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2 **Combined aerobic and resistance training decreases**
3 **inflammation markers in healthy men**

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27 **Running title:** Anti-inflammatory effects of exercise

28 **ABSTRACT**

29 **Background and aims:** Our primary aim was to study the effects of 24 weeks of combined
30 aerobic and resistance training performed on the same day or on different days on
31 inflammation markers.

32 **Methods and results:** Physically active, healthy young men were randomly divided into
33 three groups that performed: aerobic and resistance training consecutively in the same
34 training session (SS) 2-3 d·wk⁻¹ or on alternating days (AD) 4-6 d·wk⁻¹ as well as control (C).
35 The total training volume was matched in the training groups. The control group was asked to
36 maintain their habitual physical activity and exercise level. Maximal leg press strength
37 (1RM) and peak oxygen uptake (VO_{2peak}) were measured. Abdominal fat mass was estimated
38 with dual-energy absorptiometry (DXA). High-sensitivity C-reactive protein (hs-CRP),
39 interleukin 6 (IL6), monocyte chemo attractant protein 1 (MCP-1), tumor necrosis factor
40 alpha (TNF- α) and adipocytokines resistin, adiponectin and leptin were analyzed from
41 plasma samples. Training significantly reduced circulating hs-CRP, leptin and resistin in both
42 training groups (P<0.05), whereas MCP-1 and TNF- α decreased only in AD (P<0.05).
43 Significant correlations were observed between changes in abdominal fat mass and
44 corresponding changes in MCP-1, leptin, adiponectin and resistin.

45 **Conclusion:** Long-term combined aerobic and resistance training reduced markers of
46 subclinical inflammation in healthy young men. The results indicate that a higher frequency
47 of individual exercise sessions might be more beneficial with respect to the anti-inflammatory
48 effects of physical activity. The decreases in inflammation markers seem to be related to
49 decreases in abdominal fat mass.

50

51 **Keywords:** physical exercise, abdominal fat, adipokines, low-grade inflammation

52 **1 Introduction**

53 It is well recognized that the pathogenesis of chronic metabolic diseases such as type 2
54 diabetes (Pradhan et al., 2001) and atherosclerosis (Hansson, 2005) involve prolonged low-
55 grade inflammation indicated by increased circulating levels of inflammatory mediators
56 (Fantuzzi, 2005). Thus, previous studies have indicated an inverse association between
57 physical activity and low-grade inflammation (Fischer et al., 2007; Lavie et al., 2011; Pinto et
58 al., 2012). As such, lower inflammatory markers have been observed especially in individuals
59 who report performing frequent moderate intensity physical activity (Beavers et al., 2010).

60

61 Both aerobic (AT) and resistance training (RT) have been shown to be important strategies
62 for improving inflammatory profiles (Nassis et al., 2005). Interestingly, Nimmo et al. (2013)
63 concluded that the most marked improvements in the inflammatory profile are probably
64 achieved with a combination of high intensity AT and RT. While the effects of either AT or
65 RT on inflammation are relatively well studied, data regarding the effects of combined AT
66 and RT on inflammatory markers is sparse. Libardi et al. (2012) failed to observe significant
67 reductions in inflammatory markers after combined training in sedentary middle-age men,
68 while other studies have found significant improvements in inflammation markers in healthy
69 untrained men and women (Donges et al., 2013; Stefanov et al., 2014) as well as in obese men
70 (Brunelli et al., 2015) and in subjects with metabolic syndrome (Balducci et al., 2010).
71 However, combined training can be performed in multiple ways, for example by performing
72 AT and RT in the same session with different orders or separated on alternating days (Eklund
73 et al., 2016).

74

75 Training intensity and frequency have been shown to affect inflammation markers in a dose-
76 dependent manner (Fatouros et al., 2009). As changes in fat mass have previously been
77 associated with alterations in low-grade inflammation (Gleeson et al., 2011a) it can be
78 assumed that the mode of combined training could have a significant effect on the
79 inflammatory profiles as well. A previous study from our group reported a significant
80 reduction in fat mass after a training intervention, but only in a group that separated aerobic
81 and resistance exercises on alternating days thus increasing the frequency of training while
82 keeping the total training volume constant (Eklund et al., 2016). Thus, we hypothesized that
83 the combined training mode with sufficient frequency may have a beneficial effect on
84 inflammatory profiles. A secondary purpose was to assess whether training-induced changes
85 in body composition and physical performance influence inflammation markers.

86

87 2 Methods

88

89 **Participants.** This study is a part of a larger research project (Eklund et al., 2016; Schumann
90 et al., 2014). Participants were recruited through general advertisements in local newspapers
91 as well as posters and emails that were delivered to local companies and institutions. A total
92 of 150 people contacted us to express their interest towards the study (Figure 1). Of these, 93
93 people met the participation criteria: healthy non-obese ($\text{BMI} < 30 \text{ kg}\cdot\text{m}^{-2}$) men who were
94 non-smokers, free of acute and chronic illness, disease or injury and did not report use of any
95 medications (diabetes, cardiovascular diseases, cancer, hypertension, rheumatism,
96 osteoporosis). Ultimately, a total of 48 healthy men completed pre- and post-measurements
97 and were included in this study (age = 31 ± 6 yr, height = 1.79 ± 0.06 m, body mass = $80.9 \pm$
98 12.3 kg, $\text{BMI} = 25.2 \pm 3.5 \text{ kg}\cdot\text{m}^{-2}$). The subjects were moderately physically active as
99 characterized by walking, cycling or occasionally participating in team sports at light to
100 moderate intensity and a frequency of $3 \text{ d}\cdot\text{wk}^{-1}$. The subjects were informed about the
101 possible risks of all study procedures before providing a written informed consent. A
102 completed health questionnaire and resting ECG were reviewed by a cardiologist prior to
103 participation. The study was conducted according to the declaration of Helsinki, and ethical
104 approval was granted by the University of Jyväskylä Ethical Committee.

