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Modified High-Intensity Interval Training and its effects on immunometabolic regulation in sedentary young adults with overweight and obesity.

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Keywords: obesity; physical activity; lymphocytes; metabolic syndrome; visceral fat.

Abstract. Sedentary lifestyles can contribute to obesity and other diseases; while chronic low-grade inflammation associated with obesity can lead to metabolic alterations. As physical activity is an alternative to decrease excess weight and its related comorbidities, High-Intensity Interval Training (HIIT) has recently emerged as effective in regulating whole-body metabolism and inflammatory processes in people with excess weight. The objective was to compare the effects of a modified HIIT program on peripheral blood leukocytes (PBL), metabolic profile, insulin resistance (IR), and body composition (BC) in sedentary adults with excess weight. PBL, biochemical variables, IR, and BC were analyzed in 37 participants, 23 sedentary young adults (17 with overweight and six with obesity), before and after eight weeks of a modified HIIT program and compared with those of 14 healthy-weight participants**.** The results showed that after HIIT, total lymphocytes, TCD3+, and TCD8+ lymphocytes decreased; granulocytes and naïve TCD3+ cells increased in patients. Regarding partial correlations, we found that changes (Δ) in TCD8+ lymphocytes correlated positively with glucose and LDL-c, while naïve TCD3+ cells correlated with total cholesterol and LDL-c. Δ in TCD4+CD45RA+ cells correlated negatively with Δ in subcutaneous fat tissue and body fat mass. This study reports that sedentary young adults who completed the modified HIIT program showed lymphocyte levels similar to those in healthy-weight individuals and positive changes in the study variables. Such changes suggest immunometabolic regulation through the implementation of HIIT in participants with overweight and obesity.

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Entrenamiento modificado de intervalos de alta intensidad y sus efectos sobre la regulación inmunometabólica en adultos jóvenes sedentarios con sobrepeso y obesidad.

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Palabras clave: obesidad; actividad física; leucocitos; síndrome metabólico; grasa visceral.

Resumen. El sedentarismo puede contribuir a obesidad y otras enfermedades; la inflamación crónica de bajo grado asociada a la obesidad puede llevar a alteraciones metabólicas. La actividad física es una alternativa para disminuir el exceso de peso y sus comorbilidades asociadas, donde el Entrenamiento de Intervalos de Alta Intensidad (HIIT, por sus siglas en inglés) ha emergido como un regulador del metabolismo del cuerpo y del proceso inflamatorio en personas con exceso de peso. El objetivo de esta investigación fue analizar los efectos del programa de HIIT modificado sobre leucocitos en sangre periférica (LSP), perfil metabólico, resistencia a la insulina y composición corporal (CC) en 37 participantes, 23 con exceso de peso (17 con sobrepeso y 6 con obesidad), después de ocho semanas del entrenamiento y compararlos con 14 participantes con peso saludable. Se encontró que los linfocitos totales, linfocitos TCD3+ y TCD8+ disminuyeron; los granulocitos y las células TCD3+ vírgenes aumentaron. En cuanto a las correlaciones parciales, encontramos que los cambios (Δ) en los linfocitos TCD8+ se correlacionaron positivamente con la glucosa y LDL-c, mientras que las células TCD3+ vírgenes correlacionaron con colesterol total y LDL-c. Δ en células TCD4+CD45RA+ correlacionaron negativamente con Δ en tejido adiposo subcutáneo y masa grasa corporal. Estos resultados mostraron que los adultos sedentarios que completaron el entrenamiento presentaron niveles de linfocitos similares a los de los participantes con un peso saludable y cambios positivos en las variables de estudio. Tales cambios sugieren una regulación inmunometabólica en los participantes con sobrepeso y obesidad.

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INTRODUCTION

About one-third of the world population aged 15 years and older do not engage in sufficient physical activity. Consequently, health risks associated with a sedentary lifestyle are on the rise. Sedentary lifestyles and behaviors have several negative effects on the human body including, 'risks of metabolic disorders such as diabetes mellitus, hypertension, and dyslipidemia' [1].

Obesity has been considered a chronic low-grade inflammatory process characterized by an increase in the baseline number of leukocytes and an imbalance between pro- and anti-inflammatory cytokines in circulation [2-4]. Such a condition is associated with insulin resistance (IR) [5], which is characterized by impaired insulin function in metabolically essential tissues, affecting lipid homeostasis and glucose and contributing to metabolic disorders [6,7].

It has been proposed that weight gain and its related comorbidities can be reversed through caloric restriction and increased physical activity [8]. In addition, it has been found that relative health benefits can emerge even in those cases in which there is no change in total sedentary time but intermittent physical activities are included [1]. In this respect, it has been observed that regular exercise is an effective measure against chronic diseases as it improves the inflammatory profile [9].

Recently, modern lifestyles have increasingly promoted sedentary behavior also among young adults. Against this background, High-Intensity Interval Training (HIIT) has emerged as an alternative that 'burns many calories in a short time, so busy people can be more inclined to incorporate intermittent bursts of exercise into their schedule' [10]. HIIT effectively regulates whole-body metabolism [11,12], enhances insulin signaling, and improves anthropometric measures and body composition (BC) [13-14]. In addition, HIIT has been associated with immunomodulatory effects with potential anti-inflammatory benefits [4].

