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Blood Volume and Vascular Diversity in Highly Endurance  
Trained Athletes: Sport Specificity?

A Cross-sectional Study

Master's Thesis in Clinical Health Science

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## Abstract

**Background:** Blood volume (BV) is a critical component for cardiac output. However, it is unknown to what extent a high BV contributes to explain differences in maximal oxygen uptakes ( $VO_{2max}$ ) in elite athletes in different sports. Furthermore, efficient oxygen delivery to working skeletal muscles challenges the vascular system, and arterial changes are found in athletes disposed to chronic endurance training. Whether these arterial changes influence the arterial dilatory response in endurance athletes with different involvements of the upper, lower and whole body is unclear. **Objective:** The aims of this study were to compare  $VO_{2max}$ , BV, hemoglobin mass ( $Hb_{mass}$ ) and vascular function in highly trained endurance athletes primarily using whole (cross-country skiing), lower (orienteering) and upper body (flatwater kayak). **Method:** 43 male endurance athletes in the disciplines of cross-country skiing (n=17), orienteering (n=15) and flatwater kayak (n=11) participated in a cross-sectional study.  $VO_{2max}$  was measured using direct ergospirometry, carbon monoxide re-breathing method was used to assess BV variables and endothelial function was measured as flow mediated dilatation (FMD). **Results:** The cross-country skiers had higher  $VO_{2max}$  than the orienteers regarding  $mL \cdot min^{-1} \cdot kg^{-1}$  and  $L \cdot min^{-1}$  ( $p < 0.01$ ). The cross-country skiers and orienteers showed significantly higher BV than the kayakers, both relative to body mass ( $p < 0.05$ ) and FFM ( $p < 0.01$ ). The cross-country skiers had significantly higher  $Hb_{mass}$  than the kayakers relative to body mass and fat free mass ( $p < 0.05$ ), with no significant differences between the cross-country skiers and orienteers. The kayakers presented significantly larger arterial diameter than the orienteers ( $p < 0.05$ ), but there were no significant differences between groups regarding FMD. **Conclusion:** These data indicates that the whole body exercise employed by cross-country skiers induces higher  $VO_{2max}$  compared to upper and lower body sports. However, these differences did not correspond with variations in BV variables between cross-country skiers, orienteers and kayakers.

**Key words:** Hemoglobin mass, maximal oxygen uptake, endothelial function, cross-country skiing, orienteering, flatwater kayak.

## Abbreviations

a-vO<sub>2</sub> difference - arteriovenous oxygen difference

BMI - body mass index

BV - blood volume

CI - confidence interval

CO - carbon monoxide

dL - deciliter

eNOS - endothelial nitric oxide synthase

EPO - erythropoietin

FFM - fat free mass

fl - femtoliter

FMD - flow mediated dilatation

Hb - hemoglobin

Hb<sub>mass</sub> - hemoglobin mass

Hb% - hemoglobin concentration

HIT - high intensity endurance training

L - liter

LIT - low intensity endurance training

MCH - mean cell hemoglobin

MCHC - mean cell hemoglobin concentration

MCV - mean cell volume

MIT - moderate intensity endurance training

mL - milliliter

NO - nitric oxide (NO)

pg - picogram

PSR - peak shear rate

PV - plasma volume

RCV - erythrocyte volume or red blood cell volume

VO<sub>2max</sub> - maximal oxygen uptake

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# 1. Background

*Faster, higher, stronger* — the olympic motto outlines the essence of the athletic nature and the goal of the athletic performance. In the field of endurance sports, performance predominantly depends on the capacity of oxygen delivery to active skeletal muscle tissue and the skeletal muscles' ability to use oxygen — with oxygen supply as the main limiting factor (1). Blood volume (BV) and hemoglobin mass ( $Hb_{mass}$ ) have a major impact on cardiac output and oxygen transport capacity (2-4) and may, thus, be essential in determining an endurance athlete's sports performance. A well-adapted vascular function is also important in optimizing oxygen supply to working skeletal muscles because of the large cardiac output which needs to be transported by the vascular system during heavy exercise in endurance sports (5).

## 1.1 Maximal oxygen uptake

Maximal oxygen uptake ( $VO_{2max}$ ) is frequently used as an expression of cardiorespiratory fitness and is defined as the maximal amount of oxygen taken up and utilized by the body during heavy exercise (6).  $VO_{2max}$  depends on skeletal muscular oxygen consumption and the ability to transport oxygen to working skeletal muscles, the latter being the main limiting factor among endurance athletes as their muscular capacity for oxygen extraction and utilization is well adapted. Variables which influence oxygen uptake are described in Fick's principle:

$$VO_{2max} = \text{cardiac output}_{max} \times a\text{-}vO_2\text{difference}_{max}$$

Cardiac output is the product of the stroke volume and the heart rate, and is the total amount of blood pumped out by each ventricle of the heart each minute, while the arteriovenous oxygen difference (a-v $O_2$  difference) is the difference in oxygen content in arterial and mixed venous blood (7).

The Fick's principle implies that components influencing these physiological factors are possible limiting factors of  $VO_{2max}$  and thereby affect maximal physical performance. Maximal cardiac output is considered to be the main limiting factor of  $VO_{2max}$  and is primarily determined by the heart's stroke volume, since variations in maximal heart rate are small. The stroke volume depends on the venous return, which on the other hand, is affected by the heart muscle's pumping capacity and BV (6, 8). The diffusion capacity of the lungs

may be a limiting factor amongst highly trained athletes. This is due to elevated maximal cardiac output compared to untrained subjects, which further might give insufficient time for the blood to saturate with O<sub>2</sub> passing through the pulmonary capillaries (6). The O<sub>2</sub>-transport capacity is determined by the O<sub>2</sub> saturation and hemoglobin concentration (Hb%), while the venous oxygen content is affected by peripheral factors like capillary density and mitochondrial function (6, 9). Like capillary density, a well-adapted vascular function in terms of arterial dilator capacity is vital in optimizing oxygen delivery. This might be compromised during heavy exercise where arterial blood pressure is maintained at the cost of blood flow to working skeletal muscles causing vasoconstriction and further affecting oxygen delivery and (5).

It is not surprising, then, that a high positive correlation has been reported between BV, Hb<sub>mass</sub>, erythrocyte volume (RCV) and VO<sub>2max</sub>, both regarding absolute and relative values (9-13).

## **1.2 Blood volume and hemoglobin mass**

RCV and plasma volume (PV) represent together total BV and can independently change to alter total BV (14). Normal total BV for adults ranges from 5 to 6 liters for men and from 4 to 4.5 liters for women. 40-45% of the total BV consist of erythrocytes and 55-60% of plasma. Approximately 99 % of the proteins in the erythrocyte's cytosol consist of hemoglobin (Hb), which is a molecule made up of four units. Each of these units has a molecular group called heme attached to it and further, each heme group contains one atom of iron and can bind one molecule of oxygen. Thus, four oxygen molecules can attach to each of the Hb molecules. Erythrocytes are produced in the bone marrow at a speed that matches the erythrocyte destruction. This production is stimulated by erythropoietin (EPO), a hormone secreted into the bloodstream by the kidneys. The production of EPO is stimulated by hypoxia, androgens and possibly other hormones (5, 15).

