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Effect of High-Intensity Interval and Resistance Training on TNF-α Concentrations in Healthy Adolescent Males: A Randomized Controlled Trial

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ABSTRACT

Objective: This study aimed to investigate the effect of high-intensity regular exercise in reducing tumor necrosis factor-alpha (TNF-α) concentrations in adolescent males.

Methods and Materials: This randomized controlled study involved 33 adolescent males (mean age: 19.82 ± 1.79 years; BMI: 21.11 ± 1.17 kg/m²; VO_2max: 48.97 ± 4.97 mL/kg/min) with good fitness levels. Participants were randomly assigned into three groups: high-intensity resistance training (HIRT, n = 11), high-intensity interval training (HIIT, n = 11), and control (n = 11). Interventions were performed three times weekly for four weeks. Serum TNF-α concentrations were assessed pre- and post-intervention using ELISA. Data were analyzed using Wilcoxon Signed Ranks Test and Kruskal-Wallis Test (α = 0.05).

Findings: Both HIRT and HIIT groups showed statistically significant reductions in TNF-α concentrations: HIRT (mean reduction = 2.13 pg/mL, p = 0.003; effect size = 2.94), HIIT (mean reduction = 1.06 pg/mL, p = 0.003; effect size = 1.20). No significant change was observed in the control group (p > 0.05). Between-group differences were also significant (p < 0.05).

Conclusion: Both HIRT and HIIT reduced TNF-α concentrations in adolescent males, with HIIT producing greater relative improvements. Although causality cannot be firmly established due to the short duration, these findings suggest that high-intensity exercise may be a promising strategy to reduce inflammation and support metabolic health from adolescent males.

Keywords: High-intensity exercise, healthy lifestyle, inflammation, metabolic disease, sedentary lifestyle.

Introduction

Adolescence is a critical developmental phase marked by physical, social, and emotional changes, as well as being the second most significant period of brain development after infancy. This stage presents a key opportunity to establish habits that support long-term health and well-being (Cooper & Lhussier, 2025). Although often considered a generally healthy period, many non-communicable diseases (NCDs) that emerge in adulthood are rooted in risky behaviors formed during adolescence—such as smoking, poor dietary patterns, and low levels of physical activity (van Sluijs et al., 2021). Physical inactivity has become a global health concern, with approximately one-third of adults and four-fifths of adolescents (about 1.4 billion people) failing to meet recommended physical activity guidelines (Atakan et al., 2021). This situation is alarming, as many adolescents are entering adulthood with significant cardiovascular risk factors Scott et al., (2025), including elevated systemic inflammation, which may contribute to insulin resistance, hypertension, and other metabolic disorders (de F. Rocha et al., 2022). Mental health challenges are also on the rise, with data from the World Health Organization (WHO) reporting that one in seven (14%) children aged 10–19 experience mental health conditions (Organization, 2024). Given the growing complexity of adolescent health challenges, more effective approaches are needed to promote healthy living—one of which is structured physical activity that not only enhances physical fitness but also modulates the body's inflammatory response.

Tumor Necrosis Factor-alpha (TNF-α) is one of the primary pro-inflammatory cytokines involved in regulating immune responses (Jang et al., 2021). It is mainly produced by macrophages, T-cells, and adipose tissue, acting as a key mediator in both acute and chronic inflammation (Mehta et al., 2018). Although TNF-α plays a crucial role in the body's defense mechanisms, excessive or uncontrolled concentrations can lead to low-grade systemic inflammation, which has been linked to various metabolic disorders and chronic diseases, such as insulin resistance, obesity, and cardiovascular disease (Alfaddagh et al., 2020). In adolescents, elevated TNF-α levels can be influenced by several factors, including hormonal changes, increased adipose tissue growth, high-fat diets, and sedentary lifestyles(Stumper et al., 2020). Studies have shown that, unlike adolescents in high-income countries, those in low- and middle-income countries are still in the early stages of understanding the importance of physical activity, as reflected by lower levels of engagement in active behaviors (Li et al., 2024). Research on adult populations has also indicated that individuals with low physical activity tend to exhibit higher TNF-α concentrations, suggesting the presence of inflammation that could contribute to later metabolic complications (Sahabudhee et al., 2023). Furthermore, TNF-α is known to impair vascular endothelial function, increase oxidative stress, and trigger insulin resistance through the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, thereby elevating the risk of metabolic diseases from an early age (Jang et al., 2021). Therefore, effectively controlling TNF-α concentrations through targeted interventions is essential for maintaining immunological balance and metabolic health in adolescents.