Although positive effects of HIIT have been reported concerning obesity, little is known about the relationship between different variables like body composition, metabolic profile, lymphocyte subpopulations, and inflammatory response. Because the problem of obesity has grown in recent years and it is presented as a complex condition to treat and because it implies lifestyle changes, it is necessary to propose strategies to the population that, in the short term, provide them with some benefit, which is observable as physical activity. Therefore, this study aimed to investigate the effects of a modified HIIT program over eight weeks on the variables mentioned above, peripheral blood cells, metabolic profile, IR, anthropometric measures, and BC in apparently healthy sedentary patients with overweight or obesity, with or without metabolic syndrome (MS).

METHODS

Study design

A longitudinal clinical-trial study was performed with sedentary young adults in Mexico City, Mexico. The study was conducted following the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Metropolitan Autonomous University of Xochimilco (Agreement 13/16,8.1). Participants provided written informed consent before enrolling in the study.

The study population was selected according to the following criteria:

Inclusion criteria: adults aged 18-40 years apparently healthy with overweight (OW) or obesity (Ob) who did not engage in regular and sufficient physical activities.

Exclusion criteria: individuals with any infection, pregnant women, individuals with diabetes mellitus type 1 or type 2 (DM2), or autoimmune, hepatic, renal, endocrine, cancer, or heart disease, who were taking any medication and/or participating in regular exercise training, and who did not present a medical certificate to engage in regular exercise.

The program included no dietary or alimentary behavior modification to minimize the influence of additional factors. All the variables listed below were assessed before and after two months of modified HIIT workouts. Measures were taken after at least 48 h of the last exercise session to avoid data on the acute effects of exercise. All the variables listed below were evaluated at the beginning and after two months (eight weeks) under a modification of the HIIT training.

Se dentary behavior

Sedentary behavior was evaluated using an International physical activity questionnaire (IPAQ)-short form (7-day physical activity). All participants were required to complete the questionnaire in advance of the study. An IPAQ activity level of <600 MET/week was defined as sedentary behavior.

Clinical measurements

Anthropometric measurements were performed following the standardized protocol of the International Society for the Advancement of Kinanthropometry (ISAK)[15] (Although the person who made the measurements for the study is not ISAK certified, it was standardized by someone who is ISAK certified). A SECA 213 stadiometer set at 0.1 cm of precision was used to measure height. Weight was measured using InBody720 equipment (Biospace, Inc. Los Angeles, CA, USA), and waist circumference (WC) was measured employing SECA brand 201 (Chino, CA, USA) fiberglass tape. Nutritional status was assessed through body mass index (BMI) according to the World Health Organization (WHO) [16] criteria for adults.

A dual-energy X-ray absorptiometry (Hologic Discovery Wi. Hologic, Inc. Bedford, MA, USA) was used to measure body composition, obtaining data on skeletal muscle mass (SMM, in kg), body fat mass (BFM, in kg) and the percentage of body fat (BF%). A multi-frequency impedance body composition analyzer (InBody 720, equipment) was utilized to obtain visceral adipose tissue (VAT) in square meters, in which visceral obesity (increased VAT) was diagnosed in persons with ≥100 cm^2 of fat and normal VAT in persons with <100 cm^2 of fat).

Lipid and glucose measurements

For biochemical tests, 5 mL of a venous blood sample was collected using an automated clinical chemistry equipment KONTROLab, model Ikem (KONTROLab Co., Ltd. Roma, Italia), to obtain: triglycerides (TG); high-density lipoprotein-cholesterol (HDL-c), fasting glycemia (Glu), and total cholesterol (TCho). Low-density lipoprotein-cholesterol (LDL-c) was calculated based on the Friedewald formula. Samples were obtained after a 12-h fasting period. Blood pressure measurements were performed according to the guidelines of the Mexican Official Norm (NOM-030-SSA2-1999). The presence of MS was determined according to the Cholesterol Education National Program (ATP III) definition and modified according to the Hispanic population [17]. These guidelines suggest that MS is present when an association of at least three of the following factors is found: TG ≥150 mg/dL; HDL-c <40 mg/dL; blood pressure ≥130/85 mmHg or a previous diagnosis; Glu ≥100 mg/dL, and a WC in women of ≥80 cm and in men ≥90 cm.

Insulin resistance

The METabolic Scale for Insulin Resistance (METS-IR) tool was employed to measure the IR parameter. This consists of an indirect method for the detection of insulin action that takes into account blood concentrations of TG, HDL-c, glucose, and BMI as follows: (Ln((2*G0)+TG0)*IMC)/(Ln(HDL-c)); G0: fasting glucose, and TG0: fasting triglycerides [18].

Measurement of lymphocyte subpopulations

A 5 mL venous blood sample was collected in tubes containing K_2 EDTA (BD Biosciences, San Jose, CA, USA). Cells were stained with conjugated commercial antibodies (BD Biosciences, San Jose, CA, USA), and the combinations employed were the following: FITC-anti-IgG1/PE-anti-IgG2; FITC-anti-CD45/Pe-anti-CD14; FITC-anti-CD3/PE-anti-CD16+CD56/PerCP-anti-CD19; FITC-anti-CD4/PE-anti-CD62/APC -anti-CD3; FITC-anti-CD8/PE-anti-CD28/APC-anti-CD3, and FITC-anti-CD45RA/PE-anti-CD45RO/PerCP-anti-CD4/APC-anti-CD3. Peripheral blood samples were stained with conjugated antibodies for 20 min in the dark, then 3 mL of lysis buffer solution was added. Samples were washed (PBS), fixed with 1% p-formaldehyde, and analyzed within 24 h of staining [4, 19].Cell analysis was performed using a FACSCanto II Cytometer with FACSDiva software (BD Biosciences, San Jose, CA, USA). For each sample, 10,000 cells were counted.