105 **Study design.** The subjects were assigned to either of the two training interventions or the
106 control group: combined aerobic and resistance training performed in the same session (SS,
107 $n=16$) or on alternating days (AD, $n=16$) or control group (C, $n=16$). In another data set from
108 our research group, which was analyzed from the same group of previously untrained
109 subjects, we did not observe significant changes in fat mass or performance variables
110 between the participant who trained endurance and strength in a same session but with a

111 different order, thus we pooled the data of SS for the purpose of this study. The exercise
112 order of SS training was randomized with half of the group performing aerobic immediately
113 followed by resistance training and the other half performing the opposite exercise order. The
114 overall training volume was equal in the two groups but SS consisted of only 2-3 combined
115 training sessions per week, whereas AD performed 4 to 6 sessions per week (2-3 x aerobic
116 and 2-3 x resistance, respectively) for 24 weeks. Measurements were performed before
117 (PRE), during (i.e. after 12 weeks, MID) and after (i.e. after 24 weeks, POST) the training
118 intervention. The control group was measured at PRE and POST. Participants were asked to
119 keep their dietary intake constant and the dietary intake was examined by nutritional diaries.

120 **Training.** All training sessions were supervised and the detailed content has been described
121 elsewhere (Eklund et al., 2016). Briefly, the endurance training was conducted on a cycle
122 ergometer. During weeks 1-7 steady-state cycling of low to moderate intensity (below and
123 above the aerobic threshold) was performed and during the remaining weeks, additional high-
124 intensity interval sessions (below and above the anaerobic threshold) were incorporated into
125 the training program. The duration of endurance cycling progressively increased from 30 to
126 50 minutes. During the second half of the study, training volume and intensity were further
127 increased. The resistance training programme included exercises for all major muscle groups
128 with a focus on lower extremities. During the first two weeks, training was performed as a
129 circuit using low loads. Thereafter, protocols aiming for muscle hypertrophy and maximal
130 strength were performed. During the last two weeks also protocols targeting explosive
131 strength development were performed. During the subsequent 12-week period both training
132 volume and frequency were slightly increased in an attempt to avoid a training plateau. The
133 overall duration of each resistance training session was 30-50 min.

134 **Abdominal fat.** Whole body composition was estimated by Dual X-ray Absorptiometry
135 (LUNAR Prodigy, GE Medical Systems, Madison, USA). The DXA-scans were performed in

136 the morning with the participant in a fasted (12h) state. Automatic analyses (Encore-software,
137 version 14.10.022) provided total body fat mass and total body lean mass. Abdominal fat was
138 calculated manually defining a range of interest confined cranially by the upper end plate of
139 the first lumbar vertebra, laterally by the ribs and caudally by the iliac crest (Tallroth et al.,
140 2013). This customized range was then copied to the DXA scans at MID and POST,
141 respectively.

142 **Cardiorespiratory performance.** A graded protocol on a cycle ergometer (Ergometrics 800,
143 Ergoline, Bitz, Germany) was used to determine VO_{2peak} and metabolic thresholds for the
144 aerobic training. The initial load for all subjects was 50 Watts and increased by 25 Watts
145 every two minutes until volitional exhaustion. Oxygen uptake was determined continuously
146 breath-by-breath using a gas analyzer (Oxycon Pro, Jaeger, Hoechberg, Germany). Peak
147 oxygen consumption (VO_{2peak}) was averaged over 60 s periods during the test.

148 **Maximal-strength performance.** Maximal strength was measured by a one-repetition
149 maximum (1RM) test of dynamic leg press exercise performed by a David 210 leg press
150 device (David D210, David Health Solutions Ltd., Helsinki, Finland). The starting position
151 (flexed) was at a knee angle of approximately 60 degrees, and 1RM was accepted as the
152 highest loads the participants could lift to a full knee extension (180 degrees). Subjects
153 performed three warm-up sets and 3 to 5 maximal trials, after which the highest load was
154 accepted as the 1RM.

155 **Venous blood samples.** Fasting venous blood samples were drawn from an antecubital vein
156 in the morning (7:00-9:00 a.m.) after a 12 h overnight fast. Participants were instructed to
157 abstain from strenuous physical activity for 48 h before the blood samples were taken.
158 Venous blood was collected into EDTA tubes for analysis of inflammatory profiles. The
159 samples were centrifuged for 10 min at $+4^{\circ}C$ with $2000 \times g$ (Megafuge 1.0 R, Heraeus,

160 Germany). Plasma was kept at -80°C until analysed for high sensitive-C reactive protein (hs-
161 CRP) and interleukin-6 (IL-6) using the Immulite 1000 and immunoassay kits (Immulite,
162 Siemens, IL). Concentrations of monocyte chemoattractant protein-1 (MCP-1), adiponectin,
163 leptin and resistin in plasma samples were determined by enzyme-linked immunosorbent
164 assay (ELISA) with commercial reagents (R&D Systems, Europe Ltd, Abingdon, UK). The
165 detection limits and inter-assay coefficients of variation, respectively, were $0.1\text{ pg}\cdot\text{ml}^{-1}$ and
166 10 % for hs-CRP, $0.2\text{ pg}\cdot\text{ml}^{-1}$ and 3.4 % for IL-6, $3.9\text{ pg}\cdot\text{ml}^{-1}$ and 5.0 % for MCP-1, 19.5
167 $\text{pg}\cdot\text{ml}^{-1}$ and 2.2% for adiponectin, $15.6\text{ pg}\cdot\text{ml}^{-1}$ and 4.0 % for resistin and $15.6\text{pg}\cdot\text{ml}^{-1}$ and 5.1
168 % for leptin.

169 **Statistical analysis.** Data was analyzed using PASW statistic 22.0 (SPSS, Chicago, IL,
170 USA). Data is presented as mean \pm SD Before applying further statistical methods, the data
171 was checked for sphericity and normality. If a specific variable violated the assumptions of
172 parametric tests, log-transformation was used. This concerned values of adiponectin, leptin,
173 IL-6, MCP-1 and hs-CRP. Absolute changes were analysed via two-way repeated analysis of
174 variance for main (time) and interaction (group \times time) effects. For each analysis, the
175 baseline values were used as a covariate to control between-subject and between-group
176 differences at baseline. This was followed by one-way repeated measures ANCOVA on each
177 group to examine a main effect of time. If a significant main effect or interaction was
178 observed, the change from pre-values for MID and POST was compared between groups
179 using paired t-tests with Bonferroni correction. Effect sizes (ES) are given as Cohen's d with
180 an effect size of ≥ 0.20 being considered small, ≥ 0.50 medium, and ≥ 0.80 large. Spearman's
181 correlation coefficients were used to examine the associations between depending variables.
182 The level of statistical significance was set at $p \leq 0.05$.

