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Characterisation of systolic and diastolic functions with echocardiography imaging in healthy populations subjected to stress manoeuvres

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INTRODUCTION

The cardiovascular system

The cardiovascular system consists in a mechanical pump (the heart) which pumps blood volumes in a closed circuit constituted by arteries, arterioles, capillaries, venules, and veins. Venules and veins send deoxygenated blood to the lungs to receive oxygen and unload carbon dioxide. The arteries and arterioles send oxygenated blood and nutrients to the body cells while removing metabolic end products. Thus, all body tissues require circulation to survive.

To exert an effective pumping action, the contractions of heart chambers must be coordinated. Specifically, the atria contract (atrial systole) when ventricles relax (ventricular diastole); likewise, ventricles contract (ventricular systole) when atria relax (atrial diastole). Then, alternate and asynchronous periods of relaxation and contraction of atria and ventricles occur in the heart to effectively works. This complete series of events makes up a heartbeat, also known as "the cardiac cycle".

One cardiac cycle causes pressure in the heart chambers to rise and fall and cardiac valves to open and close. During early ventricular diastole, pressure in the ventricles is low, thereby opening the atrio-ventricular (AV) valves and letting the ventricles fill with blood. Nearly 70% of returning blood enters ventricles in this phase. The remaining 30% of the blood is then pushed to ventricles by atria contraction.

When ventricles contract, ventricular pressures rise and exceed atrial pressures, thus causing the AV valves to close. Also, papillary muscles contract, thus preventing the cusps of the AV valves from bulging into the atria. This phase is called "ventricular systole", during which the pressure in ventricles exceeds those of the pulmonary and aortic arteries. During ventricular systole, AV open and blood volumes are ejected from the ventricles into these arteries. With the ongoing systole, ventricular contraction and pressures progressively drop, and, when ventricular pressures are lower than in the aorta and pulmonary artery, semilunar valves close. In this phase, atrial pressures are low, and blood is flowing into the atria while the ventricles are contracting, thus filling atria before the next ventricular diastole. When ventricular pressures are lower than atrial pressures, the AV valves open, and the ventricles begin to refill.

A heartbeat makes a characteristic double thumping sound when heard through a stethoscope. This is due to the vibrations of the heart tissues related to the valves closing. The first thumping sound occurs during ventricular contraction when the AV valves close. The second sound occurs during ventricular relaxation when the pulmonary and aortic valves close.

The right side of the heart pumps oxygen-poor blood to the lungs, and the left side pumps oxygen-rich blood toward the body tissues. The right ventricle does not pump blood with as much force as

the left ventricle as the pulmonary circulation has a pressure about 6 times lower than that of the systemic circulation because the pulmonary circle is wide and relatively short.

The amount of blood ejected by ventricles during each systole is called "stroke volume" (SV), or ejection volume; cardiac output (CO) is defined as the volume of blood ejected from the heart per minute, and it is easily calculated as CO = HR × SV (where HR stands for heart rate). Therefore, anything that changes HR or SV will change CO.

Echocardiography method

The assessment of cardiac performance can be done on multiple levels using invasive methods (i.e., catheter-based hemodynamics) or non-invasive techniques such as clinical examination, echocardiography, cardiac magnetic resonance imaging, or other imaging modalities.

Among the others, echocardiography is unique as it allows the non-invasive study of the heart using high-frequency ultrasound. The method is non-invasive, reproducible, and measures can be repeated almost unlimited. In humans, echocardiography has become a well-established technique to provide real-time information on cardiac dimensions, morphology, and functions.

Quantitative information on systolic and diastolic performance can be obtained by tracing around the ventricular cavities, and end-diastolic (EDV) and end-systolic volumes (ESV) may be calculated from an assumption of standard geometric shapes, such as a truncated ellipsoid. Indices of cardiac function, such as CO, SV, and ejection fraction (EF, i.e., the fraction of blood ejected from the ventricle during one heartbeat), can also be determined.

Echocardiography is now the most routinely-employed non-invasive tool for the assessment of cardiac anatomy and function and it is routinely performed in the management of cardiovascular disease.