Intervention

HIIT group participants were supervised along three HIIT exercise sessions per

week for eight weeks. Each training session consisted of 25 min of effective exercise, and each minute was divided into 30-s intervals of activity, followed by a 30-s of active rest (Active rests refers to the participants did not stop moving altogether; they continued jogging; it was rest from the exercises involved in the session, such as squats or some other exercise). Effective exercise time and intervals increased according to the participants' capacity. Eventually, the exercise session consisted of 45 min of active exercise, while each minute consisted of 50-s intervals of activity, followed by 10-s of active rest. On the recommendation of the Centers for Disease Control and Prevention, the American College of Sports Medicine, and the American Heart Association [20], sessions were organized into three parts.: warm-up (5-9 min), aerobic part/resistance (HIIT) (25-45 min), and cooling/flexibility (8-12 min).The activity intensity was measured according to the Perceived Exertion Rating Scale (RPE), managed at moderate intensity (between 5 and 6) [21]. As HIIT works with high-intensity intervals and since we worked with sedentary subjects with overweight and obesity, a modification to this training system was introduced in order to work at moderate intensity. In addition, the Karvonen formula (Functional Capacity Evaluation [FCE]= {(FM-FR)*PI} + FR) was adapted, and the percentage of intensity (PI) at which participants worked was obtained by taking into account heart rate (FCE), resting frequency (RF), and maximal frequency (MF) during the training sessions) [22]. For the latter, the FC was taken at each session before and after the training.

The participants' adherence to the study was calculated as the percentage of complete sessions attended in relation to the total number of training sessions proposed in the present study.

Statistical analysis

The Shapiro–Wilk test was utilized to determine whether the variables presented had a Gaussian distribution. Logarithmic transformation was performed to calculate the normality in variables without Gaussian distribution. The results are presented as means ± standard deviations (SD) and medians, employing an interquartile range (IQR, 25-75). Paired Student *t*-tests were employed to compare pre-and post-exercise data. The One-way analysis of variance (ANOVA) and Bonferroni post-hoc tests were applied to determine differences between >two groups. Data were adjusted for gender and age. Bilateral partial correlation analyses adjusted for gender, age, and BMI were performed to estimate the correlation of the Δ of the lymphocyte subpopulations with the Δ of the anthropometric, BC, and biochemical variables. Then, step-wise forward linear regression models utilizing the Δ of lymphocyte subpopulations as dependent variables to evaluate the association with Δ of anthropometric, BC, and biochemical variables adjusted for gender, age, and BMI were performed. A $p<0.05$ was considered significant, using the IBM SPSS ver. 21 statistical software program.

RESULTS

Forty overweight or obese persons were accepted to participate in the training sessions; however, several individuals withdrew from the exercise (n=11). In addition, six individuals did not attend their second evaluation. Thus, the study group consisted of 23 individuals. Remarking this way, the problem that sedentary people have of adhering to a routine.

Thirty-seven people participated, and two groups were formed: 1) the control group, consisting of 14 participants with normal BMI, normal weight (NW), and VAT, without MS, matched by gender and age with the study group, who did not perform training, and 2) the study group, consisting of 23 persons with OW and Ob who underwent the training. All participants included in the study attended at least 80% of the classes

conducted. The average age of participants was 26.4±6.5 years, and 73% were female.

Baseline characteristics

Thirty-seven percent of participants (n=14) had normal BMI, 46% (n=17) people with OW, and 16% (n=6) had Ob. A prevalence of 26% (n=6) of MS was found in the study group. It was also observed that individuals with MS were mostly people with visceral obesity.

Laboratory analysis, anthropometric measurements, and BC

Regarding biochemical variables, it was observed that persons with obesity had the highest TG values compared to individuals with NW and diastolic pressure in relation to individuals with OW. It was also found that HDL-c was lower in individuals with OW than in those with NW. Concerning IR, it was found that METS-IR increased as the BMI did; therefore, individuals with NW presented the lowest values and persons with Ob the highest values; these differences were significant (Table 1).

Concerning anthropometric measurements and BC, it was found that participants with NW had the lowest values in WC, ST, BFM, and VAT compared to individuals with OW or Ob (Table 1).

Lymphocyte subpopulations

It was found that total lymphocytes (TL) increased according to BMI. On the other hand, granulocytes were lower in persons with NW when compared with individuals with OW or Ob; all of these differences were statistically significant. No significant differences were observed when analyzing the memory and naïve cells according to nutritional status (Table 2).

Intervention

Overall, participants worked at 54 ± 10.6% of their maximal level according to the formula of Karvonen. In addition, the intensity percentage was increased as the training weeks progressed, finding a significant difference between weeks 1 and 6 (46±13 vs. 61.9±10%, p<0.05).