Several cross sectional studies suggest that highly trained endurance athletes have elevated BV and Hb<sub>mass</sub> compared to untrained individuals. Kjellberg et al. described in 1949 the relationship between BV, Hb<sub>mass</sub> and physical performance, and reported relative BV and Hb<sub>mass</sub> values 20-25% higher in elite athletes compared to untrained subjects (16). Heinicke et al. (9) found that BV and Hb<sub>mass</sub> relative to body mass in endurance trained athletes in the

disciplines of cycling, running and triathlon were as much as 35-40% higher than in their untrained counterparts. Endurance athletes also exhibited higher values than athletes in sports of non-endurance nature, probably because of lower cardio-pulmonary demands during performance of their sports. These high levels are further supported by Schmidt and Prommer (10) who performed a meta-analysis of 611 subjects living at sea level and found that the group of elite athletes presented BVs and  $Hb_{mass}$  ~30% higher than the non-exercising group. Another study found 15-20% higher relative  $Hb_{mass}$  and also significantly higher BV in adult endurance trained athletes compared to controls with maximum 2 hours of endurance training per week, where the slightly smaller difference than previous studies might be explained by the control group being not completely inactive (17). The above findings are also confirmed by others (18-20).

The convincing documentation of endurance trained athletes possessing elevated BV and  $Hb_{mass}$  values is per se no evidence of this being a consequence of mode of training. Several studies (9, 10, 17, 21, 22) suggest a strong genetic component both regarding high BV and  $Hb_{mass}$ , implying that subjects with genetically high BV or  $Hb_{mass}$  might be predisposed to endurance sports. High levels of BV and  $Hb_{mass}$  have been linked to elevated  $VO_{2max}$  in inactive subjects explaining the unexpected high  $VO_{2max}$  (21). However, several intervention studies have explored the effect of endurance training on both BV and  $Hb_{mass}$ . Sawka et al. (23) presented a review of 23 studies on BV's response to endurance training. Half of the studies showed an increase both in RCV and PV after endurance training, and that the time course of these increases were different. The increase in PV could occur within 24 hours and was mainly responsible for increases in BV in the initial 2 weeks of training, while the increase in RCV occurred after 2-3 weeks of endurance training until vascular volumes reached a value ~8-10% above baseline values. Similar findings were presented by Ray et al. (24) who found a total BV increase of ~8% in previously untrained men undergoing an 8 week endurance training program. The increase was a result of elevation of both PV (~6%) and RCV (~11%).

The effect of physical training on  $Hb_{mass}$  is more inconclusive, but Schmidt and Prommer (10) found ~6% increase in  $Hb_{mass}$  and ~10% increase in BV in leisure sportsmen after following an intensive 9 month marathon training program. Worth noticing, these subjects were



relatively untrained individuals at baseline. In endurance athletes repeatedly measured throughout a year, only small oscillations were found (<6%) during a year of training (25). This is further supported by Eastwood et al. (22, 26), who found Hb<sub>mass</sub> relatively stable (~2%) over time in recreationally active men, and in response to 40 days of regular physical exercise in previously inactive men. Schmidt and Prommer (4) indicate that years of endurance training might increase Hb<sub>mass</sub> but the effects under normoxic conditions seem minor. They imply that the large difference in Hb<sub>mass</sub> between trained and untrained subjects cannot be explained by endurance training alone, and suggest that heredity plays a major role.

The BV's increase with endurance training is explained as a result of an acute decrease in PV leading to increased osmolarity in the blood, which further activates the renin-angiotensin-aldosterone system. In short, this leads to increased reabsorption of sodium and water into the blood and will thereby increase the total BV. Also, training might induce an increase in the blood's protein content increasing the blood's osmolarity and further leading to extraction of extracellular fluid into the blood (27). Increases in Hb<sub>mass</sub> are suggested to be a physiological adaptation to restore Hb% levels after an increase in PV (4).

### **1.3 Vascular function**

The interior surface of all blood vessels is lined with a single, thin layer of cells which is called the endothelium. The endothelium separates the blood from the vascular smooth muscle and the interstitial compartments. This large paracrine organ responds to both external and internal stimuli (5). Endothelial nitric oxide synthase (eNOS) is an enzyme that generates nitric oxide (NO) from the amino acid L-arginine and oxygen in blood vessels in response to receptor-dependent agonists (e.g., acetylcholin), increased blood flow and increased shear stress e.g., due to physical activity. NO diffuses through the endothelium to the vascular smooth muscles and elicits changes in vascular tone (5, 28). Normally the blood vessels dilate as a response to the endothelial release of NO, resulting in decreased vascular resistance and normalized shear stress. NO inhibits the release of vasoconstricting factors (e.g, serotonin and thromboxane) and seems to be the main mediator of flow-mediated dilatation (FMD). The flow-mediated vascular activity is influenced by many extrinsic factors like physical activity, food, drugs, sympathetic stimuli (e.g., nitroglycerin) and temperature (28-30). The menstrual

cycle, mental stress and sleep deprivation i.e. hormonal factors, may also influence the endothelial function (28).

Efficient oxygen delivery to working skeletal muscles during heavy exercise in endurance sports sets high demands on the vascular system. However, several studies of vascular adaptations in healthy subjects as a response to chronic endurance training and prolonged shear stress suggest that there exists an «athlete's artery» just as the generally acknowledged «athlete's heart» (31-33). These arteries are characterized by enlarged diameter and decreased arterial wall thickness and one speculates that the arterial function could be affected by these structural changes. Unlike what one might expect, limited evidence of improved arterial response to physiological stimuli among athletes has been found. Summarized, most short-term training studies indicate an increase in FMD whilst long-term training studies suggest arterial remodeling. This emerges as a paradox since exercise in several studies is found to improve vascular function in e.g., patients with metabolic syndrome, coronary artery disease and obesity (34-36). This phenomenon is supported by a study of highly trained male endurance athletes conducted by Rognmo et al. (37), which showed an occurrence of larger arterial diameter but similar endothelial-dependent vasodilation compared to their untrained counterparts. An intervention study looked at this discrepancy and measured FMD in 13 healthy men every second week during an 8 week endurance training program. They found increased FMD the first two weeks of training, and then FMD gradually returned to baseline. This suggests that the change in vasodilator function in response to exercise follows a time course (38).

## 2. Introduction

Endurance performance highly depends on energy delivery capacity, and correlates well with  $\text{VO}_{2\text{max}}$  and utilization of oxygen during competition (6, 39-41). Cross-country skiing is a whole body exercise endurance discipline, in which some of the highest  $\text{VO}_{2\text{max}}$  values (i.e. above  $80 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  in male athletes) have been reported (5, 42). Although  $\text{VO}_{2\text{max}}$  has not been compared directly among elite athletes in sports where the majority of exercise training performed as leg or upper body work only since the famous study by Saltin and Åstrand in 1967 (42), independent studies on orienteers and flatwater kayakers indicate that these athletes have lower levels of  $\text{VO}_{2\text{max}}$  than those found in elite cross-country skiers (43-45). Due to the possibly higher  $\text{VO}_{2\text{max}}$  levels in cross-country skiers, it might be speculated that chronic exposure to whole body exercise enhance the various factors related to  $\text{VO}_{2\text{max}}$  more than upper or lower body exercise (5, 46). For  $\text{VO}_{2\text{max}}$ , BV is a critical component for cardiac output (6); however, it is yet unknown whether higher BV may explain differences in  $\text{VO}_{2\text{max}}$  among sports with various contributions of upper and lower limbs in their training.

Previous research has suggested the existence of an «athlete's artery» with enlarged diameter and decreased arterial wall thickness compared to the general population (32). Another aspect of the arterial remodeling in athletes is the increase in arterial diameter associated with prolonged use of predominant limbs (33, 47). To what extent these arterial changes are affecting the arterial dilatory response in highly trained endurance athletes with different involvements of the upper, lower and whole body, has still not been solved.

