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1 **Summary and Keywords Page**

2 **Hemoglobin, Hematocrit and plasma volume variations following combined sprint**
3 **and strength training: Effect of advanced age**

4 **Summary**

5 **Objectives:** The study investigated the effect of combined sprint and resistance training
6 (CSRT) on red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Hct), plasma
7 volume (PV) variation at rest and during exercise.

8 **Equipment and methods:** Twenty-eight moderately trained were randomly assigned
9 into a young trained (YT), young control (YC), middle-aged trained (MAT), and middle-
10 aged control (MAC) group. Before (P1), and after (P2) CSRT, blood samples were
11 collected at rest and after exercise.

12 **Results:** At P1, Hct was significantly ($p < .05$) greater in young compared to middle-aged
13 groups. At P1, PV decrease during exercise was significantly ($p < .05$) higher in middle-
14 aged compared to young groups. Following CSRT, resting RBC count and Hb increased
15 significantly ($p < .05$) in MAT. At P2, Following CSRT, Hct decreased significantly
16 ($p < .05$) in trained groups. At P2, no significant ($p > .05$) age- effect between MAT and
17 YT was observed for Hct. In conclusion, CSRT increases RBC count and Hb in middle-
18 aged men, and ameliorates the effect of age in Hct. Such adaptations may improve
19 cardiovascular fitness of middle-aged individuals, and may be preventative of subsequent
20 declines with age.

21 **Keywords**

22 Blood viscosity; muscle blood flow; red blood cells; sports anemia; training effect.

1 **1. Introduction**

2 It is well known that older adults experience decreased total body water,
3 baroreceptor sensitivity, cell-producing marrow, and blood viscosity compared to
4 younger counterparts [1, 2]. Homeostasis observed in older adults is paralleled by
5 increases in heart rate and blood pressure and underpins a lowered exercise-induced
6 muscle blood flow [2]. During continuous exercise, oxygen delivery to the mitochondria
7 of muscle cells represents a main determinant of performance [3]. As such, age-associated
8 reductions in muscle blood flow contribute to reduced functional capacity in older adults
9 [4].

10 Acute exercise induces a variation in plasma volume (PV) because of transient
11 fluid shifts into (hemodilution), and out of (hemoconcentration), the intravascular space
12 [5]. This change in PV is dependent upon exercise intensity and type [6], posture [7],
13 ambient temperature [8], and fluid consumption [9]. A reduction in PV may limit
14 endurance capacity, via reduced blood supply to the working muscle. Moreover, PV
15 variation (PVV) has the potential to hamper interpretation of plasma biomarkers [5] as
16 specific quantities of plasma are required for common assays. In addition, PV influences
17 end diastolic volume, stroke volume, and therefore cardiac output [10].

18 Whilst the PV changes during acute exercise are well defined, the chronic
19 adaptation to exercise training is currently poorly understood. Some authors suggest that
20 magnitude of change of PV and hematocrit is dependent to exercise intensity and training
21 type (strength, aerobic, sprint) [11]. An increase in blood volume, red cell mass, and PV
22 and a decrease in hematocrit (Hct) following continuous aerobic and intermittent interval
23 exercise training in young athletes has been observed [11]. However, others found no

24 changes in PV, Hct, hemoglobin (Hb), and red blood cell (RBC) count in young and older
25 (>50 years) men after prolonged aerobic or resistance training (RT) [1].

26 Numerous studies have found greater PV variation following chronic sprint
27 training [12], and in young sprinters compared with endurance athletes [11]. In addition,
28 Vechin and colleagues [13] found greater improvements in muscle blood flow following
29 high-intensity RT when compared with low and moderate intensity training in elderly
30 participants. Ahmadizad and colleagues [14] observed slight differences in Hct, RBC
31 count, and blood viscosity between young, middle-aged, and old males following
32 endurance exercise. However, Bongers and colleagues [15] recently observed no
33 differences in PV changes between octogenarians and sexagenarians following a 30 km
34 march.