Laboratory analysis, anthropometric measurements, and BC

In individuals with OW or Ob, no significant positive changes were found in biochemical indicators after training by group (Table 1). However, when each participant was analyzed, it was found that TG decreased in 39% of the participants by 25±14.7%, Glu in 61% by 22±10%, TCho in 74% of individuals by 24±10%, LDL-c in 74% by 37±16%, and HDL-c increased in 52% of persons by 20±10% (Fig. 1).Regarding anthropometric measurements and BC after training, persons with OW presented a statistically significant decrease in WC (Table 1). Also, when each participant was analyzed, it was found that VAT decreased in 57% of the participants by 5.3±4.6cm^2, ST in 57% by 1.9±2.2%, BFM in 61% of individuals by 1.7±1.5kg, and SMM increased in 30% by 1.3±1.6kg (Fig. 2). In addition, it was observed that one person with Ob passed to OW and two with OW passed to NW; furthermore, two persons with increased VAT passed to normality.

Lymphocyte subpopulations

After training, TL and TCD8+ lymphocytes decreased in the study group. Also, granulocytes and näive TCD3+ cells increased.

When the subpopulations were analyzed according to BMI, TL, TCD3+, and TCD8+ decreased in individuals with OW, approaching the percentages of persons with NW. In addition, an increase of TCD3+CD45RA+ and monocytes was found in persons with OW. All of these differences were statistically significant (Table 2). According to MS, we found that TCD4+ lymphocytes decreased (55.6±13.2 vs. 29±13%; p<0.0001) after the training activity. In individuals without MS, the monocytes increased (6.6±3.5 vs. 8.2±2.4%; p<0.0001), while TCD8+ lymphocytes decreased (39±8.5 vs. 29.8±11.8%; p<0.05).

Table 1
Biochemical indicators, anthropometric measurements, and body composition according to Body Mass Index before and after two months of High-Intensity Integral Training.

Variable (n=37)	Normal (n=14)	Overweight (n=17)			Obesity (n=6)			p (ANOVA)	
		B	A	p#	B	A	p#	p	p (post hoc)*&
TG (mg/dL)	80 ±26.5	119.8±52.6*	150.5±70.8	0.088	163.1±42.3*	187.3±58.9	0.188	0.002	0.002
c-HDL (mg/dL)	51.9 ±13.6	39.2* ±8.1	38.2 ±8.4	0.607	43.2 ±13.3	40.5 ±3.9	0.477	0.011	0.009
Glu (mg/dL)	81.1 (77.6-85.7)	79.4 (70.2-94.3)	83.8 (57.6-95.9)	0.597	81.2 (72.9-103.8)	78.7 (72.2-88.4)	0.226	0.754	
c-LDL (mg/dL)	80.3 (64.9-93.6)	91.6 (60.9-130.3)	63.6 (46.4-88.7)	0.124	80.2 (46.7-144.1)	83.9 (48.1-99)	0.818	0.631	
TCho (mg/dL)	146.9 (133.8-166.8)	161.7 (128.4-193.9)	140.2 (114-173.5)	0.608	165.3 (115.5-228.1)	156.9 (130.8-182.5)	0.757	0.628	
SBP (mmHg)	105.5 ± 8.6	106.2 ±10.8	107 ±6.8	0.819	116.3 ±15.6	111.6 ±11.6	0.309	0.144	
DBP (mmHg)	69.5 ±7	69.5 ±7.8	73.5 ±9.9	0.151	79 ±10.3&	76.6 ±5.1	0.675	0.049	0.059
METS-IR	31.8 ±4.2	42.4 ±4.9*	43 ±43	0.494	52 ±6.6*&	51.6 ±6.4	0.712	0.000	0.001
Weight (kg)	60.9 ±9.9	76.3 ±10*	75.9 ±10.4	0.328	87.1 ±17.5*	86.7 ±18.2	0.436	0.000	0.000
BMI (kg/m^2)	22.5 ±1.7	27.3 ±1.3*	27.2 ±1.6	0.304	33.2 ±2.3*&	32.5 ±2.5	0.242	0.000	0.000
WC (cm)	81.2 ±7.4	90.9 ±6.4*	88.7 ±6.5#	0.001	102.7 ±10.5*&	99.7 ±11.2	0.187	0.000	0.000
SMM (kg)	23.8 (18.5-26.2)	26.3 (23.1-30.1)	26.6 (24.4-29.6)	0.949	23 (21.8-37)	23.6 (22-37.6)	0.131	0.150	
ST (%)	27.7 ±6	35.5 ±5.9*	35 ±5.6	0.473	43.5 ±6.4*&	42.3 ±6.2	0.187	0.000	0.000
BFM (kg)	16.8 ±3.2	27 ±5.3*	27.2 ±6.8	0.822	37.1 ±3.8*&	36 ±4.7	0.258	0.000	0.000
VAT (cm^2)	67.1 ±19	99.5 ±18*	98.5 ±18.8	0.500	139.9 ±28.6*&	138.5 ±33	0.722	0.000	0.000

Data are presented in media ± SD or median and interquartile range (IQR). p (post hoc): p adjusted with the Bonferroni test. # Statistically significant difference between before and after. * Statistically significant difference vs normal. & Statistically significant difference vs overweighs ($p<0.05$). B: before; A: after; TG: triglycerides; c-HDL: high-density lipoprotein cholesterol, Glu: glucose; c-LDL: Low-density cholesterol; TCho: total cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; WC: waist circumference; SMM: skeletal muscle mass; ST: subcutaneous adipose tissue; BFM: body fat mass; VAT: visceral adipose tissue.

