

Comparing the immunological and physiological effects between highintensity interval training and moderate-intensity continuous training in physically inactive adults

Comparación de los efectos inmunológicos y fisiológicos entre el entrenamiento por intervalos de alta intensidad y el entrenamiento continuo de intensidad moderada en adultos físicamente inactivos

Authors

Muhammad Zuhaili Suhaimi 1.2 Ayu Suzailiana Muhamad ² Chen Chee Keong ² Ooi Foong Kiew ² Marilyn Li Yin Ong ² Rabiu Muazu Musa ¹

- ¹ Universiti Malaysia Terengganu (Malaysia)
- ²Universiti Sains Malaysia (Malaysia)

Corresponding author: Ayu Suzailiana Muhamad ayu_suzailiana@usm.my

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Abstract

Introduction: High-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) are effective exercise methods for enhancing fitness and health in healthy individuals. However, research on their impact on immune responses and bone metabolism in physically inactive adults is limited.

Objective: To investigate the effects of HIIT and MICT on immune responses, bone metabolism markers and selected physiological parameters in physically inactive adults.

Methodology: Thirty-six physically inactive adults aged 18 to 35 were randomly assigned to HIIT, MICT, or control groups (n=12 each). The HIIT group performed 2 sets of 6-8 repetitions of 30 seconds of running at 85-95% of estimated HRmax, three times a week. The MICT group ran for 30 minutes at 55-70% of estimated HRmax, three times a week, while the control group did not exercise. Maximum oxygen consumption (VO $_2$ max), blood, and saliva samples were collected before and after an 8-week intervention.

Results: Both HIIT and MICT significantly increased salivary lysozyme levels compared to baseline (p < 0.05), but no significant differences were found between groups (p > 0.05). Total leukocyte counts and bone metabolism markers showed no significant changes across groups. Maximum oxygen consumption improved only in the HIIT group (p < 0.05).

Discussion: The immune response's enhancement following HIIT and MICT may be attributed to hormonal changes resulting from the production of free radicals during strenuous exercise. Conclusions: Both HIIT and MICT enhance immune responses without affecting bone metabolism markers. HIIT appears to be more effective than MICT for improving VO_2 max in physically inactive adults.

Keywords

Bone health; cardiorespiratory fitness; exercise training; immune function.

Resumen

Introducción: El Entrenamiento Interválico de Alta Intensidad (HIIT) y el Entrenamiento Continuo de Intensidad Moderada (MICT) son métodos de ejercicio eficaces para mejorar la condición física y la salud en personas sanas. Sin embargo, la investigación sobre su impacto en las respuestas inmunológicas y el metabolismo óseo en adultos físicamente inactivos es limitada. Objetivo: Investigar los efectos del HIIT y el MICT sobre las respuestas inmunológicas, los marcadores del metabolismo óseo y ciertos parámetros fisiológicos en adultos físicamente inactivos

Metodología: Treinta y seis adultos físicamente inactivos, de entre 18 y 35 años, fueron asignados aleatoriamente a los grupos de HIIT, MICT o control (n=12 en cada grupo). El grupo HIIT realizó 2 series de 6 a 8 repeticiones de 30 segundos de carrera al 85-95% de la frecuencia cardíaca máxima (FCmáx), tres veces por semana. El grupo MICT corrió durante 30 minutos al 55-70% de su FCmáx, también tres veces por semana, mientras que el grupo control no realizó ejercicio. Se tomaron muestras de consumo máximo de oxígeno (VO2máx), sangre y saliva antes y después de una intervención de 8 semanas.

Resultados: Tanto el HIIT como el MICT aumentaron significativamente los niveles de lisozima salival en comparación con los valores iniciales (p < 0.05), pero no se encontraron diferencias significativas entre los grupos (p > 0.05). Los recuentos totales de leucocitos y los marcadores del metabolismo óseo no mostraron cambios significativos entre los grupos. El consumo máximo de oxígeno mejoró únicamente en el grupo HIIT (p < 0.05).

Discusión: La mejora de la respuesta inmune después del HIIT y el MICT puede atribuirse a cambios hormonales resultantes de la producción de radicales libres durante el ejercicio extenuante.

Conclusiones: Tanto el HIIT como el MICT mejoran las respuestas inmunológicas sin afectar los marcadores del metabolismo óseo. El HIIT parece ser más efectivo que el MICT para mejorar el VO₂máx en adultos físicamente inactivos.

Palabras clave

Salud ósea; aptitud cardiorrespiratoria; entrenamiento físico; función inmunológica.





