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Effect of different exercise modalities plus a hypocaloric diet on inflammation markers in overweight patients: a randomized trial.

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Short title (Running title): Effect of different exercises on inflammation markers.

Non-standard abbreviations:

S: Strength training group

E: Endurance training group

SE: Strength + Endurance combined training group

D: Diet and physical activity recommendations group

VO_{2peak}: Peak oxygen uptake

DSI: Dynamometric Strength Index

SI: Strength Index

AV: Anthropometric Variables

TFM: Total Fat Mass

AF: Android Fat

AF/GF: Android/Gynoid fat ratio

LM: Lean Mass.

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ABSTRACT

BACKGROUND & AIMS: Inflammation markers (IM) have been associated with the development of chronic diseases. This study compares the effects on IM of three exercise programs combined with a hypocaloric diet.

METHODS: 119 overweight participants (73 women, 46 men) aged 18–50 years were randomised into four treatment groups: strength training (S; n=30), endurance training (E; n=30), combined S + E (SE; n=30), and a diet and physical activity recommendations group (D; n=29). Energy intake, anthropometric variables (AV), training variables (VO_{2peak}, strength index, dynamometric strength index [DSI]) and plasma IM were recorded at baseline and after 22 weeks of treatment.

RESULTS: 84 participants completed the study. At 22 weeks, all groups showed a significantly reduced energy intake (P<0.001) and improved AV (P<0.001). VO_{2peak} significantly increased in all groups (P<0.01). DSI increased in the exercise groups only (P<0.05). Plasma leptin fell significantly (P<0.001) in the S and E groups, but not significantly in the SE group (P=0.029) (no significant differences between these groups). Tumour necrosis factor-α (TNF-α), and C-reactive protein (CRP) concentrations decreased in all groups when examined together, but not when examined separately. No significant differences were seen in interleukin-6 (IL-6).

CONCLUSIONS: Combining strength or endurance training with a hypocaloric diet improved AV and reduced plasma leptin concentrations. No differences were seen between groups in terms of TNF-α, IL-6 or CRP reduction.

This trial was registered at clinical trials.gov as NCT01116856. http://clinicaltrials.gov/

Keywords: overweight, inflammation, strength training, endurance training, combined training.

INTRODUCTION

The majority of epidemiological studies indicate excess body weight during midlife, including overweight, to be associated with an increased risk of death[1-3]. For example, people with a BMI of 25-28.9 have a relative risk of developing cardiovascular disease twice that of people with a BMI of <21[4], while those with a BMI of ≥29 are at almost three times the risk. Further, the results of the Framingham Heart Study show that being overweight at age 40 reduces life expectancy by three years[5]. Given the increasing prevalence of obesity, finding more efficient treatments for overweight should be seen as a public health priority.

Low-grade chronic inflammation is one of the key metabolic alterations linked to excessive energy intake, physical inactivity and adiposity, and the markers of this inflammation - tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP) - have all been associated with the development of atherosclerosis and insulin resistance [6,7]. Several studies have shown that inflammation markers (IM) are reduced following weight loss [8-10]. Physical exercise may therefore be effective in reducing inflammation. Indeed, data from observational and intervention studies show that greater physical activity is associated with lower plasma IM concentrations [10-13], and regular exercise and an appropriate diet are reported to protect against all-cause mortality. This is achieved primarily through protection against atherosclerosis, type 2 diabetes, colon cancer and breast cancer [14-16].

The mechanism that underlies the anti-inflammatory response associated with acute exercise might, surprisingly, involve an *increase* in the circulating concentration of IL-6. Under exercise conditions, this cytokine appears to induce an anti-inflammatory environment by promoting the production of IL-1ra and IL-10, and by inhibiting TNF-α production [14-16]. Exercise also increases the release of epinephrine, cortisol, growth hormone, prolactin and other factors that have immunomodulatory effects [16].

A few studies have tried to determine the types of exercise intervention that produce the greatest changes in IM concentrations [13,17-20], but the results have been controversial. A recent review

indicates that increasing aerobic physical activity may be effective for reducing chronic inflammation especially in individuals with chronic diseases associated with a state of elevated inflammation. (Beavers et al., 2010). The aim of the present study was to compare the effects on plasma IM concentrations of three different exercise programs - strength, endurance, and combined strength + endurance training - all in conjunction with a hypocaloric diet, and the normal clinical practice of achieving weight loss using the same hypocaloric diet as above, plus the provision of recommendations regarding physical activity.

MATERIALS AND METHODS

Study subjects

This study was performed as part of the larger study *Nutrition and Physical Activity for Obesity* (the PRONAF study according to its Spanish initials), the aim of which was to assess the usefulness of different types of physical activity and nutrition programs for the treatment of obesity. Participants were sought via advertisements posted in newspapers and announced on the radio, the internet and TV. The eligible sample population consisted of 119 overweight subjects (73 women and 46 men; age range 18–50 years; body mass index [BMI] ≥25–<30 kg/m^2) living in the Region of Madrid, Spain. Eighty four participants completed the study (50 women and 34 men) (Figure 1). All subjects were healthy adults with no history of relevant concomitant illness, such as heart, lung or liver disease, or neoplasia. All were normoglycaemic non-smokers and took no medications or drugs, but led sedentary lifestyles. All female subjects had regular menstrual cycles. The exclusion criteria covered all physical and psychological diseases that may have precluded the performance of the requested strength or endurance training, along with the taking of any medication known to influence physical performance or that might interfere with the interpretation of the results. Subjects with a background of systematic strength or endurance training (moderate to high intensity training more than once a week) in the year before the study started were also excluded. In agreement with the guidelines

of the Declaration of Helsinki regarding research on human subjects, all participants signed an institutionally approved document of informed consent. All subjects were carefully informed about the possible risks and benefits of the study, which was approved by the Human Research Review Committee of the La Paz University Hospital (PI-643).

