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

Viviana Loria-Kohen, Ceila Fernández-Fernández, Laura M. Bermejo, Esther Morencos, Blanca Romero-Moraleda, Carmen Gómez-Candela

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1 **Effect of different exercise modalities plus a hypocaloric diet on inflammation markers in**

2 **overweight patients: a randomized trial.**

3 Viviana Loria-Kohen[1], Ceila Fernández-Fernández[1], Laura M Bermejo[1], Esther Morencos[2],

4 Blanca Romero-Moraleda[2] and Carmen Gómez-Candela[1], on behalf of the PRONAF Study

5 group.

6 [1]Nutrition Department, La Paz University Hospital, Health Research Institute IdiPAZ, Madrid,

7 Spain.

8 [2]Department of Health and Human Performance, Faculty of Physical Activity and Sport

9 Sciences, Universidad Politécnica de Madrid, Madrid, Spain.

10

11 Viviana Loria-Kohen

12 Nutrition Department, La Paz University Hospital, Health Research Institute IdiPAZ, Paseo de

13 la Castellana 268, 28043 Madrid, Spain. Tel: +34 91.727.7203 Fax: +34 91.727.70.50.

14 vloria@hotmail.com

15 Ceila Fernández-Fernández

16 Nutrition Department, La Paz University Hospital, Health Research Institute IdiPAZ, Paseo de

17 la Castellana 268, 28043 Madrid, Spain. Tel: +34 91.727.7203 Fax: +34 91.727.70.50.

18 ceila@escadem.com

19 Laura M Bermejo

20 Nutrition Department, La Paz University Hospital, Health Research Institute IdiPAZ, Paseo de

21 la Castellana 268, 28043 Madrid, Spain. Tel: +34 91.727.70.00 ext. 42199fax: +34

22 91.727.70.50. lbermejol.hulp@madrid.salud.org

23 Esther Morencos

24 Department of Health and Human Performance, Faculty of Physical Activity and Sport

25 Sciences, C/ Martín Fierro 7, 28040 Madrid, Spain. Tel: +34 91.336.4070 Fax: +034 91. 336.

26 4126.

27 esther.morencos@upm.es

28   Blanca Romero Moraleda

29   Department of Health and Human Performance, Faculty of Physical Activity and Sport

30   Sciences, C/ Martín Fierro 7, 28040 Madrid, Spain. Tel: +34 91. 336. 4070 Fax: +34

31   91.336.4126.

32   blanca.romero.moraleda@upm.es

33   Carmen Gómez-Candela

34   Nutrition Department, La Paz University Hospital, Health Research Institute IdiPAZ, Paseo de

35   la Castellana 268, 28043 Madrid, Spain. Tel: +34 91.727.7203 Fax: +34 91.727.70.50.

36   cgomez.hulp@madrid.salud.org.

37

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

39

40   **Non-standard abbreviations:**

41   S: Strength training group

42   E: Endurance training group

43   SE: Strength + Endurance combined training group

44   D: Diet and physical activity recommendations group

45   VO_{2peak}: Peak oxygen uptake

46   DSI: Dynamometric Strength Index

47   SI: Strength Index

48   AV: Anthropometric Variables

49   TFM: Total Fat Mass

50   AF: Android Fat

51   AF/GF: Android/Gynoid fat ratio

52   LM: Lean Mass.

53

54   **Address for correspondence:**

55   Laura M Bermejo:

56   Paseo de la Castellana 268. 28043. Madrid, Spain.

57   Tel: +34 91.727.7203

58   Fax: +34 91.727.70.50.

59   E-mail: laura.bermejol@salud.madrid.org.

60

61 **ABSTRACT**

62 BACKGROUND & AIMS: Inflammation markers (IM) have been associated with the

63 development of chronic diseases. This study compares the effects on IM of three exercise

64 programs combined with a hypocaloric diet.

65 METHODS: 119 overweight participants (73 women, 46 men) aged 18–50 years were

66 randomised into four treatment groups: strength training (S; n=30), endurance training (E;

67 n=30), combined S + E (SE; n=30), and a diet and physical activity recommendations group (D;

68 n=29). Energy intake, anthropometric variables (AV), training variables (VO_{2peak}, strength

69 index, dynamometric strength index [DSI]) and plasma IM were recorded at baseline and after

70 22 weeks of treatment.