35 High intensity training (HIT) involves repeated bouts of high-intensity exercise,
36 interspersed with recover periods, proclaimed as a time-efficient “healthogenic” strategy
37 [16] despite falling short of the recommended exercise volume to improve and maintain
38 cardiovascular health [16]. Given recent interest in HIT, it is imperative to determine
39 PVV following this training type to allow appropriate interpretation of serum biomarkers,
40 changes in fluids balances, and cardiac output following this exercise modality.

41 Whilst PVV has been quantified in younger cohorts following HIT [17], the effect
42 of HIT and age on PVV is yet to be examined. Therefore, the main aim of the present
43 study was to investigate the effect of HIT on PVV, Hct, RBC count, and Hb in young and
44 middle-aged participants. It was hypothesized *a priori* that an age effect would exist pre-
45 training, and this effect would be ameliorated post-training.

46

47 **2. Materials and methods**

48 **2.1. Participants**

49 Twenty-eight moderately trained men were recruited for participation in the
50 present study. Eligible participants were subsequently randomized to receive 13-weeks'
51 combined sprint and resistance training (CSRT), or control. Thus, four groups existed: a
52 young trained group (YT, age:21.2±1.2 years, height:179.5±4.2 cm; n=7), a young control
53 group (YC, age: 21.5±2.5 years, height:179.8±6.8 cm; n=7), middle-aged trained group
54 (MAT, age:40.8±1.8 years, height: 176.3±6.7 cm; n=7) and middle-aged control group
55 (MAC, age:40.9±2.1years, height:175.2±5.2 cm; n=7). Participants gave their written
56 informed consent to participate in the study after receiving a thorough explanation of the
57 study's protocol. The protocol conformed to internationally-accepted policy statements
58 regarding the use of human participants in accordance with the Declaration of Helsinki
59 and was approved by the University's Ethics Committee. Training status was assessed
60 using an adapted version of the Baecke questionnaire, to identify those with a medical
61 contraindication to performing specific assessments, participants completed medical
62 history, and 3-day-food record. Inclusion criteria included no contraindications to
63 maximal exercise testing such as cardiovascular or pulmonary risk factors, no history of
64 chronic disease, illness, surgeries, hospitalizations, and musculoskeletal or joint injuries.

65 During design of the study, statistical power analysis was performed to determine
66 sample size. This procedure showed that seven participants for each group was needed to
67 achieve a statistical power of 80% and detect a small effect ($d=0.2$) when assessed by
68 four-factor mixed analysis of variance (ANOVA) with a level of significance of 5%.

69

70 **2.2. Study design**

71 A randomized controlled trial study design was used. This study investigated the
72 effects of HIT on PVV, Hct, RBC count and Hb in young and middle-aged participants.
73 Trained participants participated in 13-weeks of CSRT. Briefly, CSRT consisted of one
74 sprint running, one sprint cycling, and one RT session per week, separated by a minimum
75 of 48 h (13 sessions of each training unit). Each age group (young and middle-aged) was
76 randomly divided between control (n = 7) and trained (n = 7) groups. Data were collected
77 before starting training, and immediately after the 13th week. On both occasions, data
78 were collected in the same conditions, at the same time of day. The protocol included the
79 Astrand-Ryhming test, a repeated sprint cycling test, the Wingate Anaerobic Test with
80 concomitant heart rate measurement, a lactate threshold test, and systolic and diastolic
81 blood pressure and hematological markers levels (more details below).

82

83 **2.3. Evaluation and Procedures**

84 Sessions were performed during the morning and lasted no longer than 70 min,
85 inclusive of 15 min warm-up (jogging and stretching) and 15 min cool-down (jogging
86 and stretching). Sprint running sessions entailed 3-5 sets of 3-5 short bouts at maximum
87 velocity. A recovery of 2-3 min was permitted between each set. Sprint cycling sessions
88 comprised 3-5 repetitions of 10-30 s. The 10-30 s trials were performed maximally.
89 Participants recovered actively (50% $\text{VO}_{2\text{max}}$) for 3-5 min between each sprint. RT
90 sessions entailed 5-6 exercises targeting all major muscle groups. The load used during
91 exercise was progressively increased from 40% to 65% of one-repetition maximum (1-
92 RM) [18]. To produce maximal power output (in other words; velocity \times load), the

93 concentric phase of each exercise was performed as fast as possible [19]. Repetitions were
94 maintained at 10-15 per sets and the number of sets increased from 3 to 4 during the
95 training period. Hence, training volume increased progressively during the CSRT
96 program. Rest periods between sets were 3-5 min for upper body muscles [19] and a
97 minimum of 1 min for lower limbs. To adjust load during RT session and monitor
98 adaptation, we determined strength using a 1-RM for the six resistance exercises, pre-
99 training (P1), during the sixth week, and post-training (P2).

