duration of the high intensity load is also a matter of debate. The protocols in the following sections will be referred to as HIIT if one session does not exceed 4–5 min during the total exercise time. Sprint interval training (SIT) will be referred to as “all out” maximal effort (greater than PVO_{2max}) if the duration is less than 1 min per sprint. Our nomenclature will follow the terminology used by MacInnis and Gibala in their review. The present paper aims to discuss the molecular adaptive response of HIIT and emphasizes the differences from moderate intensity exercise with long duration.

What is high intensity interval training?

Interval training can be defined simply as discontinuous, periodic (demanding) exercise loads separated by periods of recovery. However, the intensity level which defines an exercise as “high intensity” varies in different research publications. There is no accurate consensus threshold from which point the training load is categorized as different research publications. There is no accurate consensus threshold, the intensity level which de...
HIIT and MICT training impact on skeletal muscle oxidative capacity

The different adaptations to exercise stimuli of skeletal muscle fiber types may help to answer the question: why is HIIT exercise capable of increasing aerobic capacity in a relative short time? Performing physical exercise at high intensity activates type II fiber contraction, obeying the size principle. Data suggest that mitochondrial content of Type I fibers can be increased by low intensity exercise of long duration, while in case of Type II fibers, mitochondrial biogenesis is induced by high intensity exercise. Since VO$_{2\text{max}}$ is dependent on arterio-venous oxygen differences, which is influenced by mitochondrial content, it is easy to understand why HIIT-induced mitochondrial biogenesis is associated with enhanced VO$_{2\text{max}}$. In addition, the induction of HIF1 and VEGF have been reported, and this adaptive response may be also important to create greater arterio-venous oxygen differences and VO$_{2\text{max}}$. In contrast, after eight weeks of MICT a superior increase in capillary density was detected when compared to HIIT with similar total energy expenditure. Data with similar vascular adaptations is also known.

Fig. 1. The energy demand of exercise with different intensities. The suggested energy cost of MICT, HIIT and SIT. The discrepancies of the results in various studies are due to the different energy costs of exercise training. Matched energy or power cost are required to valid evaluation.
Calcium mediated regulation

Another important event in skeletal muscle functional activity is the muscle contraction-initiated calcium release from the sarcoplasmic reticulum by a ryanodine receptor-mediated mechanism. Inside skeletal muscle cells, calcium sensitive proteins evolve to mediate metabolic and electrophysiological pathways, during physical exercise. Place and colleagues showed that a 30s all-out cycling exercise (≤3 min total exercise time) with 4 min rest between bouts in recreationally active subjects caused the fragmentation of ryanodine receptor type 1 (RyR1) 24 h after the exercise stimuli. After SIT, only 15% of the RyR1 remained intact and ~375, 80, and 60 kDa fragments were detected. However, this response was not present in elite well trained athletes nor in athletes pretrained marathon race. The authors suggest the involvement of reactive oxygen species (ROS), since superoxide dismutase 2 (SOD2) and catalase (CAT) expression are at least two-fold higher in vastus lateralis muscle of elite athletes at baseline, indicating better antioxidant capacity. Endogenous antioxidants and Ca2+ handling are associated with enhanced mitochondrial function and recurrent high Ca2+, AMP and ROS levels during high intensity exercise may contribute to mitochondrial biogenesis by activating peroxisome proliferator-activated receptor α isoform in exercise dependent AMPK regulation. The available information indicates that AMPK isoform-specific activity may play an important role in HIIT-induced metabolic adaptation, however, the adaptive response to HIIT is very complex (Table 1).