71 RESULTS: 84 participants completed the study. At 22 weeks, all groups showed a significantly

72 reduced energy intake ($P<0.001$) and improved AV ($P<0.001$). VO_{2peak} significantly increased

73 in all groups ($P<0.01$). DSI increased in the exercise groups only ($P<0.05$). Plasma leptin fell

74 significantly ($P<0.001$) in the S and E groups, but not significantly in the SE group ($P=0.029$)

75 (no significant differences between these groups). Tumour necrosis factor-α (TNF-α), and C-

76 reactive protein (CRP) concentrations decreased in all groups when examined together, but not

77 when examined separately. No significant differences were seen in interleukin-6 (IL-6).

78 CONCLUSIONS: Combining strength or endurance training with a hypocaloric diet improved

79 AV and reduced plasma leptin concentrations. No differences were seen between groups in

80 terms of TNF-α, IL-6 or CRP reduction.

81

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

83

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

85

86 **INTRODUCTION**

87 The majority of epidemiological studies indicate excess body weight during midlife, including

88 overweight, to be associated with an increased risk of death[1-3]. For example, people with a BMI

89 of 25-28.9 have a relative risk of developing cardiovascular disease twice that of people with a

90 BMI of <21[4], while those with a BMI of ≥29 are at almost three times the risk. Further, the

91 results of the Framingham Heart Study show that being overweight at age 40 reduces life

92 expectancy by three years[5]. Given the increasing prevalence of obesity, finding more efficient

93 treatments for overweight should be seen as a public health priority.

94 Low-grade chronic inflammation is one of the key metabolic alterations linked to excessive

95 energy intake, physical inactivity and adiposity, and the markers of this inflammation - tumour

96 necrosis factor-α (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP) - have all been

97 associated with the development of atherosclerosis and insulin resistance [6,7]. Several studies

98 have shown that inflammation markers (IM) are reduced following weight loss [8-10]. Physical

99 exercise may therefore be effective in reducing inflammation. Indeed, data from observational

100 and intervention studies show that greater physical activity is associated with lower plasma IM

101 concentrations [10-13], and regular exercise and an appropriate diet are reported to protect against

102 all-cause mortality. This is achieved primarily through protection against atherosclerosis, type 2

103 diabetes, colon cancer and breast cancer [14-16].

104 The mechanism that underlies the anti-inflammatory response associated with acute exercise

105 might, surprisingly, involve an *increase* in the circulating concentration of IL-6. Under exercise

106 conditions, this cytokine appears to induce an anti-inflammatory environment by promoting the

107 production of IL-1ra and IL-10, and by inhibiting TNF-α production [14-16]. Exercise also

108 increases the release of epinephrine, cortisol, growth hormone, prolactin and other factors that

109 have immunomodulatory effects [16].

110 A few studies have tried to determine the types of exercise intervention that produce the greatest

111 changes in IM concentrations [13,17-20], but the results have been controversial. A recent review

112   indicates that increasing aerobic physical activity may be effective for reducing chronic

113   inflammation especially in individuals with chronic diseases associated with a state of elevated

114   inflammation. (Beavers et al., 2010). The aim of the present study was to compare the effects on

115   plasma IM concentrations of three different exercise programs - strength, endurance, and

116   combined strength + endurance training - all in conjunction with a hypocaloric diet, and the

117   normal clinical practice of achieving weight loss using the same hypocaloric diet as above, plus

118   the provision of recommendations regarding physical activity.

119

120   **MATERIALS AND METHODS**

121

122   *Study subjects*

123   This study was performed as part of the larger study *Nutrition and Physical Activity for Obesity*

124   (the PRONAF study according to its Spanish initials), the aim of which was to assess the

125   usefulness of different types of physical activity and nutrition programs for the treatment of

126   obesity. Participants were sought via advertisements posted in newspapers and announced on

127   the radio, the internet and TV. The eligible sample population consisted of 119 overweight

128   subjects (73 women and 46 men; age range 18–50 years; body mass index [BMI] ≥25–<30

129   kg/m^2) living in the Region of Madrid, Spain. Eighty four participants completed the study (50

130   women and 34 men) (Figure 1). All subjects were healthy adults with no history of relevant

131   concomitant illness, such as heart, lung or liver disease, or neoplasia. All were normoglycaemic

132   non-smokers and took no medications or drugs, but led sedentary lifestyles. All female subjects

133   had regular menstrual cycles. The exclusion criteria covered all physical and psychological

134   diseases that may have precluded the performance of the requested strength or endurance

135   training, along with the taking of any medication known to influence physical performance or

136   that might interfere with the interpretation of the results. Subjects with a background of