183 3 Results

184 **Training adherence.** The training adherence was $99\pm 2\%$ and $100\pm 1\%$ in SS and AD
185 respectively. All subjects completed at least 90% of the overall training volume.

186 **Circulating inflammatory markers.** Circulating hs-CRP is presented in figure 2. For hs-
187 CRP a significant main effect of time was observed ($p = 0.010$, $ES = 0.785$). Circulating
188 concentrations of hs-CRP decreased significantly in the SS ($p = 0.021$) and in the AD ($p =$
189 0.004) from PRE to POST.

190 Figure 3 illustrates the changes in circulating adipocytokine and cytokine concentrations. A
191 significant main effect of time ($p = 0.010$, $ES = 0.942$) was observed in concentrations of
192 circulating resistin. Significant reductions in concentrations of circulating resistin were
193 observed in SS ($p = 0.031$, $ES = 0.582$) and AD ($p = 0.022$, $ES = 0.661$) but remained
194 unaltered in C. At POST, significant changes in concentrations of circulating leptin were
195 observed in SS ($p = 0.031$) and AD ($p = 0.019$) at POST. Significant changes in adiponectin
196 concentrations were not observed.

197 In the inflammatory cytokines, a significant main effect of time ($p = 0.02$, $ES = 0.869$) and
198 interaction ($p = 0.027$, $ES = 0.760$) was observed in the levels of MCP-1. At POST a
199 significant reduction was observed in AD ($p = 0.02$, $ES = 0.840$) but not in SS and the control
200 groups. In addition, the reduced concentration of MCP-1 in AD was significantly lower than
201 in SS and C ($p = 0.019$ and $p = 0.007$ respectively). A significant main effect of time was
202 observed in circulating concentrations TNF- α ($p = 0.001$, $ES = 0.926$). Slight but statistically
203 significant reduction in TNF- α concentration was observed in AD at POST ($p = 0.048$, $ES =$
204 0.418), while no changes in SS or C were found ($p = 0.056$ and $p = 0.218$, respectively).
205 Significant main effects of time or interaction in IL-6 were not observed.

206 **Body composition, aerobic performance and strength.** Changes in body composition,
207 1RM and $VO_{2\text{peak}}$ are summarized in Table 1 and have been partly published elsewhere
208 (Eklund et al. 2015; Eklund et al. 2016; Schumann et al. 2015). No significant changes were
209 observed in body weight. A significant main effect of time ($p < 0.001$, $ES = 0.974$) and
210 interaction ($p = 0.014$, $ES = 0.789$) was observed in abdominal fat mass. After 12 weeks of
211 training, fat mass did not decrease in either of the two experimental groups. However, a
212 significant decrease in abdominal fat mass from PRE to POST was observed in SS ($-7.4 \pm$
213 15.4 %, $p = 0.041$, $ES = 0.445$) and AD (-21.1 ± 17.6 %, $p < 0.001$, $ES = 0.997$). No
214 significant changes in abdominal fat mass was observed in C. Abdominal fat mass in AD at
215 POST was significantly lower compared to SS and C group ($p = 0.050$, $p = 0.019$
216 respectively).

217 A significant main effect of time ($p = 0.015$, $ES = 0.748$) and interaction ($p = 0.007$, $ES =$
218 0.877) was observed in $VO_{2\text{peak}}$. Both the SS and AD groups increased $VO_{2\text{peak}}$ significantly
219 from PRE to MID (6.80 ± 8.28 % $p = 0.001$ and 13.2 ± 11.9 % $p < 0.001$, respectively) and
220 from PRE to POST (9.3 ± 8.85 % $p < 0.001$ and 18 ± 10.3 % $p < 0.001$, respectively), while
221 no significant change was observed in C ($p = 0.637$, $ES = 0.081$). A significant main effect of
222 time ($p < 0.001$, $ES = 0.989$) and interaction ($p = 0.003$, $ES = 0.918$) in 1RM was observed.
223 1RM increased in all groups ($p < 0.001$). Both training groups as well as C increased 1RM
224 from PRE to MID ($p < 0.001$) and from PRE to POST ($p < 0.001$). The increase in 1RM was
225 significantly larger in SS and AD groups ($+14.1 \pm 11.4$ %, $p < 0.01$ and $+12.7 \pm 7.24$ %, $p < 0.01$;
226 respectively) than in C group ($+4.7 \pm 4.65$ %).

227 **Associations between changes in performance, body composition and inflammatory**
228 **markers.**

229 Leptin correlated significantly with abdominal fat mass at all measurement points (PRE R =
230 0.732, $p < 0.001$, MID R = 0.650, $P < 0.001$ and POST R = 0.522 $p < 0.001$) when all the
231 subjects were pooled. In addition, in the pooled data, the changes from PRE to POST in
232 abdominal fat mass correlated positively with the change in leptin (R = 0.433, $p = 0.002$),
233 MCP-1 (R = 0.581, $P = 0.023$) and resistin (R = 0.343, $P = 0.016$) and negatively with
234 adiponectin (R = -0.290, $p = 0.043$). Changes in inflammation markers and performance
235 variables were not associated but a significant negative correlation was observed between
236 TNF- α and $VO_{2\text{peak}}$ as well as between leptin and $VO_{2\text{peak}}$ at PRE (R = -0.389, $R = 0.018$ and
237 $p = -0.654$, all $p < 0.05$). In the experimental groups, an inverse relationship between change
238 in concentration of circulating adiponectin and change in maximal strength from PRE to
239 POST was observed (R = -0.459, $p = 0.014$).

240

241

242

243 **4 Discussion**

244

245 The present study assessed the effects of 24 weeks of combined aerobic and resistance
246 training on inflammation markers in young, healthy men. Herein, we provide evidence that
247 combined AT and RT reduces inflammation as demonstrated by lowered circulating
248 concentrations of hs-CRP, leptin and resistin. The special focus of the present study,
249 however, was to investigate whether the performing AT and RT in the same session (SS) or
250 on alternating days (AD) affected the inflammation markers differently. The main finding of
251 the study was that combined training performed on alternating days elicited the largest
252 reductions in circulating levels of TNF- α and MCP-1. Furthermore, the beneficial effects of
253 exercise on inflammation markers were achieved without concomitant weight loss, however,
254 a decrease in abdominal fat mass was associated with reductions in the inflammation
255 markers, which emphasizes meaningfulness of this change in body composition.