100

101 2.3.1. Testing Schedule

102 During experimental period, participants completed anthropometric
103 measurements (pre-, mid-, and post training) and a dietary assessment using a 3-day food
104 record by a sports nutritionist. One week before training-cycle, participants were
105 familiarized with testing procedures to minimize learning effect. Participants avoided
106 physical activity for 48 h preceding each test. The testing period was divided into two
107 phases: before (P1), and after (P2) training and included three consecutive laboratory
108 visits separated by 48h. P2 commenced 48 h after training cessation and finished 7-days
109 later.

110 On day 1, participants performed the Astrand-Ryhming test on a cycle ergometer
111 to estimate maximal oxygen uptake (VO_{2max}). On day 2, participants performed a repeated
112 sprint cycling test on a cycle ergometer. It consisted of five short trials (6 s) against
113 increasing resistance (2 kg per sprint) until exhaustion and when the velocity began to
114 decrease during the 6 s trials. Recovery time between each trial was 5 min. On day 3,
115 participants performed the WAnT on a mechanically braked Monark cycle ergometer.

116

117 *2.3.2. Physiological parameters*

118 Systolic (SBP) and diastolic (DBP) blood pressure were measured in a sitting
119 position. Heart rate variability during WAnT was also measured continuously using Heart
120 rate monitor.

121 During day 3, blood samples were collected to determine hematological markers.
122 Upon arriving, a heparinized catheter (Insyte-W, 1.1 mm o.d. × 30 mm) was inserted into
123 an antecubital vein, following 20-min sitting. Blood was drawn 8:00-9:00 h following
124 overnight fasting. Venous blood samples were drawn at four times: rest (₀ [after 20 min
125 sitting on the bike]), after warm-up, immediately post-WAnT (_{end}) and 10 min post-
126 WAnT (₁₀). Hct and [Hb] were determined directly in quadruplicate, automatically by
127 using standard laboratory procedures. PVV was calculated using Dill and Costill [20]
128 method.

129

130 *2.4. Statistical Analysis*

131 Data analyses were performed using SPSS version 23.0 for Windows (SPSS, Inc.
132 Chicago, IL, USA). Means and SD were calculated after verifying the normality of
133 distributions using the Kolmogorov-Smirnov procedure. For anthropometric,
134 physiological, and physical performances indices, data were analyzed using a
135 multifactorial three-way (time [P1, P2] × age [young, middle-aged] × group [trained,
136 control]) ANOVA and Fisher “F” value was given. Blood variables changes were
137 analyzed using a four-factor ANOVA (time [P1, P2] × Wingate time [warm-up,
138 immediately post-WAnT and 10 min post-WAnT] × age [young, middle-aged] × group
139 [trained, control]). To help protect against type II errors, an estimate of power ($\hat{\omega}$) and

140 effect size (η^2_p) were calculated. Bonferroni-adjusted pairwise post hoc comparisons were
141 performed where appropriate. Pearson's product-moment correlation coefficients were
142 calculated to assess relationships between variables. Significance level was fixed to
143 $p < .05$.

144

145 3. Results

146 3.1. Morphological Data and Physical Performances

147 For body mass ((BM) kg), there was no significant age-effect ($F=1.61$, $p=0.26$,
148 $\eta^2_p=0.11$) at P1 or P2. Following CSRT, both training groups experienced a decrease in
149 BM (72.8 ± 6.3 to 70.9 ± 6.7 kg for YT and 73.0 ± 12.5 to 72.3 ± 10.6 kg for MAT respectively
150 with $F= 8.79$, $p < 0.001$, $\eta^2_p=0.27$).