137   systematic strength or endurance training (moderate to high intensity training more than once a

138   week) in the year before the study started were also excluded. In agreement with the guidelines

139 of the Declaration of Helsinki regarding research on human subjects, all participants signed an

140 institutionally approved document of informed consent. All subjects were carefully informed

141 about the possible risks and benefits of the study, which was approved by the Human Research

142 Review Committee of the La Paz University Hospital (PI-643).

143 *Study design*

144 Subjects who fulfilled the inclusion criteria and passed a baseline physical examination were

145 stratified by age and sex and assigned (using a randomisation table) to a strength training group

146 (S), endurance training group (E), combined strength + endurance training group (SE), or diet

147 and physical activity recommendations group (D).

148 This study design was that of an intervention trial of 22 weeks duration. Baseline measurements

149 for all subjects were made before starting the intervention period. The final measurements were

150 taken once the intervention period was over (within 48-72 h of the last training session for the

151 exercise groups).

152 *Exercise training programs*

153 The different exercise groups followed their corresponding training programs, which in all cases

154 involved training 3 times/week for 22 weeks. All training sessions were carefully supervised by

155 certified personal trainers. An adherence to training of 90% was demanded.

156 The S group followed a circuit involving the following eight exercises: shoulder press, squat,

157 barbell row, lateral split, bench press, front split, biceps curl, and French press for triceps. E

158 group training involved the use of an exercise bike or cross trainer. The SE group performed a

159 combination of cycle ergometry, treadmill or cross trainer work, plus weight training with the

160 following exercises intercalated between lift sets (15 lifts per set): squat, rowing machine, bench

161 press and front split. The D group subjects followed the hospital's habitual clinical practice for

162 achieving weight loss, i.e., the same dietary intervention as the training groups plus being made

163   aware of the general recommendations of the American College of Sports Medicine (ACSM)

164   regarding physical activity [22].

165   All subjects were instructed to keep their daily physical activity habits unchanged. These habits

166   were carefully checked with a dairy registry by personal trainers in all training sessions to the

167   groups S, E and SE. Group D subjects were not supervised, although they were subjected to

168   activity monitoring using an armband accelerometer, just as they would be in normal practice.

169   The exercise programs were designed taking into account each subject's muscular strength (MS)

170   and heart rate reserve (HRR). MS was measured in the strength program subjects (S, SE)

171   using the 15-repetition maximum (15 RM) testing method [21] every other day during the week

172   before the intervention period. The intraclass correlation coefficient of reliability for all

173   exercises was ICCr=0.995 for the men and ICCr=0.994 for the women (groups S and SE

174   subjects together). The HRR was calculated to set the exercise intensity ([maximum heart rate-

175   resting heart rate] x 50% to 60%) and resting heart rate for the E and SE programs.

176   The intensity of exercise was increased over the study period. In weeks 2-5, exercise was at an

177   intensity of 50% of the 15RM and HRR, and lasted an overall 51 min and 15 s (twice around the

178   circuit, lasting 7 min 45 s each lap). In weeks 6-14, exercise was performed at an intensity of

179   60% of 15RM and HRR, again with a duration of 51 min and 15 s (again, twice around the

180   circuit). Finally, in weeks 15-23, exercise was performed at an intensity of 60% of 15RM and

181   HRR, with a duration of 64 min (three times around the circuit). The recovery period between

182   circuits was set at 5 min. Participants performed 15 repetitions (45 s) of each exercise with a rest

183   period of 15 s between them. Each training session for the S, E and SE subjects commenced

184   with a 5 min aerobic warm-up, followed by the main session exercises, and concluded with 5

185   min of cooling down and stretching exercises. In all sessions the exercise rhythm was controlled

186   by instructions recorded on a compact disk. The cadence for the resistance exercises was fixed

187   at 1:2 (concentric-eccentric phase).

188   *Hypocaloric diet program*

189 Hypocaloric diets (between 5028 and 12570 KJ) were prescribed individually for all

190 participants by expert dieticians at the Department of Nutrition, La Paz University Hospital,

191 Madrid. The diet was designed to provide 25% less energy than the baseline daily energy

192 expenditure (DEE), as measured using a SenseWear Pro Armband™ (Body Media, USA).