Introduction

Physical inactivity is defined as a state that does not meet the minimum physical activity guidelines (Thivel et al., 2018). It is a significant concern globally, with detrimental effects on health. Research has highlighted its association with obesity and non-communicable diseases (NCDs), leading to millions of deaths annually (Amine, 2003). In 2023, the World Health Organization (WHO) reported that 41 million people died per year from NCDs, equivalent to 74% of all deaths globally (WHO, 2023). Global Health Observatory (GHO) data in 2016 showed that 28.0% of adults above 18 years globally and specifically 34.6% of Malaysian males were insufficiently physically active (WHO, 2016). In Malaysia, the recent survey by the National Institute of Health (NIH) (2024) showed that one in three adults in Malaysia are physically inactive (NIH, 2024). These figures reflect the disturbing fact that physical inactivity is one of Malaysia's health issues.

Regular participation in physical activity offers numerous health benefits. It enhances physical fitness, reducing vulnerability to chronic diseases like cardiovascular disease (Anderson & Durstine, 2019). A study by Marques et al. showed that physical activity is associated with a lower risk of chronic diseases such as heart problems, hypertension, type 2 diabetes, allergies, and obesity (Marques et al., 2018). The recent physical activity guideline for individuals 18-64 years of age is at least 150-300 minutes of moderate-intensity aerobic physical activity per week or at least 75-150 minutes of vigorous-intensity aerobic physical activity per week or an equivalent combination of moderate and vigorous intensity activity throughout the week, for substantial health benefits (WHO, 2020).

Maintaining physical fitness and overall health is crucial and various exercise programmes have been proposed to achieve this goal. Traditionally, aerobic or endurance training, often referred to as moderate-intensity interval training (MICT), has been the mainstay recommendation by health organizations (Riebe et al., 2018). MICT typically involves 30-60 minutes of sustained activity at moderate intensity (64-76% of peak heart rate) and has demonstrably improved cardiorespiratory fitness and other health-related markers (Abd El-Kader & Al-Shreef, 2018; Khammassi et al., 2020). However, recent years have witnessed a surge in the popularity of high-intensity interval training (HIIT) as a potential alternative for fitness and health improvement (Dolci et al., 2020; Martin-Smith et al., 2020). HIIT involves alternating short bursts of intense activity and brief recovery periods, typically lasting less than 30 minutes in total (Buchheit & Laursen, 2013).

HIIT has emerged as a promising exercise modality with benefits extending beyond the general population. Recent research suggests its effectiveness in improving health outcomes for individuals with chronic conditions and sedentary older adults. Studies like that by Sawyer et al. demonstrate that HIIT can significantly enhance cardiorespiratory fitness and exercise capacity in patients with Chronic Obstructive Pulmonary Disease (COPD), producing results comparable to traditional continuous exercise (Sawyer et al., 2020). Similarly, Putro et al. propose HIIT as a therapeutic approach for individual with type 2 diabetes, highlighting its potential to improve metabolic health (Putro et al., 2024). Furthermore, Cabrera Linares et al. found HIIT to be a safe and effective exercise prescription for overweight and obese preschool children, promoting cardiorespiratory fitness and metabolic capacity (Cabrera Linares et al., 2024). Collectively, these findings underscore the versatility and broad applicability of HIIT across diverse populations and age groups.

Moreover, in sports, HIIT has transcended its popularity in general fitness and is now recognised as a valuable tool for athlete seeking to enhance their performance (Dolci et al., 2020; García-Pinillos et al., 2017; Rafael et al., 2020). Research by Susiono et al. demonstrated that HIIT significantly boosted the aerobic performance of middle-distance runners (Susiono et al., 2025). Similarly, Rafael et al. found that HIIT led to improvements in both aerobic and anaerobic performances in amateur athletes (Rafael et al., 2020). Furthermore, Ní Chéilleachair et al. compared HIIT to traditional long slow distance training (LSD) in trained rowers (Ní Chéilleachair et al., 2017). Their study revealed that HIIT produced greater gains in aerobic power and endurance performance, suggesting its potential as a superior training method for athlete seeking to optimise their efficiency and power output.

