International Journal of Cardiology 221 (2016) 77–80

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Arterial stiffness is strongly and negatively associated with the total volume of red blood cells

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ARTICLE INFO

Article history:
Received 3 April 2016
Received in revised form 24 May 2016
Accepted 27 June 2016
Available online 28 June 2016

Keywords:
Arterial stiffness
Pulse wave velocity
Blood volume
Anemia

ABSTRACT

Background: Erythropoiesis is partly regulated through classic feedback pathways that govern blood volume (BV) as sensed by veno-atrial but also arterial stretch receptors. Hence, the total volume of red blood cells (RBCV) could be associated with arterial stiffness (AS), although such hypothesis has not yet been tested. Therefore, we sought to investigate the association of AS with hematological variables including RBCV.
Methods: Fourteen healthy physically active individuals volunteered for the study (age = 23 ± 2). RBCV, plasma volume (PV), and BV were calculated from measures of hematocrit and total hemoglobin mass (Hb_{mass}) determined by CO-rebreathing. Carotid compliance with ultrasonography and carotid-ankle pulse wave velocity (PWV) were determined at rest and immediately after a maximal exercise test. The rationale for assessment of AS after exercise derives from the potential marked role of AS in the regulation of erythropoiesis in the setting of reduced central venous pressure.
Results: At rest, carotid compliance was positively associated with Hb_{mass}, RBCV, BV, but not PV, with coefficients of determination (R^2) ranging from 0.39 to 0.57. Following exercise, closer positive associations were observed between carotid compliance and Hb_{mass}, RBCV, or BV. Moreover, carotid-ankle PWV was negatively associated with all hematological variables after exercise except for PV, with R^2 ranging from 0.49 to 0.75. Similar results were observed when adjusted by body weight.
Conclusions: AS is strongly and inversely associated with RBCV in healthy individuals. These findings suggest that AS may adversely intercede in the regulation of erythropoiesis through the alteration of mechanisms that control BV.

1. Introduction

The total volume of red blood cells (RBCV) fundamentally determines cardiorespiratory capacity [1]. Thus, understanding the regulation of RBCV is particularly relevant for athletic performance as well as the management of individuals with anemia and exercise intolerance. While the hypoxic feedback control of kidney erythropoietin (EPO) synthesis and thereby erythropoiesis have been extensively studied, relatively little is known with respect to the regulation of RBCV under basal (non-kidney-hypoxic) conditions [2]. In this regard, previous studies have shown that plasma EPO concentration fluctuate inversely with changes in central (intrathoracic) venous pressure (CVP) [3–6], which reflects the filling state of the cardiovascular system. This suggests that EPO synthesis and RBCV may be regulated through classic feedback pathways that govern blood volume (BV) as sensed by veno-atrial but also arterial baroreceptors [6–8]. Accordingly, we speculate that RBCV could be impaired in proportion to alterations of baroreflex sensitivity due to arterial stiffness (AS) in central elastic arteries in which baroreceptors are located (e.g., carotid artery) [9,10]. The relationship between baroreflex sensitivity and AS is only natural given that baroreceptor firing rate is proportional to changes in arterial circumference [11]. In this line, Monahan et al. demonstrated that carotid AS independently explains the majority of the variance of baroreflex sensitivity across the lifespan [9]. Interestingly, carotid AS is inversely associated with cardiorespiratory capacity [12,13], which takes us back to the initial argument of this article.

Provided that EPO synthesis is stimulated with decreases in CVP [3–5], any potential association between AS and RBCV should be prominent if AS is assessed in conditions of reduced CVP. In this regard, CVP is transitorily reduced following exercise involving a substantial fraction of total muscle mass (e.g., running, cycling) [14,15]. The question arises as to whether AS measured immediately after exercise could be a closer predictor of RBCV than resting AS in physically active individuals. However, this hypothesis has not yet been tested.

Therefore, we sought to investigate the association of AS, as determined by carotid compliance and carotid-ankle PWV at rest and after

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http://dx.doi.org/10.1016/j.ijcard.2016.06.265
0167-5273/

acute exercise, with hematological variables including RBCV in healthy, young, physically active individuals.