256

257 In the present study, we showed that the baseline levels of hs-CRP allowed us to classify the
258 participants as having “moderate cardiovascular risk” (1.0 to 3.0 mg·L⁻¹) prior to
259 commencement of the study in all groups. At POST the mean hs-CRP was reduced to the
260 level of “low cardiovascular risk” (< 1.0 mg·L⁻¹) in both experimental groups (Pearson et al.
261 2003). These findings are in line with a study by Stewart et al. (Stewart et al., 2007a), who
262 suggested that a combination of AT and RT reduced the risk of cardiovascular disease
263 development, as defined by a decrease in hs-CRP concentrations in healthy populations.
264 While C-reactive protein (CRP) concentrations are generally determined by genetic factors,
265 centrally located adiposity is also considered to be a major determinant of CRP levels (Perry
266 et al., 2008). Cross-sectional studies have found an inverse relationship between physical

267 activity and CRP (Ford, 2002) and training studies have reported reductions in CRP (Stewart
268 et al., 2007a). Interestingly, Libardi et al. (2012) did not find any significant differences in
269 CRP, IL-6 or TNF- α in sedentary middle age men after 16 weeks of concurrent training in
270 which AT and RT were performed in the same session, three times a week. These findings
271 were opposed to those of Stewart et al. (Stewart et al., 2007b), who found a significant
272 improvement in CRP concentrations after a 12-wk concurrent training period in young and
273 old sedentary subject. Interestingly, in the present study we did not observe any significant
274 changes in circulating inflammation markers after 12 weeks, but only after 24 weeks of
275 training. In contrast to the studies by Stewart et al. (2007) and Libardi et al. (2012), the
276 subjects in the present study were young and healthy and reported to be moderately active.
277 Thus, our findings indicate that even moderately active young healthy subjects benefit from
278 prolonged combined AT and RT, but adaptations may be delayed in comparison to inactive
279 and/or elderly subjects. However, it is notable that the training in the present study was
280 progressive as both training volume and frequency were increased during the training
281 intervention. Therefore, it is also possible that the training was not intensive enough to elicit
282 anti-inflammatory effect during the first 12 weeks of training.

283

284 Beavers et al. (Beavers et al., 2010) concluded that AT interventions for healthy individuals
285 are beneficial for reducing inflammatory biomarkers, although reductions in body weight are
286 small. In the present study, we did not observe significant reductions in body weight.
287 Interestingly, the abdominal fat mass decreased significantly only when combined training
288 was performed on alternating days as opposed to AT and RT in the same session. This group
289 difference in abdominal fat mass could be due to the greater frequency of exercise that
290 probably resulted in increased overall energy expenditure (Almuzaini et al., 1998). Intra-
291 abdominal obesity has been shown to be an important risk factor for low-grade inflammation.

292 The distribution of excess fat in the abdominal region is known to modify the health risk
293 profile, whereas excess adiposity in the periphery does not appear to increase the risk of
294 developing cardiovascular disease (Strasser et al., 2012). In the present study, we observed a
295 significant association between the change in abdominal fat mass and all measured
296 circulating adipocytokine concentrations. Previous studies suggest that physically active
297 individuals or subjects with higher fitness level have more favorable adipocytokine profiles
298 compared to sedentary populations (Lavie et al., 2011). This was supported by our findings as
299 the initial VO_{2peak} was significantly associated with circulating leptin concentration at
300 baseline. However, we did not observe a significant correlation between changes in VO_{2peak}
301 and changes in adipocytokine concentrations. Interestingly, we observed a significant
302 reduction in circulating MCP-1 concentrations after 24 weeks when the training was
303 separated into alternating days as opposed to AT and RT in the same session. Moreover,
304 reductions in MCP-1 are associated with the changes in abdominal fat mass, irrespective of
305 intervention group, which indicates that fat mass in the abdominal area has a significant
306 effect on MCP-1 concentration.

307

308 We observed that the circulating resistin levels were reduced in both experimental groups
309 after 24 weeks of training, even if we did not observe a significant reduction in visceral fat
310 mass in SS group. Resistin is a signaling protein that has been linked to inflammation and
311 coronary heart disease (Zhang et al., 2010), and, consequently, a reduction in resistin
312 concentrations may be interpreted as a beneficial biological adaptation. Our data indicate that
313 long-term combined AT and RT alters the concentrations of circulating resistin regardless of
314 changes in abdominal fat mass. Gleeson et al. (Gleeson et al., 2011b) suggested that both the
315 reduction of visceral fat mass and the anti-inflammatory environment induced by each
316 exercise session might elicit long-term anti-inflammatory effects. One of the possible

317 mechanisms behind the anti-inflammatory effect of exercise has been suggested to be the
318 acute IL-6 release following an exercise session, possibly stimulating the accumulation of
319 anti-inflammatory cytokines, such as interleukin-10 and interleukin-1 receptor antagonist
320 (Gleeson et al., 2011c). IL-6 has been shown to be related to circulating resistin levels, but if
321 IL-6 releases are mechanistically linked to reductions in circulating resistin levels awaits
322 further investigation. Nevertheless, we observed no significant changes in circulating IL-6
323 concentration in the experimental groups.

324

325 Changes in body composition, or more precisely, changes in abdominal fat mass seem to be
326 an important factor when an exercise intervention for reducing inflammation markers is
327 planned. In the present study we showed that a significant reduction in adipokines is possible
328 also in the absence of change in abdominal fat mass, as seen in the decrease in resistin levels.
329 However, significant reductions in leptin levels seem to be dependent on a significant
330 reduction in fat mass (Baile et al., 2000). There are several mechanisms involved in the
331 beneficial effects of exercise on immunological function, and recent research has focused on
332 its role in the improvement of the inflammatory profile. However, further studies are needed
333 to identify the molecular mechanisms underlying the anti-inflammatory effect of exercise and
334 what the role of skeletal muscle is in this action.