As people are increasingly concerned with the health benefits of MICT and HIIT, numerous studies have investigated the comparative effects of these exercise modalities on various health components, including immune response, bone health, and other physiological parameters. For example, Khammassi et al. (2018) conducted a study examining the impacts of HIIT and MICT on aerobic capacity and immune

CALIDADA NIDVISTAS CENTÍFICAS ESIMACAS function in active young men. Their findings revealed no significant differences between the two groups in terms of maximal aerobic velocity and estimated VO_2 max. However, the MICT group demonstrated significantly greater improvements in immune parameters, including leukocyte, lymphocyte, neutrophil, and monocyte counts, compared to the HIIT group.

Regarding bone health, both HIIT and MICT have been shown to enhance bone metabolism, but HIIT often yields superior benefits when matched for workload. An 8-week intervention study by Lu et al. reported that participants engaging in HIIT experienced a notable 8.5% increase in total body bone mineral density (BMD), compared to a 5.5% increase in the MICT group. This result is supported by additional research demonstrating that HIIT not only enhances BMD but also positively influences bone turnover markers. For instance, levels of 1,25-dihydoxyvitamin D3 increased by 42.8% in the HIIT group, compared to a 24.9% increase in the MICT group, suggesting that the higher intensity of HIIT may provoke a more robust hormonal response beneficial to bone health (Lu et al., 2022).

Despite these promising findings, some studies have reported contradictory results, highlighting inconsistencies in the literature. These discrepancies underscore the need for further research to clarify the differential effects of HIIT and MICT on immune responses, bone health, and other physiological components. Therefore, the present study was conducted to investigate the effects of these two training methods on these aspects to determine their effectiveness in improving health outcomes among physically inactive adults.

Method

Study design

A randomized controlled trial was employed as the study design for this research endeavour. The participants were randomly assigned (age, gender and BMI-matched) into one of the three groups (HIIT, MICT, control) with 12 participants in each group (n=12). Participants in the HIIT group performed the HIIT protocol, while participants in the MICT group followed the MICT protocol, and those in the control group did not engage in any exercise training.

Pre- and post-assessments including anthropometric measurements, blood and salivary sample collection, and physiological parameters evaluations, were conducted both before and after an 8-week training intervention. The primary outcomes include the salivary lysozyme concentration, total leukocyte count, serum total calcium, and serum carboxyl terminal telopeptide of type I collagen (1 CTP), while the secondary outcome is the maximum oxygen consumption (VO_2 max). The research took place at the Exercise and Sports Science Laboratory, Universiti Sains Malaysia, Health Campus, Kelantan.

Participants

Thirty-six physically inactive adults were recruited from the students and staff population at Universiti Sains Malaysia, Health Campus. Recruitment efforts involved the dissemination of research information through posters. The inclusion criteria comprised adults aged between 18 and 35 years, categorised as physically inactive (category 1 assessed via International Physical Activity Questionnaire-Short form (IPAQ-SF)), not undergoing pharmacological treatments or supplement intake, answering 'NO' to all Physical Activity Readiness Questionnaire (PARQ+), and being in good health. Exclusion criteria included individual unable to run at the prescribed intensity during the familiarisation trial and those with body mass index (BMI) exceeding 30 (classified as obese).

Procedures

In initial meeting, participants were briefed on the experimental protocols' nature and associated risks. They completed the PARQ+ to assess their health status and the IPAQ-SF to gauge their physical activity level. Subsequently, anthropometric measurements including body weight, height, BMI, and BF% were taken and recorded. Participants meeting the inclusion criteria were chosen for the study and provided written consent before participation. The selected participants were then randomly allocated into three groups: control groups (C), moderate-intensity continuous training groups (MICT) and high-intensity interval training groups (HIIT). Matching was done based on gender, age, and BMI to ensure a balanced distribution across the groups.





Prior to commencing the training intervention, participants' saliva and blood samples were collected, and they underwent pre-test measurements, including VO_2 max tests. These evaluations were conducted under the supervision of proficient laboratory technologists at the Exercise and Sport Science Laboratory, Universiti Sains Malaysia. The data obtained were meticulously recorded in the data collection form.

Training program

The training program consisted of three phases: Phase 1 (Weeks 1 – 2), Phase 2 (Weeks 3 – 4) and Phase 3 (Weeks 5 – 8). The participants underwent progressive training sessions three times per week on alternate days for a total of 8 weeks on the treadmill in Exercise and Sports Science Laboratory, Universiti Sains Malaysia, Health Campus, Kelantan. The training sessions began with a 10-minute warm-up and static stretching, followed by treadmill running. At the end of each session, participants cooled down for about 10 minutes by running at low intensity and performed static stretching.