Study design

Subjects who fulfilled the inclusion criteria and passed a baseline physical examination were stratified by age and sex and assigned (using a randomisation table) to a strength training group (S), endurance training group (E), combined strength + endurance training group (SE), or diet and physical activity recommendations group (D).

This study design was that of an intervention trial of 22 weeks duration. Baseline measurements for all subjects were made before starting the intervention period. The final measurements were taken once the intervention period was over (within 48-72 h of the last training session for the exercise groups).

Exercise training programs

The different exercise groups followed their corresponding training programs, which in all cases involved training 3 times/week for 22 weeks. All training sessions were carefully supervised by certified personal trainers. An adherence to training of 90% was demanded.

The S group followed a circuit involving the following eight exercises: shoulder press, squat, barbell row, lateral split, bench press, front split, biceps curl, and French press for triceps. E group training involved the use of an exercise bike or cross trainer. The SE group performed a combination of cycle ergometry, treadmill or cross trainer work, plus weight training with the following exercises intercalated between lift sets (15 lifts per set): squat, rowing machine, bench press and front split. The D group subjects followed the hospital's habitual clinical practice for achieving weight loss, i.e., the same dietary intervention as the training groups plus being made

aware of the general recommendations of the American College of Sports Medicine (ACSM) regarding physical activity [22].

All subjects were instructed to keep their daily physical activity habits unchanged. These habits were carefully checked with a dairy registry by personal trainers in all training sessions to the groups S, E and SE. Group D subjects were not supervised, although they were subjected to activity monitoring using an armband accelerometer, just as they would be in normal practice.

The exercise programs were designed taking into account each subject's muscular strength (MS) and heart rate reserve (HRR). MS was measured in the strength program subjects (S, SE) using the 15-repetition maximum (15 RM) testing method [21] every other day during the week before the intervention period. The intraclass correlation coefficient of reliability for all exercises was ICCr=0.995 for the men and ICCr=0.994 for the women (groups S and SE subjects together). The HRR was calculated to set the exercise intensity ([maximum heart rate-resting heart rate] x 50% to 60%) and resting heart rate for the E and SE programs.

The intensity of exercise was increased over the study period. In weeks 2-5, exercise was at an intensity of 50% of the 15RM and HRR, and lasted an overall 51 min and 15 s (twice around the circuit, lasting 7 min 45 s each lap). In weeks 6-14, exercise was performed at an intensity of 60% of 15RM and HRR, again with a duration of 51 min and 15 s (again, twice around the circuit). Finally, in weeks 15-23, exercise was performed at an intensity of 60% of 15RM and HRR, with a duration of 64 min (three times around the circuit). The recovery period between circuits was set at 5 min. Participants performed 15 repetitions (45 s) of each exercise with a rest period of 15 s between them. Each training session for the S, E and SE subjects commenced with a 5 min aerobic warm-up, followed by the main session exercises, and concluded with 5 min of cooling down and stretching exercises. In all sessions the exercise rhythm was controlled by instructions recorded on a compact disk. The cadence for the resistance exercises was fixed at 1:2 (concentric-eccentric phase).

Hypocaloric diet program

Hypocaloric diets (between 5028 and 12570 KJ) were prescribed individually for all participants by expert dieticians at the Department of Nutrition, La Paz University Hospital, Madrid. The diet was designed to provide 25% less energy than the baseline daily energy expenditure (DEE), as measured using a SenseWear Pro Armband™ (Body Media, USA). Some 29–34% of energy came from fat, 12–18% from protein, and 50–55% from carbohydrates, according to the recommendations of the Spanish Society of Community Nutrition (SENC, according to its Spanish initials) [23]. The hypocaloric diet program was followed during the 22-week interventional period. Dietary counselling was given at baseline and at 12 weeks to resolve questions and to motivate participants sufficiently to comply with dietary advice. All subjects were instructed on how to record their dietary intake using a daily log, and given recommended portion sizes and information on possible food swaps. In addition, voluntary group nutrition education sessions were given by the dieticians. The goal was to equip the participants with the knowledge and skills necessary to achieve gradual but permanent behavioural changes.

Analytical methods, measurement of training variables, dietetic study, and anthropometric variables

The following analyses and measurements were made at baseline and at the end of the study period.

- *Blood analysis:* Blood samples were taken early in the morning at the La Paz University Hospital Extraction Unit after a 12 h overnight fast. Samples were kept at 4-6ºC until analysis, which was always performed within 48 h. Plasma C-reactive protein concentrations (CRP) were determined using a BNII nephelometer (Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). Plasma leptin, IL-6 and TNF-α concentrations were determined using a Luminex®-LX200 Analyzer (Millipore Corp, Billerica, Massachusetts, USA) and a MILLIPLEX MAP human circulating cancer biomarker magnetic bead panel (HCCBP1MAG-58K) (Millipore, St. Charles, Missouri, USA). All samples were analysed in duplicate. Data were analysed using

xPONENT v.3.1 software (Millipore). The intra- and interassay coefficients of variation for the cytokine assays all fell in the 5-10% range.