Cross-country skiing, orienteering and flatwater kayak are all sports in which Norwegian athletes are performing at high international levels. Elite endurance athletes that are experts in their movement, have had chronic endurance adaptations to that movement for several years of training, and will in that respect function as a unique research model for determining the possible adaptations to exercise training in humans. Assuming that the genetic component does not differ in its contribution in such elite athletes, the differences in the physiological adaptations on BV,  $\text{Hb}_{\text{mass}}$  and vascular function, between elite athletes specially trained for the arms, legs or whole body, may give further insight into the upper limits of the human's ability to adapt to such training. Also, how these highly specialized subjects implement their

way of training might be of interest considering the mechanisms potentially leading to physiological diversities.

The primary aim of this study was to compare the level of  $VO_{2max}$  and BV in a cross sectional study of highly trained endurance athletes primarily using whole (cross-country skiing), lower (orienteering) and upper body (flatwater kayak).

The secondary aim was to compare the level of  $Hb_{mass}$  and the vascular function (FMD) in the same groups of athletes.

It was hypothesized that the cross-country skiers being a whole body exercise endurance discipline, had higher  $VO_{2max}$  than disciplines where the majority of exercise training is performed as leg (orienteers) or upper body work only (flatwater kayakers) and also, that the higher  $VO_{2max}$  corresponded with a higher BV in the same group.

## 3. Methodology

### 3.1 Subjects

Forty-three male elite endurance athletes on National and Regional teams were recruited through Olympiatoppen Mid Norway. All subjects were competing at national or international level in one of the following disciplines; cross-country skiing (n=17), orienteering (n=15) or flatwater kayaking (n=11). Exclusion criteria for participation were significant blood loss ( $\geq 500$  mL) within last 3 months, anemia ( $\leq 13$ g/dL), 10 days or more of altitude training the last 3 months, kidney failure (reduced production of EPO), EPO use, cardiovascular or pulmonary disease, or medication limiting maximal endurance performance.

Four cross-country skiers were not measured by Bioelectrical impedance analysis (BIA) and are thus excluded in analyses related to these measurements.

All experimental procedures were approved by the Regional Committees for Medical and Health Research Ethics. All subjects received oral and written information about the study before signing a written consent. For subjects under the age of 18, an additional written consent was obtained from their parents.

### 3.2 Study design

The study had a cross sectional design with 3 groups of elite endurance athletes. One group of cross-country skiers using whole body, a second group of orienteers primarily using lower body and a third group of flatwater kayakers primarily using their upper body during performance of their sports.

The data were collected right after each disciplines competition phase from spring to late fall 2012 and took place at the Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway (NTNU), and at the Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway (NIH). Some tests of  $VO_{2max}$  were conducted at Olympiatoppen Norway and Olympiatoppen Mid Norway. To avoid methodological errors, identical medical equipment were used. Two subjects were tested by the carbon monoxide (CO) re-breathing method in Trondheim and Oslo to estimate test-retest reliability, with a coefficient of variation of 0.26.

Ergospirometry systems were regularly calibrated using a high precision gas ( $16.00\pm 0.04\%$   $O_2$  and  $5.00\pm 0.1\%$   $CO_2$ , Riessner-Gase GmbH & Co, Lichtenfels, Germany) and the inspiratory flowmeter was calibrated using a 3 l volume syringe (Hans Rudolph Inc., Kansas City, MO, USA). Ergospirometry systems were tested against each other, and showed identical results.

Measurements were conducted over two days for each subject.  $VO_{2max}$  measurements were conducted with minimum 24 hours' and maximum 15 days' distance to other measurements. Blood samples, anthropometry, measurement of vascular function,  $Hb_{mass}$  and BV were performed in the morning with 12 hours fasting, avoiding caffeine, alcohol, tobacco, vitamins and minerals in addition to food. Subjects were asked to drink 500 ml water 2 hours prior to the investigation, and avoid or only exercise at light or moderate intensity the last 24 hours.

### **3.3 Measurements**

#### **3.3.1 Maximal oxygen uptake**

Measurement of  $VO_{2max}$  was conducted as incremental tests, using a direct ergospirometry system with a mixing chamber (Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany). The tests were performed on a treadmill (Woodway USA Inc., Waukesha, WI, USA) at a fixed inclination of 10.5% and an increase in speed of 1 km/h every minute until exhaustion. Warm ups were set to at least 15-20 minutes and initial speed was individualized.

A leveling off of oxygen uptake despite increased workload and a respiratory exchange ratio  $\geq 1.05$  were used as criteria for  $VO_{2max}$  (48). Peak heart rate ( $HR_{peak}$ ) was measured by the use of a heart rate monitor (Polar RS400, Polar Electro Oy, Kempele, Finland). The mean of the three highest 10 second measurements recorded continuously, was used to determine  $VO_{2max}$ .  $HR_{peak}$  was defined as the highest recorded value during the test.



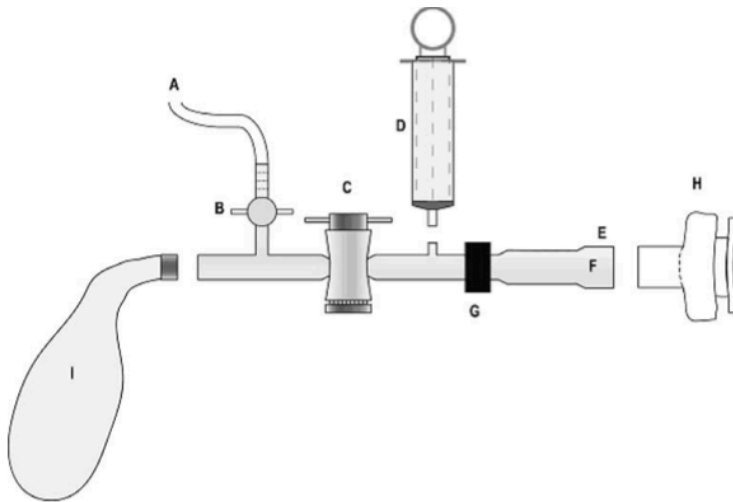
*Figure 1. Measuring maximal oxygen uptake (picture used with permission).*

### **3.3.2 The optimized carbon monoxide re-breathing method**

Carbon monoxide re-breathing spirometry (Bayreuth, Germany) as described by Schmidt and Prommer (49) and later modified by Prommer and Schmidt (50), was used to determine  $Hb_{mass}$ , BV, PV and RCV. This is an indirect method using CO as a marker of Hb, with its affinity to the Hb molecule 250 times stronger than oxygen (5). The  $Hb_{mass}$  is estimated according to the difference in carboxyhemoglobin concentration (HbCO%) before and after rebreathing, a known volume of CO, and the binding capacity of Hb for CO ( $1.39 \text{ mL} \cdot \text{g}^{-1}$ ).

Gore et al. (51, 52) have showed that the CO re-breathing method is both valid and reliable for estimating  $Hb_{mass}$  and has a low error of measurement ( $\sim 2.2\%$ ) similar to the method considered to be the gold standard; the radioactive method using the isotopic tracer radioactive chromium ( $^{51}\text{Cr}$ ) ( $\sim 2.8\%$ ).

A dose (milliliter) of CO (99.9%) was estimated in accordance to gender, physical fitness and body mass. A 100 ml syringe was filled with an individualized dose of CO gas from a CO gas cylinder and connected to a closed spirometer with a 3 liter anaesthetic bag containing 100% oxygen. Soda lime was added inside the adapter for the mouthpiece to absorb CO<sub>2</sub>. Ambient temperature and the current barometric pressure were recorded during the measurement. Spirometer shown in Figure 2.



