

## Short Term HIIT increase VO<sub>2</sub>max, but can't decrease Free Fatty Acids in Women Sedentary Lifestyle El HIIT a corto plazo aumenta el VO<sub>2</sub>máx, pero no puede disminuir los ácidos grasos libres en mujeres con estilo de vida

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**Abstract** Previous studies have investigated the effects of high-intensity interval training (HIIT) on cardiorespiratory fitness and body composition in overweight populations. However, short-term effect of HIIT and free fatty acids (FFA) serum in women sedentary lifestyle is not clearly understood. The aim of this study was to investigate the effect of 2-week high intensity interval training on body fat percentage, free fatty acids, and VO<sub>2</sub>max in overweight sedentary adult women. The fourteen sedentary young women (18–30 years old) randomly divided to control and HIIT groups. The HIIT group performed two-weeks training program, 20 min of repeated 10 s cycling interspersed with 50 s rest intervals per session, four days in a week. The variables were taken before and after intervention. The result showed the VO<sub>2</sub>max improved significantly ( $p < 0.05$ ) and the change of body fat percentage was decreased significantly ( $p < 0.05$ ) in HIIT compared to control. The FFA levels were raised in HIIT and reduced in control, but they were not significantly different between groups ( $p \geq 0.05$ ). The conclusion of this study reveals that the HIIT can be a reliable strategy for countering obesity in the adult population for improving fitness level and lessen body fat percentage. It may cause by increase energy metabolism. However, its underlying mechanism seem not via FFA. Then the further study needs to be conducted to figure out the other metabolic substances in human body.

**Keywords:** HIIT; VO<sub>2</sub>max; Free Fatty Acids; SGDs; Healthy Lifestyle

**Resumen** Estudios previos han investigado los efectos del entrenamiento interválico de alta intensidad (HIIT) sobre la aptitud cardiorespiratoria y la composición corporal en poblaciones con sobrepeso. Sin embargo, el efecto a corto plazo del HIIT y el suero de ácidos grasos libres (FFA) en ayunas en mujeres con estilo de vida sedentario no está claro. El objetivo de este estudio fue investigar el efecto del entrenamiento interválico de alta intensidad de 2 semanas sobre el porcentaje de grasa corporal, los ácidos grasos libres y el VO<sub>2</sub>máx en mujeres adultas sedentarias con sobrepeso. Las catorce mujeres jóvenes sedentarias (de 18 a 30 años) se dividieron aleatoriamente en grupos de control y HIIT. El grupo HIIT realizó un programa de entrenamiento de dos semanas, 20 min de ciclismo repetido de 10 s intercalados con intervalos de descanso de 50 s por sesión, cuatro días a la semana. Las variables se tomaron antes y después de la intervención. El resultado mostró que el VO<sub>2</sub>max mejoró significativamente ( $p < 0,05$ ) y el cambio en el porcentaje de grasa corporal disminuyó significativamente ( $p < 0,05$ ) en HIIT en comparación con el control. Los niveles de FFA aumentaron en HIIT y se redujeron en el control, pero no fueron significativamente diferentes entre los grupos ( $p \geq 0.05$ ). La conclusión de este estudio revela que el HIIT puede ser una estrategia confiable para contrarrestar la obesidad en la población adulta para mejorar el nivel de condición física y disminuir el porcentaje de grasa corporal. Puede causar por aumento del metabolismo energético. Sin embargo, su mecanismo subyacente no parece a través de FFA. Luego, se debe realizar un estudio adicional para descubrir las otras sustancias metabólicas en el cuerpo humano.

**Palabras clave:** HIIT; VO<sub>2</sub>máx; ácidos grasos libres; SGD; Estilo de vida saludable

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### Introduction

Physical inactivity is a contributor to multiple pathological conditions and has recently been highlighted as a leading cause of death in the world (Åsvold et al. 2017; Phillips et al. 2016; Yosnengsih et al. 2023). Among the chronic diseases most affected by physical inactivity are cardiovascular and metabolic diseases. Indeed, prior evidence indicates that physical inactivity contributes to multiple features of metabolic dysfunction including large postprandial glucose excursions and hyperinsulinemia, which have been directly linked to alterations in lipid metabolism, increased oxidative stress, and long-term micro- and macrovascular complications (Wen et al. 2019; Winn et al. 2019).