335

336 The strengths of this study include its careful measurement of a wide range of potential
337 confounding variables and a prolonged supervised training intervention. However, several
338 limitations should be considered when interpreting our results. First, the participants in this
339 study were young healthy men and therefore a generalization of our results to other
340 populations might be problematic. Secondly, although in the present study several different

341 factors are suggested to be important markers and/or regulators of inflammation, there are
342 many other pro- or anti-inflammatory factors that could have been measured. Nevertheless,
343 CRP, in particular, has proven to be a relatively useful marker of systemic inflammation and
344 predictor of clinically relevant outcomes and is the most commonly measured inflammatory
345 marker (Pearson et al. 2003). Lastly, we cannot determine the directions of the associations
346 nor causality observed in this study with absolute certainty.

347 **4.1 Perspectives**

348 Combined AT and RT without concomitant body weight loss may induce anti-inflammatory
349 effects, leading to improvements in levels of circulating inflammation markers in men. These
350 effects could be enhanced with a reduction in visceral fat mass that was observed only when
351 AT and RT were performed on alternating days. The findings of this study indicate that a
352 higher frequency of exercise sessions should be recommended in the prevention of
353 inflammation related diseases. The improvement in the inflammatory profile achieved in the
354 present study may be an effective strategy for reduction in low-grade systemic inflammation
355 and improving the health trajectory of young men.

356

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364

365 **CONFLICT OF INTEREST**

366 The authors do not have conflicts of interests and state that the results of the present study do
367 not constitute endorsement by ACSM. The authors alone are responsible for the content and
368 writing of the manuscript.

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References

- 372 Almuzaini K.S., Potteiger J.A., and Green S.B. Effects of Split Exercise Sessions on Excess
373 Postexercise Oxygen Consumption and Resting Metabolic Rate. *Canadian Journal of Applied*
374 *Physiology* 1998; 23: 433-443.
- 375 Baile C.A., Della-Fera M.A., and Martin R.J. Regulation of Metabolism and Body Fat Mass
376 by Leptin. *Annu Rev Nutr* 2000; 20: 105-127.
- 377 Balducci S., Zanuso S., Nicolucci A., Fernando F., Cavallo S., Cardelli P., Fallucca S., Alessi
378 E., Letizia C., and Jimenez A. Anti-Inflammatory Effect of Exercise Training in Subjects
379 with Type 2 Diabetes and the Metabolic Syndrome is Dependent on Exercise Modalities and
380 Independent of Weight Loss. *Nutrition, Metabolism and Cardiovascular Diseases* 2010; 20:
381 608-617.
- 382 Beavers K.M., Brinkley T.E., and Nicklas B.J. Effect of Exercise Training on Chronic
383 Inflammation. *Clinica Chimica Acta* 2010; 411: 785-793.
- 384 Brunelli D.T., Chacon-Mikahil M.P.T., Gáspari A.F., Lopes W.A., Bonganha V., Bonfante
385 I.L.P., and Cavaglieri C. Combined Training Reduces Subclinical Inflammation in Obese
386 Middle-Aged Men. *Med Sci Sports Exerc* 2015; 47: 2207-2215.
- 387 Donges C.E., Duffield R., Guelfi K.J., Smith G.C., Adams D.R., and Edge J.A. Comparative
388 Effects of Single-Mode Vs. Duration-Matched Concurrent Exercise Training on Body
389 Composition, Low-Grade Inflammation, and Glucose Regulation in Sedentary, Overweight,
390 Middle-Aged Men. *Applied Physiology, Nutrition, and Metabolism* 2013; 38: 779-788.
- 391 Eklund D., Häkkinen A., Laukkanen J.A., Balandzic M., Nyman K., and Häkkinen K.
392 Fitness, Body Composition and Blood Lipids Following Three Concurrent Strength and
393 Endurance Training Modes. *Applied Physiology, Nutrition, and Metabolism* 2016.
- 394 Fantuzzi G. Adipose Tissue, Adipokines, and Inflammation. *J Allergy Clin Immunol* 2005:
395 115: 911-919.
- 396 Fatouros I.G., Chatzinikolaou A., Tournis S., Nikolaidis M.G., Jamurtas A.Z., Douroudos I.I.,
397 Papassotiriou I., Thomakos P.M., Taxildaris K., Mastorakos G., and Mitrakou A. Intensity of
398 Resistance Exercise Determines Adipokine and Resting Energy Expenditure Responses in
399 Overweight Elderly Individuals. *Diabetes Care* 2009; 32: 2161-2167.
- 400 Fischer C.P., Berntsen A., Perstrup L.B., Eskildsen P., and Pedersen B.K. Plasma Levels of
401 Interleukin-6 and C-Reactive Protein are Associated with Physical Inactivity Independent of
402 Obesity. *Scand J Med Sci Sports* 2007; 17: 580-587.
- 403 Ford E.S. Does Exercise Reduce Inflammation? Physical Activity and C-Reactive Protein
404 among US Adults. *Epidemiology* 2002; 13: 561-568.
- 405 Gleeson M., Bishop N.C., Stensel D.J., Lindley M.R., Mastana S.S., and Nimmo M.A. The
406 Anti-Inflammatory Effects of Exercise: Mechanisms and Implications for the Prevention and
407 Treatment of Disease. *Nature Reviews Immunology* 2011a; 11: 607-615.