**Figure 2.** Spirometer used for CO-rebreathing method. A; O<sub>2</sub> tube (disconnected during test), B; O<sub>2</sub> port (closed during test), C; valve to anaesthetic bag (open during test), D; CO syringe, E; adapter for mouthpiece, F; netting bag of soda lime, G; sleeve, H; mouthpiece, I; 3 liter anaesthetic bag. From Schmidt and Prommer, 2005 (49) (illustration used with the approval of Prof. Dr. Walter Schmidt).

Capillary blood samples were collected prior to the test, after 15 minutes of seated rest, allowing the PV to stabilize, and 6 and 8 minutes after the rebreathing of CO, ensuring that CO was equally distributed in the body. Capillary blood samples were analyzed for Hb% and HbCO% by the ABL800 FLEX analyzer (Radiometer Medical ApS, Brønshøj, Denmark).

After receiving thorough information about the procedure, the subject put on a nose clip and exhaled most of the air, put the spirometer's mouthpiece to the mouth and continued to exhale. When instructed, the subject performed a maximal inhalation through the spirometer at the same time as the CO bolus was injected from the syringe and the stopcock to the oxygen bag was opened. After the inhalation the subject held his breath for 10 seconds. The



subject then continued breathing normally through the spirometer for 1 minute and 50 seconds before performing a maximal expiration of air into the 3 liter bag, whereupon the bag valve was closed trapping the remaining mixed air in the bag. The inhalation procedure makes it likely that the CO bolus is inhaled in the first part of the inspiration and that a large part diffuses into the blood during the first seconds of inspiration.



*Figure 3. CO-rebreathing method (picture used with permission).*

The end-tidal CO concentration was measured with a CO gas-tester (Draeger<sup>®</sup>, Luebeck, Germany) prior to and 4 minutes after the CO-inhalation in order to estimate the amount of CO exhaled until the time of blood sampling. A 3 liter calibration syringe (Hans Rudolph inc., Shawnee, KS, USA) and the CO tester were used to measure gas volume and CO concentration in the spirometer and the bag after the procedure was completed by the athletes. The CO tester was also used to check for gas leaks around the syringe, valves and the test person's nose and mouth during the test. The values for BV, Hb<sub>mass</sub>, RCV and PV were estimated using Spico Calculation Software (Blood tec, GbR, Bayreuth, Germany), which also corrects for loss of CO to myoglobin.

### **3.3.3 Flow mediated dilatation**

Endothelial function was measured as FMD in the brachial artery according to current guidelines (29, 30, 53, 54). The principle of the FMD method is to create a stimulus to shear stress in the artery by occlusion of the limb for 5 minutes, causing an ischemic condition. A release of the occlusion results in a reactive hyperemia and increased shear stress, which will decrease during a period of 1-2 minutes.

The measurements were conducted in a quiet and temperature stable room after 10 minutes of rest in the supine position. FMD was measured in the brachial artery 4.5 cm above the antecubital fossa, using high-resolution vascular ultrasound (14MHz Doppler probe, Vivid 7 and Vivid I, GE Vingmed Ultrasound AS, Horten, Norway). Baseline images were taken before an occlusion was made on the distal part of the forearm by inflating a pneumatic cuff (SC10, D.E. Hokanson, Inc., Bellevue, WA, USA) to 200-250 mm Hg (25-50 mm Hg over systolic pressure) for 5 minutes. The cuff was then deflated to create a high-flow state in the artery. A longitudinal image of the brachial artery's internal diameter was recorded continuously for 3 minutes after cuff-release.

Arterial diameter varies during the cardiac cycle. An integrated electrocardiogram (ECG) was therefore used to secure assessment of the diameter according to the cardiac cycle. Diameter was calculated as an average of 3 measures synchronized with the R-wave peak and measured from intima to intima. Mean flow velocity was measured at baseline and 15 seconds after cuff-release (53, 55).

FMD was calculated as percent increase at 30, 60 and 90 seconds in the arterial diameter post deflation from baseline vessel size. Peak shear rate (PSR) was calculated as the difference between mean flow velocity at baseline and 15 seconds after cuff-release, divided by baseline diameter. The FMD was normalized by dividing FMD by PSR, multiplied by 1000 (55). Ultrasound images were analyzed using EchoPACtm (GE Vingmed Ultrasound AS, Horten, Norway).

### **3.3.4 Blood samples**

Blood was collected by venous puncture in a fasted state ( $\geq 12$  hours), and EDTA and serum vacutainers collected for the biochemical analysis including; hematocrit, Hb%, mean cell

volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), leucocytes, erythrocytes and thrombocytes. Venous blood samples were analyzed by the XE-2100 analyzer (Sysmex Co., Kobe, Japan) according to standard local procedures at the Department of medical biochemistry, St. Olavs University Hospital, Trondheim, Norway.

### **3.3.5 Anthropometric measurements**

Body mass and body height were measured, and BMI was calculated as body mass (kilogram) divided by the body height (meter) squared. Bioelectrical impedance analysis (BIA) (InBody 720, Biospace CO, Ltd, Seoul, Korea) was used to assess body composition.

### **3.3.6 Training history**

Training history the last 6 months prior to measurements was recorded based on the athletes' training diaries and classified into intensity zones according to the session goal method as previously done by Sandbakk et al. (56). Endurance training was registered by a heart rate monitor and categorized into three intensity zones according to the Norwegian Olympic system's intensity scale; low intensity (LIT) (60-81% of  $HR_{max}$ ), moderate intensity (MIT) (82-87% of  $HR_{max}$ ) and high intensity (HIT) (>88% of  $HR_{max}$ ). Speed/resilience and strength training were also recorded. Measurements were performed right after each disciplines competition phase, thus, training hours were recorded in a period with relatively low training volumes (i.e. during the competitive season).

## **3.4 Sample size**

With a mean difference in total BV of 0.65 liter and a standard deviation of 0.5 liter between expected values in the disciplines of cross-country skiing versus orienteering and kayak (9), it was estimated that 15 subjects were needed in each group with 80 % power and alpha level set to 5%.

## **3.5 Statistical analysis**

All statistical analyses were performed using IBM SPSS Statistics software program version 20 (SPSS Inc. Chicago, IL., USA), and GraphPad Prism version 6 (GraphPad Software Inc., San Diego, CA, USA) was used for graphic illustrations. Variables are presented as mean with 95% confidence interval. Histograms with normality curves, error bars and Q-Q plots were

used to explore assumptions of normality. Homogeneity of variances was checked with Lavené's test. One-way ANOVA was used for comparisons between the three groups. The Welch and Brown-Forsythe robust tests of equality of means were used if the assumption of the homogeneity of variance was violated. Post hoc comparisons were made using Tukey HSD. The independent-samples t-test was used to look at differences between subjects aged  $\leq 20$  and subjects aged  $\geq 21$  regarding training volumes. Level of significance was set to  $< 0.05$ .

## 4. Results

### 4.1 Subject characteristics

Subject characteristics are presented in Table 1. Mean age amongst the cross-country skiers was 3.1 years higher than in the kayak group ( $p<0.05$ ). Concerning body composition the kayak group possessed approximately 8% higher BMI and total body FFM (kg) than the orienteering group ( $p<0.05$ ). FFM in the upper body was significantly higher in the kayak group than the other groups ( $p<0.01$ ), with about 17% higher values than the orienteering group and 11% higher values than the cross-country skiing group. Relating FFM upper body to total body FFM, the kayakers still presented about 8% higher values than the orienteers and 4% higher values than the cross-country skiers ( $p<0.01$ ). The cross-country skiing group also presented about 4% higher values than the orienteering group ( $p<0.01$ ). FFM in the legs (%) related to total body FFM showed that the orienteers had approximately 9% higher values than the kayakers and 6% higher values than the cross-country skiers ( $p<0.01$ ).