HIIT protocol

In Phase 1, the participants ran for 30 seconds at 85% of their estimated maximum heart rate (HRmax) which is equivalent to 81% of maximum aerobic speed (MAS) followed by active rest (50% of estimated HRmax) for 30 seconds. This was repeated 6 times for 2 sets. A rest interval of 4 minutes was given to the participants between sets. In Phase 2, the participants ran for 30 seconds at 90% of estimated HRmax, which is equivalent to 87% of MAS followed by active rest (50% of estimated HRmax) for 30 seconds. This was repeated 8 times for 2 sets. Finally, in Phase 3, the participants ran for 30 seconds at 95% of their estimated HRmax which is equivalent to 95% of MAS followed by active rest (50% of HRmax) for 30 seconds. This was repeated 8 times for 2 sets. The training protocol was adapted from the previous study by de Souza et al. and Racil et al. (de Souza et al., 2018; Racil et al., 2013). In this study, age-predicted maximum heart rate (220-age) was used for determining the running intensity of the participants. The running speed of the participants was determined by plotting a graph (the running speed against heart rate) of the submaximal test.

To ensure participants reached the target heart rate within the 30-second work interval, the running speed was not determined solely by heart rate but was instead predetermined based on their MAS obtained from prior fitness testing. Specifically, the running speed was set at 100-120% of MAS, which allowed participants to rapidly elevate their heart rate to the target zone within the short interval duration. During each high-intensity bout, participants' heart rate was continuously monitored using chest-strap heart rate monitors. Adjustment to the running speed were made if participants consistently failed to reach or overshot the target HR zone within the first few sessions.

MICT protocol

The participants performed 30 minutes of running at 55%, 60% and 70% of estimated HRmax in Phase 1, Phase 2 and Phase 3 respectively. The training session begun with 5 minutes of warm-up and 5 minutes of static stretching. At the end of the training session, participants performed cool down activities by running at low intensity for 5 minutes and perform 5 minutes of static stretching. The post-exercise heart rate of the participants was determined immediately after each running session. The training protocol was adapted from the previous study by Sahrir et al. (2017).

Submaximal and maximal oxygen consumption test

Following familiarisation with treadmill running, participants underwent sub-maximal tests to establish the relationship between running speed and heart rate and VO_2 max tests to determine their cardiorespiratory fitness. For the sub-maximal test, the participants engaged in a 16-minute incremental sub-maximal running test to assess the relationship between speed and heart rate. Before starting the sub-maximal test, a heart rate monitor sensor (Sport Tester PE3000, Polar, Finland) was attached to the chests of the participants to monitor their heart rates. Participants then ran on the motorised treadmill (Quinton 18-60, USA) in four stages with incremental test speed (5, 6, 7 and 8 km.h⁻¹) over the 16-minute duration. The running speed increased by 1 km.h⁻¹ at each 4-minute interval, and the average heart rate of the participants was recorded at the end of each increment.

In the VO_2 max test, a modified Astrand protocol was employed to determine the VO_2 max of the participants. Participants ran on a motorised treadmill continuously until reaching volitional exhaustion. Prior





to the test, participants engaged in 5 minutes of stretching and ran at a moderate speed (6 km.h⁻¹) for 5 minutes. Subsequently, they were equipped with headgear, a mouthpiece, and a nose clip in preparation for the test. The test commenced at an initial speed of 8-12 km.h⁻¹ and a 3.5% grade for 3 minutes. After that, the grade was increased 2.5% for every 2 minutes and the participants were encouraged to run until volitional exhaustion. Expired air was analysed every 2 minutes, while heart rate and ratings of perceived exertion were recorded at the of each 2-minute stage. Data collection continued until participants reached their VO_2 max and HRmax. VO_2 max was determined when there was a plateau in oxygen uptake (VO_2) despite escalating workload. Additional criteria indicating VO_2 max attainment included the failure of heart rate to increase with exercise intensity and a respiratory exchange ratio exceeding 1.15.

Data analysis

In the current study, statistical analysis was conducted using Statistical Package for Social Science (SPSS) version 28.0 and all data were reported as mean ± standard deviation (SD). Before performing parametric or non-parametric tests, all assumptions for ANOVA, including normality distribution, were verified. In this study, Mixed ANOVA with repeated measures was employed to determine the significance of differences within and between groups. The independent variables included one between-subject factor (3 groups: HIIT, MICT and C) and one within-subject factor (pre-test and post-test).