The mitral inflow velocities can be obtained in most patients using the pulsed-wave (PW) Doppler at the apical 4-chamber view. The primary measures include the peak diastolic early filling (E wave) and the diastolic late atrial filling (A wave) velocities, the ratio between these (E/A), and the peak velocity deceleration time (DT). Normal values of the mitral inflow parameters vary with age, with the E wave velocity decreasing, and the DT and A wave increasing amplitude with aging, resulting in a decrease in the E/A ratio. Heart rate and rhythm, PR interval, CO, mitral annular size, and left atrium (LA) function can affect the mitral inflow (Nagueh et al. 2009). It is well established that the mitral E-wave velocity primarily reflects the LA-left ventricular (LV) pressure gradient during the early stage of diastole and thus it is amenable to changes in the preload and alterations in LV relaxation (Appleton et al. 1988, Nagueh et al. 2009). The mitral A-wave velocity reflects the LA-LV pressure gradient during the late stage of diastole, which is affected by LV compliance and

the LA contractile function. The DT of the mitral E-wave is influenced by the LV relaxation, LV diastolic pressure after mitral valve opening, and the LV compliance. Alterations in the LV end-systolic and/or end-diastolic volumes, LV elastic recoil, and/or LV diastolic pressures directly affect the mitral inflow velocities (E-wave) and the time intervals (DT) (Nagueh et al. 2009). The use of these parameters, especially the E/A ratio and DT, is useful to evaluate the echocardiographic filling patterns as normal or impaired.

Conventional echocardiographic and Doppler measures such as SV, CO, EF, end-diastolic volume (EDV), end-systolic volume (ESV), blood flow velocities, and times measures have recently been supplemented by tissue Doppler imaging (TDI). TDI is a robust and reproducible echocardiographic tool that has permitted a quantitative assessment of both global and regional function and timing of myocardial events (Gorcsan J et al 1996; Yu CM et al 2003). Most published studies have examined the long-axis function of the heart by TDI using the apical view, and several parameters have been proposed as valid tools to investigate hemodynamics of several cardiac diseases.

During systole, potentially prognosticators obtained by TDI include peak systolic velocity during ejection measured at mitral annulus (Sm) (Fig. 1). In diastole, potentially important prognosticators include peak myocardial early and late diastolic velocities measured at the mitral annulus (Ea and Em respectively) (Fig. 1) as well as their ratio (Em/Ea). These measures have been demonstrated useful in various diseases, including heart failure, hypertension, and acute myocardial infarction, and in patients undergoing stress echocardiography for suspected coronary heart disease (Sanderson JE et al 2004).

TDI velocities assessed at the mitral annulus reflect the long-axis motion of the ventricle, which is an important component of ventricular systolic and diastolic functions. Subendocardial fibres make a substantial contribution to long-axis function, and these are particularly susceptible to disturbance by various diseases. The amplitude of long-axis motion during systole also correlates well with EF, and this is also the case for the right ventricle. The peak systolic velocity is also a sensitive marker of mildly impaired LV systolic function, even in those with a normal EF or apparently preserved systolic function, such as the “diastolic heart failure”, or in diabetic subjects without overt heart disease.

In healthy individuals, increasing cardiac pre-load increases and transmitral gradient, increases Ea. This happens in situations such as during exertion or when there is a sympathetic activation. Differently, in patients with impaired myocardial relaxation Ea is reduced and can not increase to the same extent as in normal subjects.

It is to be noted that echocardiography measurements can be performed during rest, but recently new devices, implemented with robust software and probes, has allowed the use of

echocardiography also during physiological stresses, such as exercise and acute hypoxia, which increases sympathetic tone.

Exercise as a stressor for the cardiovascular system

During exercise, neural signals of both central and peripheral origin provide information to the cardiovascular control areas responsible for hemodynamic regulation. Such information aims at adjusting the cardiovascular system to muscle requirements. In detail, the activation of regions of the brain responsible for motor unit recruitment also activates the cardiovascular control areas; this establishes a basal level of sympathetic and parasympathetic efferent activity to the cardiovascular apparatus closely linked to the intensity of the effort (Strange et al. 1993, Thornton et al. 2002). This basic pattern of autonomic activity, commonly known as "central command," is in turn modulated by peripheral signals originating from receptors within muscle that reflexively activate the cardiovascular control areas located in the Medulla Oblongata. The current thinking is that, when O_2 delivery does not match the requirements of contracting muscles, then the metabolic end products of the muscle metabolism accumulate and activate muscle metaboreceptors - free nerve endings of group III and IV afferents -, which in turn augment arterial blood pressure through a reflex of nervous origin commonly called "metaboreflex." This pressure effect is thought to be mostly mediated by reflex vasoconstriction, i.e., by a systemic vascular resistance (SVR) increase that augment blood pressure to restore blood flow to the hypo-perfused muscle (Sheriff et al. 1987, O'Leary and Sheriff 1995, O'Leary et al. 1999). Whilst the effect of metaboreflex on SVR has been well established, less is known about its action on central hemodynamics, i.e., myocardial contractility, cardiac preload, SV, and CO.