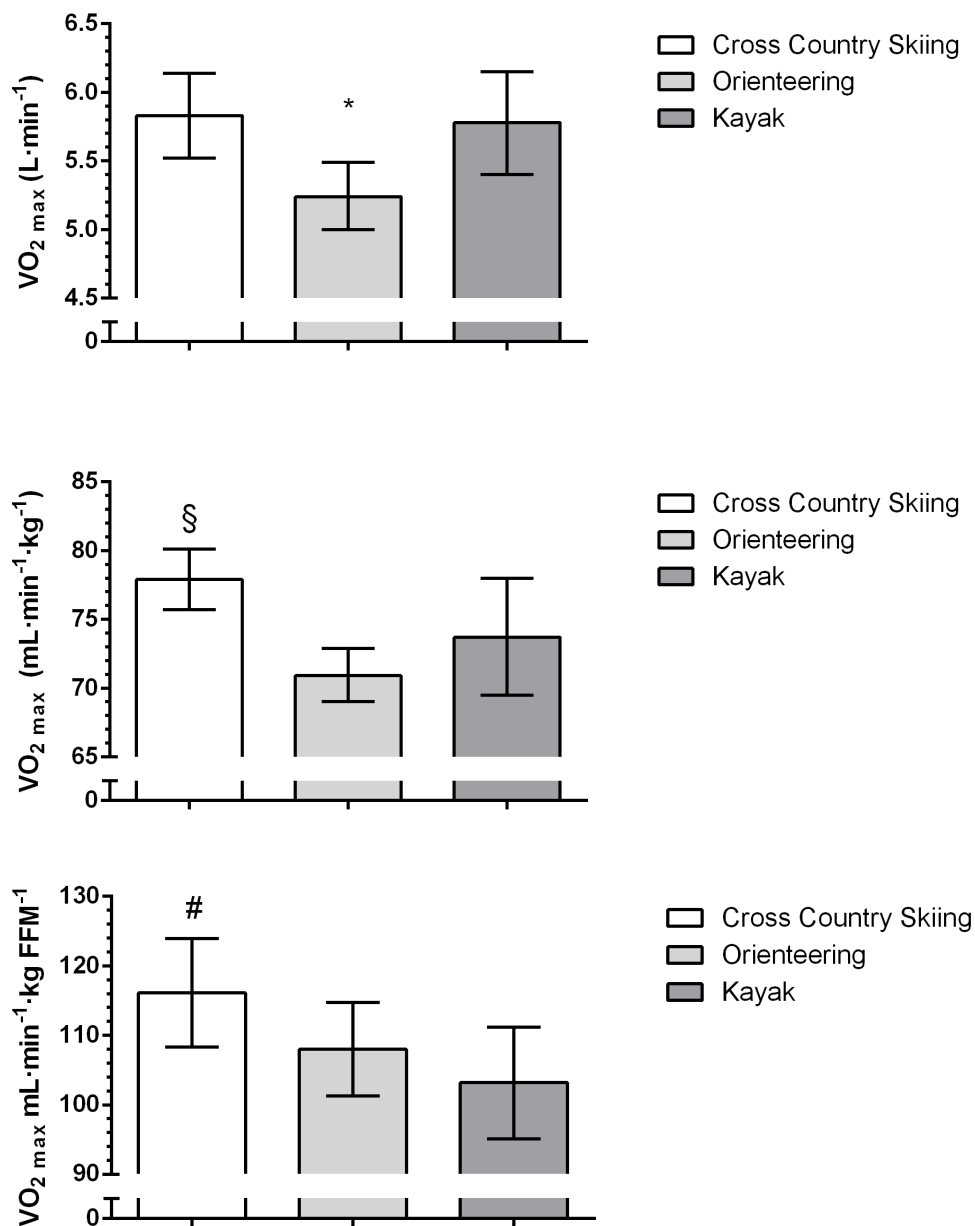
**Table 1.** Subject characteristics

Subjects	Cross-country skiing	Orienteering	Kayak
Age (years)	22.7 (20.7-24.6)*	22.5 (21.3-23.6)	19.6 (18.0-21.2)
<b>Anthropometric variables</b>			
Body height (cm)	181.1 (178.6-183.5)	184.4 (181.0-187.8)	183.2 (181.1-185.3)
Body mass (kg)	74.9 (70.7-79.1)	74.0 (70.5-77.4)	78.4 (75.1-81.7)
BMI (kg/m <sup>2</sup> )	22.8 (21.9-23.7)	21.7 (20.9-22.5)	23.4 (22.6-24.1)§
<b>Bioelectrical impedance analysis (BIA)</b>			
Fat mass (%)	9.7 (7.6-11.8)	10.1 (8.7-11.4)	8.7 (7.4-10.1)
Fat mass (kg)	7.4 (5.5-9.3)	7.4 (6.3-8.5)	6.9 (5.7-8.1)
Fat free mass total body (kg)	67.0 (63.3-70.6)	66.3 (62.9-69.6)	71.8 (68.7-74.9)§
Fat free mass upper body (kg)	36.6 (34.8-38.4)	34.9 (32.9-36.8)	40.7 (38.7-42.7)#
Fat free mass upper body (%)	54.8 (53.7-55.8)	52.6 (51.8-53.4)**	56.7 (55.8-57.5)#
Fat free mass legs (kg)	20.5 (19.4-21.6)	21.6 (20.4-22.7)	21.4 (20.4-22.5)
Fat free mass legs (%)	30.6 (30.0-31.2)	32.5 (31.8-33.3)#	29.9 (29.3-30.5)

Data presented as mean (95% CI). \*Significantly higher than the kayak group ( $p<0.05$ ), §significantly higher than the orienteering group ( $p<0.05$ ), #significantly higher than other groups ( $p<0.01$ ), \*\*significantly lower than other groups ( $p<0.01$ ). Fat free mass of upper body and legs (%) is calculated as % of fat free mass total body (kg). CI; confidence interval, cm; centimeter, kg; kilogram, BMI; body mass index, m; meter, %; percent.

## 4.2 Maximal oxygen uptake

Data on  $VO_{2max}$  are presented in Figure 4. The cross-country skiing group had 9.9% higher  $VO_{2max}$  ( $mL \cdot min^{-1} \cdot kg^{-1}$ ) than the orienteering group ( $p < 0.01$ ) and 5.7% higher than kayak group.  $VO_{2max}$  ( $mL \cdot min^{-1} \cdot kg^{-FFM}$ ) in cross-country skiing group was 12.5% higher than the kayak group ( $p < 0.05$ ) and 7.5% higher than orienteering group.  $VO_{2max}$  ( $L \cdot min^{-1}$ ) was 11.3% and 10.3% lower in the orienteering group than in the cross-country skiing and orienteering groups, respectively ( $p < 0.01$ ).



**Figure 4.** Data presented as mean (95% CI). \*Significantly lower than other groups ( $p < 0.01$ ), §significantly higher than orienteering group ( $p < 0.01$ ), #significantly higher than kayak group ( $p < 0.05$ ).

### 4.3 Blood volume and total hemoglobin mass

Data on BV variables are presented in Table 2. As can be seen from the table, the cross-country skiing and orienteering group presented 10.4% and 9.9% higher BV than the kayak group, respectively ( $p < 0.05$ ), relative to body mass (kg). Related to FFM ( $\text{kg}^{\cdot\text{FFM}^{-1}}$ ), the orienteering and cross-country skiing group held respectively a BV 11.8% and 10.6% higher than the kayak group ( $p < 0.01$ ). No significant differences were detected between the orienteering and cross-country skiing groups regarding relative values. Concerning total BV there were no significant differences between the three groups.