Physical activity has been widely recognized as one of the most effective strategies to modulate TNF-α levels and control systemic inflammation Ghojazadeh et al., (2024), including among healthy adolescents. Two prominent exercise modalities extensively studied in relation to inflammatory and metabolic responses are High-Intensity Interval Training (HIIT) and High-Intensity Resistance Training (HIRT) (Nikseresht & Nikseresht, 2024; Soltani et al., 2023). HIIT, which involves short bursts of high-intensity exercise alternated with brief recovery periods, has been shown to significantly reduce TNF-α concentrations Ramadhansyah et al., (2025), through mechanisms such as improved antioxidant capacity, enhanced insulin sensitivity Avansar, (2017), reduced oxidative stress, and the inhibition of NF-κB pathway activation (Freitas et al., 2018). Meanwhile, resistance training also demonstrates potent anti-inflammatory effects, particularly by stimulating the release of anti-inflammatory myokines like interleukin-6 (IL-6), which suppress TNF-α production by macrophages and adipose tissue (Pinckard et al., 2019). However, while both exercise types have shown promising effects on inflammatory responses, their specific mechanisms differ, and there remains a lack of research focusing on healthy adolescent populations—who often present distinct inflammatory profiles compared to individuals

with metabolic conditions. This gap is important, as healthy adolescents may not display overt clinical signs of inflammation but may still experience low-grade systemic inflammation due to sedentary habits or poor nutrition.

This study aims to explore the effects of HIIT and HIRT on serum TNF-α concentrations in healthy adolescent males. The findings are expected to provide deeper scientific insights into the role of physical training in the early prevention of metabolic diseases and serve as a foundation for developing more targeted, exercise-based interventions for adolescent populations.

Methods and Materials

Research Design and Participants

This study employed a true experimental, pretest-posttest control group design. A total of 33 healthy male participants aged 18–23 years (mean: 19.82 ± 1.79 years)—classified as late adolescents or young adults based on WHO/CDC guidelines—were recruited. Eligibility criteria included: normal body mass index (21.11 ± 1.17 kg/m^2), normal blood pressure (systolic: 117.52 ± 3.75 mmHg; diastolic: 72.66 ± 4.93 mmHg), normal oxygen saturation (98.09 ± 0.98%), a "good" physical fitness level (VO_2max: 48.97 ± 4.97 mL/kg/min), and no history of chronic disease, alcohol consumption, or smoking in the past five years. These inclusion criteria were used to minimize confounding variables that might affect inflammatory marker levels.

Participants were randomly allocated using a simple randomization technique into three groups: control (n = 11), high-intensity resistance training (HIRT; n = 11), and high-intensity interval training (HIIT; n = 11). Randomization was performed using a computer-generated number sequence by an independent researcher. Allocation concealment and blinding were not implemented due to logistical constraints; however, participants were blinded to the study hypothesis. Outcome assessors were not blinded.

Before participation, all subjects received detailed information about the study's purpose, procedures, benefits, and potential risks. Written informed consent was obtained from each participant. This study was conducted under the Declaration of Helsinki and received ethical approval from the appropriate institutional review board.

High-Intensity Training Protocol

Intervention programs were conducted under the supervision of certified coaches from Olympus Training Surabaya, Indonesia. Both HIIT and HIRT protocols were implemented three times per week (Monday, Wednesday, Friday) for four consecutive weeks between 6:00–8:00 AM. Each session included a 5-minute warm-up and cool-down using dynamic and static stretching at low intensity.

The HIIT group performed running at 100–120% of Maximal Aerobic Speed (Freitas et al.) for 15–20 seconds, followed by 15 seconds of running at 70% MAS, repeated for 10 cycles per set, for 4 sets, with a 3-minute rest between sets. Exercise intensity was monitored using the Borg Rating of Perceived Exertion (RPE) scale (target RPE: 17–19).

The HIRT group performed bench press and leg extension exercises at 75–85% of one-repetition maximum (1RM), in 3–4 sets of 8–10 repetitions. Each repetition followed a 1:2 concentric–eccentric tempo. Active rest (light walking or low-intensity cycling) was applied between sets for 1–2 minutes. This protocol was designed to mimic high-intensity resistance while remaining within sustainable training volumes for the given intensity. Progressive overload was not applied due to the short study duration and the focus on immediate inflammatory response rather than long-term adaptation.