Several studies suggest that metaboreflex activation can also affect central hemodynamics by modulating chronotropism, contractility, and pre-load. For example, it has been found in dogs that the muscle metaboreflex can increase ventricular performance (O'Leary and Augustyniak 1998, Sala-Mercado et al. 2006). This observation has been confirmed also in humans, where it was found that myocardial contractility and SV can be improved during metaboreflex activation elicited by postexercise muscle ischemia (Bonde-Petersen et al. 1978, Bonde-Petersen and Suzuki 1982, Crisafulli et al. 2003). Moreover, it has been proposed that the muscle metaboreflex can enhance diastolic filling pressure through splanchnic and general venoconstriction that propels blood volume toward the central circulation (Bastos et al. 2000, Sheriff et al. 1998).

From what previously exposed it can be gleaned that exercise is a challenge for the cardiovascular system and that, in normal individuals, the hemodynamic response to exercise encompasses

complex changes in HR, myocardial contractility, preload, and afterload (Crisafulli et al. 2006, Higginbotham et al. 1984, Michelini et al. 2015, Nobrega et al. 2014, Plotnick et al. 1986).

This response is influenced by many factors, including ageing, which substantially reduces the capacity to exercise and to augment CO (Hossack et al. 1982, Farinatti et al. 2009, Farinatti et al. 2018, Fleg et al. 2005, Moreira et al. 2020). Several concurring phenomena are responsible for the age-related reduction in the capacity of the heart as a pump. It is well known that maximal HR progressively decreases with aging and this in turn causes a proportional reduction in maximal CO, maximal oxygen uptake ($\dot{V}O_{2max}$), and maximal workload (W_{max}) (Fleg et al. 2009, Christou et al. 2008). Less conclusive results have been reported for SV, which in some studies has been reported to be well preserved with ageing, whereas in others not (Farinatti et al. 2018, Fleg et al. 1995, Fleg et al. 2012, Rodeheffer et al. 1984).

In some investigations conducted in healthy elderly individuals of both sexes, it was found that the decline in maximal CO during dynamic exercise was entirely due to the reduction in HR, since SV did not decline with age. However, aging affected the process by which the SV level was achieved. Specifically, older individuals showed a blunted capacity to reduce ESV and to increase EF in response to effort, but this deficit was offset by recruiting the Frank-Starling mechanism, that is, by increasing EDV. This, in turn, was possible thanks to a longer diastolic interval due to the slower HR as compared to young subjects (Fleg et al. 2012). The underlying mechanisms for the age associated reduction in maximal EF are multifactorial and probably include an impaired myocardial performance, an increased arterial afterload, a decreased effectiveness of the autonomic nervous system to modulate myocardial contractility, and in intrinsic and only partially known impairment in cardiomyocytes contractile function (Farinatti et al. 2018, Fleg et al. 2012, Moreira et al. 2021).

Moreover, available data suggest that also diastolic function deteriorates with age, probably because aging is associated with an increase in cardiac stiffness and in ventricular relaxation (Arbab-Zadeh et al. 2004, Carrick-Ranson et al. 2012, De Souza 2002, Lakatta 2002, Milia et al. 2015).

While the hemodynamic differences between elderly and young subjects during incremental exercise tests up to exhaustion have been extensively studied, less in known about the hemodynamic differences during submaximal steady-state exercise. Specifically, we could not find any information about the effect of ageing during effort at constant workload at the intensity of the gas exchange threshold (GET), which represents a useful tool to discriminate the transition from moderate to heavy exercise. Exercises performed above the intensity of GET do not allow a steady state in cardio-pulmonary and metabolic variables and cannot be sustained for long periods (Poole et al. 2021). More important, while $\dot{V}O_{2max}$ declines with age and parallels that of HR and CO, the reduction in GET is slower, thus suggesting that the cardiovascular response is well preserved

during submaximal exercise in aging, although its hemodynamic mechanisms are still to be described (Neder et at. 1999, Poole et al. 2021).