- 408 The Anti-Inflammatory Effects of Exercise: Mechanisms and Implications for the Prevention
409 and Treatment of Disease. *Nature Reviews Immunology* 2011b: 11: 607-615.
- 410 Hansson G.K. Inflammation, Atherosclerosis, and Coronary Artery Disease. *N Engl J Med*
411 2005: 352: 1685-1695.
- 412 Lavie C.J., Church T.S., Milani R.V., and Earnest C.P. Impact of Physical Activity,
413 Cardiorespiratory Fitness, and Exercise Training on Markers of Inflammation. *J Cardiopulm*
414 *Rehabil Prev* 2011: 31: 137-145.
- 415 Libardi C.A., De Souza G.V., Cavaglieri C.R., Madruga V.A., and Chacon-Mikahil M. Effect
416 of Resistance, Endurance, and Concurrent Training on TNF-a, IL-6, and CRP. *Med Sci*
417 *Sports Exerc* 2012: 44: 50-56.
- 418 Nassis G.P., Papantakou K., Skenderi K., Triandafillopoulou M., Kavouras S.A.,
419 Yannakoulia M., Chrousos G.P., and Sidossis L.S. Aerobic Exercise Training Improves
420 Insulin Sensitivity without Changes in Body Weight, Body Fat, Adiponectin, and
421 Inflammatory Markers in Overweight and Obese Girls. *Metab Clin Exp* 2005: 54: 1472-1479.
- 422 Nimmo M., Leggate M., Viana J., and King J. The Effect of Physical Activity on Mediators
423 of Inflammation. *Diabetes, Obesity and Metabolism* 2013: 15: 51-60.
- 424 Perry C.D., Alekel D.L., Ritland L.M., Bhupathiraju S.N., Stewart J.W., Hanson L.N.,
425 Matvienko O.A., Kohut M.L., Reddy M.B., Van Loan M.D., and Genschel U. Centrally
426 Located Body Fat is Related to Inflammatory Markers in Healthy Postmenopausal Women.
427 *Menopause* 2008: 15: 619-627.
- 428 Pinto A., Di Raimondo D., Tuttolomondo A., Buttà C., Milio G., and Licata G. Effects of
429 Physical Exercise on Inflammatory Markers of Atherosclerosis. *Curr Pharm Des* 2012: 18:
430 4326-4349.
- 431 Pradhan A.D., Manson J.E., Rifai N., Buring J.E., and Ridker P.M. C-Reactive Protein,
432 Interleukin 6, and Risk of Developing Type 2 Diabetes Mellitus. *Jama* 2001: 286: 327-334.
- 433 Schumann M., Küüsmaa M., Newton R.U., Sirparanta A., Syväoja H., Häkkinen A., and
434 Häkkinen K. Fitness and Lean Mass Increases during Combined Training Independent of
435 Loading Order. 2014.
- 436 Stefanov T., Blüher M., Vekova A., Bonova I., Tzvetkov S., Kurktschiev D., and Temelkova-
437 Kurktschiev T. Circulating Chemerin Decreases in Response to a Combined Strength and
438 Endurance Training. *Endocrine* 2014: 45: 382-391.
- 439 Stewart L.K., Flynn M.G., Campbell W.W., Craig B.A., Robinson J.P., Timmerman K.L.,
440 McFarlin B.K., Coen P.M., and Talbert E. The Influence of Exercise Training on
441 Inflammatory Cytokines and C-Reactive Protein. *Med Sci Sports Exerc* 2007a: 39: 1714.
- 442 The Influence of Exercise Training on Inflammatory Cytokines and C-Reactive Protein. *Med*
443 *Sci Sports Exerc* 2007b: 39: 1714.

- 444 Strasser B., Arvandi M., and Siebert U. Resistance Training, Visceral Obesity and
445 Inflammatory Response: A Review of the Evidence. *Obesity Reviews* 2012; 13: 578-591.
- 446 Tallroth K., Kettunen J.A., and Kujala U.M. Reproducibility of Regional DEXA
447 Examinations of Abdominal Fat and Lean Tissue. *Obes Facts* 2013; 6: 203-210.
- 448 Zhang M.H., Na B., Schiller N.B., and Whooley M.A. Resistin, Exercise Capacity, and
449 Inducible Ischemia in Patients with Stable Coronary Heart Disease: Data from the Heart and
450 Soul Study. *Atherosclerosis* 2010; 213: 604-610.
- 451

TABLES WITH HEADINGS

Table 1. Physical fitness and body composition at before (pre) after 12 weeks (mid) and after 24 weeks (post) of training. AD = Different-day training, SS = Same-session training, C = Controls. *=-difference from PRE value (p<0.05) #-difference between the AD and SS. Mean \pm SD.

	PRE			MID		POST		
	SS (n=16)	AD (n=15)	CONT (n=18)	SS (n=16)	AD (n=15)	SS (n=16)	AD (n=15)	CONT (n= 18)
Physical fitness								
1RM (kg)	151 \pm 32.2	145 \pm 18.3	159 \pm 29.9	164 \pm 26.5	159 \pm 16.7	170 \pm 26.2	163 \pm 16.0	167 \pm 28.5
VO ₂ _{peak} (L·min ⁻¹)	3.13 \pm 0.40	2.82 \pm 0.32	3.07 \pm 0.53	3.33 \pm 0.42	3.17 \pm 0.26	3.41 \pm 0.49	3.34 \pm 0.36	3.11 \pm 0.53
Body composition								
Height (m)	1.78 \pm 0.06	1.80 \pm 0.08	1.78 \pm 0.06	1.78 \pm 0.06	1.80 \pm 0.08	1.78 \pm 0.06	1.80 \pm 0.08	1.78 \pm 0.06
Body weight (kg)	80.1 \pm 13.2	81.8 \pm 10.3	80.7 \pm 11.7	80.1 \pm 11.9	81.9 \pm 10.3	80.4 \pm 11.1	80.6 \pm 10.4	81.7 \pm 11.5
BMI (kg·m ⁻²)	25.2 \pm 3.00	25.3 \pm 2.60	25.2 \pm 3.9	25.2 \pm 2.50	25.3 \pm 2.93	25.4 \pm 2.34	24.9 \pm 2.85	25.5 \pm 3.8 ⁹
Body fat mass (kg)	20.8 \pm 8.12	22.9 \pm 6.11	19.2 \pm 7.42	20.0 \pm 7.27	21.6 \pm 6.67	19.0 \pm 7.00	19.5 \pm 7.28	20.4 \pm 7.66
Body Fat-% (%)	25.4 \pm 7.1	27.0 \pm 4.3	23.1 \pm 8.3	24.5 \pm 6.6*	27.6 \pm 4.4	23.2 \pm 6.2 **	25.9 \pm 5.5 **	24.4 \pm 8.9
Abdominal fat mass (g)	2571 \pm 1190	3060 \pm 993	2310 \pm 1210	2340 \pm 1060	2810 \pm 1040**	2330 \pm 1080	2490 \pm 1120***	2450 \pm 1361
Lean mass (kg)	53.3 \pm 6.13	55.9 \pm 5.12	59.5 \pm 5.85	54.1 \pm 5.74	57.2 \pm 5.73	54.8 \pm 5.93*	58.0 \pm 5.22*	58.7 \pm 5.87

FIGURE LEGENDS

FIGURE 1. Flowchart of study participants.