Table 2
Leukocyte distribution before and after High-Intensity Integral Training according to Body Mass Index

Variable (n=34) (%)	Normal (n=11)	Overweight (n=17)			Obesity (n=6)			
		B	A	p#	B	A	p#	P*&
Total lymphocytes	29.3 ±10.9	40.3 ±9.6*	32.2 ±8.5#	0.048	53.5 ±9*&	33.1±7.5#	0.029	0.000
Monocytes	7.8 ±2.4	7.1 ±3.1	8.4 ±2.2#	0.044	5.6 ±2.5	5.9 ±1.3	0.893	0.256
Granulocytes	62.6±11.1	52.4 ±8.5*	59.1±8.2	0.064	40.9±8.7*	60.7 ±6.6#	0.014	0.000
Lymphocytes B	7.5 (5-12.6)	7.4 (5.4-10.5)	9.2 (6.1-1.7)	0.148	11.6 (7.7-18.8)	15.2 (9.9-18.6)	0.293	0.202
Lymphocytes NK	19.5 (12.8-32.7)	14.3 (10.6-19.9)	16.6 (14.2-24)	0.171	13 (9.4-19.8)	20 (16.2-26.7)	0.199	0.430
TCD3+	68.4 ±10.6	76.5 ±8.1	68.9±10.5#	0.043	71.2±10.9	62 ±9.3	0.244	0.112
TCD4+	56.9 (46-60.7)	52.2 ±7.7	43.5 ±16.5	0.139	51.4±13.7	38.4 ±19.5	0.568	.763
TCD8+	29.8 ±14.9	38.7 ±7.8	29.6±12.1#	0.002	34 ±7.4	24.1±11.1	0.147	0.198
TCD3+CD45RA+	54.2 ±13	48.5 ±11.6	53.4 ±8.7#	0.050	44.5±18.7	59 ±15.6	0.330	0.456
TCD3+CD45RO+	32.1 ±9.4	31.6 ±8.7	31.4 ±5.8	0.792	39.7±14.9	26.1±9.5	0.230	0.358
TCD3+CD45RO+ CD45RA+	15.7 ±6	16.7 ±5.9	12.7 ±6.6	0.454	14.6 ±5.8	11.6 ±6.7	0.558	0.828
TCD4+CD45RA+	36.4 ±14.7	20.3 ±7.2	35 ±17.3	0.438	33.9 ±5.1	52.5 ±15.2	0.160	0.521
TCD4+CD54RO+	43.2 ±13.4	48.1 ±8.8	53.1 ±20.6	0.712	43 ±20.6	36 ±12.7	0.661	0.796
TCD4+ CD45RO+CD45RA+	17.6 ±5.8	16.7 ±6.1	11.5 ±3.8	0.250	24.4±15.4	11.8 ±5.7	0.444	0.395

Data are presented in means ± SD or median and interquartile interval (IIC).* Statistically significant difference vs normal. & Statistically significant difference vs. overweight. # Statistically significant difference before vs. after ($p<0.05$). B: before; A: after; Lymph B: Lymphocytes B; Lymph NK: Lymphocytes natural killer.

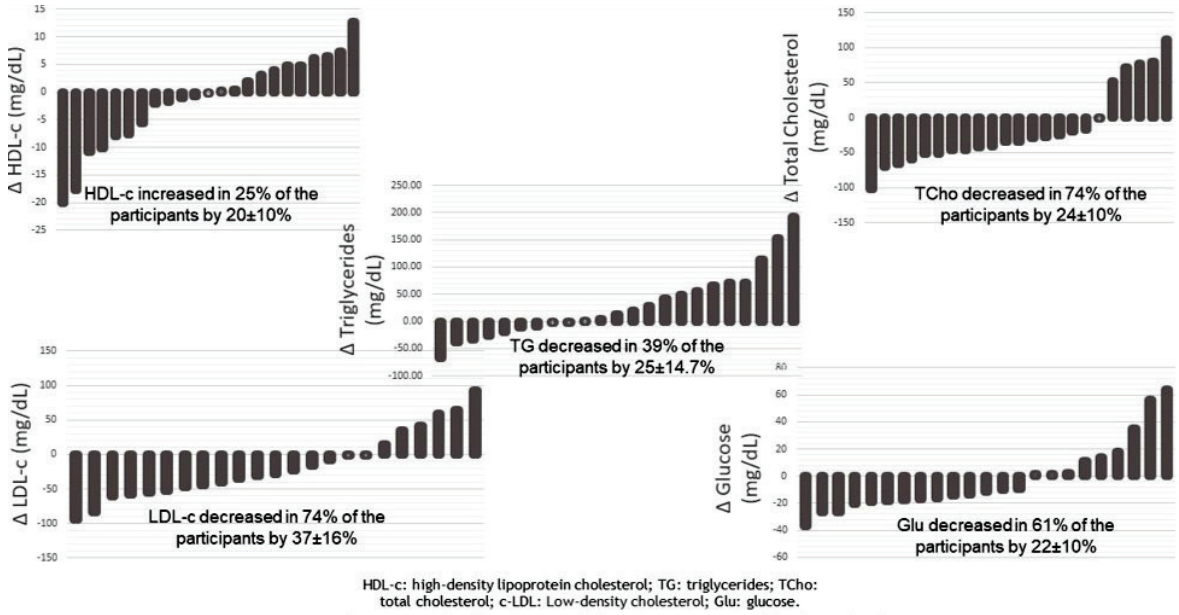

Fig. 1. Distribution and improvement of biochemical indicators after training.