The control group received no exercise intervention and was instructed to maintain their usual lifestyle without altering physical activity or diet.

Blood Collection and Biochemical Analysis

Participants fasted overnight (~10 hours) before blood collection at baseline and post-intervention. Venous blood samples (4 mL) were drawn from the antecubital vein into plain vacutainer tubes, centrifuged at 3000 rpm for 15 minutes to separate serum, which was then transferred to Eppendorf tubes and immediately analyzed.

Serum TNF-α concentrations were measured using a commercial ELISA kit (BT-Lab BT-E0082Hu, Biossay Technology Laboratory, Shanghai, China) with a detection range of 3–900 ng/L and sensitivity of 1.52 ng/L. All samples were analyzed in duplicate, and average values were used. Intra-assay and inter-assay

coefficient of variation (CV) were maintained below 10% and 12%, respectively. Quality control samples were included in each run, and calibration was performed according to the manufacturer's instructions.

Statistical Analysis

Descriptive statistics were used to describe participant characteristics. Normality and homogeneity of data were assessed using the Shapiro-Wilk and Levene's tests, respectively. For normally distributed and homogeneous data, one-way ANOVA was performed. If data were non-normally distributed, non-parametric tests were used: Wilcoxon Signed Ranks Test for within-group comparisons, and Kruskal-Wallis followed by Mann-Whitney U test for between-group differences. Effect size (ES) assessment by using Cohen's d. All statistical analyses were conducted using SPSS version 20 (Chicago, IL, USA), with a significance level set at 5%. Graphical presentation using GraphPad Prism version 9.0.0 (GraphPad Software, San Diego, California USA).

Findings and Results

The results of the Normality and Homogeneity analysis show that all data has a normal and homogeneous distribution (all $p > 0.05$). No significant differences were observed among the groups at baseline in terms of resting heart rate (RHR), blood pressure (BP), oxygen saturation (SpO_2), body temperature (BT), maximal oxygen uptake (VO_2max), age, anthropometric variables, and body composition (all $p > 0.05$) (Table 1). Meanwhile, the assessment of TNF-α concentration pre- and post-exercise in each group are presented in Figure1.

Table 1

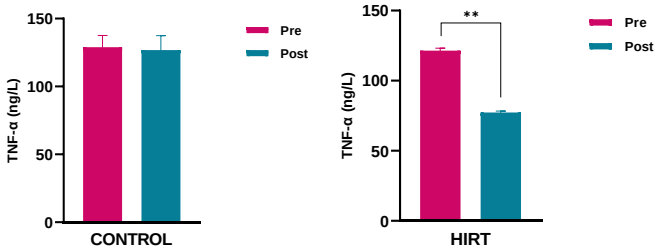
General characteristics of study participants

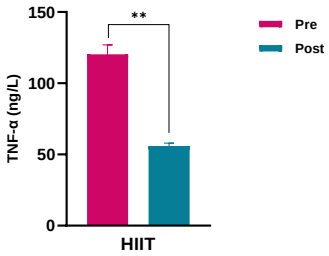
Parameters	CONTROL (n=11)	HIRT (n=11)	HIIT (n=11)	Normality			Homogeneity	p-value
RHR (bpm)	66.91 ± 6.68	62.73 ± 4.12	63.18 ± 5.74	0.116	0.199	0.121	0.790	0.177
SpO_2 (%)	98.27 ± 0.78	98.09 ± 1.04	97.91 ± 1.13	0.122	0.123	0.109	0.680	0.698
SBP (mmHg)	117.91 ± 2.02	116.46 ± 3.32	118.18 ± 5.28	0.107	0.521	0.823	0.230	0.524
DBP (mmHg)	73.55 ± 3.47	70.82 ± 5.91	73.64 ± 5.02	0.145	0.342	0.178	0.244	0.324
VO_2max (mL/kg/min)	49.02 ± 3.67	48.48 ± 3.42	49.39 ± 7.31	0.110	0.174	0.278	0.144	0.917
Age (yrs)	19.73 ± 1.79	19.82 ± 2.08	19.91 ± 1.64	0.241	0.111	0.188	0.332	0.974
Weight (kg)	60.91 ± 6.04	60.18 ± 4.72	60.36 ± 3.52	0.508	0.725	0.254	0.547	0.936
Height (m)	1.70 ± 0.06	1.69 ± 0.05	1.68 ± 0.03	0.175	0.328	0.165	0.132	0.964
BMI (kg/m²)	21.17 ± 1.41	20.99 ± 1.15	21.15 ± 1.01	0.318	0.205	0.100	0.354	0.930
BF (%)	15.36 ± 2.71	13.93 ± 1.94	16.14 ± 3.01	0.362	0.649	0.267	0.269	0.145
TBW (%)	55.48 ± 3.59	57.54 ± 2.71	56.89 ± 4.56	0.353	0.165	0.964	0.545	0.423
SM (kg)	48.93 ± 4.34	49.24 ± 5.72	50.55 ± 3.69	0.741	0.418	0.498	0.154	0.691