151 At P1, there was no age-effect for body fat percentage (BF %) ($11.6 \pm 3.1\%$,
152 $10.4 \pm 2.4\%$, $12.3 \pm 1.6\%$ and $12.5 \pm 1.4\%$ for YT, YC, MAT and MAC respectively with
153 $F=2.33$, $p=0.16$ and $\eta^2_p=0.09$). Following CSRT, both training groups experienced a
154 decrease in body fat from P1 ($10.3 \pm 5.5\%$ and $10.4 \pm 1.1\%$ for YT and MAT respectively
155 with $F= 10.32$, $p < 0.001$, $\eta^2_p=0.28$), while the control groups' body fat percentages were
156 not significantly different from P1 ($p > .05$).

157 At P1, there was an age-effect for fat-free mass ($F=??$, $p < 0.001$, $\eta^2_p=??$).
158 Following CSRT, fat-free mass increased significantly ($F= 8.21$, $p=0.03$) only in MAT
159 and was 63.9 ± 5.3 kg.

Commented [LH1]: Please change as according.

160 For estimated VO_{2max} , there was no significant age-effect ($F= 2.64$, $p=0.15$,
161 $\eta^2_p=0.32$), but we observed a significant effect of time ($F=17.35$, $p < 0.001$, $\eta^2_p=0.30$). In
162 fact, estimated VO_{2max} increased significantly ($p < .001$) after CSRT in both trained
163 groups, but not in control groups ($p > .05$).

Commented [LH2]: From what at P1?

164 W_{peak} during the WAnT exhibited a significant effect of age for at P1 ($F= 8.32$,
165 $p<.001, \eta^2_p= 0.99$), which was ameliorated at P2 ($p>.05$). W_{peak} was significantly ($F=5.88$,
166 $p=0.02, \eta^2_p=0.25$) higher after training in both YT (1025 ± 187 to 1187 ± 165 W) and MAT
167 (934 ± 178 to 1096 ± 145 W).

168 W_{mean} increased significantly only in MAT after CSRT (422 ± 56 to 560 ± 67 W).

169 Only at P2, $[\text{La}]_{\text{peak}}$ increased significantly ($F=20.12, p<0.001, \eta^2_p=0.89$) in both
170 trained participants (YT and MAT), while remained stable in their control matched groups
171 ($16.7\pm 2.1, 16.3\pm 3.6, 14.8\pm 2.8$, and $13.1\pm 3.1 \text{mmol}\cdot\text{l}^{-1}$ respectively for YT, YC, MAT and
172 MAC.

173

174 **3.2. Blood Pressure, Heart Rate Characteristics and Hematological Markers**

175 At P1, a significant age effect ($F=5.43, p=0.02, \eta^2_p=0.64$) was observed in systolic
176 blood pressure (SBP). After CSRT, there was a training effect ($F=9.43, p=0.03, \eta^2_p=0.98$)
177 decrease in SBP in MAT at rest and at the end of exercise. Post-hoc and pairwise
178 comparisons were represented in Table 1.

179 ****Insert Table 1 here****

180 RBC ($10^{12}\cdot\text{L}^{-1}$) levels are described in Table 2. There was no significant age-
181 effect ($F=2.09, p=0.16, \eta^2_p=0.06$) at P1 and P2. In addition, a significant ($F=14.50$,
182 $p=0.04, \eta^2_p=0.86$) effect of training was observed in MAT but not ($p>.05$) in the other
183 three groups. Post-hoc and pairwise comparisons were represented in Table 2.

184 ****Insert Table 2 here****

185 Hemoglobin (Hb) concentration (g/100ml) levels are represented in Table 3.
186 There was no significant age-effect ($F=1.87, p>.05, \eta^2_p=0.15$). However, a slight increase
187 ($F=9.10, p<.001, \eta^2_p=0.34$) in basal Hb concentration were observed in MAT after CSRT.