193 Some 29–34% of energy came from fat, 12–18% from protein, and 50–55% from

194 carbohydrates, according to the recommendations of the Spanish Society of Community

195 Nutrition (SENC, according to its Spanish initials) [23]. The hypocaloric diet program was

196 followed during the 22-week interventional period. Dietary counselling was given at baseline

197 and at 12 weeks to resolve questions and to motivate participants sufficiently to comply with

198 dietary advice. All subjects were instructed on how to record their dietary intake using a daily

199 log, and given recommended portion sizes and information on possible food swaps. In addition,

200 voluntary group nutrition education sessions were given by the dieticians. The goal was to equip

201 the participants with the knowledge and skills necessary to achieve gradual but permanent

202 behavioural changes.

203

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

205 ***variables***

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

207 period.

208 - *Blood analysis:* Blood samples were taken early in the morning at the La Paz University

209 Hospital Extraction Unit after a 12 h overnight fast. Samples were kept at 4-6ºC until analysis,

210 which was always performed within 48 h. Plasma C-reactive protein concentrations (CRP) were

211 determined using a BNII nephelometer (Siemens Healthcare Diagnostics GmbH, Eschborn,

212 Germany). Plasma leptin, IL-6 and TNF-α concentrations were determined using a Luminex®-

213 LX200 Analyzer (Millipore Corp, Billerica, Massachusetts, USA) and a MILLIPLEX MAP

214 human circulating cancer biomarker magnetic bead panel (HCCBP1MAG-58K) (Millipore, St.

215 Charles, Missouri, USA). All samples were analysed in duplicate. Data were analysed using

216   xPONENT v.3.1 software (Millipore). The intra- and interassay coefficients of variation for the

217   cytokine assays all fell in the 5-10% range.

218   - *Training variables:* Peak oxygen uptake (VO_{2peak}) was measured using the protocol described

219   by Bruce[24]. The test was conducted on an H/P/COSMOS 3P 4.0 computerised treadmill

220   (H/P/Cosmos Sports & Medical, Nussdorf-Traunstein, Germany). The volume and composition

221   of expired gas were measured using a Jaeger Oxycon Pro gas analyser (Erich Jaeger, Viasys

222   Healthcare, Germany) The general strength index (SI) was calculated following the method of

223   Jurca et al. (2004)[25]. This method measures the strength of the leg and arm with respect to body

224   weight via two exercises: the bench press and squat. The dynamometric strength index (DSI)

225   was determined by measuring muscular strength using a Tecsymp Tkk5002 hand and leg

226   dynamometer (Tecsymp, Barcelona, Spain) and a Tecsymp Tkk5401 back dynamometer

227   (Tecsymp, Barcelona, Spain). The DSI value was calculated as the sum of the values obtained

228   with both apparatuses divided by subject body weight.

229   - *Dietetic study*: All food and beverages consumed by the participants were recorded using a

230   food frequency questionnaire and a "3-day food and drink record", validated for the Spanish

231   population [26], at the beginning and end of the intervention. Participants were instructed to record

232   the weights of food consumed whenever possible, and to use household measurements

233   (tablespoons, cups, etc.) when not. The energy and nutritional content of the foods consumed

234   were then calculated using DIAL software (Alce Ingeniería, 2004). The Healthy Eating Index

235   (HEI) was calculated according to Kennedy et al., [27] taking into account the number of servings

236   recommended for the Spanish population[28]. Compliance with recommended intakes was

237   assessed for the different food groups (cereals, vegetables/greens, fruits, dairy products, meat/

238   fish/eggs, expressed in servings per day), and also from the point of view of meeting nutritional

239   objectives (intake of lipids, saturated fatty acid, cholesterol and sodium, and dietary variety).

240   Each of these 10 factors was awarded a maximum of 10 points when the intake was the same as

241   that recommended, and a minimum of 0 points when the difference was very great. Intermediate

242   values were awarded proportionally [27]. Diet quality was deemed "'good" when more than 80

243　total points were scored, as "needing improvement" when the score was 51-80, and "poor" when

244　below 51 [27].

245　- *Anthropometric variables (AV)*:　Height was measured using a SECA stadiometer (range 80-

246　200 cm).　Body weight was measured using a TANITA BC-420MA balance (Bio Lógica

247　Tecnología Médica S.L, Barcelona, Spain).　The BMI was calculated as [body weight

248　$(kg)/(height (m))^2$]. The waist circumference (WC) was measured using a Seca 201 steel tape

249　(Quirumed, Valencia, Spain). Dual-energy x-ray absorptiometry (DXA) was used to measure

250　the percentage total fat mass (TFM%), percentage android fat (AF%), the android/gynoid fat

251　ratio (AF/GF), and the lean mass (kg), employing a GE Lunar Prodigy apparatus (GE

252　Healthcare, Madison, Wisconsin, USA).　All DXA scans were performed making use of GE

253　Encore 2002 software v. 6.10.029.

254　Percentage changes from baseline were calculated for the studied variables as: (final value -

255　baseline value/ baseline value) X 100.