According to WHO, 60 to 85% of people in the world—from both developed and developing countries—lead sedentary lifestyles, making it one of the more serious

yet insufficiently addressed public health problems of our time (WHO. 2002), meta-analysis research says that the greatest sedentary time compared with the lowest was associated with a 112% increase in the respiratory rate of diabetes, a 147% increase in the respiratory rate of cardiovascular events, a 90% increase in the risk of cardiovascular mortality and a 49% increase in the risk of all-cause mortality (Biswas et al. 2015; Warren et al. 2010; Wilmot et al. 2012).

It has been generally acknowledged that the concentration of circulating free fatty acids (FFA), often also referred to as non-esterified fatty acids, is increased in obesity and that this constitutes an important causal factor for the association between obesity and type 2 diabetes.

Free fatty acids which are the same as non-esterified fatty acids have a higher tendency in sedentary lifestyle people compared to normal people in general (Karpe,

Dickmann, and Frayn 2011; Pate, O'Neill, and Lobelo 2008). Generally, overweight patients have free fatty acid levels 26% higher than normal BMI in general, this high FFA is the cause of insulin resistance (Arner and Rydén 2015; Boden 2011).

Regular physical activity is good for heart, body and mind. It is a key component of national action on the prevention and management of noncommunicable diseases, including heart disease, stroke, type-2 diabetes and some cancers (Gavin et al. 2019; Ramos et al. 2015). It helps in the prevention and management of hypertension and the maintenance of a healthy weight, and can improve mental health, cognitive function and overall well-being (Kipping et al. 2014; Solomon et al. 2009; Syamsudin, Wungu, et al. 2021).

Exercise is the solution to these problems, HIIT is a high-intensity exercise with pauses between each attack of the movement (Kriel, Askew, and Solomon 2019; Syamsudin, Syaifullah, et al. 2021; Vella, Taylor, and Drummer 2017). HIIT does not take a long time, has the same and even greater benefits than sustainable types of exercise as recommended by WHO in general (De Nardi et al. 2018; Reljic, Wittmann, and Fischer 2018; Syamsudin, Wungu, et al. 2021).

Previous research (Syamsudin, Herawati, et al. 2021) has shown that HIIT can increase VO<sub>2</sub>Max in sedentary lifestyle women with various training durations and tempos, (Lanzi et al. 2015) has proven that exercise Gfatmax and Ghiit in obese men > 30 can reduce NEFA levels and reduce insulin resistance. Research (Fisher et al. 2015) has shown that HIIT and MICT in obese young men > 29 for 6 weeks can increase insulin sensitivity, reduce blood fat, and reduce body fat percentage. Research (Madsen et al. 2015) shows that hiit for 8 weeks in subjects with type 2 diabetes can reduce high levels of FFA. However, HIIT research on overweight female subjects who have a sedentary lifestyle has never been conducted.

## Materials and Methods

### Study Design

This Study used cross-sectional experimental research with randomized pre- and post-test control group design. There were 2 groups in this study: the control group (without intervention) and the experimental group (with HIIT).

This article was a randomization control trial that compared the pretest and posttest in the HIIT experimental group with the control group, their effect on VO<sub>2</sub>max, FFA, Body Fat %, Fat Mass, and Visceral Fat.

### HIIT protocol

The exercise program given is based on previous research on sedentary subjects by Alansare (Alansare et al. 2018), exercise was carried out consistently for 20 repetitions, each repetition pedaling a cycle ergometer (Monark 894 E, Sweden) with a ratio of 10: 50 seconds,

active 10 seconds at a speed of 100 rpm with a maximum effort of up to intensity 90% HRM (heart rate maximum) and 50 seconds passive at 50 rpm with 55-65% HRM. Subjects wore a heart rate monitor (Model T 34, Polar Electro Oy, Finland), to monitor HR during training sessions. The duration of the training was 2 weeks with 8 meetings.

### Free Fatty Acids

The subjects fasted at night at 21.00, then blood was taken in the morning with 12 hours of fasting. One day before the blood was drawn, they ordered not drink alcohol and caffeinated drinks. Before taking blood, the subject sat and rested for 30 minutes, as much as 4mL of blood was taken from the brachial vein. After the blood was taken, the blood was centrifuged for 15 minutes at a speed of 2500 rpm to collect blood serum. Then, it was analyzed using the ELISA KIT method (Cat.NO E2013Hu, Bioassay Technology Laboratory, Shanghai, China).