The cross-country skiing group had 9.2% higher  $\text{Hb}_{\text{mass}}$  relative to body mass (kg) than the kayak group ( $p < 0.05$ ). Though, values for the orienteering group were 7.7% higher than the kayak group, statistical significance was not reached. There was also no significant difference between the cross-country skiing and orienteering groups. Related to FFM, the cross-country skiing group possessed 10.6% higher and the orienteering group 9.9% higher  $\text{Hb}_{\text{mass}}$  than the kayak group ( $p < 0.05$ ), while there was no statistical difference between the cross-country skiing and orienteering groups. No statistical differences were present when looking at total  $\text{Hb}_{\text{mass}}$  values.

Concerning RCV relative to body mass (kg), there were no significant differences between groups, but the orienteers had RCV relative to FFM 9.0% higher than the kayak group ( $p < 0.05$ ). The cross-country skiing group also presented RCV 7.9% above the kayak group, though significance was not reached ( $p = 0.1$ ). There was no statistical difference between the cross-country skiing and orienteering groups, and either between groups looking at total values.

As for PV relative to body mass, the cross-country skiing group had 13.2% higher values, and the orienteering group 11.8% higher values than the kayak group ( $p < 0.05$ ). No significant difference was detected between the cross-country skiing and orienteering groups. The orienteering and cross-country skiing groups also presented PV relative to FFM 13.7% and 12.5% higher than the kayak group, respectively ( $p < 0.01$ ), where there was no significant difference between the orienteering and cross-country skiing groups. Statistical analysis on total BV variables showed no statistical differences.

**Table 2.** Blood volume variables

<b>Subjects</b>	<b>Cross-country skiing</b>	<b>Orienteering</b>	<b>Kayak</b>
BV (mL)	7984 (7360-8608)	7733 (7166-8300)	7482 (6833-8132)
BV (mL·kg <sup>-1</sup> )	105.3 (100.5-110.0)	104.8 (100.4-109.2)	95.4 (89.1-101.6)*
BV (mL·kg <sup>FFM-1</sup> )	115.2 (108.5-122.0)	116.5 (112.1-120.9)	104.2 (97.1-111.2)§
Hb <sub>mass</sub> (g)	1079 (992-1166)	1034 (968-1100)	1022 (947-1096)
Hb <sub>mass</sub> (g·kg <sup>-1</sup> )	14.2 (13.5-15.0)**	14.0 (13.6-14.5)	13.0 (12.5-13.5)
Hb <sub>mass</sub> (g·kg <sup>FFM-1</sup> )	15.7 (14.6-16.8)	15.6 (15.1-16.1)	14.2 (13.6-14.9)*
RCV (mL)	3103 (2848-3357)	3039 (2828-3251)	3019 (2782-3257)
RCV (mL·kg <sup>-1</sup> )	40.9 (38.8-42.9)	41.2 (39.7-42.8)	38.4 (36.7-40.2)
RCV (mL·kg <sup>FFM-1</sup> )	45.3 (42.2-48.3)	45.8 (44.2-47.4)	42.0 (39.8-44.2)§§
PV (mL)	4882 (4490-5273)	4694 (4317-5070)	4462 (4003-4922)
PV (mL·kg <sup>-1</sup> )	64.4 (61.2-67.5)	63.6 (60.3-66.9)	56.9 (51.9-61.9)*
PV (mL·kg <sup>FFM-1</sup> )	70.0 (65.7-74.3)	70.7 (67.3-74.1)	62.2 (56.7-67.6)§

Data presented as mean (95% CI). \*Significantly lower than other groups (p<0.05), \*\*significantly higher than kayak group, §significantly lower than other groups (p<0.01), §§significantly lower than orienteering group (p<0.05). BV; blood volume, Hb<sub>mass</sub>; hemoglobin mass, RCV; red cell volume, PV; plasma volume, FFM; fat-free mass, mL; milliliter, g; gram, kg; kilogram.



Hematological variables are presented in Table 3. Comparisons between groups of the results of the blood sample analysis showed that all groups had similar hematological values except for MCHC, which is the concentration of Hb in a given volume of packed red blood cells. Here, the cross-country skiers presented 2.3% higher values than the orienteering group and 3.0% higher than the kayak group ( $p < 0.01$ ).

**Table 3.** Hematological variables

	<b>Cross-country skiing</b>	<b>Orienteering</b>	<b>Kayak</b>
[Hb] (g/dl)	14.9 (14.5-15.3)	14.7 (14.3-15.2)	15.1 (14.4-15.7)
Hematocrit (%)	42.7 (41.6-43.8)	43.3 (42.1-44.4)	44.5 (42.6-46.3)
Leukocytes ( $10^9 \cdot l^{-1}$ )	4.9 (4.5-5.3)	5.5 (4.8-6.1)	4.5 (3.7-5.4)
Erythrocytes ( $10^{12} \cdot l^{-1}$ )	4.8 (4.7-5.0)	4.9 (4.7-5.0)	4.9 (4.7-5.2)
MCV (fl)	88.4 (87.3-89.6)	89.2 (87.9-90.5)	90.5 (88.1-92.8)
MCH (pg)	30.8 (30.5-31.2)	30.4 (29.8-30.9)	30.5 (29.7-31.3)
MCHC ( $g \cdot dl^{-1}$ )	348.4 (345.2-351.6)*	340.6 (336.0-345.3)	338.3 (335.2-341.3)
Thrombocytes (g/L)	215.6 (188.6-242.6)	206.8 (189.3-224.3)	211.9 (193.7-230.1)

Data presented as mean (95% CI). \*Significantly higher than other groups ( $p < 0.01$ ). MCV; mean cell volume, MCH; mean cell hemoglobin, MCHC; mean cell hemoglobin concentration, fl; femtoliters; pg; picograms, g; gram, dl; deciliter, L; liter.

#### 4.4 Flow-mediated dilatation

The kayak group presented diameter values 15% higher than the orienteering group at baseline and 12% higher at both 60 and 90 seconds ( $p < 0.05$ ). At 30 seconds the kayak group had 13.5% higher values than the orienteering group ( $p < 0.01$ ). The cross-country skiing group on the other hand, had 11.8%, 10.8% and 12% higher diameter than the orienteers at baseline, 60 seconds and 90 seconds, respectively ( $p < 0.05$ ). The difference between the cross-country skiing and orienteering group was 13.5% at 30 seconds ( $p < 0.01$ ). There were no significant differences between the cross-country skiing and kayak groups. Neither, there were any significant differences observed for FMD and shear rate. Normalizing FMD for shear rate did not change the significant differences.

## 4.5 Training history

The training distribution is shown in Table 4. Regarding total training hours, the kayak group trained 29% more than the cross-country skiing group and 73% more than the orienteering group ( $p<0.01$ ). Also, the cross-country group presented total training hours 34% above the orienteering group ( $p<0.01$ ). Regarding LIT, the cross-country skiing and kayak group had values 42% and 39% above the orienteering group, respectively ( $p<0.01$ ). The kayak group presented MIT values 100% above the cross-country skiing and 89% above the orienteering group ( $p<0.01$ ), and for HIT their values were 88% and 96% above the cross-country skiing and orienteering group, respectively ( $p<0.01$ ). Also regarding speed/resilience and strength training, the kayakers performed as much as a threefold or more, than the other groups ( $p<0.01$ ).