256

257　*Statistical analysis*

258　Means and standard deviations (SD) were calculated for normally distributed continuous

259　variables, and medians and interquartile ranges (IQR) for non-normally distributed continuous

260　variables. The Kolmogorov-Smirnov test was used to determine whether or not the data were

261　normally distributed among groups. For variables that were not normally distributed, differences

262　between the four groups at baseline and after the intervention were compared using the Kruskal-

263　Wallis test. Adopting a closed testing procedure, *post hoc* pairwise comparisons were performed

264　using the Mann-Whitney test if the results of the Kruskal-Wallis test showed significant

265　differences. For the variables that were normally distributed, differences between groups were

266　compared by one way ANOVA plus Bonferroni's *post hoc* adjustment. The change within each

267　group was determined using the Wilcoxon test or paired Student t test depending on the data

268　distribution. Significance was generally set at $P<0.05$; however, Bonferroni adjustments were

269　used to take into account multiple comparisons. Thus, the *P* values for intergroup comparisons

270　were considered significant when $P<0.0083$ (0.05 divided by six possible comparisons for each

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

272   group changes).

273   Linear and multiple regression analyses were performed to determine the potential role of

274   changes in AV, energy intake and training variables as predictors of change in IM. Dummy

275   variables were used to represent the S, SE and E groups in the multiple regression analysis.

276   The sample size of the PRONAF study was calculated to detect any effect of training and diet

277   on TFM% (with 80% statistical power, with significance set at $P<0.05$, assuming a correlation

278   of 0.80 between repeated measures, [29] and assuming an estimated drop out of 20%); it was not

279   explicitly determined to analyse the influence on IM.

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

281

282   **RESULTS**

283   All groups showed similar baseline characteristics except for the leptin concentration which

284   differed between the S and SE group, the IL-6 concentration which differed between the S and

285   E subjects, and between the E group and SE group, the CRP concentration which differed

286   between the E and SE groups, and the BMI which differed between the S and SE groups

287   (P=0.011) (Table 1).

288   Tables 1 and Table 2 show, by group, the changes in body composition and inflammatory and

289   training variable values after the intervention. Significant reductions were seen in the BMI, WC,

290   TFM and AF in all groups. Lean mass did not change in any of the exercise groups but showed

291   a trend towards a significant reduction in group D (P=0.029). The change in TFM% from

292   baseline was -11.6%, -11.5%, -19.4% and -9.7% in the S, E, SE and D group respectively.

293   VO_{2peak} significantly increased in all groups. DSI increased in the three exercise groups, but not

294   in group D. The SI increased in the S and SE groups, the increase in the latter being

295   significantly greater ($P<0.01$) (Table 1).

296   Good compliance with the diets was achieved over the 22-week diet intervention period (Table

297   3). All groups significantly reduced their energy intake: S group -2530±2497, E group -

298   2266±1495, SE group -2057±2459, D group: -2497±2040 KJ (P<0.001), with no significant

299 differences between groups. At baseline, all groups had a diet "needing improvement" and at the

300 end all had a significantly improved HEI index, the diet quality now being "good" (S: 66.8±12.7

301 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,

302 *P*<0.001; D: 62.8±10.8 to 83.2±11.3, *P*<0.001).

303 The reduction in the plasma leptin concentration was significant in the S and E groups

304 and tended towards significance in the SE group (P=0.016); no change was seen in the

305 D group (Table 2). The percentage change in leptin after the intervention was -27.2%, -

306 37.5%, -32.6% and -2.5% for the S, E, SE and D groups respectively. A significant

307 difference in the change in leptin concentration was observed between the S and D

308 groups (-6.70±1.89 vs. -0.86±1.78 pg/mL, p=0.011) and between the E and D groups (-

309 3.33±2.17 vs. -0.86±1.78 pg/mL, p=0.016) after the intervention. No significant

310 difference in plasma leptin changes were seen among the three exercise groups (Table

311 2).

312 When taking the subjects of all four groups together, the plasma TNF-α and CRP

313 concentrations decreased between baseline and the end of the intervention (4.48±2.06

314 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).