To test the null hypothesis of no difference in changeover time in response to the training intervention (main effect of time) and the null hypothesis of no difference in the changeover time between training groups (training intervention x time interaction), the Mixed ANOVA was utilised. Additionally, effect sizes were calculated using partial eta squared to enhance the interpretation of the results. Values of 0.01, 0.06 and > 0.15 were considered small, medium, and large, respectively. The level of statistical significance was set at p < 0.05.

Results

The mean age, body height, body weight, body mass index (BMI), and body fat percentage (BF%) of the participants in each group are detailed in Table 1. Descriptive statistics were employed to analyse all the data, which is presented as the mean \pm standard deviation (SD). There were no significant differences in physical characteristics between groups at the baseline. Following 8 weeks of the training intervention, there were no significant differences in any physical characteristics of each group from the baseline except the BF% in the HIIT group (p < 0.05).

Table 1. Physical characteristics of the participants (mean±SD)

Variables		C (n=12)	MICT (n=12)	HIIT (n=12)
Age (year)		23.1 ± 0.1	22.5 ± 0.1	23.2 ± 0.1
Body height (cm)		170.7 ± 0.1	168.4 ± 0.1	169.3 ± 0.1
Body weight (kg)	Pre-test	56.3 ± 12.4	54.5 ± 11.6	58.9 ± 9.8
	Post-test	56.1 ± 12.2	54.5 ± 10.8	59.2 ± 9.7
Body mass index (kg/m²)	Pre-test	22.0 ± 4.0	22.0 ± 3.6	23.1 ± 3.2
	Post-test	21.9 ± 3.9	22.1 ± 3.5	23.2 ± 3.3
Body fat percentage (%)	Pre-test	28.3 ± 6.5	25.8 ± 9.6	29.4 ± 8.9
	Post-test	28.1 ± 6.3	25.4 ± 8.4	31.4 ± 9.1+

Note: *significantly different from respective pre-test (p < 0.05); C, control group, MICT, moderate-intensity continuous training, HIIT, high-intensity interval training

Maximum oxygen consumption

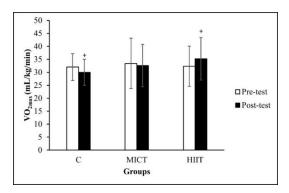
The mean maximum oxygen consumption (VO_2 max) of the participants during pre- and post-test is presented in Figure 1. A significant interaction between group and time was observed, with Wilks' Lambda = 0.785, F (2,33) = 4.514, p = 0.019, and a partial eta squared of 0.215. However, there was no significant main effect for time, as indicated by Wilks' Lambda = 1.000, F (1,33) = 0.003, p = 0.953, with a partial eta squared of 0.000. Further analysis was conducted to compare the differences between pre-value and post-value of the training intervention of each group. The result revealed a significant increase in





 VO_2 max among physically inactive adults in the HIIT group at the end of the training intervention compared to baseline (p < 0.05). In contrast, there was no significant difference in the VO_2 max between groups in the test of between-subjects effects, with F (2,33) = 0.464, p = 0.633, and a partial eta squared = 0.027. This finding indicates that there was no differential effect between groups on the mean VO_2 max of physically inactive adults.

Figure 1. Mean Maximum oxygen consumption (mean ± SD)

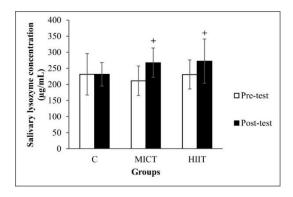


Note: \pm significantly different from respective pre-test (p < 0.05)

Salivary lysozyme concentration

The mean salivary lysozyme concentration of the participants during pre- and post-test are detailed in Figure 2. The analysis revealed no significant interaction between groups and time, with Wilks' Lambda = 0.854, F (2,33) = 10.537, p = 0.075, and a partial eta squared = 0.146. However, a significant main effect for time was observed, as indicated by Wilks' Lambda = 0.757, F (1,33) = 10.573, p = 0.003, with a partial eta squared = 0.243. Both groups exhibited an increase in mean salivary lysozyme concentration among physically inactive adults at end of the training intervention compared to the baseline levels. Conversely, in the test of between-subjects effect, no significant difference in mean salivary lysozyme concentration was found, F (2,33) = 0.678, p = 0.515, and a partial eta squared of 0.039. These results suggest that there was no differential effect between groups on the mean salivary lysozyme concentration of physically inactive adults.