**Table 4.** Total self-reported training performed 6 months before testing

Subjects	Cross-country skiing		Orienteering		Kayak	
	Hours	% of total	Hours	% of total	Hours	% of total
LIT	216 (188-244)	76.6	152 (119-186)*	72.4	212 (191-233)	58.2
MIT	17 (11-22)	6.0	18 (12-24)	8.6	34 (26-42) <sup>§</sup>	9.3
HIT	26 (20-32)	9.2	25 (19-31)	11.9	49 (24-73) <sup>§</sup>	13.5
Speed/resilience	5 (3-8)	1.8	1 (0-3)	0.5	19 (2-35) <sup>§</sup>	5.2
Strength	20 (14-26)	7.1	12 (7-16)	5.7	61 (46-77) <sup>§</sup>	16.8
Total	282 <sup>§</sup> (251-313)	100.0	210 (177-243)*	100.0	364 (325-404) <sup>§</sup>	100.0

Data presented as mean (95% CI). \*Significantly lower than other groups ( $p<0.01$ ), <sup>§</sup>significantly higher than other groups ( $p<0.01$ ). LIT; low-intensity endurance training, MIT; moderate-intensity endurance training, HIT; high-intensity endurance training.

Due to significant differences in mean age, an analysis of total training hours was also conducted separating the total sample size into two different age groups (age $\leq$ 20 and age $\geq$ 21). This showed no significant difference between the two groups.

## 5. Discussion

This study aimed to compare the levels of  $VO_{2max}$ , BV,  $Hb_{mass}$  and vascular function in three groups of highly trained endurance athletes primarily using whole body (cross-country skiing), lower body (orienteering) and upper body (flatwater kayak), respectively.

The main findings of the present study were: 1) the cross-country skiing group presented significantly higher  $VO_{2max}$  than the orienteering group regarding total ( $L \cdot min^{-1}$ ) and relative  $VO_{2max}$  ( $mL \cdot min^{-1} \cdot kg^{-1}$ ), 2) the cross-country skiing group presented significantly higher relative BV and  $Hb_{mass}$  than the kayak group, but the cross-country skiing and orienteering groups did not differ regarding BV variables, 3) the kayak and cross-country skiing group had significantly higher arterial diameter than the orienteering group, but there were no significant differences in FMD between groups.

### 5.1 Maximal oxygen uptake

The cross-country skiers had the highest  $VO_{2max}$  in our study and by this, we confirmed our hypothesis of higher oxygen uptakes amongst cross-country skiers than in orienteers and kayakers. The higher  $VO_{2max}$  in the cross-country group was present whether comparing total values or values related to body mass or FFM, though total values were about identical in the cross-country skiing and kayak groups. Mean  $VO_{2max}$  for the cross-country skiing group relative to body mass was  $77.9 mL \cdot min^{-1} \cdot kg^{-1}$  which is in line with previous studies (42, 56-58). Our findings are supported by Saltin and Åstrand (42) who found values well above  $80 mL \cdot min^{-1} \cdot kg^{-1}$  in a study comprising 5 male cross-country skiers. However, their total  $VO_{2max}$  value of  $5.6 L \cdot min^{-1}$  was  $0.2 L \cdot min^{-1}$  lower than in our study. In the same study, kayakers were found to have substantially lower  $VO_{2max}$  values than the cross-country skiers, with values of  $70 mL \cdot min^{-1} \cdot kg^{-1}$ . Only four kayakers were tested and testing was sport specific. The kayak group in our study presented values of  $73.7 mL \cdot min^{-1} \cdot kg^{-1}$  during treadmill running. Tesch (43) found in his study of 5 kayakers that  $VO_{2max}$  during kayak exercise accounted for 87% of  $VO_{2max}$  during running, suggesting that values in the study of Saltin and Åstrand (42) might be underestimated. The 9 orienteers tested in Saltin and Åstrand's study (42) had  $VO_{2max}$  values in between the other groups, with about  $77 mL \cdot min^{-1} \cdot kg^{-1}$ , while the orienteers in the present study had  $70.9 mL \cdot min^{-1} \cdot kg^{-1}$ , significantly

lower than the cross-country skiing group. A large epidemiological study conducted on the general population by Aspenes et al. (59), showed  $VO_{2max}$  values of  $54 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  in corresponding age groups (20-29 years). Our groups of endurance athletes as a whole, presented a mean value of  $74.4 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ , which is 38% above the general population, while the cross-country skiing group presented values as much as 44% above the general population.

Kayaking is a non weight-bearing sport and hence, this study is conducted in disciplines with different requirements for weight-bearing during sport performance. Thus, we found it appropriate to normalize  $VO_{2max}$  values for fat free mass (FFM). By this, the kayak group presented lower  $VO_{2max}$  values ( $\text{mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-FFM-1}$ ) than the other two groups. Though the actual age difference between groups was small, mean age in the kayak group was significantly lower than in the cross-country skiing group. Rusko (60) suggested that  $VO_{2max}$  increased more in athletes 15-20 years of age than in athletes above 20, where they seemed to stabilize around their upper limit. On the other hand, Ingjer (58) suggested that  $VO_{2max}$  expressed in  $\text{mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  seemed to level off at the age of 15, reaching similar values as adult elite skiers. Thus, the effect of growth on  $VO_{2max}$  seems inconclusive. Notwithstanding, the difference between groups in our study shows a pattern matching previous explanatory mechanisms of high  $VO_{2max}$  as a physiological adaptation to the huge demand for oxygen supply during heavy exercise utilizing large muscle volumes, as in cross-country skiing (5).

## **5.2 Blood volume variables**

Results from the present study showed that the cross-country skiers and orienteers possessed significantly higher relative BV than the kayakers. Interestingly, no differences were detected between the cross-country skiing and orienteering group notwithstanding the significantly higher  $VO_{2max}$  amongst cross-country skiers. The same pattern was also seen when looking at relative  $Hb_{mass}$ , RCV and PV. The kayak group tended to show lower values than the cross-country skiing and orienteering group, not always reaching statistical significance, but nevertheless showing a tendency. Also, regarding these relative values, the cross-country skiing and orienteering group did not differ. In contrast to Heinicke et al. (9), we found no differences between the different disciplines of endurance athletes regarding total BV variables in the present study.

Previous studies of  $Hb_{mass}$  and BV have presented different values for trained individuals. Values have been ranging respectively from  $14.0 \text{ g}\cdot\text{kg}^{-1}$  and  $99.6 \text{ mL}\cdot\text{kg}^{-1}$  in a study of elite long distance runners (61),  $15.0 \text{ g}\cdot\text{kg}^{-1}$  and  $105.4 \text{ mL}\cdot\text{kg}^{-1}$  in a study of 92 elite athletes with a  $VO_{2max} > 67 \text{ (mL}\cdot\text{min}\cdot\text{kg}^{-1})$  (10), to  $15.4 \text{ g}\cdot\text{kg}^{-1}$  and  $107 \text{ mL}\cdot\text{kg}^{-1}$  in a study of professional cyclists (18). Corresponding values from the present study were  $Hb_{mass}$  of 14.2, 14.0 and  $13.0 \text{ g}\cdot\text{kg}^{-1}$ , and BV of 105.3, 104.8 and  $95.4 \text{ mL}\cdot\text{kg}^{-1}$  in the cross-country skiing, orienteering and kayak group, respectively. Thus, BV values in the present study were in line with previous findings (10, 61). In contrast to some studies (10, 18),  $Hb_{mass}$  in our study was somewhat lower. Still, values for the cross-country skiers and orienteers were in accordance with values from the study of long distance elite runners (61), and matched the values from a study of 30 elite cross-country skiers and triathletes presenting values of  $15.2\text{-}15.6 \text{ mL}\cdot\text{kg}^{FFM^{-1}}$  (17). Our findings relative to FFM were 15.7, 15.6 and  $14.2 \text{ mL}\cdot\text{kg}^{FFM^{-1}}$  in the cross-country skiing, orienteering and kayak group, respectively. These data show that the cross-country skiing and orienteering group in our study is in line with previous findings in the endurance athletic population regarding BV and  $Hb_{mass}$ , 20-35% and 20-30% higher than in untrained individuals, respectively (9).