315 No significant changes were seen, however, in plasma IL-6.

316 Linear regression analysis was used to determine the extent to which improvements in the AV,

317 training variables and energy intake may have contributed to the changes in IM (Table 4).

318 Changes in CPR were not significantly associated with any other variable. Changes in the

319 leptin concentration were significantly associated with several variables. Multiple regression

320 analysis was therefore performed to identify those with the greatest influence on this change.

321 The model included five variables: change in TFM% baseline leptin concentration and training

322 group (S, E or SE). BMI was the anthropometric variable with the greatest coefficient of

323 determination (R^2=0.177), but change in TFM% was included in analyses since BMI is not a

324 measure of body composition or fat distribution. Baseline leptin concentrations were included in

325   the model due to the baseline intergroup differences observed. The results showed that the

326   change in TFM% (β=0.492; P=0.0001) plus the difference in baseline leptin concentration (β=-

327   0.464; P=0.0001) explained 38.3% of the change in leptin concentrations (R^2=0.383,

328   P=0.0001). The type of exercise regimen, however, had no influence on this change (S group,

329   β=-0.172, P=0.159; E group, β=-0.209, P=0.077; SE group, β=-0.030, P=0.802).

330

331   **DISCUSSION**

332   The combination of the different exercise modalities plus a hypocaloric diet produced slight

333   improvement in subjects' IM values. Groups whose regimen involved following an exercise

334   program showed greater reductions in leptin concentrations, with no differences seen among

335   these groups. Moreover, CRP levels tended towards a significant reduction with the E program.

336   Overweight individuals commonly have high plasma IM concentrations [30]. Indeed, the baseline

337   IM values recorded in the present study were higher than those observed in the normal weight

338   population by other authors [31], and similar to or higher than the values observed in the

339   overweight population [32-34]. The most recent literature suggests that adipose tissue macrophage

340   density increases with weight gain, reducing the production of anti-inflammatory adipokines

341   and increasing the secretion of pro-inflammatory cytokines [35]. This chronic inflammatory

342   situation contributes to the development of atherosclerosis, insulin resistance, tumour growth

343   and neurodegeneration [3,14,35-38]. Knowledge of therapeutic strategies that might reduce

344   inflammation are therefore important when deciding on the treatment of overweight/obesity and

345   the prevention of its associated complications. Several studies have documented that weight

346   loss in conjunction with energy restriction can improve IM concentrations [8-10]. However, the

347   effect of exercise on IM is controversial and not well documented [13,17-20].

348   During exercise, IL-6 is produced by muscle fibres. IL-6 is normally associated with low grade

349   inflammation, but under conditions of physical exercise it appears to stimulate the appearance in

350   the blood of *anti*-inflammatory cytokines such as interleukin-1 receptor antagonist (IL-1ra) and

351   IL-10, and to *inhibit* the production of proinflammatory cytokine TNF-α [14,16]. Typically, IL-6 is

352 the first cytokine present in the circulation during exercise, the concentration attained related to

353 the intensity and duration of exercise, the muscular mass, and the subject's endurance capacity

354 [14,16,36]. It declines in the post-exercise period. In the present work, no significant changes were

355 observed in IL-6 between the beginning and end of the intervention period. It may be that more

356 than 22 weeks are necessary for any change to be noticed, as suggested by Libardi et al. (2011)

357 [39]. In other studies, times of 10 [12] or even 12 months [17] have been required. However, some

358 studies with long intervention periods (1 year [32,40], 18 months [41]) report no alterations in IM.

359 Short-term (12-week) low to moderate intensity aerobic exercise has been reported not

360 to change IL-6, TNF-α or CRP levels in obese women [42]. However, longer-term (7-

361 month) training at higher intensity and frequency has been indicated to reduce body

362 weight, body fat, CRP and TNF-α, and to increase adiponectin levels in obese young

363 women [43]. In the present study, increasing the intensity and frequency of training intervention

364 beyond the levels set was rejected due to the previous sedentary lifestyles of the subjects, and

365 because of the increased the risk of injuries and dropout that this might entail.

366 No association was seen between changes in IL-6 (which were not significant) and that of any

367 other variable, except for TNF-α: as IL-6 increased, so did TNF-α.   (Table 3). The literature

368 suggests, however, that IL-6 produced during exercise exerts an inhibitory effect on TNF-α.

369 Concentrations of TNF-α are markedly elevated in anti-IL-6-treated mice and in IL-6-deficient

370 knockout mice[14]. Further work is needed to determine the cause of this discrepancy.