- *Training variables:* Peak oxygen uptake (VO_{2peak}) was measured using the protocol described by Bruce[24]. The test was conducted on an H/P/COSMOS 3P 4.0 computerised treadmill (H/P/Cosmos Sports & Medical, Nussdorf-Traunstein, Germany). The volume and composition of expired gas were measured using a Jaeger Oxycon Pro gas analyser (Erich Jaeger, Viasys Healthcare, Germany) The general strength index (SI) was calculated following the method of Jurca et al. (2004)[25]. This method measures the strength of the leg and arm with respect to body weight via two exercises: the bench press and squat. The dynamometric strength index (DSI) was determined by measuring muscular strength using a Tecsymp Tkk5002 hand and leg dynamometer (Tecsymp, Barcelona, Spain) and a Tecsymp Tkk5401 back dynamometer (Tecsymp, Barcelona, Spain). The DSI value was calculated as the sum of the values obtained with both apparatuses divided by subject body weight.

- *Dietetic study*: All food and beverages consumed by the participants were recorded using a food frequency questionnaire and a “3-day food and drink record”, validated for the Spanish population [26], at the beginning and end of the intervention. Participants were instructed to record the weights of food consumed whenever possible, and to use household measurements (tablespoons, cups, etc.) when not. The energy and nutritional content of the foods consumed were then calculated using DIAL software (Alce Ingeniería, 2004). The Healthy Eating Index (HEI) was calculated according to Kennedy et al., [27] taking into account the number of servings recommended for the Spanish population[28]. Compliance with recommended intakes was assessed for the different food groups (cereals, vegetables/greens, fruits, dairy products, meat/ fish/eggs, expressed in servings per day), and also from the point of view of meeting nutritional objectives (intake of lipids, saturated fatty acid, cholesterol and sodium, and dietary variety). Each of these 10 factors was awarded a maximum of 10 points when the intake was the same as that recommended, and a minimum of 0 points when the difference was very great. Intermediate values were awarded proportionally [27]. Diet quality was deemed "'good" when more than 80

total points were scored, as "needing improvement" when the score was 51-80, and "poor" when below 51 [27].

- *Anthropometric variables (AV)*: Height was measured using a SECA stadiometer (range 80-200 cm). Body weight was measured using a TANITA BC-420MA balance (Bio Lógica Tecnología Médica S.L, Barcelona, Spain). The BMI was calculated as [body weight (kg)/(height (m))^2]. The waist circumference (WC) was measured using a Seca 201 steel tape (Quirumed, Valencia, Spain). Dual-energy x-ray absorptiometry (DXA) was used to measure the percentage total fat mass (TFM%), percentage android fat (AF%), the android/gynoid fat ratio (AF/GF), and the lean mass (kg), employing a GE Lunar Prodigy apparatus (GE Healthcare, Madison, Wisconsin, USA). All DXA scans were performed making use of GE Encore 2002 software v. 6.10.029.

Percentage changes from baseline were calculated for the studied variables as: (final value - baseline value/ baseline value) X 100.

Statistical analysis

Means and standard deviations (SD) were calculated for normally distributed continuous variables, and medians and interquartile ranges (IQR) for non-normally distributed continuous variables. The Kolmogorov-Smirnov test was used to determine whether or not the data were normally distributed among groups. For variables that were not normally distributed, differences between the four groups at baseline and after the intervention were compared using the Kruskal-Wallis test. Adopting a closed testing procedure, *post hoc* pairwise comparisons were performed using the Mann-Whitney test if the results of the Kruskal-Wallis test showed significant differences. For the variables that were normally distributed, differences between groups were compared by one way ANOVA plus Bonferroni's *post hoc* adjustment. The change within each group was determined using the Wilcoxon test or paired Student t test depending on the data distribution. Significance was generally set at $P<0.05$; however, Bonferroni adjustments were used to take into account multiple comparisons. Thus, the *P* values for intergroup comparisons were considered significant when $P<0.0083$ (0.05 divided by six possible comparisons for each

variable) and $P<0.0125$ for within-group comparisons (0.05 divided by four possible within-group changes).

Linear and multiple regression analyses were performed to determine the potential role of changes in AV, energy intake and training variables as predictors of change in IM. Dummy variables were used to represent the S, SE and E groups in the multiple regression analysis.

The sample size of the PRONAF study was calculated to detect any effect of training and diet on TFM% (with 80% statistical power, with significance set at $P<0.05$, assuming a correlation of 0.80 between repeated measures, [29] and assuming an estimated drop out of 20%); it was not explicitly determined to analyse the influence on IM.

All analyses were performed using SPSS v.17.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

All groups showed similar baseline characteristics except for the leptin concentration which differed between the S and SE group, the IL-6 concentration which differed between the S and E subjects, and between the E group and SE group, the CRP concentration which differed between the E and SE groups, and the BMI which differed between the S and SE groups ($P=0.011$) (Table 1).