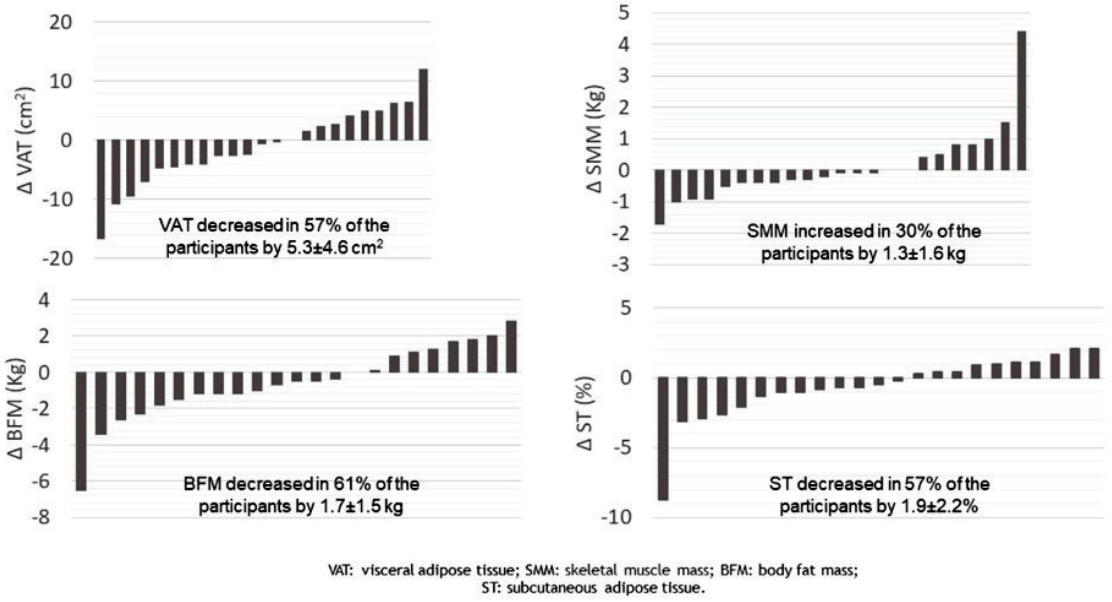

Fig. 2. Distribution and improvement of anthropometric measurements and body composition after training.

Partial correlations were made of the observed variations in leukocyte cells after the intervention concerning the changes (Δ) in the different variables, adjusting the analysis by gender, age, and BMI. We found that Δ at the peripheral level of the granulocytes and TCD8+ lymphocytes correlated negatively with Δ in WC; additionally, the Δ of TCD8+ lymphocytes correlated positively with glucose and LDL-c. The Δ of the total näive cells correlated positively with TCho and LDL-c, and the Δ of the positive double cells of TCD3+ (TCD3+CD45RA+CD45RO+) positively correlated with the Δ in MERS-IR. Last, the Δ of the näive cells of TCD4+ lymphocytes correlated negatively with the Δ

percentage and kilograms of fat, all of these differences statistically significant (Table 3). No correlation was observed between exercise intensity and change in lymphocyte subpopulations.

Furthermore, we decided to perform a linear regression adjusted for gender, age, and BMI among the changes in lymphocyte subpopulations statistically correlated with the changes in the different variables. A positive correlation was found between the changes of TCD8+ lymphocytes and TCD3+CD45RA+ lymphocytes with changes in LDL-c, where a decrease of 1 mg/dl of this cholesterol was associated with a decrease of 3% (range, 0.8-4.5%) of TCD8+ lymphocytes and an increase of 6% (range, 2.8-9.6%) of TCD3+CD45RA+ lymphocytes. Also, TCD3+CD45RA+ lymphocytes correlated positively with TCho, where a decrease of 1 mg/dL of this type of cholesterol was associated with an increase of 7% (range, 1-11%) of TCD3+CD45RA+ lymphocytes. Finally, TCD3+CD45RA+CD45RO+ lymphocytes correlated positively with METS-IR, where a decrease of 1 unit of METS-IR was associated with a decrease of 1% (range, 0.5-2%) of TCD3+CD45RA+CD45RO+ lymphocytes (Fig. 3).

DISCUSSION

Obesity is caused by a genetic predisposition and environmental and lifestyle factors, including physical inactivity and poor eating habits [23]. Physical inactivity and obesity are associated with visceral fat accumulation, leading to chronic low-grade inflammation and the pathogenesis of IR, MS, DM2, cardiovascular diseases, and cancer [24,25]. Total sedentary time and moderate-to-vigorous physical activity have been reported to be negatively correlated. Waist circumference, body mass index, triglyceride level, and plasma glucose level have also been reported to decrease while the number of breaks in sedentary time increased [1]. On that basis, the present study aimed at determining the impact of a modified HIIT on metabolic, an-

Table 3
Partial correlation between changes in leukocyte cells and changes in the anthropometric, biochemical, and body composition variables.

Leukocyte cells	Variables	p	P
Δ of Total lymphocytes	Δ of WC	0.491	0.053
Δ of granulocytes	Δ of WC	-0.514	0.041*
Δ of lymphocytes TCD8+	Δ of WC	-0.612	0.007*
	Δ of Glu	0.474	0.047*
	Δ of DBP	0.458	0.056
	Δ of c-LDL	0.617	0.006*
	Δ of Total Cho.	0.460	0.055
Δ of TCD3+CD45RA+	Δ of c-LDL	0.959	0.010*
	Δ of Total Cho.	0.941	0.017*
Δ of TCD3+CD45RA+CD45RO+	Δ of METS-IR	0.962	0.009*
Δ of TCD4+CD45RA+	Δ of ST	-0.950	0.050*
	Δ of BFM	-0.974	0.026*
	Δ of c-HDL	0.938	0.062

WC: waist circumference; Glu: glucose; DBP: diastolic blood pressure; c-LDL: Low-density cholesterol; Total Cho: total cholesterol; ST: subcutaneous adipose tissue; BFM: body fat mass; c-HDL: high-density lipoprotein cholesterol. p: p value adjusted by gender, age, and BMI ($p<0.05$).