Figure 2. Mean salivary lysozyme concentration (mean ± SD)



Note: \pm significantly different from respective pre-test (p < 0.05)

Total leukocyte count

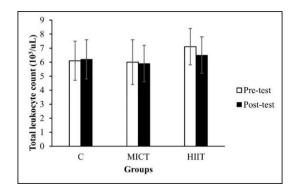
The mean total leukocyte count of the participants during pre- and post-test are detailed in Figure 3. The analysis revealed no significant interaction between group and time, with Wilks' Lambda = 0.911, F (2,33) = 1.614, p = 0.214, and a partial eta squared of 0.089. Similarly, there was no significant main effect for time, as indicated by Wilks' Lambda = 0.951, F (1,33) = 1.703, p = 0.201, with a partial eta





squared of 0.049. These findings suggest that all group interventions had no discernible effects on the mean total leukocyte count of physically inactive adults. Furthermore, in the test of between-subjects effects, no significant differences in mean total leukocyte count between groups were observed, with F (2,33) = 1.343, p = 0.275, and a partial eta squared = 0.075. This outcome indicates that there was no differential effect between groups on the mean total leukocyte count of physically inactive adults.

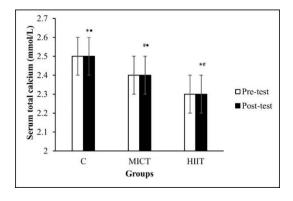
Figure 3. Mean total leukocyte count (mean ± SD)



Serum total calcium

The mean bone formation marker of serum total calcium concentration of the participants during preand post-test is presented in Figure 4. The analysis revealed no significant interaction between group and time, with Wilks' Lambda = 0.967, F (2,33) = 0.565, p = 0.574, and a partial eta squared of 0.053. Additionally, there was no significant main effect for time, as indicated by Wilks' Lambda = 0.947, F (1,33) = 1.837, p = 0.185, with a partial eta squared of 0.053. These results suggest that all group interventions had no discernible effects on the mean bone formation marker of serum total calcium concentration of physically inactive adults. However, there was a significant difference in the mean bone formation marker of serum total calcium concentration between groups in the test of between-subjects effects, with F (2,33) = 12.435, p = 0.000, and a substantial partial eta squared = 0.430. This finding indicates that there was a distinct effect between groups on the mean bone formation marker of serum total calcium concentration of physically inactive adults.

Figure 4. Mean serum total calcium concentration (mean \pm SD)



Note: *significantly different from MICT group (p < 0.05); *significantly different from control group (p < 0.05); *significantly different from HIIT group (p < 0.05)

Serum carboxy-terminal telopeptide of type 1 collagen concentration

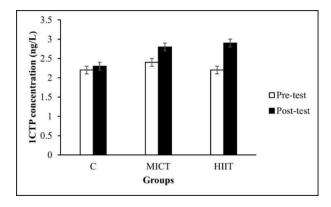
The mean bone formation marker of serum 1CTP concentration of the participants during pre- and posttest is presented in Figure 5. The statistical analysis revealed no significant interaction between group and time, with Wilks Lambda = 0.879, F (2,33) = 2.269, p = 0.119, and a partial eta squared of 0.121. Additionally, there was no significant main effect for time, as indicated by Wilks' Lambda = 1.000, F





(1,33) = 0.001, p = 0.977, with a partial eta squared = 0.000. These results suggest that all group interventions had no discernible effects on the bone formation marker of serum 1CTP concentration in physically inactive adults. Similarly, in the test of between-subjects effects, no significant differences in the mean bone formation marker of serum 1CTP concentration between groups were observed, with F (2,33) = 1.432, p = 0.253, and a partial eta squared of 0.080. This outcome indicates that there was no differential effect between groups on the mean bone formation marker of serum 1CTP concentration of physically inactive adults.

Figure 5. Mean carboxy-terminal telopeptide of type 1 collagen concentration (mean ± SD)