Tables 1 and Table 2 show, by group, the changes in body composition and inflammatory and training variable values after the intervention. Significant reductions were seen in the BMI, WC, TFM and AF in all groups. Lean mass did not change in any of the exercise groups but showed a trend towards a significant reduction in group D ($P=0.029$). The change in TFM% from baseline was -11.6%, -11.5%, -19.4% and -9.7% in the S, E, SE and D group respectively. VO_{2peak} significantly increased in all groups. DSI increased in the three exercise groups, but not in group D. The SI increased in the S and SE groups, the increase in the latter being significantly greater ($P<0.01$) (Table 1).

Good compliance with the diets was achieved over the 22-week diet intervention period (Table 3). All groups significantly reduced their energy intake: S group -2530±2497, E group -2266±1495, SE group -2057±2459, D group: -2497±2040 KJ ($P<0.001$), with no significant

differences between groups. At baseline, all groups had a diet "needing improvement" and at the end all had a significantly improved HEI index, the diet quality now being "good" (S: 66.8±12.7 to 81.14±8.9, *P*<0.001; E: 68.8±87.1 to 87.1±4.9, *P*<0.01; SE: 62.72±11.30 to 83.5±8.0, *P*<0.001; D: 62.8±10.8 to 83.2±11.3, *P*<0.001).

The reduction in the plasma leptin concentration was significant in the S and E groups and tended towards significance in the SE group (P=0.016); no change was seen in the D group (Table 2). The percentage change in leptin after the intervention was -27.2%, -37.5%, -32.6% and -2.5% for the S, E, SE and D groups respectively. A significant difference in the change in leptin concentration was observed between the S and D groups (-6.70±1.89 vs. -0.86±1.78 pg/mL, p=0.011) and between the E and D groups (-3.33±2.17 vs. -0.86±1.78 pg/mL, p=0.016) after the intervention. No significant difference in plasma leptin changes were seen among the three exercise groups (Table 2).

When taking the subjects of all four groups together, the plasma TNF-α and CRP concentrations decreased between baseline and the end of the intervention (4.48±2.06 vs. 4.18±1.94 pg/ml, *P*<0.05; and 3.19±3.94 vs. 2.85±4.44 mg/dl, *P*<0.05 respectively). No significant changes were seen, however, in plasma IL-6.

Linear regression analysis was used to determine the extent to which improvements in the AV, training variables and energy intake may have contributed to the changes in IM (Table 4). Changes in CPR were not significantly associated with any other variable. Changes in the leptin concentration were significantly associated with several variables. Multiple regression analysis was therefore performed to identify those with the greatest influence on this change. The model included five variables: change in TFM% baseline leptin concentration and training group (S, E or SE). BMI was the anthropometric variable with the greatest coefficient of determination (R^2=0.177), but change in TFM% was included in analyses since BMI is not a measure of body composition or fat distribution. Baseline leptin concentrations were included in

the model due to the baseline intergroup differences observed. The results showed that the change in TFM% (β=0.492; P=0.0001) plus the difference in baseline leptin concentration (β=-0.464; P=0.0001) explained 38.3% of the change in leptin concentrations (R^2=0.383, P=0.0001). The type of exercise regimen, however, had no influence on this change (S group, β=-0.172, P=0.159; E group, β=-0.209, P=0.077; SE group, β=-0.030, P=0.802).

DISCUSSION

The combination of the different exercise modalities plus a hypocaloric diet produced slight improvement in subjects' IM values. Groups whose regimen involved following an exercise program showed greater reductions in leptin concentrations, with no differences seen among these groups. Moreover, CRP levels tended towards a significant reduction with the E program.

Overweight individuals commonly have high plasma IM concentrations [30]. Indeed, the baseline IM values recorded in the present study were higher than those observed in the normal weight population by other authors [31], and similar to or higher than the values observed in the overweight population [32-34]. The most recent literature suggests that adipose tissue macrophage density increases with weight gain, reducing the production of anti-inflammatory adipokines and increasing the secretion of pro-inflammatory cytokines [35]. This chronic inflammatory situation contributes to the development of atherosclerosis, insulin resistance, tumour growth and neurodegeneration [3,14,35-38]. Knowledge of therapeutic strategies that might reduce inflammation are therefore important when deciding on the treatment of overweight/obesity and the prevention of its associated complications. Several studies have documented that weight loss in conjunction with energy restriction can improve IM concentrations [8-10]. However, the effect of exercise on IM is controversial and not well documented [13,17-20].

During exercise, IL-6 is produced by muscle fibres. IL-6 is normally associated with low grade inflammation, but under conditions of physical exercise it appears to stimulate the appearance in the blood of *anti*-inflammatory cytokines such as interleukin-1 receptor antagonist (IL-1ra) and IL-10, and to *inhibit* the production of proinflammatory cytokine TNF-α [14,16]. Typically, IL-6 is

the first cytokine present in the circulation during exercise, the concentration attained related to the intensity and duration of exercise, the muscular mass, and the subject's endurance capacity [14,16,36]. It declines in the post-exercise period. In the present work, no significant changes were observed in IL-6 between the beginning and end of the intervention period. It may be that more than 22 weeks are necessary for any change to be noticed, as suggested by Libardi et al. (2011) [39]. In other studies, times of 10 [12] or even 12 months [17] have been required. However, some studies with long intervention periods (1 year [32,40], 18 months [41]) report no alterations in IM.