Table 1

| Reference | Population characteristics (n, M/F, age, VO2max) | Training period | Exercise details | Main physiological and molecular changes |
|-----------|-----------------------------------------------|-----------------|-----------------|-----------------------------------------|
| 47        | 6, 6/0, 25 ± 2.9 yr, 55.5 ± 1.3 mL/kg/min     | Single bout, cycling | HIIT: ~40-45 min 2 min at 90% and 2 min at 25% VO2peak; MICT: ~60 min of at 50% VO2peak. | Postprandial fat oxidation was increased similarly in both exercise groups with a higher increase in HIIT. |
| 116       | 10, 10/0, 20 ± 1 yr., 52 ± 7 mL/kg/min        | Single bout, running | HIIT: 3 min bouts at 90% VO2max and 3 min at 50% VO2max; MICT: 50 min at 70% VO2max. | Heart rate, RPE, and blood lactate was significantly higher in HIIT compared with MICT. Phosphorylation of AMPK Thr172 and p38MAPK Thr180/Tyr182 increased after exercise with no difference between exercise protocols. Muscle (vastus lateralis) PGC-1α mRNA content increased after hrs. of exercise with no difference between protocols. Plasma norepinephrine and interleukin-6 increased similarly. Plasma insulin decreased during recovery in both HIIT and MICE. |
| 24        | 10, 10/0, 23.2 ± 6.7 yr., 4.8 ± 0.3 L/min (~61 mL/kg/min) | Single bout, cycling | HIIT: 10 × 4 min cycling at 81.6 ± 3.7% VO2max and 2 min with at 50 W (11.4 ± 0.6% peak power output); MICT: cycling at 65% VO2max for a time corresponds to total HIIT work. | HIIT was superior in improving norepinephrine, endothelin-1 (ET-1) and (nitrite/nitrate) NOx response to exercise than MICT. HIIT and MICT were similarly effective in improving ABP and insulin sensitivity. HIIT was superior in improving cardiovascular fitness. |
| 117       | 44,0/44, ~25 yr., ~29 mL/kg/min                | 3 times a week for 16 weeks, running/walking | HIIT: 40 min with 1 min at 80–90% VO2max and 2 min at 50–60% VO2max; MICT: 40 min at 60–70% VO2max. | In HIIT group there was a significantly greater improvement in vastus lateralis muscle buffer capacity (lim in vitro) than the MICT group. VO2peak increased in both group similarly. |
| 118       | 16, 0/16, HIIT: 20 ± 1 yr, MICT: 19 ± 1 yr, HIIT: 43.7 ± 6.8 mL/min/kg, MICT: 42.1 ± 7.2 mL/min/kg | 5 weeks 3 days per week, cycling | HIIT: 120% (week 1), 130% (weeks 2 and 3) and 140% (weeks 4 and 5) of the lactate threshold, 2 min duration, with 1 min recovery. MICT: 80% (week 1), 90% (weeks 2 and 3) and 95% (weeks 4 and 5) of the lactate threshold. Work matched to HIIT. | After HIIT and SIT VO2max and stroke volume increased significantly |
| 119       | 40, 40/0, 24.6 ± 3.8 yr, ~55–60 mL/kg/min     | 8 weeks 3 days per week, running | HIIT: 4 × 4 min at 90–95% HRmax with 3 min active recovery at 70% HRmax. SIT: 47 × 15 s at 90–95% HRmax with 15 s active recovery at 70% HRmax. MICT: 4 × 4 min at 85–90% HRpeak with 3 min active recovery at 65–75% HRpeak. MICT: 32 min at 65–75% HRpeak. | VO2peak, ejection fraction and insulin resistance (HOMA-IR) improved in HIIT. |
| 120       | 43, 17/26, 55.7 yr., ~23–25.9 mL/kg/min        | 8 weeks 4 days per week, all-extremity ergometer | HIIT: 4 × 4 min at 85–90% HRpeak with 3 min active recovery at 65–75% HRpeak. MICT: 32 min at 65–75% HRpeak. | VO2peak, ejection fraction and insulin resistance (HOMA-IR) improved in HIIT. |
| 121       | 10, 10/0, 23 ± 1 yr, 46 ± 2 mL/kg/min         | 2 week 6 session, One-leg cycling | HIIT: 4 × 5 min at 65% Wmax and 2.5 min recovery with 20% Wmax. MICT: 30 min with 50% Wmax | CS maximal activity and mass specific O2 flux oxidative phosphorylation capacities in HIIT vs. MICT. In whole muscle, the COXIV, NDUF49 and mitofustin 2 (MFN2) increased similarly in both groups. VO2max elevated only in the MICT group. SDH activity increased in both group, but no difference found in PK levels. VO2max increased in the 4HIIT group compared to the other two groups. Calculated stroke volume increased only in the 4HIIT group. Muscle CS activity and TTE (time to exhaustion) improved in all exercise group with a difference between 4HIIT and MICT in TTE. VO2max and maximal exercise ventilation (VEmax) increased in all protocol with a higher increase of VEmax in HIIT to other MICT groups. |
| 122       | 9,n.d., 20–28 yr., ~25.7–61.3 mL/kg/min        | 7–8 weeks 3 days/ week, cycling | HIIT: 5 × 4 min 2 min recovery, 101% of VO2max matched by W. MICT: average 27 min, 79% of VO2max | VO2max increased in the 4HIIT group compared to the other two groups. Calculated stroke volume increased only in the 4HIIT group. Muscle CS activity and TTE (time to exhaustion) improved in all exercise group with a difference between 4HIIT and MICT in TTE. VO2max and maximal exercise ventilation (VEmax) increased in all protocol with a higher increase of VEmax in HIIT to other MICT groups. |
| 123       | 26, 10/16, obese, 41 ± 9 yr, ~31–36.2 mL/kg/min | 18 session 3/ week, running | 4HIIT: 4 × 4 min at 85%–95% HRmax with 3 min recovery at 70% HRmax. 4HIIT: 10 × 1 min 90% HRmax with active recovery time n.d. MICT: 45 min at 70% HRmax. | Muscle SDH activity increased in both exercise groups. |
| 124       | 17, 17/0, ~24.6 ± 3 yr, 3046–3757 mL/min       | 8 weeks 3 day/ week, cycling | HIIT: 10 × 2 min at 105% with 2 min recovery. 1MCT: 55 min of continuous exercise at ~70%. VO2max: 2MCT: 35 min at 70% VO2max; | |
receptor-gamma coactivator (PGC)-α and the downstream nuclear respiratory factor 1–2 (NRF1–NRF2) systems. However, it is not clear why individuals with various levels of fitness respond differently to HIIT-like exercise stimuli. One possible explanation for this phenomenon is that training causes optimal biological responses at a distinct range of intensity for each person and, presumably, it follows a hormetic trajectory in groups with different training backgrounds.