188 ***Insert Table 3 here***

189 Hematocrit (Hct) changes (%) are represented in Table 4. A significant effect of
190 time ($F=13.50$, $p<0.001$, $\eta^2_p=0.92$), Wingate time ($F=14.12$, $p<0.001$, $\eta^2_p=0.10$), age
191 ($F=8.55$, $p<0.001$, $\eta^2_p=0.06$) and also group ($F=9.21$, $p<0.001$, $\eta^2_p=0.85$) was present.
192 For YT, Hct₀, Hct_w, and Hct_{end} were significantly lower at P2 as compared to P1
193 ($p<0.001$). For MAT, Ht_w, and Hct_{end} were significantly lower at P2 as compared to P1
194 ($p<0.001$). Moreover, Hct₀ were significantly higher ($p<0.001$) in YT as compared to YC
195 at P2 (see Table 4).

196 ***Insert Table 4 here***

197 During WAnT, plasma volume decreased significantly ($p<0.001$) from warm-up
198 (PVV_w) to the end of the WAnT (PVV_{end}) in all groups, then increased from the WAnT
199 to recovery time (PVV₁₀) at P1 and P2 ($p<.05$) (Table 5). This decrease of PVV during
200 exercise was significantly ($p<.05$) greater in middle-aged groups compared to younger
201 groups at P1.

202 During warm-up and WAnT, the PVV decrease was significantly ($p=0.04$) higher
203 in MAT as compared to young groups at P1 (in other words; PVV_w: $-8.19\pm 2.88\%$ for YT
204 vs. $-12.75\pm 7.41\%$ for MAT, $F=10.31$, $p=0.02$, $\eta^2_p=0.14$). Significant increases in PVV
205 were observed in YT and MAT following CSRT ($p<0.001$). The age effect was not
206 present at P2 ($F=1.25$, $p=0.35$, $\eta^2_p=0.03$) between YT and MAT, whilst, for the control
207 groups, the age-effect remained statistically significant ($F=8.46$, $p<0.001$, $\eta^2_p=0.12$).

208 ***Insert Table 5 here***

209

210 4. Discussion

211 The main finding of the current study was the increased resting RBC count and
212 Hb in MAT after CSRT. A decrease in Hct in response to WAnT was observed in trained
213 groups following CSRT with a reduction in age-related difference between age-groups.
214 PVV changes suggest a moderate to high increase in PV after warm-up, at the end of the
215 WAnT, and during recovery in the trained groups. Furthermore, the age-related effect on
216 PVV during exercise was not seen between groups after training. In addition, we observed
217 decreased SBP in MAT with diminution in age-related difference between trained groups
218 after CSRT. Although the increase in PV following exercise training is well known [21,
219 22], we believe we are the first to describe a change to acute PVV pre- and post-training
220 in middle-aged men after training exercise. Ben Abderrahman and colleagues [22],
221 described increased PV in 15 young males following interval training, which is consistent
222 with the present investigation. Moreover, they observed greater PVV following training,
223 which is consistent with the present study in observing increases in PVV following CSRT.
224 Our finding that the age-effect was not present post-CSRT suggests that CSRT can
225 improve haematological regulation in middle-aged individuals, to the point where it is
226 similar to young adults.

227 An aim of the present study was to provide insight into the effect of acute and
228 chronic intense exercise on hematological profiles in young and middle-aged men. Sosner
229 and colleagues [23] reported that training in general resulted in an increase of vagal
230 parasympathetic activity in the myocardium, an improvement in endothelial function,
231 resulting in decreased arterial resistances, and improved aortic compliance. As we age,
232 this aortic compliance declines and arterial resistance increases, leading to higher blood
233 pressure [24]. Hence, to mediate blood pressure disturbance, the American College of
234 Sports Medicine (ACSM) Position Stand [25], suggested that aerobic activities and

235 resistance exercises performed 3 times per week are the best alternative to counteract age-
236 related blood disorders. Rezk and colleagues [26] measured a decrease in SBP in
237 normotensive young participants following 90-minutes of low intensity RT. RT reduced
238 BP by 3.2-3.5 mmHg in young trained men [27]. Interestingly, results of the present study
239 reported greater decreased in SBP (~10 mmHg) in trained groups after CSRT. Typically,
240 attenuated effects of aerobic exercise on blood pressure are observed in trials lasting 3-6
241 months, because of poor adherence. As such, Weston and colleagues [28], suggested that
242 short term HIT (2-3 months) allow greater decline in SBP in individuals with
243 hypertension.