Short-term (12-week) low to moderate intensity aerobic exercise has been reported not to change IL-6, TNF-α or CRP levels in obese women [42]. However, longer-term (7-month) training at higher intensity and frequency has been indicated to reduce body weight, body fat, CRP and TNF-α, and to increase adiponectin levels in obese young women [43]. In the present study, increasing the intensity and frequency of training intervention beyond the levels set was rejected due to the previous sedentary lifestyles of the subjects, and because of the increased the risk of injuries and dropout that this might entail.

No association was seen between changes in IL-6 (which were not significant) and that of any other variable, except for TNF-α: as IL-6 increased, so did TNF-α. (Table 3). The literature suggests, however, that IL-6 produced during exercise exerts an inhibitory effect on TNF-α. Concentrations of TNF-α are markedly elevated in anti-IL-6-treated mice and in IL-6-deficient knockout mice[14]. Further work is needed to determine the cause of this discrepancy.

At the end of the 22-week intervention period, no differences were seen between the different exercise groups in terms of TNF-α reduction. This may have been a consequence of the small sample size; more subjects may be required in each group for differences to be detected. However, other authors who have compared different exercise types have found no differences either [7,12]. Nonetheless, some studies employing similar methodology but using patients with diabetes, and of a wider age range, have reported just the opposite. For example, Balducci et al. (2010) observed a significant reduction in a group

subjected to a combination of supervised aerobic exercise and resistance training [17]. In contrast, in a smaller number of hospitalised patients with diabetes following a strict hypocaloric diet for 21 days, Lucotti et al. (2011) observed that while the members of a group assigned to endurance training experienced significant reductions (some 20%) in TNF-α, those assigned to combined strength + endurance training experienced an increase in this IM.

No association was found between the changes in TNF-α and any AV or training variable. The reduction in energy intake and the changes in IL-6 concentration (though not significant in themselves) were the only variables independently associated with changes in the TNF-α concentration.

No significant reduction in CRP levels was seen for any group. The great dispersion of the results may explain this. Other authors have reported conflicting information. For example, Jorge et al. (2011) reported reductions in subjects following strength, endurance, and strength + endurance programs [13]. Donges et al. (2010) and Kohut et al. (2006) reported reductions only in those groups undertaking endurance exercise [18,12], and Balducci et al. (2010) reported reductions only in groups assigned to combined strength + endurance training [17]. Both the dispersion observed in the present work, and the discrepancies in the results of other authors, might be due to the nonspecific nature of CRP as an IM [13]. Being an acute phase reactant, its circulating concentrations increase rapidly whenever a situation of inflammation, infection or immune dysfunction develops [44]. In fact, in the present work, no association was found between changes in CRP and any other variable.

Leptin is synthesized and secreted primarily by adipocytes, and the amount in plasma is proportional to that present in the adipose tissue. Its main role is to provide the central nervous system a signal of energy intake and of the energy stores in the body, thus

allowing the hypothalamus to keep the body weight stable [45,46]. After an energy-restricted diet, leptin concentrations fall in proportion with the amount of fat lost [47,48]. In the present work, leptin concentrations showed a significant decline in the S and E groups. Despite showing the greatest reductions in BMI and TFM%, the SE group showed no significant reduction in leptin (although it tended towards a significant reduction). It should be remembered that this group started with a considerably more favourable baseline leptin concentration; in fact it was significantly lower than that of the S group, which may have limited the identification of possible differences. The latter finding disagrees with that reported by other authors [47,48]. However, it agrees with that reported by Kondo et al. (2010) who examined eight obese subjects who performed strength exercises more than 30 min four to five times week for seven months, and in whom a significant reduction in circulating leptin of 25% was observed [47]. In the present study, the percentage reduction in leptin was higher in all exercise groups than in the D group (S group: 27.2%, E group: 37.5%, SE group: 32.6%). Fisher et al. (2010) and Lucotti et al. (2011) reported significantly reduced leptin levels in endurance training groups, while those of subjects who had undergone combined strength + endurance training experienced no such change [7,19]. In a study of longer duration, Balducci et al. (2010) reported that groups that undertook supervised endurance and combined endurance + strength exercises experienced significant reductions in leptin of 27% and 47% respectively. The discrepancies between the present results and those of the above authors may be associated with differences in the change in TFM%, the length of the intervention, the presence/absence of a dietary intervention, or the training methodology employed in each [17].

Finally, since improvements in AV, energy intake and training variables were seen, linear regression analysis was performed to identify the main variables associated with

changes in the serum leptin concentration. Changes in BMI, TFM%, AF%, undertaking a supervised exercise program (S, E or SE) and gender (see Table 3) were all found to be independent predictors of changes in the leptin level. Multiple regression attributed 38.3% of the change in leptin concentration to variation in the percentage of TFM%, plus the undertaking of supervised exercise, plus the baseline leptin concentration. However, within this model, neither undertaking such exercise nor the type of exercise undertaken (S, E or SE) had a significant effect on leptin concentration, accounting for just 5.9% and 4.4% of this change respectively. These results highlight the great interest in identifying other independent variables (e.g., other IM, visceral fat mass, cortisol and growth hormone concentrations, as well as other dietary variables not investigated in the present work) that might be associated with this change.