Note: carboxy-terminal telopeptide of type 1 collagen, 1CTP

Discussion

The primary focus of the present study was to investigate the impact of 8 weeks of HIIT and MICT interventions on cardiorespiratory fitness, immune response, and bone metabolism markers in physically inactive adults. Initial analysis revealed no significant differences in anthropometric measurements at baseline across the groups, indicating similar physical characteristics among all participants at the start of the intervention. In terms of cardiorespiratory fitness, the analysis revealed no significant main effect of time on the VO₂max of participants in any of the groups. However, a significant interaction between time and group was observed. Specifically, the VO₂max of participants in the HIIT group showed a significant increase at the end of the training intervention, while no significant changes were noted in the MICT and control groups. These findings are consistent with existing research that highlight the superiority of HIIT over MICT in enhancing participants' cardiorespiratory fitness (Marterer et al., 2020; Martin-Smith et al., 2020; Su et al., 2019). A comprehensive review study indicated that even a short duration of low-volume HIIT intervention, as brief as 4 weeks, can lead to improvements cardiorespiratory fitness (Sultana et al., 2019). Conversely, a separate study reported that while cardiorespiratory fitness did not change following 12-week HIIT intervention, participants were able to enhance their workload on the treadmill (Andersen et al., 2020). There could be multiple explanations for the absence of a noteworthy impact on VO₂max. Nonetheless, the workout regimen itself can be the most likely cause. It is plausible that insufficient adherence to the protocol by participants or inadequate total exercise volume (including factors such as frequency of sessions per week, number of intervals per session and exercise intensity) may have contributed to the absence of a notable effect on VO₂max.

In the current investigation, a significant main effect for time was observed, showing an increase in the mean salivary lysozyme concentration of physically inactive adults in both HIIT and MICT at the end of the training intervention compared to the baseline. This increase in mean salivary lysozyme concentration following exercise is a noteworthy finding that aligns with existing literature (Bartlett et al., 2020; Werner et al., 2019). For example, Bartlett et al. demonstrated in their study HIIT can enhance immune function to a similar degree as MICT (Bartlett et al., 2017). Their research revealed that ten weeks of low-volume HIIT yielded comparable improvements to MICT in enhancing neutrophil and monocyte bactericidal capacity in sedentary men and women. While the specific relationship between exercise and salivary lysozyme concentration may not have been directly addressed in the literature, the broader





impact of exercise on immune function supports the plausibility of the study's finding. Therefore, it is crucial to acknowledge the potential implications of these results. The increase in mean salivary lysozyme concentration observed in both exercise group may indicate a general enhancement of the participants' immune response, which could have been positive health implication. However, further research is needed to fully understand the underlying mechanism and long-term effects of these changes.

In another immune response marker, the results of the statistical analysis indicate that there were no significant interactions between group and time, as well as no significant main effect of time on total leukocyte count. This suggests that the group interventions had no significant effects on the mean total leukocyte count of physically inactive adults. Additionally, the absence of significant differences in the mean total leukocyte counts between groups further supports the conclusion that there was no distinct effect of the interventions on the mean total leukocyte count of physically inactive adults. These findings are in line with growing body of research that has examined the impact of group interventions on immune parameters. For instance, a study by Souza et al. found similar results when reviewed multiple studies and concluded that while group interventions had some positive chronic effects on immune functions, their impact on biological markers such as leukocyte count was not significant (Souza et al., 2021). Conversely, other studies conducted by Khammassi et al. observed increased in immune response markers (leukocyte, lymphocyte, neutrophil and monocytes) in the MICT group but not in the HIIT group (Khammassi et al., 2018). Similarly, other study discovered that HIIT supressed the immune system and raised the risk of infectious diseases (Nieman & Wentz, 2019). The main mechanism behind this phenomenon remains unclear, but several potential explanations may elucidate the changes of the immunological markers following HIIT. One of the possible mechanisms is the hormonal changes during and after strenuous exercise. Several studies found that a few hormones including stimulating hormones (cortisol, epinephrine, norepinephrine) increased following exhaustive exercise (Gleeson et al., 1995). A theoretical model of "J-shape" curve proposed by Nieman speculated that the intensity of exercise is associated with the risk of respiration infections. The occurrence of respiration infection was higher among people who involved with strenuous exercise. This may be due to an increment of free radicals (reactive oxygen species and reactive nitrogen species) productions during strenuous and exhaustive exercise (Nieman, 1994).

For the bone metabolism marker, there was no significant effect of time and no differences between groups on bone formation marker of serum total calcium of the participants. Serum total calcium of the participants did not alter in any of the groups following 8 weeks of intervention training. The current study's findings are consistent with a prior study in which the amount of calcium of active women did not alter after 8 weeks of intensive HIIT intervention (Abbasi et al., 2018). Conversely, a study by Jalalifarahani demonstrated that serum calcium of martial arts women was significantly increased following 6 weeks of high-intensity exercise training (Jalalifarahani, 2021). In addition, a recent study by Lu et al. discovered that sedentary young females who received 8 weeks of HIIT and MICT interventions had increases in various bone metabolism markers such as total bone mineral density and 1,25-dihydroxyvitamin D3 (Lu et al., 2022). However, a study conducted by Julian et al. discovered that HIIT was superior to MICT in enhancing multisite bone density after 16 weeks of training interventions (Julian et al., 2022). This indicated that HIIT is more osteogenic than MICT, which suggests that HIIT may have a positive effect on bone health, potentially affecting serum calcium level. These discrepancies highlight the complexity of interpreting research findings between HIIT versus MICT on bone metabolism markers. Several factors contribute to the inconsistent results such as study designs, variations in participant characteristics, training protocols and duration of intervention. The discrepancies highlight the need for further research employing standardised methodologies, diverse participant population and a wider range of bone metabolism markers.