### Body Composition Analysis

The body fat percentage, fat mass and visceral fat were assessed using the scanning clients' body method using Dual Frequency Bioelectrical Impedance Analysis (BIA), standing on the weighing board for 15 seconds to determine the body composition analyzer (TANITA DC-360 P, Ireland).

### Ethics

This research had accepted the ethical feasibility from the Health Research Ethics Committee, Faculty of Medicine, Airlangga University, Surabaya, Indonesia with approval number 227/EC/KEPK/FKUA/2020. This research was conducted during the Covid-19 pandemic, although we implemented strict protocols to avoid transmission of Covid-19.

### Participants

Subjects were young adult women 18-30 years old with a sedentary category determined based on IPAQ (Innerd, Harrison, and Coulson 2018) and PAR-Q, declared healthy, not following a regular physical exercise program, normal BMI - obese 2, not pregnant and not taking medication within 24 hours the last day of the blood draw. Recruitment using electronic brochures distributed on social media, 55 subjects enrolled in our study. A total of 14 subjects were assigned to groups that were randomly assigned using a randomized subject application from a computer. Participants were grouped into 2 groups, the HIIT experimental group, and the control group.

### Statistical Analysis

Statistical analysis was carried out using SPSS software (Version 20.0, IBM, New York, USA). All variables were checked their normal distribution by the Kolmogorov-Smirnov test. Independent sample t-tests were performed to determine the differences in training parameters between the two groups.

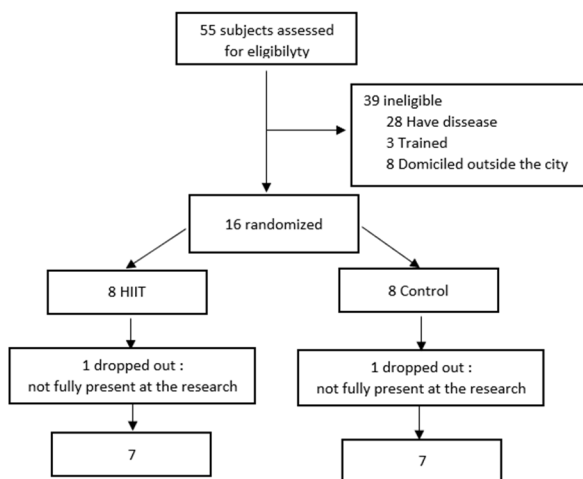


Figure 1. The scheme of selecting subjects for analysis.

## Result

The data were presented as mean ± SE for all variables (age, height, weight, BMI, VO2max, and body composition).

Table 1.

Comparison of criteria between groups before being given training

Variable	HIIT (n = 7)	Control (n = 7)	p-Value
	Mean ± SE	Mean ± SE	
Age (years)	23.0 ± 0.7	21.5 ± 0.8	0.136
Height (cm)	156.2 ± 2.2	151.5 ± 3.1	0.249
Weight (kg)	60.7 ± 4.6	51.9 ± 2.6	0.225
BMI (kg · m <sup>-2</sup> )	24.9 ± 1.6	22.9 ± 1.3	0.406
VO2max (mL · kg <sup>-1</sup> · min <sup>-1</sup> )	27.7 ± 3.9	26.5 ± 1.8	0.277
Body Composition			
% Body fat	39.8 ± 2.1	35.3 ± 1.7	0.064
Fat Mass (kg)	23.4 ± 3.1	18.6 ± 1.6	0.406
Visceral Fat (kg)	5.7 ± 0.8	4.2 ± 0.7	0.300

HIIT, High Intensity Interval Training; BMI, Body Mass Index. No difference ( $p \geq 0.05$ ) between groups

The average values for all groups showed in table 1. The baseline characteristics between groups were not significant in both groups ( $p \geq 0.05$ ). Therefore, the subjects were in the similar condition before treatment.

Table 2.