BF: Body fat; BMI: Body mass index; DBP: Diastolic blood pressure; SBP: Systolic blood pressure; SMM: Skeletal muscle mass; TBW: Total body water. Data presented as Mean ± Std. Deviation (SD). Normality and homogeneity of data were assessed using the Shapiro-Wilk and Levene's tests. p-value was obtained using one-way ANOVA.

Figure 1

*TNF-α concentration assessment at pre- and post-exercise in each group. **Significant difference at pre in HIT and HIIT ($p < 0.01$). Data presentation as Mean ± Std. Error (SE). All data are not normally distributed (all $p < 0.05$). The p-value was obtained using the Wilcoxon Signed Ranks Test.*

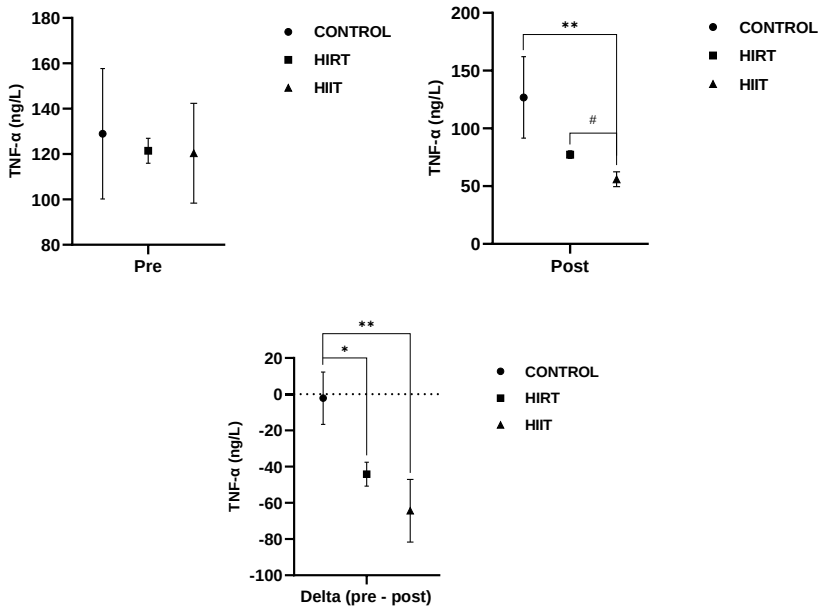




We observed a significant reduction in TNF-α concentrations between pre- and post-exercise in HIRT (121.47 ± 5.51 to 77.33 ± 3.28 ng/L; p=0.003; Effect Size [ES]: 2.937) and HIIT (120.34 ± 21.99 to 55.99 ± 6.37 ng/L; p=0.003; ES: 1.198), while in CONTROL no significant change in TNF-α concentration was observed (128.95 ± 28.76 to 126.78 ± 35.25 ng/L; p > 0.05). The results of the analysis of differences in TNF-α concentrations between groups are presented in Figure2.

Figure 2

*Assessment of TNF-α concentration in the three groups at each observation. *Significant difference at CONTROL (p < 0.05). **Significant difference at CONTROL (p < 0.01). #Significant difference at HIRT (p < 0.05). Data presentation with Mean ± Std. Error (SE). All data are not normally distributed and homogeneous (all p < 0.05). The p-value was obtained using the Kruskal-Wallis Test and continued with the Mann-Whitney Test.*



Discussion and Conclusion

This study examined the effects of High-Intensity Interval Training (HIIT) and High-Intensity Resistance Training (HIRT) on serum TNF-α concentrations in late adolescent males. Both interventions were associated with statistically significant reductions in TNF-α levels over four weeks, with the HIIT group showing a greater average reduction. Although this pattern suggests a more pronounced anti-inflammatory response to HIIT, caution is warranted in interpreting these findings due to the small sample size and short intervention duration.