Additional interesting finding is AMPK can be regulated by the Ca^2+ sensitive protein calcium/calmodulin-dependent kinase kinase β (CaMKKβ). Beside AMPK, PGC-1α gene expression is also subjected to Ca^2+ dependent signaling.

Mitochondrial biogenesis

It has been shown that AMPK can activate PGC-1α nuclear coactivator, which is referred to as the master regulator protein of mitochondrial biogenesis. PGC-1α plays an integrative role in governing mitochondrial biogenesis. In fact, it has a pivotal role in establishing the connection between cell energy sensing mechanisms and physiological signal conduction to nuclear transcription factors. The pinnacle aspect of PGC-1α related molecular pathways is its sensitivity to many intracellular stimuli and by co-activating NRF1, NRF2, PPARy, ERR, YY1, it regulates the adaptation of the cellular oxidative metabolism. However, in a rather provocative review Islake et al. questioned the dominance of PGC-1α in coordination with mitochondrial biogenesis. The authors main argument is that the sequential regulation of PGC-1α, NRF1/2, TFAM, mitochondrial proteins does not always follow this temporal pattern. They propose that in humans, it is unlikely to have only one “master regulator” and they point to the possible regulatory role of PPAR-β, p53 and LRPPRC or LRPL10. On the other hand, PGC-1α mRNA transcription increases with exercise intensity and the PGC-1α mRNA abundance is associated with the activation of upstream protein kinases. Only after high intensity exercise was induced did the downstream activating transcription factor-2 (ATF-2) phosphorylation increase.