The average data between pre & post

Variable	Pre test	Post test	P (between pre & post)	P (between groups post intervention)
	Mean ± SE	Mean ± SE		
VO2max (mL · kg <sup>-1</sup> · min <sup>-1</sup> )				
HIIT	27.7 ± 3.9	30.6 ± 3.5	0.000 *	0.011 *
Control	26.5 ± 1.8	25.7 ± 2.4	0.168	
Free Fatty Acids				
HIIT	161.7 ± 22.7	181.3 ± 31.7	0.357	0.506
Control	203.0 ± 78.2	201.7 ± 71.7	0.945	
Body fat (%)				
HIIT	39.8 ± 5.5	39.5 ± 5.5	0.191	0.253
Control	35.3 ± 4.7	36.2 ± 4.4	0.122	
Fat Mass				
HIIT	23.4 ± 8.3	23.0 ± 8.4	0.295	0.235
Control	18.6 ± 4.2	18.6 ± 4.0	0.955	
Visceral Fat				
HIIT	5.7 ± 2.2	5.5 ± 2.3	0.356	0.174
Control	4.2 ± 1.9	4.0 ± 1.6	0.356	

Note : Paired T test and Independent T test, followed by VO2max, Free Fatty Acids, Body Fat Percentage, Fat Mass and Visceral Fat. The data were presented as Mean ± SE. Pre = before intervention; Post = after intervention; SE = Standard Error; HIIT = High Intensity Interval Training. Insignificant ( $p > 0.05$ ) between groups (Independent T Test) and between pre & post in the same group (paired T test). VO2max was a significant increase in the HIIT group  $p > 0.005$ , but not in the Control group  $p < 0.168$

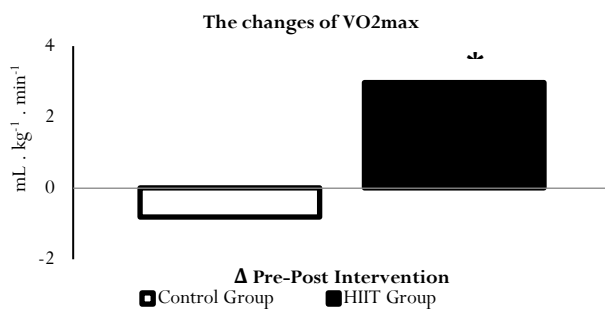


Figure 2. presentase perubahan VO2max pada HIIT dan Control \*sig. difference ( $p < 0.05$ ) compared to the control group (independent T-test). Based on the figure 2, there was a significant increase in VO2max in the HIIT group, and in the Control group it tended to decrease.

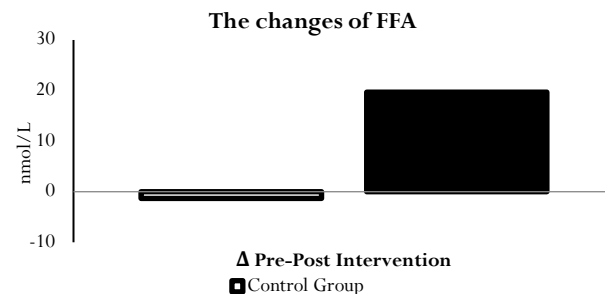


Figure 3. presentase perubahan FFA pada HIIT dan Control No. sig. difference ( $p \geq 0.05$ ) compared to the control group (independent T-test). Based on the figure 3, there was an increase in FFA in the HIIT group, and in the Control group it tended to decrease and both showed no significance.

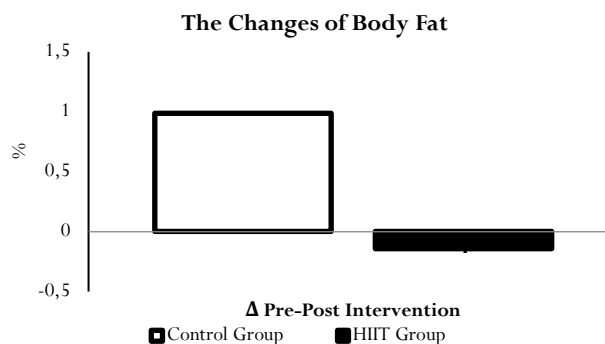


Figure 4. presentase perubahan body fat pada HIIT dan Control \*sig. difference ( $p < 0.05$ ) compared to the control group (independent T-test). Based on the figure 4, there is a significant decrease in body fat percentage in the HIIT group, and in the Control group it tends to increase but is not significant.