FIGURE 2. Mean (SD) in hs-CRP at weeks 0, 12 and 24. * significant within-group change. AD = alternating days training, SS = same session training, C = controls.

FIGURE 3. Mean (SD) changes in adipocytokines (left) and cytokines (right). * significant within-group change. SS = same session training, AD = alternating days training, C = Controls.

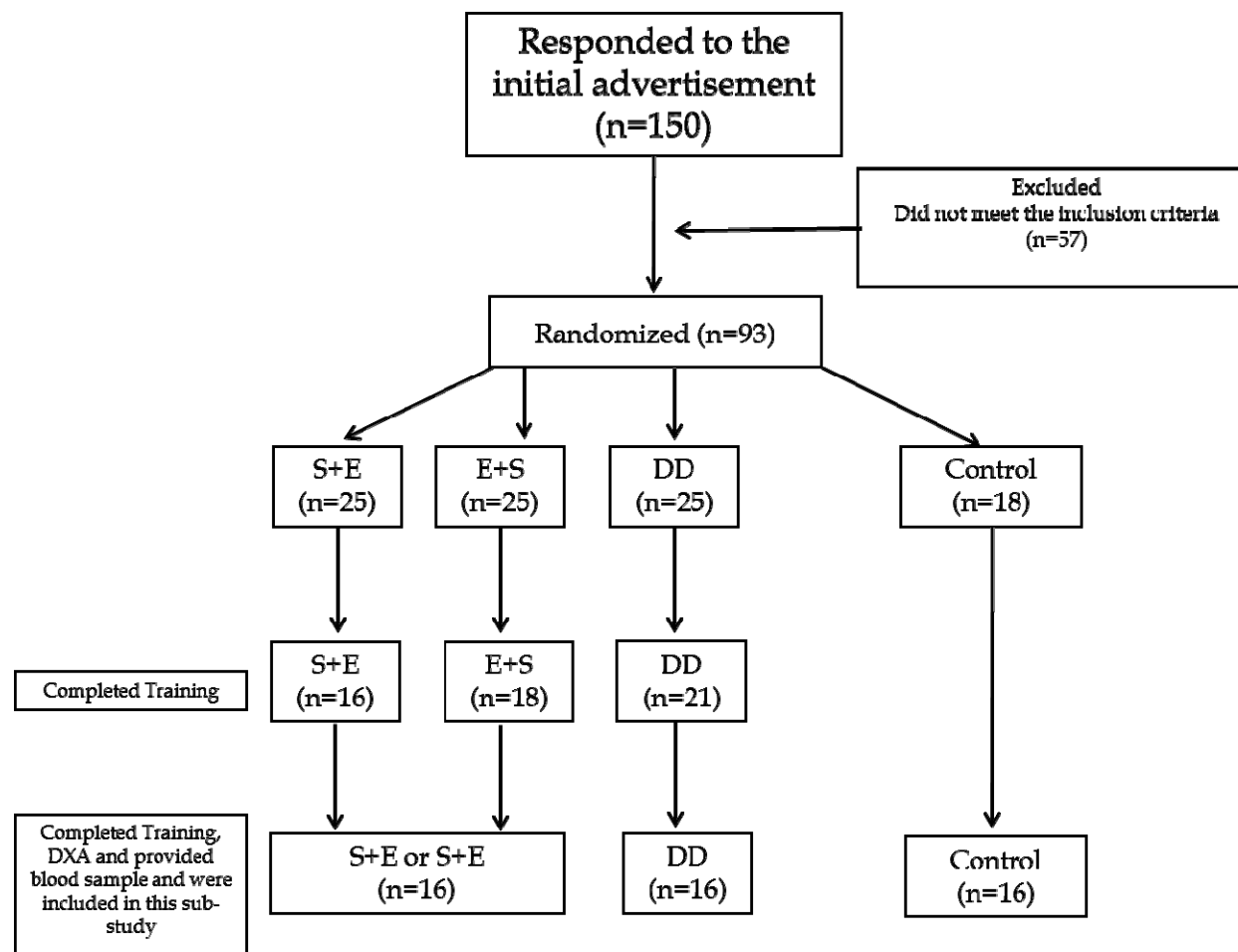


Fig 1. Flowchart of study participants.