2. Methods

2.1. Study subjects

Fourteen healthy, physically active individuals volunteered to participate in the study. The main characteristics of the study subjects are illustrated in Table 1. All participants were normotensive (systolic blood pressure (SBP) < 130 and diastolic blood pressure (DBP) < 85 mmHg), medication free, and with no history of renal or cardiovascular diseases. The study was approved by the local Ethics Committee and conducted in accordance with the Declaration of Helsinki. Prior to the start of the experiments, informed oral and written consents were obtained from all participants.

2.2. Experimental design

Participants were required to report to our laboratory on two occasions. All individuals avoided strenuous exercise, alcohol, and caffeine from 24 h prior to testing. All measurements described below were performed in the morning after fasting overnight in a quiet room with controlled temperature between 22 and 24 °C. Vascular and hematological variables were respectively assessed in the first and second testing sessions, respectively. AS variables were determined (i) following 15 min of supine rest and (ii) immediately (within 5 min) after a conventional incremental maximal exercise test performed in a cycle ergometer [1]. Hematological measurements were performed only at rest since our primary outcome, RBCV, is not altered with acute exercise in healthy individuals [4]. Time of day of testing sessions was kept consistent for each participant with a minimum of 48 h and a maximum of 7 days between sessions.

2.3. Experimental measures

2.3.1. Blood

RBCV, plasma volume (PV), and BV were measured as previously described [16], using carbon monoxide (CO) rebreathing. In brief, all subjects rested for 20 min in a semi-recumbent position before each measurement. Thereafter, 2 mL of blood was sampled from an antecubital vein via a 20-G venflon (BD, USA) and analyzed immediately in quadruplicate for (i) percent carboxyhemoglobin (%HbCO) and Hb concentration ([Hb]) using a hemoximeter (ABL800, Radiometer, Denmark), and (ii) hematocrit (Htc) with the micromethod (4 min at 13,500 rpm). Subsequently, the subject breathed 100% oxygen for 4 min to flush the nitrogen from the airways. After closing the oxygen input, a bolus 1.5 ml/kg of 99.997% chemically pure CO (CO N47, Air Liquide, France) was administrated into the breathing circuit. The subjects rebreathed this gas mixture for 10 min. Then, an additional 2 ml blood sample was obtained and analyzed in quadruplicate. The change in %HbCO was used to calculate hemoglobin mass (Hb_{mass}), taking into account the amount of CO that remained in the rebreathing circuit at the end of the procedure (2.2%) [16]. RBCV, PV, and BV were derived from measures of Hb_{mass} and hematocrit [16].

Table 1
Main characteristics, hematology, and AS in study participants.

Main characteristics	
Age (yr)	23.3 ± 1.5
Sex (% female)	7 (50)
Height (cm)	172.9 ± 9.6
Weight (kg)	66.6 ± 9.7
SBP (mmHg)	116.3 ± 6.9
DBP (mmHg)	66.9 ± 6.2
Maximal heart rate (bpm)	182.6 ± 5.9
W_{peak} (W)	269.3 ± 59.5
Hematological variables	
Hb_{mass} (kg)	0.71 ± 0.20
Hb_{mass} ($g \cdot kg^{-1}$)	10.3 ± 1.8
RBCV (L)	2.1 ± 0.6
PV (L)	3.6 ± 0.7
BV (L)	5.7 ± 1.2
Rest	
Carotid compliance ($mm^2\ kPa^{-1}$)	0.13 ± 0.04
Carotid-ankle PWV ($m \cdot s^{-1}$)	5.87 ± 0.36
Following maximal exercise	
Carotid compliance ($mm^2\ kPa^{-1}$)	0.09 ± 0.03
Carotid-ankle PWV ($m \cdot s^{-1}$)	5.84 ± 0.79

Data are presented as mean ± SD or *n* (%).
AS, arterial stiffness; BV, blood volume; DBP, diastolic blood pressure; Hb_{mass}, hemoglobin mass; PV, plasma volume; RBCV, red blood cell volume; SBP, systolic blood pressure; W_{peak}, maximal cycling power output.