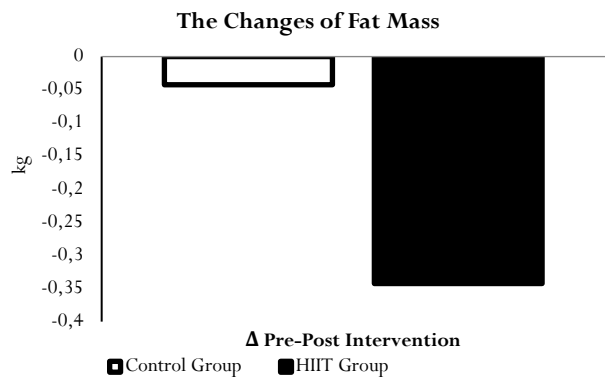


Figure 5. presentase perubahan fat mass pada HIIT dan Control No. sig. difference ( $p \geq 0.05$ ) compared to the control group (independent T-test). Based on the picture 5, there is a decrease in Fat Mass in the HIIT group, and in the Control group it tends to decrease but not as much as HIIT.

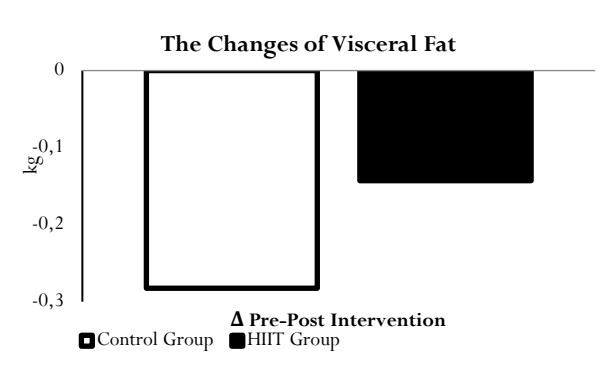


Figure 6. presentase perubahan fat mass pada HIIT dan Control  
No. sig. difference ( $p \geq 0.05$ ) compared to the control group (independent T-test).  
Based on the figure 6, there was a decrease in both groups, but more in the control group.

## Discussions

We investigated the effects of two different group exercise modalities on  $VO_{2max}$  and FFA metabolism in sedentary, overweight women. The first group was given HIIT and the other group was Control (without any intervention), so it was useful to see if there really was a difference between the experimental group with short-term HIIT and the control group.

This study aimed to examine the effects of HIIT on other markers of physical fitness and health ( $VO_{2max}$ , body composition parameter) in young women with a sedentary lifestyle, but there was no significant change in FFA, Body Fat%, Visceral Fat, and Fat Mass.

We had hypothesized that  $VO_{2max}$  would be increased and FFA plasma would be decreased and that would be reduced after an exercise bout. The 2-week HIIT intervention led to a significant increase in  $VO_{2max}$ . This is the first study to examine the effect of short-term HIIT on FFA. This work is in line with the recommendations of physical activity recommended by WHO, as much as 75-150 minutes of high-intensity physical activity. Because this training is only 80 minutes done in one week.

### Change in $VO_{2max}$

$VO_{2max}$  is known to fall into the low category for sedentary lifestyle subjects, and low  $VO_{2max}$  is closely related to the risk of cardiovascular disease (Fernström et al. 2017; Su et al. 2019; Syamsudin, Wungu, et al. 2021). In this study, HIIT significantly increased  $VO_{2max}$  by 1.45% ( $p > 0.005$ ), but not in the control group ( $p < 0.168$ ). These findings suggest that HIIT may be equally effective in increasing maximum aerobic capacity in sedentary lifestyle women. In line with previous research (Arboleda Serna et al. 2016; Kong et al. 2016) where a similar increase was observed in  $VO_{2max}$  in the HIIT group, after 4 and 5 weeks of training in both men and women. In line with research from (Cocks et al. 2013) that SIT increased  $VO_{2max}$  after 6 weeks of training in sedentary men. Research systematic review and meta-analysis also states that each HIIT can increase  $VO_{2max}$  (Batacan et al. 2017; Engel et al. 2018; Milanović, Sporiš, and Weston 2015; Wen et al. 2019).

This increase in  $VO_{2max}$  increases due to two factors, the first is the central factor, which states that maximal

stroke volume and maximal peak  $O_2$  respond to an increase in  $VO_{2max}$  (Naranjo Orellana and Muela Galán 2021; Oliveira et al. 2009), the second is due to peripheral mechanisms, which show increased mitochondrial enzyme activity, skeletal muscle oxidative capacity, and other metabolic adaptations (Ruffino et al. 2017; Zhidan Wu; Pere et al. 1999). The meta-analysis conducted by (Costigan et al. 2015) concluded clearly that HIIT is a feasible and time-efficient approach for improving cardiorespiratory fitness or included  $VO_{2max}$  and body composition in adolescent populations.