Acute hypoxia as a stressor for the cardiovascular system

In physiological conditions, hypoxia is experienced by ascending at high altitudes. At sea level, air pressure (P_{atm}) is 760 mmHg with a fixed percentage distribution of gases [oxygen (O_2) 20.93%, carbon dioxide (CO_2) 0.03%, and Nitrogen (N_2) 79.04%]. At higher altitudes, air pressure decreases and reaches 380 mmHg at 5840 m and 250 mmHg on the summit of Mount Everest (8878mt), but the percentage distribution of gases does not change. According to Dalton's Law, this fact implies a reduction in the partial pressure of O_2 from 159 mmHg at sea level (i.e., 20.93% of 760 mmHg), to 79 mmHg at 5840 m (i.e., 20.93% of 380 mmHg), and 52 mmHg at the top of Mount Everest (i.e., 20.93% of 250 mmHg). Moreover, as we breathe, the inspired air is moistened by the upper airways with water vapor at a pressure (P_{H2O}) of 47 mmHg that doesn't change at high altitudes.

Since oxygen blood content decreases in hypoxic conditions, the regulation of cardiac functions become fundamental to guarantee a sufficient oxygen delivery to peripheral tissues. Normally, at rest after acute exposure to hypoxia CO increases. This adjustment is related to an increase in HR, whereas SV has been reported to be unchanged (Talbot et al. 2005) or even decreased (Stembridge et al 2015).

Arterial blood pressure remains unchanged, or slightly increases after acute hypoxia exposure (Naeije 2010), while SVR decreases. The effect on SVR is the result of the balance between the hypoxia-mediated local vasodilation and the vasoconstriction due to the sympathetic nervous system activation, with the former prevailing on the latter.

Recently, echocardiographic studies contributed to shed light on the SV reduction after 10 days of exposure to high altitude (5050 m) (Stembridge et al. 2014, Stembridge et al. 2015b). The authors reported a decreased left ventricular EDV, an increased pulmonary systolic pressure (PSP), an enhanced left ventricular systolic function (assessed as EF and as strain, rotation, and twist obtained by tissue Doppler imaging), and a preserved diastolic function. These results appear to indicate that the main responsible for the SV reduction is an afterload dependent depression in right ventricular function that reduces left ventricular filling.

Maximal HR in hypoxia has been reported to be reduced (Lundby et al. 2001). This effect is related to an increased parasympathetic drive since maximal HR is restored after administration of glycopyrrolate, a parasympathetic blocking agent. The increased HR is accompanied by a decrease in maximal SV, so that CO was unchanged in this experimental setting (Boushel et al. 2001). This finding suggests that the regulated variable at maximal effort is CO, which is likely adjusted by

reducing SV to allow an appropriate transit time of red blood cells in the pulmonary circulation and more efficient blood oxygenation (Boushel et al. 2001). The sympathetic nervous system plays a pivotal role in systemic cardiovascular adjustment to hypoxia. The increase in HR that occurs in acute hypoxic conditions depends on both sympatho-excitation and vagal withdrawal. Indeed, it has been observed that muscle sympathetic nerve activity, which is a marker of global sympathetic nervous system activity, is increased after hypoxia exposure (Saito et al. 1988). It appears that carotid chemoreceptors are the main responsible for the hyperventilation and vasoconstriction response to acute hypoxia, while aortic chemoreceptors are the main determinant of hypoxia-mediated tachycardia (Niewinski et al. 2014). However, other studies indicate that carotid chemoreceptors activation exerts an important indirect effect on HR, since hyperventilation triggers the pulmonary stretch receptors and reduces the vagal traffic to the heart (Kato et al. 1988). Moreover, carotid chemoreceptors increase the level of circulating catecholamines, further influencing HR when activated (Siebenmann et al. 2015).

Moreover, acute hypoxia induces a shift of the baroreflex set point to a higher pressure level, thereby inducing an increase in sympathetic tone and a vagal withdrawal (Halliwill et al. 2003, Querido et al. 2011). The precise mechanism of this phenomenon remains unclear, but it seems related to peripheral chemoreceptors activation (Halliwill et al. 2003). It should also be considered that hypoxia triggers baroreflex thanks to its vasodilator effect on peripheral arteries, and this further increases the sympathetic tone and inhibits the parasympathetic activity (Calbet et al. 2014).

From what exposed, it can be gleaned that acute hypoxia is a challenge for the human circulation, which experiences rapid changes in the main hemodynamic modulators (i.e., pre-load, after-load, contractility, and chronotropism) which potentially impact on cardiovascular function and regulation.

Aim of the studies

Starting from the above-described background, I set two investigations to study the cardiovascular response in healthy individuals subjected to two different cardiovascular stressors:

1) dynamic exercise,
2) acute hypoxia.