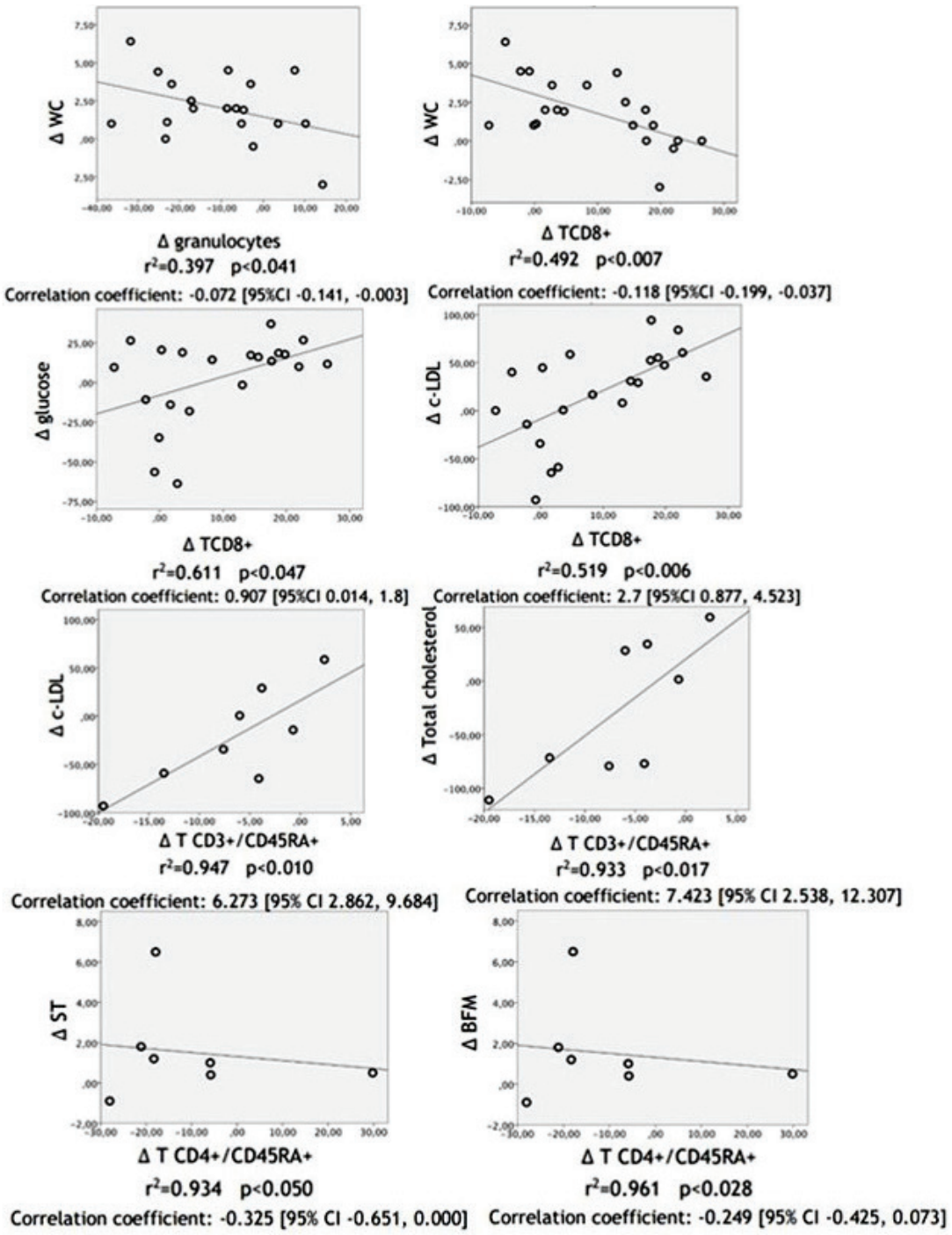

Fig. 3. Linear regression between changes in leucocytes cells and changes of the different variables.

thropometric, BC, and PBL measures in sedentary patients with OW and Ob.

HIIT has been proposed as a better-suited activity for people with OW and Ob than traditional continuous exercise [4]. Some studies have reported a reduction in fasting glucose, insulin, diastolic blood pressure (DBP), and systolic blood pressure (SBP) and improvements in insulin sensitivity after 16, 14, 12, or 2 weeks of HIIT [3, 26-29]. In the present study, about 74% of participants showed improvements in TG, Glu, TCho, LDL-c, and HDL-c levels after training. Concerning BC, other studies that implemented HIIT in persons with OW or Ob for two and 12 weeks, found a reduction in weight, WC, fat mass, and BMI [27, 29]. In this study, a significant statistical reduction was observed only in WC in persons with OW, as well as about 50% of participants showed decreased VAT (5.3±4.6cm^2), ST (1.9±2.2%), and BFM (1.7±1.5kg), and increased SMM (1.3±1.6kg).