244 Age-related differences in body fat and fat free mass increase risk of hypertension
245 and aortic stiffness in older individuals [29]. In the current study, BP improvements
246 following CSRT were associated with decreased BM and increased FFM in MAT. These
247 improvements occurred alongside improved anaerobic (W_{peak} and W_{mean}) and aerobic
248 (VO_{2max}) performance following CSRT.

249 Greater acidosis is usually detected in patients with severe depletion of body fluids
250 [30]. At the end of WAnT, the higher blood lactate was associated with higher PV
251 decrease in young and middle-aged men. Before intervention, the PV decrease was higher
252 in middle-aged compared to young groups suggesting a greater fluid depletion during the
253 WAnT. Interestingly, PV increased after CSRT in both YT and MAT with a reduction in
254 the age effect on PV. Hence, the PV increase in trained groups after CSRT intervention
255 suggests 1) improvements in water balance in the extracellular compartment driven
256 indirectly by the lower blood pressure detected in MAT and 2) better nutrient exchanges
257 through the compartment leading to low blood viscosity.

258 A decrease in post-training Hct has been detected in trained (endurance or
259 resistance) participants when compared to untrained ones [11]. Hct decreases are usually
260 associated with higher red cell mass as well as plasma volume in young endurance-
261 trained individuals [11] but not following strength training in young and middle-aged men
262 [31]. In our study, we found that the combination of strength and sprint training improved
263 resting RBC count and Hb in middle-aged trained group. However, further research is
264 required to determine underlying mechanisms that decrease Hct and increase RBC count
265 and Hb following the HIT.

266 The present study is not without limitations. For example, evaluation of water and
267 sodium status, antidiuretic hormone (ADH) and aldosterone, would have furthered our
268 understanding of the fluid movements during acute and chronic exercise. However, this
269 was outside the scope of the present study. Moreover, although changes in the present
270 study reached statistical significance, they may not be considered clinically meaningful.
271 However, in the present investigation where RBC count at rest increased by ~25% in
272 MAT from P1 to P2, this exceeds the critical difference of ~9% determined using flow
273 cytometry [32]. When resting RBC count decreased by ~20% in YC from P1 to P2 it
274 exceeded the critical difference, but did not reach statistical significance, suggesting that
275 an increased sample size should be used in future investigations. Moreover, the difference
276 between biological and statistical significance should be considered.

277

278 **5. Conclusion**

279 In summary, 13 weeks' sprint and resistance training appears to reduce the age-
280 related decline in substrate metabolism (in other words; lactate) with increased
281 performance levels during strenuous exercise in middle-aged men. In addition, this

282 training intervention reduced systolic blood pressure in middle-aged trained men at rest
283 and in response to exercise. These results occurred alongside increased resting RBC
284 count, Hb, and PV in MAT. Moreover, the age-related differences among groups RBC
285 count and PV changes following short-term exercise, were reduced at P2. Hence, short-
286 term intense training with mixed exercises (sprints and resistances) prescription would
287 allow lower blood pressure for a short period. Typically, individuals with hypertension
288 have been dissuaded from engaging in long duration interventions and a poor adherence
289 is usually registered. However, from this study, it appears 13-weeks' exercise training
290 may be recommended as part of a program that reduces cardiovascular disease risk.

291

292 **Disclosure of interest**

293 The authors declare that they have no competing interest.

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396 **Illustrations**

397 **Tables**

398 **Table 1.** Blood pressure and heart rate variation determined before (P1) and after (P2)
399 training

400 **Table 2.** Red blood cell count ($10^{12}/L$) determined before (P1) and after (P2) training

401 **Table 3.** Hemoglobin concentration (g/100 ml) determined before (P1) and after (P2)

402 training

403 **Table 4.** Hematocrit variation (%) determined before (P1) and after (P2) training

404 **Table 5.** Plasma volume variation (%) determined before (P1) and after (P2) training