The same pattern was observed in bone resorption marker, where there was no significant effect of time on serum 1CTP concentration. After 8 weeks of MICT and HIIT interventions, there was no discernible impact on the serum 1CTP concentration. Even though none of the studies directly evaluated the effects of HIIT on serum 1CTP concentration, the current findings are in line with a prior study conducted by Karabulut et al. which found that after 6 weeks of high-intensity resistance training, the level of the bone resorption marker, known as carboxyl-terminal cross-linking telopeptide of Type I collagen (CTX) level, did not alter from the baseline (Karabulut et al., 2011). Despite the growing body of research, studies on





comparing the effects of HIIT versus MICT on 1CTP concentration still limited. Therefore, further research is needed to determine the effects of HIIT versus MICT on serum 1CTP concentration and the underlying mechanisms.

The present study has several limitations that should be considered. Firstly, the short duration of the intervention, lasting only 8 weeks, may not be sufficient to observe long-term effects on immune responses, bone metabolism markers, and physiological parameters. Longer studies could provide insights into the sustainability of benefits and any delayed effects that may occur with extended training periods. Furthermore, the study only focuses on a specific age group (18 – 35 years) and physical activity level (physically inactive), hence limits its applicability to other age groups or individuals with different fitness levels or health conditions. These limitations highlight areas for further research to confirm findings and explore broader implications for exercise prescriptions in various populations.

Future research should prioritise longitudinal designs to evaluate the long-term effects of HIIT and MICT on various health parameters. These studies could provide critical insights into the sustainability of health benefits over time and the potential necessity for ongoing interventions. Additionally, expanding research to include diverse populations such as older adults, individuals with chronic diseases, and those from varied socio-economic background will enhance the generalisability of HIIT and MICT effects. Tailoring exercise prescriptions to meet specific health needs based on demographic factors could significantly improve outcomes, particularly for individuals with chronic conditions. Finally, future studies should examine a wider range of health outcomes, extending beyond traditional fitness parameters to include mental health indicators, quality of life assessment, and metabolic health markers like insulin sensitivity and lipid profiles. By addressing these areas in future research, we can gain a more comprehensive understanding of the benefits and limitations of HIIT and MICT, ultimately leading to more effective exercise prescriptions tailored to individual needs and enhancing public health outcomes across diverse populations.

Conclusions

The findings from this study demonstrate that both HIIT and MICT significantly enhanced immunological response, specifically affecting salivary lysozyme concentration, while not impacting total leukocyte count. Despite an 8-week intervention of HIIT and MICT, there were no notable alterations observed in bone formation or resorption markers such as serum total calcium and serum 1CTP concentration. Notably, HIIT exhibited superiority over MICT in enhancing cardiorespiratory fitness among physically inactive adults. These underscore the potential of tailored exercise programmes to elicit physiological responses, highlighting the efficacy of HIIT in improving key health parameters in this population.

The findings of this study hold significant practical implications for the population, particularly in the realm of tailored exercise programmes for physically inactive adults. By showing the effectiveness of both HIIT and MICT in enhancing immunological response and improving cardiorespiratory fitness, this research underscores the potential for targeted exercise interventions to positively impact health outcomes. Specifically, the superiority of HIIT over MICT in certain aspects highlights the importance of selecting appropriate exercise modalities based on individual needs and goals. These results can guide healthcare professionals, fitness trainers, and individual seeking to improve their health through structured exercise programmes.

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Authors and translators' details:

Muhammad Zuhaili Suhaimi m.zuhaili@umt.edu.my Author Ayu Suzailiana Muhamad ayu_suzailiana@usm.my Author Chen Chee Keong ckchen@usm.my Author Ooi Foong Kiew fkooi@usm.my Author Marilyn Li Yin Ong marilynong@usm.my Author Rabiu Muazu Musa rabiu.muazu@umt.edu.my Author Muhammad Zulhusni Suhaimi zulhusni@utar.edu.my Translator



