Summary and Keywords Page

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

Summary

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

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

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

Keywords

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

1. Introduction

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

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

Whilst the PV changes during acute exercise are well defined, the chronic adaptation to exercise training is currently poorly understood. Some authors suggest that magnitude of change of PV and hematocrit is dependent to exercise intensity and training type (strength, aerobic, sprint) [11]. An increase in blood volume, red cell mass, and PV and a decrease in hematocrit (Hct) following continuous aerobic and intermittent interval exercise training in young athletes has been observed [11]. However, others found no changes in PV, Hct, hemoglobin (Hb), and red blood cell (RBC) count in young and older (>50 years) men after prolonged aerobic or resistance training (RT) [1].

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

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

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

2. Materials and methods

2.1. Participants

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

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

2.2. Study design

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

2.3. Evaluation and Procedures

Sessions were performed during the morning and lasted no longer than 70 min, inclusive of 15 min warm-up (jogging and stretching) and 15 min cool-down (jogging and stretching). Sprint running sessions entailed 3-5 sets of 3-5 short bouts at maximum velocity. A recovery of 2-3 min was permitted between each set. Sprint cycling sessions comprised 3-5 repetitions of 10-30 s. The 10-30 s trials were performed maximally. Participants recovered actively (50% VO_{2max}) for 3-5 min between each sprint. RT sessions entailed 5-6 exercises targeting all major muscle groups. The load used during exercise was progressively increased from 40% to 65% of one-repetition maximum (1-RM) [18]. To produce maximal power output (in other words; velocity × load), the concentric phase of each exercise was performed as fast as possible [19]. Repetitions were maintained at 10-15 per sets and the number of sets increased from 3 to 4 during the training period. Hence, training volume increased progressively during the CSRT program. Rest periods between sets were 3-5 min for upper body muscles [19] and a minimum of 1 min for lower limbs. To adjust load during RT session and monitor adaptation, we determined strength using a 1-RM for the six resistance exercises, pre-training (P1), during the sixth week, and post-training (P2).

2.3.1. Testing Schedule

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

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

2.3.2. Physiological parameters

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

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

2.4. Statistical Analysis

Data analyses were performed using SPSS version 23.0 for Windows (SPSS, Inc. Chicago, IL, USA). Means and SD were calculated after verifying the normality of distributions using the Kolmogorov-Smirnov procedure. For anthropometric, physiological, and physical performances indices, data were analyzed using a multifactorial three-way (time [P1, P2] × age [young, middle-aged] × group [trained, control]) ANOVA and Fisher “F” value was given. Blood variables changes were analyzed using a four-factor ANOVA (time [P1, P2] × Wingate time [warm-up, immediately post-WAnT and 10 min post-WAnT] × age [young, middle-aged] × group [trained, control]). To help protect against type II errors, an estimate of power (ώ) and effect size (η^2_p) were calculated. Bonferroni-adjusted pairwise post hoc comparisons were performed where appropriate. Pearson's product-moment correlation coefficients were calculated to assess relationships between variables. Significance level was fixed to $p<.05$.

3. Results

3.1. Morphological Data and Physical Performances

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

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

At P1, there was an age-effect for fat-free mass (F=??, p<0.001, η2P=??). Following CSRT, fat-free mass increased significantly (F= 8.21, $p=0.03$) only in MAT and was 63.9±5.3 kg.

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

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

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

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

3.2. Blood Pressure, Heart Rate Characteristics and Hematological Markers

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

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

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

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

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

****Insert Table 3 here****

Hematocrit (Hct) changes (%) are represented in Table 4. A significant effect of time (F=13.50, *p<0.001*, η^2_P*=0.92*), Wingate time (F=14.12, p<*0.001*, η^2_P=0.10), age (F=8.55, *p<0.001*, η^2_P=0.06) and also group (F=9.21, *p<0.001*, η^2_P=0.85) was present. For YT, Hct_0, Hct_w, and Hct_{end} were significantly lower at P2 as compared to P1 (*p<0.001*). For MAT, Ht_w, and Hct_{end} were significantly lower at P2 as compared to P1 (*p<0.001*). Moreover, Hct_0 were significantly higher (*p<0.001*) in YT as compared to YC at P2 (see Table 4).

****Insert Table 4 here****

During WAnT, plasma volume decreased significantly (*p<0.001*) from warm-up (PVV_w) to the end of the WAnT (PVV_{end}) in all groups, then increased from the WAnT to recovery time (PVV_{10}) at P1 and P2 (*p<.05*) (Table 5). This decrease of PVV during exercise was significantly (*p<.05*) greater in middle-aged groups compared to younger groups at P1.

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

****Insert Table 5 here****

4. Discussion

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

An aim of the present study was to provide insight into the effect of acute and chronic intense exercise on hematological profiles in young and middle-aged men. Sosner and colleagues [23] reported that training in general resulted in an increase of vagal parasympathetic activity in the myocardium, an improvement in endothelial function, resulting in decreased arterial resistances, and improved aortic compliance. As we age, this aortic compliance declines and arterial resistance increases, leading to higher blood pressure [24]. Hence, to mediate blood pressure disturbance, the American College of Sports Medicine (ACSM) Position Stand [25], suggested that aerobic activities and resistance exercises performed 3 times per week are the best alternative to counteract age-related blood disorders. Rezk and colleagues [26] measured a decrease in SBP in normotensive young participants following 90-minutes of low intensity RT. RT reduced BP by 3.2-3.5 mmHg in young trained men [27]. Interestingly, results of the present study reported greater decreased in SBP (~10 mmHg) in trained groups after CSRT. Typically, attenuated effects of aerobic exercise on blood pressure are observed in trials lasting 3-6 months, because of poor adherence. As such, Weston and colleagues [28], suggested that short term HIT (2-3 months) allow greater decline in SBP in individuals with hypertension.

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

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

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

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

5. Conclusion

In summary, 13 weeks' sprint and resistance training appears to reduce the age-related decline in substrate metabolism (in other words; lactate) with increased performance levels during strenuous exercise in middle-aged men. In addition, this training intervention reduced systolic blood pressure in middle-aged trained men at rest and in response to exercise. These results occurred alongside increased resting RBC count, Hb, and PV in MAT. Moreover, the age-related differences among groups RBC count and PV changes following short-term exercise, were reduced at P2. Hence, short-term intense training with mixed exercises (sprints and resistances) prescription would allow lower blood pressure for a short period. Typically, individuals with hypertension have been dissuaded from engaging in long duration interventions and a poor adherence is usually registered. However, from this study, it appears 13-weeks' exercise training may be recommended as part of a program that reduces cardiovascular disease risk.

Disclosure of interest

The authors declare that they have no competing interest.

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Illustrations

Tables

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

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

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

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

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