The observed TNF-α reductions align with prior research indicating that high-intensity exercise can suppress pro-inflammatory signaling pathways Jang et al., (2021), potentially through modulation of oxidative stress and cytokine release (Jiménez-Maldonado et al., 2019; Pinckard et al., 2019). One hypothesized mechanism involves inhibition of the NF-κB pathway, a

key transcription factor in TNF-α regulation. Additionally, HIIT has been associated in the literature with increases in anti-inflammatory cytokines such as IL-10, while HIRT may stimulate IL-6 release from contracting muscles, leading to metabolic and immune adaptations (Cerqueira et al., 2020; Zunner et al., 2022). However, these mechanisms remain speculative in our context, as neither IL-6 nor IL-10 were measured in this study. Therefore, while biologically plausible, the proposed pathways cannot be confirmed based on our data.

Our findings add to a growing body of literature on the anti-inflammatory effects of high-intensity training, though most prior studies have focused on adult or clinical populations (Khalafi & Symonds, 2020; Liu et al., 2022). Adolescents may exhibit unique cytokine profiles due to hormonal fluctuations, growth processes, and baseline metabolic states, and thus require distinct consideration. It is also worth noting that conflicting evidence exists in the literature, with some studies reporting minimal cytokine response to short-term exercise interventions or inter-individual variability in inflammatory biomarkers among youth (Nara & Watanabe, 2021). This heterogeneity underscores the need for cautious interpretation of general trends, especially in populations without overt metabolic dysfunction.

While this study suggests that structured high-intensity exercise may contribute to inflammation control in healthy adolescents, the concept of "early prevention" of metabolic disease remains theoretical in the absence of long-term tracking. Future studies should incorporate longitudinal designs to examine whether exercise-induced reductions in TNF-α persist over time and translate into meaningful health outcomes. Comprehensive cytokine profiling, including markers such as IL-6, IL-10, and C-reactive protein (CRP), would also allow for a more mechanistic understanding.

This study is not without limitations. First, the four-week duration may have been insufficient to observe stable or lasting immunological adaptations. Second, the small sample size (n = 33) limited statistical power, and no power analysis was conducted. Third, the inclusion of only healthy male adolescents with relatively high fitness levels restricts generalizability to other populations. In addition, although intervention sessions were supervised and participants reported full attendance, no objective adherence tracking was conducted. There were no dropouts during the study period, which reduced attrition bias but also limited the evaluation of tolerability across more diverse adolescent profiles.

In summary, high-intensity training protocols—particularly HIIT—were associated with short-term reductions in serum TNF-α in a sample of healthy late-adolescent males. While promising, these findings should be interpreted with caution and validated through longer, more robust studies involving broader biomarker panels and diverse populations.

This study found that both high-intensity interval training (HIIT) and high-intensity resistance training (HIRT) significantly reduced serum TNF-α concentrations in healthy late-adolescent males over four weeks, with HIIT showing relatively greater effects. These findings support the potential of high-intensity exercise as a non-pharmacological strategy to modulate low-grade systemic inflammation during late adolescence. However, given the short intervention duration, small sample size, and lack of mechanistic biomarker data, these results should be interpreted with caution. Practical implementation of HIIT, such as short running intervals 2–3 times per week under supervision, may be feasible and safe within structured environments like schools or youth fitness programs, but further evaluation of adherence, safety, and long-term sustainability is needed. Future research should include larger, more diverse cohorts (including females), explore long-term inflammatory outcomes, and incorporate cytokine profiling (e.g., IL-6, IL-10, CRP) to clarify the underlying mechanisms driving exercise-induced changes in inflammation among youth.

Conflict of interest

The author declares that there is no conflict of interest in this study.

Acknowledgments

The authors express their gratitude and appreciation to all participants.

Declaration of Interest

The authors of this article declared no conflict of interest.

Ethical Considerations

The study protocol adhered to the principles outlined in the Helsinki Declaration, which provides guidelines for ethical research involving human participants. Ethical considerations in this study were that participation was entirely optional.

Transparency of Data

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

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Authors' Contributions

All authors equally contribute to this study.

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