A point of interest of the present study is that it is the first to include normoglycaemic, young to middle-aged men and women. Other studies that have included different types of exercise have only looked at women, the elderly, and patients with diabetes. A limitation of the present study was the reduced number of subjects in each exercise group, a consequence of a larger number of withdrawals than anticipated. The sample size of the PRONAF study was selected to detect an effect of training and diet on TFM%, but, unfortunately, not to detect an effect on IM. This prevented determining which of the three exercise regimens had the greatest effect on serum IM concentrations. The results of the present study could, however, assist researchers in the calculation of appropriate sample sizes for future clinical trials focused on clarifying the effect of exercise and hypocaloric diet programs on IM.

The present study is of interest since the state of chronic inflammation that accompanies overweight and obesity, manifested as high concentrations of non-muscle-produced IL-6, TNF-α and CRP, contributes towards the development of atherosclerosis, insulin

resistance and other chronic diseases. A reduction in the concentrations of these markers might help prevent the appearance of these complications.

In conclusion, the present results show that strategies combining supervised physical exercise and a hypocaloric diet can provide benefits in terms of body composition and slight improvements in IM, especially in leptin, with no differences among the physical exercise program. Further studies with larger sample sizes are required to clarify the specific influence of different exercise types on IM concentrations.

STATEMENT OF AUTHORSHIP

The contributions of the authors to the manuscript are as follows. V.L-K: study design, data collection, data analysis and writing of the manuscript; C.F-F: study design and data collection; L.B, EM and BRM reviewing the manuscript; C.G-C: study design and reviewing the manuscript. All authors read and approved the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

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Figure 1: Participation flow diagram for the PRONAF study. Recruitment methods included all types of media advertisement. A total of 1568 candidates were screened, of whom 119 were randomised into the PRONAF study. The dropout rates in the groups were: endurance group (E) 16.6 %, strength group (S) 26.6 %, combined group (SE) 23.3%, and diet and physical activity recommendations group (D) 31.03 %.

Table 1. Anthropometric and training variables in each treatment group before and after the intervention (expressed as mean values ± (SD)).

	S n=19		**E** n=25		**SE** n=22		**D** n=18	
	Baseline	Final	Baseline	Final	Baseline	Final	Baseline	Final
Gender (male/female)	7/12		10/15		10/12		7/11	
Age (years)	36.46(8.9)		35.69 (8.07)		36.71 (6.99)		36.77 (9.24)	
BMI (kg/m^2)	29.51 (2.00) $^{a2^o}$	27.43 (2.08) †***	28.91 (1.78)	26.41 (2.04) †***	28.32 (1.54)	25.36 (1.84) †***	28.502 (1.29)	26.19 (1.91) †***
WC (cm)	97.03(6.54)	88.72 (7.94) †***	95.76(8.32)	88.05 (8.04) †***	95.51(7.23)	85.39 (6.545) †^{***b6**}	95.13 (6.08)	86.47(6.59) †***
TFM (%)	40.27 (6.76)	36.10 (7.77) †$^{***b3^o}$	39.89 (5.63)	35.32 (6.80) †$^{***b4^o}$	37.05 (6.00)	30.04 (7.68) †^{***b6**}	40.20 (5.90)	36.63 (3.26) †***
AF (%)	46.30 (6.57)	40.77 (8.59) †$^{***b2^o}$	44.82 (7.19)	38.19 (9.37) †***	43.11 (5.58)	32.56 (8.77) †$^{***b6^o}$	45.48 (7.41)	39.99 (7.83) †***
A/G (%)	1.10 (0.17)	1.06 (0.16)	1.07 (0.16)	1.02 (0.19) †**	1.16 (0.26)	1.07 (0.24) †***	1.06 (0.19)	1.01 (0.17) †^{o}
LM (kg)	46.78 (10.29)	46.82 (11.10) $^{b3^o}$	46.04 (8.71)	45.60 (8.61)	48.40 (9.14)	48.24 (9.51)$^{b6^o}$	46.44 (8.21)	45.46 (7.47) †^{o}
VO_{2peak} (ml/min/kg)	2476 (692)	2742 (921) †**	2473 (622)	2745 (655) †***	2768 (767)	3134 (888) †***	2746 (734)	2468 (664) †**
SI	0.99 (0.32)	1.20 (0.52) †^{**b2**}	-	-	1.14 (0.25)	1.59 (0.39) †***	-	-
DSI	3.20 (0.86)	3.73 (1.32) †^{*b3**}	3.36 (0.81)	3.72 (0.84) †*	3.74 (0.74)	4.25 (0.69) †***	3.27 (0.54)	3.55 (0.71)

S = Strength group; E = Endurance group; SE = Combined strength and endurance group; D = Diet and physical activity recommendations group. WC: waist circumference; TFM: Total Fat Mass; AF: Android Fat; A/G: Android/Gynoid ratio; LM: Lean mass.

VO_{2peak}: Peak oxygen uptake; SI: Strength Index; DSI: Dynamometric Strength Index.

[a] Intergroup baseline differences (^{a1} S-E; ^{a2} S-SE; ^{a3} S-C; ^{a4} E-SE) ; [b] Intergroup final differences (^{b1} S-E; ^{b2} S-SE; ^{b3} S-C; ^{b4} E-SE; ^{b5} E-C; ^{b6} SE-C); † Intragroup differences after 22 weeks of intervention. Significance of differences: ^{o} Trend towards significance: $P<0.029$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 2. Inflammatory marker concentrations in each treatment group before and after the intervention (expressed as median (IQR)).