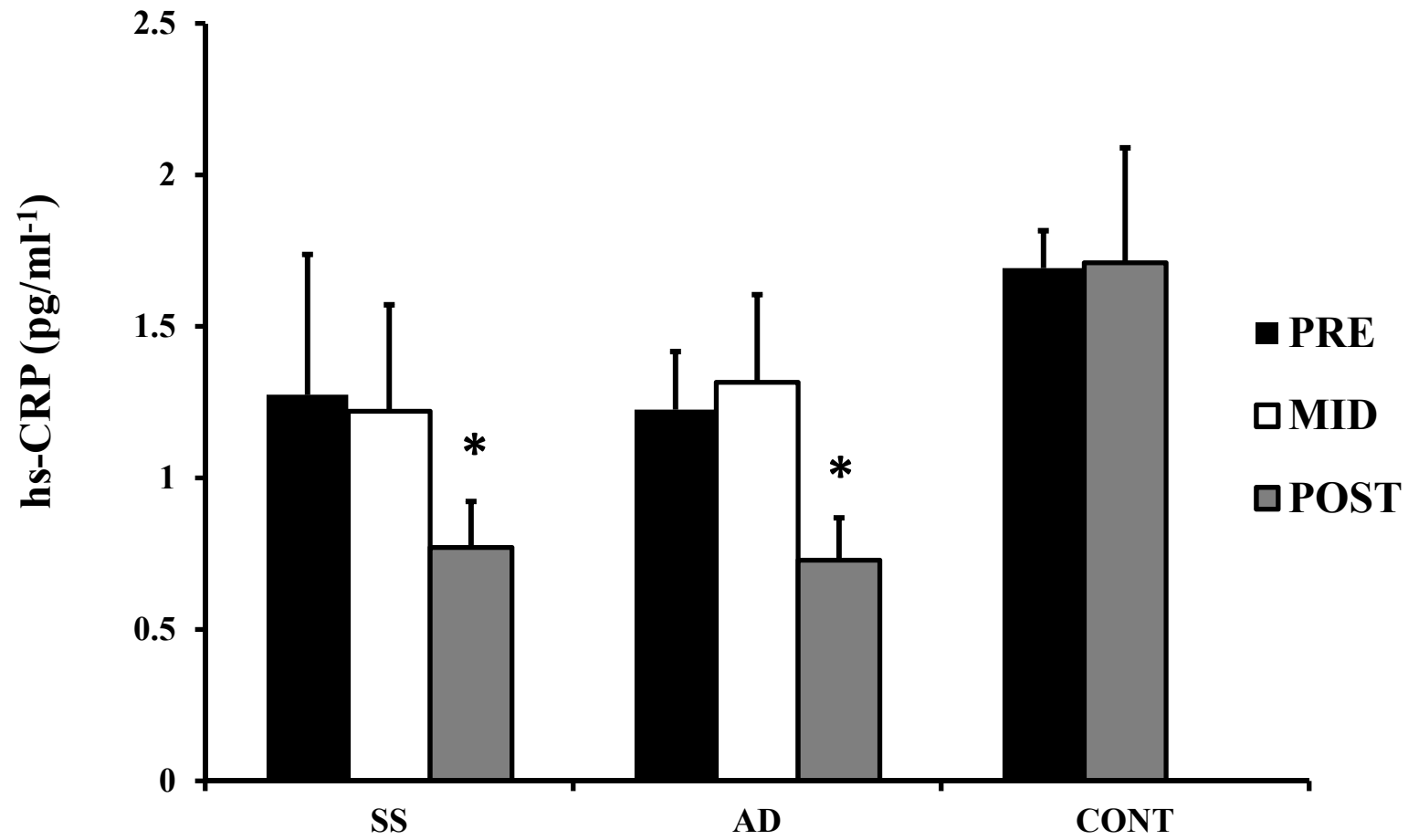


Fig 2. Mean (SD) in hsCRP at weeks 0, 12 and 24. * significant within-group change. AD = alternating days training, SS = same session training, C = controls

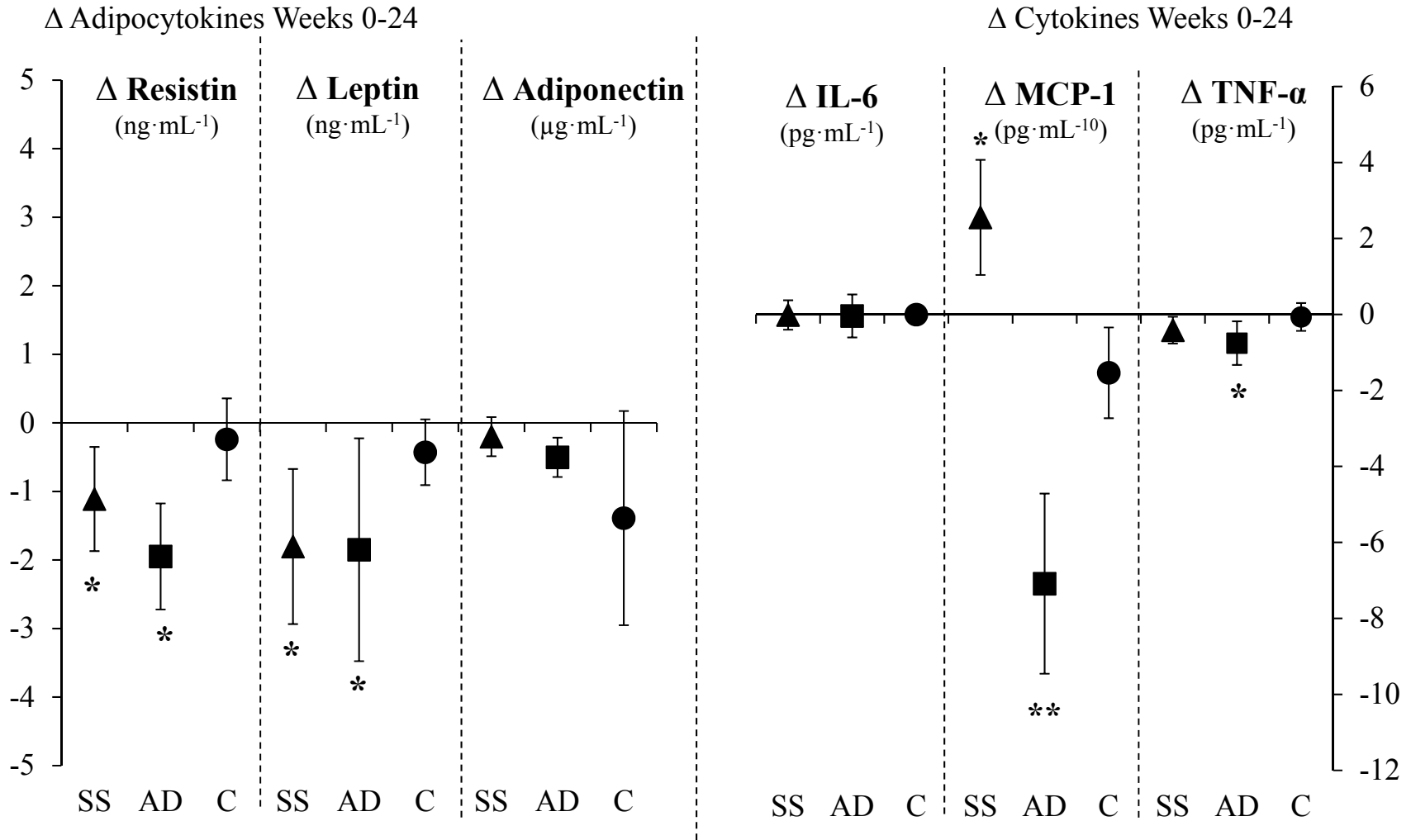


Fig 3. Mean (SD) changes in adipocytokines (left) and cytokines (right). * significant within-group change. SS = same session training, AD = alternating days training, C = Controls