High BV and  $Hb_{mass}$  among endurance athletes have been explained by adaptations in RCV and PV due to modes of training (9) and one might expect that the cross-country skiers possessed the largest BV due to the huge demand of blood supply to working skeletal muscles during performance of their sports, and hence the need of a larger cardiac output. Our study did not fully confirm this assumption since there were no significant differences between the cross-country skiing and orienteering group, which do not recruit the same amount of muscle volume when running. Neither were there any significant differences between the two groups concerning  $Hb_{mass}$ . This might imply other explanatory mechanisms affecting the factors of Fick's principle, such as e.g., enhanced cardiac chamber compliance, as suggested by Levine et al. (62). But still, the significant difference in BV and  $Hb_{mass}$  between the cross-country skiers and the kayakers indicates a different physiological adaptation concerning blood constituents — adaptations which seem more explicit between sports using whole body and sports primarily using upper body, than between sports using whole body and sports primarily using lower body. This is not surprising, considering that the highest  $VO_{2max}$  during arm exercise is found to be 20-30% lower than leg exercise, and the demand for oxygen delivery

thus must be lower (7). Secher et al. (63, 64) found that when arms and legs were used simultaneously in an exercise,  $VO_{2max}$  was not significantly elevated compared to leg work only. These investigators (63, 64) therefore suggested a central or cardiac limitation to  $VO_{2max}$ . This might, to some extent, reflect our results showing similar BV variables in orienteers and cross-country skiers, but higher  $VO_{2max}$  in the latter, also implying other explanatory mechanisms of the higher  $VO_{2max}$ .

As previously mentioned, the kayakers were significantly younger than the cross-country skiers, though the actual age difference was small. Steiner et al. (17) studied  $Hb_{mass}$  in 45 male endurance athletes at age 16, 21 and 28 and found approximately 15% lower relative  $Hb_{mass}$  values in the youngest group compared with adult athletes (age 21 and 28). They identified no significant difference in  $Hb_{mass}$  between age 21 and 28. Mean age in the kayak group was 19.6 years and the youngest participant was close to 18 years, suggesting that the influence of age on  $Hb_{mass}$  in the present study was minimal.

### **5.3 Vascular function**

In studies of endurance athletes with predominant use of upper or lower limbs, changes in arterial diameter were associated with prolonged use of the predominant limb (33, 47). Also, a significant inverse correlation between arterial diameter and flow mediated dilatation (FMD) has been found (33, 47). These findings are supported by our study where the cross-country skiers and kayakers had significantly larger brachial artery diameter than the orienteers.

Even though we found no significant difference between the groups concerning FMD, our study showed enhanced FMD in the cross-country skiing and orienteering groups if we compared them with estimated normal values from larger general populations (65). On the other hand, the kayak group which had the largest arterial diameter among our groups had lower FMD compared with normal values. This might be explained by structural remodeling in arterial diameter apparent in this group. This finding was in accordance with previous data suggesting an inverse correlation between arterial diameter and FMD response (33, 47). Why the same phenomenon was not present in the cross-country skiing group is unclear, since this group also presented enlarged arterial diameter compared to the orienteering group. One might speculate that the kayakers use their upper body more predominantly than the cross-

country skiers. Hence, this makes their brachial arteries even more exposed to shear stress than cross-country skiers who use both their upper and lower body.

#### **5.4 Training history**

The kayak group trained significantly more total hours than other groups, mainly due to a higher amount of MIT, HIT and strength training. Cross-country skiing, orienteering and flatwater kayaking are disciplines in which Norwegian athletes are performing at high international levels, suggesting that the participating subjects' training are optimized for performing well in their sports. Why they still differ so much in training hours might be explained by several factors. The action of moving the kayak in itself requires a certain work load due to the constant resistance of water. This might contribute to more training hours at moderate and high intensities in the kayak group. Also, the upper body does not profit from lifestyle activities to the same extent as the lower body. Hence, the kayakers might be in need of additional training hours compared to athletes participating in weight-bearing activities like cross-country skiing and orienteering.

There might also be a difference between the three groups regarding the amount of competitions during the period in question. Cross-country skiers seem to compete more than the orienteers and kayakers. The difference in «base training» might not be so pronounced as the training data in our study, since training hours during the period in question (i.e. the competition phase) are likely to constitute approximately one third of the total annual amount of training.

Frequent anaerobic workloads exerted on large muscle groups mostly located in the lower body, might require longer time to recover than the smaller muscle groups in the upper body. Avoiding overloads becomes essential in preventing injuries and limits training hours at high intensities to some extent. This might reflect the distribution in training hours in our study where the kayakers reported the highest amount of training hours and the orienteers the lowest. Using both upper and lower body, the cross-country skiers position themselves in between.

Many studies suggest HIT as the most effective training to increase  $VO_{2max}$  not only in untrained subjects, but also in highly trained endurance athletes (66-68). Despite presenting

significantly lower amounts of HIT than the kayakers and values equal to the orienteers, the cross-country skiers were still the group with the highest  $VO_{2max}$  in our study. Training data are recorded in a period characterized by low training hours (i.e. competition phase and just after) and might be misleading due to different procedures regarding training volumes and intensities in the period in question. However, our findings might support the perception of whole body training as an important factor of high oxygen uptakes per se.

## **5.5 Study limitations**

Conducting a study on top athletes strongly reduces the population relevant for recruitment. Due to these challenges we have a relatively small sample size. Though, in the context of other studies conducted on elite athletes, the sample size might not be considered small. This study might as other studies with limited sample size, be subject to selection biases regarding the representativeness in our study population. Results must therefore be used carefully when applied to larger populations. Providing an age matched control group might have strengthened the study in the way of more accurate comparisons with untrained peers.

Since it is not possible to standardize training protocols in studies of performing top athletes and training procedures are likely to differ between disciplines during the period in question, we collected training diaries 6 months prior to measurements. These athletes have extensive experience in reporting training diaries and do so routinely. The influence of potential recall biases must therefore be considered minimal.

Controlling for blood iron status, EPO concentration, distribution of the age profile of red blood cells, would have strengthened the study with information of the hormonal and red cell status that potentially could affect measurements of  $Hb_{mass}$  and BV. Also controlling for urine density at the time of measurements would have secured that all athletes were in the same state of hydration.

## **5.6 Conclusion**

The present study indicates that whole body exercise employed by cross-country skiers induces higher  $VO_{2max}$  compared to upper and lower body sports like orienteering and flatwater kayak. However, these differences did not correspond with differences in BV variables between cross-country skiers, orienteers and kayakers and suggest other



physiological adaptations to whole body exercise which might explain the higher  $VO_{2max}$  in cross-country skiers.

## **5.7 Perspectives**

The present study indicates different physiological adaptations especially to whole body and upper body exercise on BV variables. Sports involving whole body and lower body exercise do not seem to share the same differential adaptations. Yet, we found an elevated  $VO_{2max}$  in the cross-country skiers compared to orienteers. Exploring other explanatory mechanisms of higher  $VO_{2max}$  as potential adaptations to whole body exercise e.g., heart size, cardiac chamber compliance and peripheral factors in athletes specially trained for the arms, legs or whole body may give further insight into the upper limits of the human's ability to adapt to such training.

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