Although participants were untrained people, they reached moderate intensity during weeks 6 and 7 (62% of their intensity percentage). The intensity percentage increased as weeks progressed, observing a significant difference between week 1 and week 6 ($p<0.05$).

On the other hand, physical activity has been often recognized as a powerful countermeasure to inflammation [30]. A study carried out with untrained young adults who participated in HIIT for three days found that the percentages of TCD4+, TCD8+, and CD19+ lymphocytes increased significantly after training [31]. Another study carried out over two weeks with inactive persons with OW or Ob revealed that training did not affect the blood concentration of total lymphocytes (TL), monocytes, and neutrophils [27]. Both data do not coincide with those reported in the present study, as it was found that in persons with OW, TL, TCD3+, and TCD8+ decreased (averages were similar to those in persons with NW). On the other hand, in individuals with Ob, TL decreased, and granulocytes increased statistically, getting closer to those values in persons with NW. With these results, it can be observed that only TL had the same behavior both in individuals with OW and in those with Ob. These data suggest differences in the mobilization of leukocytes at the peripheral blood level according to the nutritional status after physical activity.

In addition, it has been reported that T lymphocytes and TCD8+ increase with obesity [32, 33] and that they infiltrate adipose tissue and promote the classical pro-inflammatory activity of M1 macrophages and the production of pro-inflammatory cytokines. These phenomena can trigger a metabolic imbalance, such as an increase in Glu, TCho, LDL-c, and TG and a decrease in HDL-c, among others [34-36]. The finding in the present study of diminished TL, TCD3+, and TCD8+ might indicate a decrease of the inflammatory process at the peripheral blood level, improving the metabolic profile, because the improvement was found in the different metabolic variables in a little more than 70% of the participants, in addition, in different correlations and linear regressions, it was found a relationship between improvements in lymphocyte subpopulations and both biochemical and anthropometric variables.

Furthermore, in other studies, it has been observed that monocytes, B cells, NK cells, and T cells (CD4+ and CD8+) are found in higher proportions in people with obesity and provide a link between systemic inflammation and IR [37-40], which would guide us to reiterate that having found some of these cells decreased after physical activity means an improvement of the inflammatory process that is associated with IR and metabolic alterations, representing a better metabolic state for the participants.

On the other hand, in persons with obesity, immunosenescent behavior has been reported, similar to that appearing in the elderly. This has been denominated as "premature immunosenescence", an imbalance between senescent, naïve, and mem-

ory cells that renders the individual with obesity more susceptible to the disease [19, 43]. The specific increase in memory cells in obese patients could somehow reflect what some authors have pointed out about the capacity for proliferation and activation of memory cells, revealing the high degree of chronic adaptive immune activation [41,42], which has been associated, as has already been mentioned, with metabolic alterations in these patients.

In this sense, it has been observed that exercise may counteract immunosenescence and its associated diseases by limiting the accumulation of senescent T cells and repopulating the blood with naïve T cells [30], mainly through promoting the expansion of the naïve cell repertoire as a consequence of the apoptosis of senescent T cells [44-47], this apoptotic process is thought to induce hematopoietic stem cells production in the bloodstream, which may move to the thymus and stimulate the development of naïve T cells [30]. In the present study, in addition to finding an increase in naïve cells after training (TCD3+ ($p<0.05$) in persons with OW), a correlation between such an increase and the reduction of total lymphocytes, IR and LDL- cholesterol was observed. These results suggest that this training could promote immunometabolic regulation in these patients. Furthermore, it has been found that senescent T lymphocytes are related to high percentages of body fat [44, 46, 48]. It would be valuable and interesting to investigate the presence of senescent T cells in persons with obesity.

Strengths, limitations and conclusions

Exercise is one of the therapies that accompany the treatment of obesity; However, on some occasions, depending on the period and the routine that is studied, it has been observed that "there is no change", but with this work, we can realize that the physical activity (modified HIIT with moderate intensity, in this case) carried out, is influencing the inflammatory process that triggers obesity and, in turn, these changes were related to immunometabolic improvements, thus highlighting the relevance of this study.

Some of the study's strengths were that it was possible to implement an exercise routine in a sedentary population; immunometabolic changes were obtained in only eight weeks of exercise. Also, the presence of comorbidities (for example, dyslipidemias, hypertriglyceridemias, hyperglycemia, MS, among others) was detected and treated in 25% of the participants with exercise; All international standards were followed to evaluate the selected variables.

Certain limitations of the present study were: The lack of analysis of other inflammation markers that could give us more information about the inflammatory process before and after routine physical activity and the voluntary desertion of some patients due to the problem of adherence to a routine in sedentary people.

In conclusion, this study reports that an eight-week modified HIIT program brought about positive changes in peripheral lymphocyte subpopulations in sedentary individuals with OW or Ob, as it observed a reduction in TL and TCD8+ and an increase in naïve cells, bringing the values closer to those in persons with NW. These changes correlated with healthier metabolic variables. Also, differential leukocyte changes were observed according to BMI. These results represent novel knowledge about the positive effects of HIIT, not only regulating whole-body metabolism but also regulating immunomodulatory effects with anti-inflammatory benefits. All these results reinforce the benefits of HIIT as an exercise strategy to promote the regulation of immunometabolism.

As shown in this study, exercising is essential, and obese patients should be made aware that they should make changes in their lifestyle in general, particularly in terms of having a more active life. For this reason, it is essential to continue conducting studies of this nature.

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Conflict of interest

None

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