2.3.2. Arterial stiffness (AS)

AS was assessed by means of high-resolution ultrasonograpy equipped with a 7 MHz linear array probe (Mindray M7, China) in the right carotid artery as well as two pressure transducers (Complior, France) placed at the neck and left ankle to obtain carotid compliance and carotid-ankle pulse wave velocity (PWV), respectively, according to established guidelines [17]. Carotid-ankle PWV measurements before and after exercise were available in 10 subjects. Blood pressure and heart rate were measured on the left arm with an automated system (Dinamap, GE Medical Systems, USA).

2.3.3. Maximal exercise test

Maximal power output (W_{peak}) was determined on an electronically braked bicycle ergometer (Monark, Sweden). The test started with a warm-up period of 5 min at 70–100 W workloads. Thereafter, the workload was increased by 30 W every 60 s until exhaustion. W_{peak} was calculated as $W_{compl} + 30$ (t/60); W_{compl} is the last fully completed workload and t is the number of seconds in the final workload.

2.4. Statistical analysis

Statistical analysis was performed using IBM SPSS v. 20 (Chicago, USA) software package. Data were tested for normal distribution with the Kolmogorov–Smirnov test and for homogeneity of variances with Levene's test. Paired *t*-tests were used to compare carotid compliance and carotid-ankle PWV prior to and after exercise. Linear regression analyses were performed to determine the association between AS and hematological variables. In all analyses, measures of AS were considered as independent variables. In addition, these analyses were performed with inclusion of BW or gender as covariates (i.e., adjusting for BW or gender). A two-tailed *P*-value less than 0.05 was considered significant. Data are reported as mean (±SD) unless otherwise stated. A two-tailed *P*-value less than 0.05 was considered significant.

3. Results

Hb_{mass}, RBCV, PV, and BV ranged from 7.7 to 12.6 $g \cdot kg^{-1}$, 1.2 to 2.8 L, 2.5 to 4.3 L, and 3.7 to 7.1 L, respectively. With regard to AS measures, carotid compliance and carotid-ankle PWV ranged from 0.06 to 0.19 $mm^2 \cdot kPa$ and 5.35 to 6.37 $m \cdot s^{-1}$ in resting conditions, and from 0.03 to 0.13 $mm^2 \cdot kPa$ and 4.98 to 7.18 $m \cdot s^{-1}$ after maximal exercise, respectively. Carotid compliance, but not carotid-ankle PWV ($P > 0.05$), was decreased after exercise compared with resting conditions ($P < 0.05$).

Linear regression analyses regarding the association of AS (carotid compliance, carotid-ankle PWV) with hematological variables (Hb_{mass}, $Hb_{mass} \cdot kg^{-1}$, RBCV, PV, BV) are presented in Table 2. At rest, carotid compliance was positively associated ($P < 0.05$) with all hematological variables except for PV, with coefficients of determination (R^2) ranging from 0.39 to 0.57. Likewise, following exercise carotid compliance was positively associated ($P < 0.05$) with all hematological variables and R^2 ranged from 0.43 to 0.48. In addition, carotid-ankle PWV was negatively associated ($P < 0.05$) with all hematological variables after exercise except for PV, with R^2 ranging from 0.49 to 0.75. Similar results were observed when regression analyses were adjusted by body weight (Fig. 1), while closer associations of measures of AS with RBCV were found when adjusted by gender.

4. Discussion

The main findings of this study are 1) AS, as determined by carotid compliance and carotid-ankle PWV, is inversely associated with Hb_{mass}, RBCV, and BV; 2) these associations present higher correlation coefficients when AS is measured following acute exercise. These data are consistent with a central role of AS in the regulation of erythropoiesis.

Beyond the universally accepted role of kidney tissue hypoxia on EPO synthesis, the regulation of erythropoiesis remains uncertain [2,18]. In this regard, the fact that blood pressure independently influences BV raises the question of whether erythropoiesis and thus RBCV could be modulated by hemodynamic stimuli [19–24]. In support hereof are reports of augmented plasma EPO concentration during experiments inducing central hypovolemia and vice versa [3–6]. Indeed, EPO has been postulated as a blood-volume-regulated hormone, in line with known hormones regulating fluid homeostasis such as natriuretic peptides, vasopressin (VPN), and those pertaining to the renin–angiotensin–aldosterone system [6]. These hormones control BV through feedback loops including veno-atrial and central arterial stretch receptors [7,8]. Since