	S n=19		**E** n=25		**SE** n=22		**D** n=18	
	Baseline	End	Baseline	End	Baseline	End	Baseline	End
TNF α (pg/ml)	4.96 (4.18-5.48)	4.44 (3.98-5.21)	3.60 (3.14-4.87)	3.31 (2.66-4.38)	4.68 (0.91-7.33)	4.41 (0.73-6.17)	4.51 (4.15-4.99)	4.47 (4.14-4.96)
Leptina (ng/ml)	27.78 (8.87-39.17) ^{a2**}	17.65 (7.48-32.28) †$^{***\ b3^{o}b5^{o}}$	21.20 (5.81-36.64)	5.81 (3.95-23.62) †***	5.07 (3.34-18.44)	3.67(1.64-11.30) †^{o}	16.46 (8.96-26.16)	13.86 (7.05-26.49)
CRP (mg/dl)	1.89 (0.69-3.62)	1.45 (0.79-3.17)	2.09 (1.00-5.07) ^{a4**}	1.02 (0.79-4.05) †^{o}	0.96 (0.79-2.08)	0.79 (0.79-2.01)	2.45 (1.14-5.61)	1.28 (0.79-4.12)
IL-6 (pg/ml)	2.60 (2.28-3.75) ^{a1**}	2.70 (1.97-4.90)	4.89 (3.42-7.89) ^{a4***}	4.44 (3.32-5.34)	2.45 (0.13-3.65) 2.45 (0.13-3.65)	2.11 (0.11-3.67)	2.29 (1.80-2.83)	2.02 (1.78-2.3)

S group = Strength group; E group = Endurance group; SE group = Combined strength + endurance group; D group = Diet and physical activity recommendations group.

IQR: interquartile range; TNF α: Tumor necrosis factor; IL-6: Interleukin 6; CRP: C-reactive protein.

^{a} Intergroup baseline differences (^{a1} S-E; ^{a2} S-SE; ^{a3} S-C; ^{a4} E-SE) ; ^{b} Intergroup final differences (^{b1} S-E; ^{b2} S-SE; ^{b3} S-C; ^{b4} E-SE; ^{b5} E-C; ^{b6} SE-C); † Intragroup differences after 22 weeks of intervention. Significance of differences: ^{o} Trend towards significance: $P<0.029$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 4. Dietetic parameters in each treatment group before and after the intervention (expressed as means ± (SD)).

	S n=22		**E** n=25		**SE** n=23		**D** n=19	
	Before	After	Before	After	Before	After	Before	After
Carbohydrates (%)	40.53 (7.20)	49.13 (7.27) †***	37.57 (5.03)	49.97 (7.17) †***	37.99 (6.98)	46.25 (3.93) †***	36.45 (8.06)	44.43 (4.84) †***
Proteins (%)	20.49 (4.12)	18.40 (2.36)	20.51(3.58)	18.94 (2.91)	20.51 (3.84)	18.51 (2.17)	22.18 (9.75)	19.74 (3.09)
Fat (%)	38.24 (6.11)	32.45 (6.17) †**	41.92 (4.87)	31.08 (0.81) †**	41.49 (6.75)	35.22 (3.56) †***	41.35 (6.69)	35.81 (5.24) †***
SAF (%)	12.10 (2.66)	9.76 (2.42)	13.61 (2.60)	10.14 (3.58) †***	14.14 (3.11)	9.54 (1.87) †***	14.03 (3.23)	10.19 (2.39) †***
HEI	66.78 (12.65)	66.90 (5.37)	68.78 (14.28)	87.14 (4.87) †**	62.72 (11.30)	83.51 (8.02) †***	62.76 (10.76)	83.24 (11.29) †***

S group = Strength group; E group = Endurance group; SE group = Combined strength + endurance group; D group = Diet and physical activity recommendations group. SAF: saturated fatty acids. HEI: Healthy Eating Index.

† Intragroup differences after 22 weeks of intervention. Significance of differences: ** $P<0.01$; *** $P<0.001$