### Free Fatty Acids

This study showed that there was no significant difference in the FFA of the HIIT and control groups, in fact the HIIT group tended to increase slightly. However, the increase in fasting FFA is unexpected, it seems that the increase in FFA is due to eating not being controlled directly, there is a possibility that the subject has a diet that increases fat intake. The HIIT we did hoping for a reduction in FFA regardless of the caloric deficit is the wrong thing to do (As'ad, Liben, and Herawati 2021; Bagheri et al. 2020; Lutfi, Herawati, and Sari 2021; Viana et al. 2019).

The results that do not match this hypothesis may also be due to the short-term training provided, many studies say that HIIT can reduce FFA, but it is at training 8 weeks and over (Astorino et al. 2013). Food also has a big role in FFA levels, and from us researchers did not give rules on the number of calories that should be eaten or calorie restrictions that should be, this is the researchers' evaluation that calorie deficit is very important in FFA reduction programs (Lutfi et al. 2021).

In a meta-analysis study carried out by (Batacan et al. 2017) indeed could not change the body composition of the subjects if only given the intervention in a relatively short time, recent research, in a more in-depth discussion, FFA is not always the main cause of insulin resistance. This is considered too to be a simple story, while insulin resistance is caused by several factors (Karpe et al. 2011; Sahlin et al. 2010).

### Fat metabolism during exercise

Fat is an extremely important substrate for muscle contraction, both at rest and during exercise. Triglycerides (TGs), stored in adipose tissue and within muscle fibres, are considered to be the main source of the free fatty acids (FFAs) oxidised during exercise. It is still unclear, however, how the use of these substrates is regulated during exercise (Ranallo and Rhodes 1998).

It is known for a long time that fatty acids are an important fuel for contracting muscle. After lipolysis, fatty acids from adipose tissue have to be transported through the blood to the muscle. Fatty acids derived from circulating TG may also be used as a fuel but are believed to be less important during exercise. In the muscle the IMTG stores may also provide fatty acids for oxidation after stimulation of hormone sensitive lipase. In the muscle cell, fatty acids

will be transported by carrier proteins (FABP), and after activation, fatty acyl CoA have to cross the mitochondrial membrane through the carnitine palmytoyl transferase system, after which the acyl CoA will be degraded to acetyl CoA for oxidation. The two steps that are most likely to limit fat oxidation are fatty acid mobilization from adipose tissue and transport of fatty acids into the mitochondria along with mitochondrial density and the muscles capacity to oxidize fatty acids (Bennàsser Torrandell and Vidal Conti 2021; Jeukendrup, Saris, and Wagenmakers 1998).

Fat and carbohydrate are the principal substrates that fuel aerobic ATP synthesis in skeletal muscle. Endogenous carbohydrates, mainly stored as muscle and liver glycogen, represent less than 5 % of total energy storage in an average man. The vast majority of our energy reserves is stored as fat, mainly deposited as triacylglycerol (TG) in subcutaneous and deep visceral adipose tissue (Van Loon et al. 2003). Regardless of training status, intramuscular triglyceride use accounted for about 90% of the oxidized fatty acids that were not supplied from adipose tissue via the plasma. Intramuscular triglycerides were the source of virtually all of the additional fatty acids oxidized in the trained state. Both before and after physical conditioning they explained the discrepancy between the rates of plasma FFA and total fat oxidation during moderate-intensity exercise of up to 2 hr in duration (Van Loon et al. 2003).

## Conclusions

Based on the results of the study, it can be concluded that HIIT for 2 weeks increased VO<sub>2</sub>max in the intervention group, but there were no significant differences in FFA, visceral fat, % body fat and fat mass between the control and experimental groups in overweight women with a sedentary lifestyle. HIIT shows the necessary potential to become a reliable strategy for countering obesity in the adults population, given not its impact on the reduction of body fat in the individuals involved. But, The short workouts mean that it can be introduced simply and frequently into physical education sessions at university or interludes with daily exercise. Require longer training duration if you want big VO<sub>2</sub>max impact and changes in body compositions with less fat.

It is necessary to conduct research with a long duration and monitor the diet of research subjects, the need for calorie calculations and isolation of subjects so that they can be controlled during the study.

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## Conflict of Interest

The authors declared that there is no conflict of interest

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