371 At the end of the 22-week intervention period, no differences were seen between the different

372 exercise groups in terms of TNF-α reduction. This may have been a consequence of the small

373 sample size; more subjects may be required in each group for differences to be detected.

374 However, other authors who have compared different exercise types have found no

375 differences either [7,12]. Nonetheless, some studies employing similar methodology but

376 using patients with diabetes, and of a wider age range, have reported just the opposite.

377 For example, Balducci et al. (2010) observed a significant reduction in a group

378   subjected to a combination of supervised aerobic exercise and resistance training [17]. In

379   contrast, in a smaller number of hospitalised patients with diabetes following a strict

380   hypocaloric diet for 21 days, Lucotti et al. (2011) observed that while the members of a

381   group assigned to endurance training experienced significant reductions (some 20%) in

382   TNF-α, those assigned to combined strength + endurance training experienced an increase in

383   this IM.

384   No association was found between the changes in TNF-α and any AV or training

385   variable. The reduction in energy intake and the changes in IL-6 concentration (though

386   not significant in themselves) were the only variables independently associated with

387   changes in the TNF-α concentration.

388   No significant reduction in CRP levels was seen for any group. The great dispersion of

389   the results may explain this. Other authors have reported conflicting information. For

390   example, Jorge et al. (2011) reported reductions in subjects following strength,

391   endurance, and strength + endurance programs [13]. Donges et al. (2010) and Kohut et al.

392   (2006) reported reductions only in those groups undertaking endurance exercise [18,12],

393   and Balducci et al. (2010) reported reductions only in groups assigned to combined

394   strength + endurance training [17]. Both the dispersion observed in the present work, and

395   the discrepancies in the results of other authors, might be due to the nonspecific nature

396   of CRP as an IM [13]. Being an acute phase reactant, its circulating concentrations

397   increase rapidly whenever a situation of inflammation, infection or immune dysfunction

398   develops [44]. In fact, in the present work, no association was found between changes in

399   CRP and any other variable.

400   Leptin is synthesized and secreted primarily by adipocytes, and the amount in plasma is

401   proportional to that present in the adipose tissue. Its main role is to provide the central

402   nervous system a signal of energy intake and of the energy stores in the body, thus

403   allowing the hypothalamus to keep the body weight stable [45,46]. After an energy-

404   restricted diet, leptin concentrations fall in proportion with the amount of fat lost [47,48]. In

405   the present work, leptin concentrations showed a significant decline in the S and E

406   groups. Despite showing the greatest reductions in BMI and TFM%, the SE group

407   showed no significant reduction in leptin (although it tended towards a significant

408   reduction). It should be remembered that this group started with a considerably more

409   favourable baseline leptin concentration; in fact it was significantly lower than that of

410   the S group, which may have limited the identification of possible differences. The

411   latter finding disagrees with that reported by other authors [47,48]. However, it agrees with

412   that reported by Kondo et al. (2010) who examined eight obese subjects who performed

413   strength exercises more than 30 min four to five times week for seven months, and in

414   whom a significant reduction in circulating leptin of 25% was observed [47]. In the

415   present study, the percentage reduction in leptin was higher in all exercise groups than

416   in the D group (S group: 27.2%, E group: 37.5%, SE group: 32.6%). Fisher et al. (2010)

417   and Lucotti et al. (2011) reported significantly reduced leptin levels in endurance

418   training groups, while those of subjects who had undergone combined strength +

419   endurance training experienced no such change [7,19]. In a study of longer duration,

420   Balducci et al. (2010) reported that groups that undertook supervised endurance and

421   combined endurance + strength exercises experienced significant reductions in leptin of

422   27% and 47% respectively. The discrepancies between the present results and those of

423   the above authors may be associated with differences in the change in TFM%, the

424   length of the intervention, the presence/absence of a dietary intervention, or the training

425   methodology employed in each [17].

426   Finally, since improvements in AV, energy intake and training variables were seen,

427   linear regression analysis was performed to identify the main variables associated with

428 changes in the serum leptin concentration. Changes in BMI, TFM%, AF%, undertaking

429 a supervised exercise program (S, E or SE) and gender (see Table 3) were all found to

430 be independent predictors of changes in the leptin level. Multiple regression attributed

431 38.3% of the change in leptin concentration to variation in the percentage of TFM%,

432 plus the undertaking of supervised exercise, plus the baseline leptin concentration.