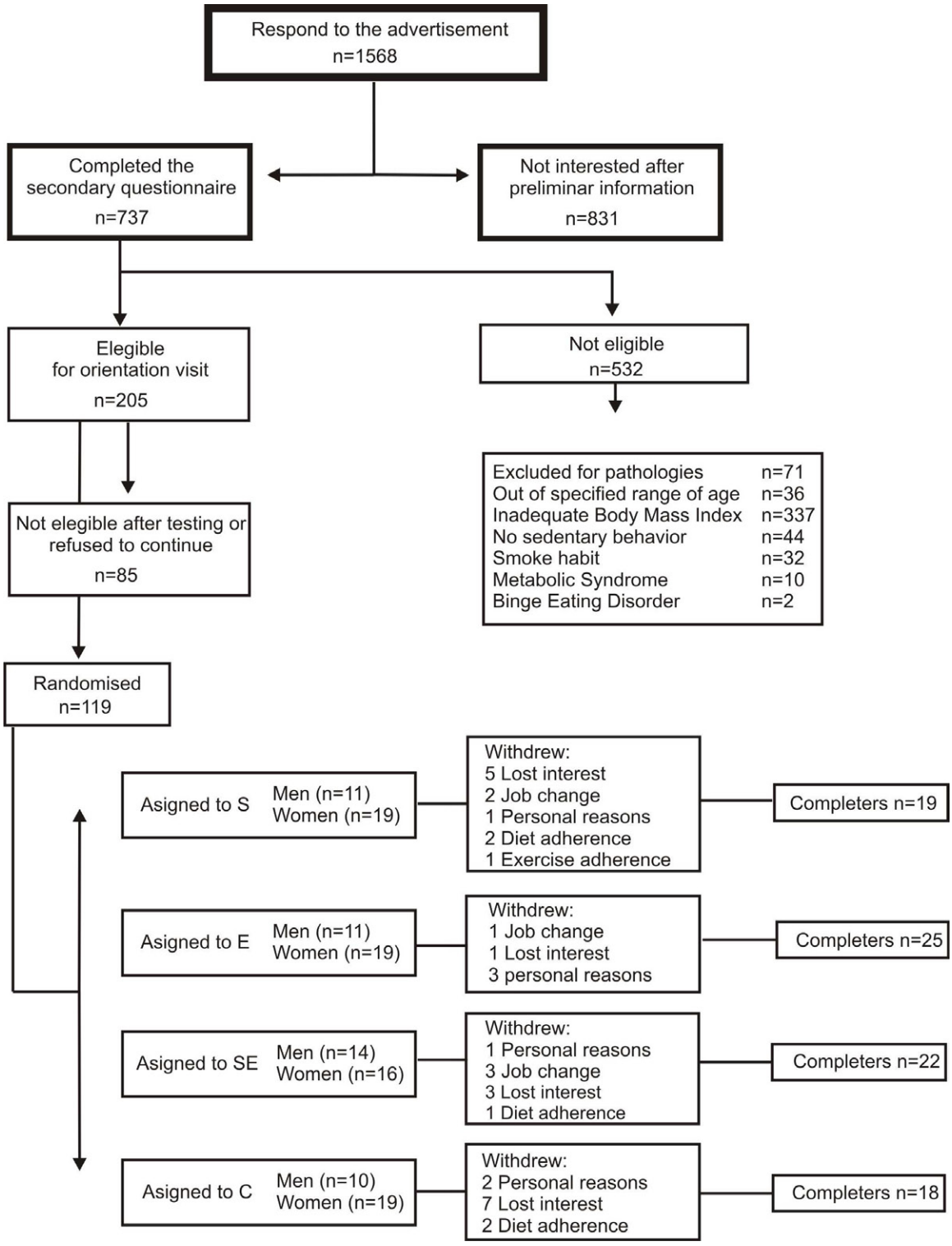
Table 3. Regression analysis of change in body composition, training variables and energy intake over the treatment period as predictors of changes in inflammation markers.

Inflammation marker	Variable	R^2	B	95% confidence interval	Standard β-coefficient	*P* value
Δ Leptin	Δ BMI (kg/m^2)	0.177	2.60 (0.64)	1.31-3.88	0.420	0.000
	Δ WC (cm)	0.093	0.60 (0.21)	0.17-1.03	0.305	0.007
	Δ TFM (%)	0.092	0.81 (0.29)	0.22-1.39	0.303	0.007
	Δ AF (%)	0.069	0.39 (0.16)	0.06-0.72	0.263	0.021
	Supervised exercise program (S + E+ SE)	0.061	-5.45 (2.42)	-10.29-(-0.62)	-0.247	0.027
	Gender (male/female)	0.127	6.66 (1.97)	2.72-10.60	0.357	0.001
Δ TNFα	Δ Energy (KJ/day)	0.060	0.001(0.00)	0.00-0.01	0.244	0.045
Δ IL-6	Δ TNFα (pg/ml)	0.415	1.00 (0.135)	0.77-1.23	0.644	0.000

Δ= Final value – Baseline value.

S group = Strength group; E group = Endurance group; SE group = Combined strength + endurance group

BMI: body mass index; WC: waist circumference; TFM: Total Fat Mass; AF: Android Fat


Respond to the advertisement
n=1568
Completed the secondary questionnaire
n=737
Not interested after preliminar information
n=831
Elegible for orientation visit
n=205
Not eligible
n=532
Excluded for pathologies n=71
Out of specified range of age n=36
Inadequate Body Mass Index n=337
No sedentary behavior n=44
Smoke habit n=32
Metabolic Syndrome n=10
Binge Eating Disorder n=2
Not elegible after testing or refused to continue
n=85
Randomised
n=119
Asigned to S Men (n=11) Women (n=19)
Withdrew:
5 Lost interest
2 Job change
1 Personal reasons
2 Diet adherence
1 Exercise adherence
Completers n=19
Asigned to E Men (n=11) Women (n=19)
Withdrew:
1 Job change
1 Lost interest
3 personal reasons
Completers n=25
Asigned to SE Men (n=14) Women (n=16)
Withdrew:
1 Personal reasons
3 Job change
3 Lost interest
1 Diet adherence
Completers n=22
Asigned to C Men (n=10) Women (n=19)
Withdrew:
2 Personal reasons
7 Lost interest
2 Diet adherence
Completers n=18