Another level of complexity – which may contribute to the above-mentioned issue - comes from the promoter structure of the PGC-1α (PPARGC1A) gene. Alternative promoter usage coupled with alternative splicing gives rise to eight PGC-1α isoforms ranging from 257 to 797 amino acids in human samples. One prominent transcript from the PGC-1α gene alternative promoter is marked as PGC1α-b, also known as NT-PGC1α-b (Martinez-Redondo, Pettersson, & Ruas, 2015). This isoform, together with other NT (lacking the N-terminal RS and RR domains) transcripts, has been shown to be an inducible form. The recently mentioned issue - comes from the promoter structure of the PGC-1α gene. Alternative promoter usage coupled with alternative splicing gives rise to eight PGC-1α isoforms ranging from 257 to 797 amino acids in human samples. The promoter structure of the PGC-1α gene is complex and consists of multiple alternative promoters which can lead to the production of different isoforms. The alternative promoters are regulated by various transcription factors and can be activated under different physiological conditions. Therefore, the expression of PGC-1α is highly dependent on the promoter structure and may vary depending on the cell type and metabolic state.

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Oxidative stress and the antioxidant system

Exercise-derived oxidative challenge makes a major contribution to exercise adaptation and reactive oxygen and nitrogen species are important signaling molecules in skeletal muscle. The most prominent reactive oxygen species (and nitrogen) in skeletal muscle are nitric oxide (NO), superoxide anion, hypoxia inducible factors (HIIF), and uncoupled nitric oxide synthases (NOS). Mitochondrial respiratory enzymes are also subject to oxidative stress and the antioxidant system

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production in a rat model in permeabilized fibers of tibialis anterior and gastrocnemius but not in soleus muscle.\textsuperscript{100} We have reported a linear relationship between blood lactate levels and activity of XO after a single bout of exhausted running on the treadmill in a murine model.\textsuperscript{101} Indeed, it was also reported that high intensity exercise is associated with excess production of ROS.\textsuperscript{102} Conversely, in diseases where inactive lifestyle plays an important role, tissue specific and systemic ROS elevation is commonly detected.\textsuperscript{103,104} However, ROS production seems to be important in insulin regulated glucose uptake\textsuperscript{105,106} and GLUT4 translocation.\textsuperscript{107,108} In vitro studies found that H$_2$O$_2$ treatment increased PGC-1$\alpha$ promoter activity and mRNA expression in C2C12 cells.\textsuperscript{109} Interestingly, HIIT is found to positively regulate GLUT4 protein content.\textsuperscript{42,110} ROS are important players in exercise-associated adaptation,\textsuperscript{111} since they modulate the Ca channels and force generation,\textsuperscript{112} mitochondrial biogenesis,\textsuperscript{102} and housekeeping processes.\textsuperscript{113} Interestingly, the excess of ROS generation during exercise can be partly controlled by PGC-1$\alpha$, since activation of this co-activator activates the expression of antioxidant genes such as SOD2 and GPX.\textsuperscript{114} Indeed, HIIT (5 x 4-min 75% of Wmax), SIT (4 x 30 s all-out), MICT (30 min at 50% of Wmax) both high intensity exercise, increased peak plasma hydrogen peroxide values during exercise.\textsuperscript{115} But, significant elevation of peak catalase activity was measured only in the SIT group (with a main effect of time in catalase and SOD activity). It is important to note, that exercise-associated increases in ROS generation very rarely, if ever, can reach the level which could have a negative effect on health. Mounting data support the health promoting effects of regular exercise, regardless of the intensity of exercise.

**Conclusion**

HIIT-associated increases in cellular metabolism of skeletal muscle results in fiber specific responses, since Type II fibers with higher recuitral thresholds are fully active during HIIT. The HIIT-induced enhanced mitochondrial biogenesis is mediated by the AMPK, PGC-1$\alpha$, SIRT1 and ROS pathway as well as by the modulation of Ca$^{2+}$ homeostasis. The fact that the time saving HIIT associated adaptive response at least comparable or superior to MICT associated adaptation, guarantees further increases of HIIT in the preparation of elite sport and health promotion physical activities.

**Conflict of interest**

The authors have no conflict of interest to report.

**Submission statement**

The manuscript has not been published and is not under consideration for publication elsewhere.

**Each Author’s contributions**

FT, ZG, MJ, MT, TM and ZR contributed by searching and discussions on the relevant literature. ZR drafted the final version of the paper, but all authors were involved in the correction of the original paper. FT and ZG designed and drew the figures.
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