433 However, within this model, neither undertaking such exercise nor the type of exercise

434 undertaken (S, E or SE) had a significant effect on leptin concentration, accounting for

435 just 5.9% and 4.4% of this change respectively. These results highlight the great interest

436 in identifying other independent variables (e.g., other IM, visceral fat mass, cortisol and

437 growth hormone concentrations, as well as other dietary variables not investigated in the

438 present work) that might be associated with this change.

439 A point of interest of the present study is that it is the first to include normoglycaemic,

440 young to middle-aged men and women. Other studies that have included different types

441 of exercise have only looked at women, the elderly, and patients with diabetes. A

442 limitation of the present study was the reduced number of subjects in each exercise

443 group, a consequence of a larger number of withdrawals than anticipated. The sample

444 size of the PRONAF study was selected to detect an effect of training and diet on

445 TFM%, but, unfortunately, not to detect an effect on IM. This prevented determining

446 which of the three exercise regimens had the greatest effect on serum IM

447 concentrations. The results of the present study could, however, assist researchers in the

448 calculation of appropriate sample sizes for future clinical trials focused on clarifying the

449 effect of exercise and hypocaloric diet programs on IM.

450 The present study is of interest since the state of chronic inflammation that accompanies

451 overweight and obesity, manifested as high concentrations of non-muscle-produced IL-

452 6, TNF-α and CRP, contributes towards the development of atherosclerosis, insulin

453   resistance and other chronic diseases. A reduction in the concentrations of these

454   markers might help prevent the appearance of these complications.

455   In conclusion, the present results show that strategies combining supervised physical

456   exercise and a hypocaloric diet can provide benefits in terms of body composition and

457   slight improvements in IM, especially in leptin, with no differences among the physical

458   exercise program. Further studies with larger sample sizes are required to clarify the

459   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.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

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624   **Figure 1**: Participation flow diagram for the PRONAF study. Recruitment methods

625   included all types of media advertisement. A total of 1568 candidates were screened, of

626   whom 119 were randomised into the PRONAF study. The dropout rates in the groups

627   were: endurance group (E) 16.6 %, strength group (S) 26.6 %, combined group (SE)

628   23.3%, and diet and physical activity recommendations group (D) 31.03 %.

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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) $^{a2°}$	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°	39.89 (5.63)	35.32 (6.80) †***b4°	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°	44.82 (7.19)	38.19 (9.37) †***	43.11 (5.58)	32.56 (8.77) †***b6°	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) †$^{°}$
LM (kg)	46.78 (10.29)	46.82 (11.10) b3°	46.04 (8.71)	45.60 (8.61)	48.40 (9.14)	48.24 (9.51)$^{b6°}$	46.44 (8.21)	45.46 (7.47) †$^{°}$
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: ° 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°b5°]	21.20 (5.81-36.64)	5.81 (3.95-23.62) †[***]	5.07 (3.34-18.44)	3.67(1.64-11.30) †[°]	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) †°	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: ° 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       │◄──────────────────►│  Not interested after    │
│  secondary question- │                    │  preliminar information  │
│  naire n=737         │                    │         n=831            │
└──────────────────────┘                    └──────────────────────────┘
         │
    ┌────┴──────────────────────────────────────┐
┌──────────────────────┐              ┌──────────────────────────┐
│   Elegible           │              │      Not eligible        │
│ for orientation visit│              │         n=532            │
│       n=205          │              └──────────────────────────┘
└──────────────────────┘                         │
         │                           ┌──────────────────────────────────────┐
         │                           │ Excluded for pathologies      n=71   │
┌──────────────────────────┐         │ Out of specified range of age n=36   │
│ Not elegible after testing│        │ Inadequate Body Mass Index    n=337  │
│ or refused to continue    │        │ No sedentary behavior         n=44   │
│        n=85               │        │ Smoke habit                   n=32   │
└──────────────────────────┘         │ Metabolic Syndrome            n=10   │
         │                           │ Binge Eating Disorder         n=2    │
         │                           └──────────────────────────────────────┘
┌──────────────────┐
│  Randomised      │
│     n=119        │
└──────────────────┘
```

| Assigned 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 |

| Assigned to E | Men (n=11) Women (n=19) | Withdrew: 1 Job change 1 Lost interest 3 personal reasons | Completers n=25 |

| Assigned to SE | Men (n=14) Women (n=16) | Withdrew: 1 Personal reasons 3 Job change 3 Lost interest 1 Diet adherence | Completers n=22 |

| Assigned to C | Men (n=10) Women (n=19) | Withdrew: 2 Personal reasons 7 Lost interest 2 Diet adherence | Completers n=18 |