Specifically, in the first experiment I compared hemodynamics of healthy elderly and young subjects during an exercise bout conducted at the same relative submaximal workload, i.e., at the GET intensity. I hypothesized that elderly individuals have a reduced capacity to increase SV because of their impaired diastolic and systolic functions as compared to young individuals. This

would lead to a reduced capacity to increase ventricular filling and emptying rates to sustain CO and may, at least in part, explain their lower workload at GET.

The aim of the second experiment was to investigate the effect of acute dynamic exercise during normobaric hypoxia on echocardiographic parameters related left ventricular volumes, systolic, and diastolic functions. Specifically, this investigation was devised to verify whether classical echocardiographic measures of ventricular volume confirm or reject our previous hypothesis of a reduced pre-load during the recovery from mild exercise in AH. Moreover, the study aimed to verify whether Doppler and tissue Doppler measures confirmed or rejected the hypothesis that systolic and diastolic functions were affected by mild exercise conducted in acute normobaric hypoxia.

METHODS

These investigations were approved by the Independent Ethical Committee of the A.O.U. of Cagliari. Each participant was informed about the purposes and methods of the research and signed consent to participate in the investigations, which were carried out in accordance with the Declaration of Helsinki.

First study

For the first study, two groups of subjects were enrolled:

1) Elderly group (EG), which was composed by 11 healthy subjects (7 females and 4 males) older than 60 years [range of age 60-72 yrs.; mean ± standard deviation (SD) 65.18 ± 4.71]. At the time of the study, all were physically active and were practicing Tai Chi Chuan for an average of six years. They trained three times a week.

2) Young group (YG), which was composed by 13 healthy subjects (7 females and 6 males), younger than 35 years (range of age 18-35 yrs.; mean ± SD 29.84 ± 4.06). All subjects were physically active and were regularly involved in exercise activities in a gym for at least three times a week.

In both groups, recruitment was conducted after a medical visit to exclude the presence of cardiac, pulmonary, and metabolic problems. Subjects under medications for any known disease were excluded. Smoking was also considered as exclusion criterion. We decided to enrol physically active individuals since, especially in ageing, inactivity is often linked to cardiovascular and metabolic diseases. Thus, physically active people should be considered as the "nomality" either in youth or in aging (Lees et al. 2005). Specifically, we enrolled Tai Chi Chuan practitioners as in a recent paper this discipline was demonstrated able to keep aged individuals in a good nutritional status, body composition, and muscle functionality (Stagi et al. 2020).

To calculate the required sample size, we used a calculator free available on the web (https://clincalc.com/stats/samplesize.aspx). The criteria set to calculate the sample size were a power of 85%, an overall type 1 error of 0.05, a SD of 20%, and a 25% difference between groups in the studied variable. Ten subjects/group were needed to obtain adequate statistical power.

Experimental protocol.

The experimental protocol consisted in a preliminary cardiopulmonary test (CPT) and in the experimental session. Participants were asked for abstaining from drinking alcohol or coffee for at least 24 hours before scheduled tests. All experiments were conducted in a room with controlled temperature and humidity (22 °C, relative humidity 50%).

Cardiopulmonary test.

Each participant underwent a CPT on an electromagnetically braked cycle-ergometer (CUSTO

Med, Ottobrunn, Germany). Data of oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and pulmonary ventilation (V_E) were collected breath by breath with a gas analyser (Ultima CPX, MedGraphics St. Paul, MN, USA). Respiratory exchange ratio (RER) was calculated as $\dot{V}CO_2/\dot{V}O_2$. The gas analyser was calibrated immediately before the CPT, accordingly to the manufacturer. The exercise consisted of a linear increase of workload (20 or 30 $W \cdot min^{-1}$ for the EG and the YG respectively, starting at 20 or 30 W), keeping a pedalling frequency of 60 rpm until exhaustion, which was considered as the point at which the subject was unable to maintain a pedalling rate of at least 50 rpm. W_{max}, $\dot{V}O_{2max}$, maximum carbon dioxide production ($\dot{V}CO_{2max}$), maximum ventilation (V_{Emax}), and maximum heart rate (HR_{max}) were measured as the average of the last 15 s of exercise. Achievement of $\dot{V}O_{2max}$ was considered as the attainment of at least 2 of the following criteria: 1) a plateau in $\dot{V}O_2$ despite increasing workload (<80 ml·min-1); 2) RER above 1.10; and 3) HR ± 10 $beats \cdot min^{-1}$ of predicted maximum HR calculated as 220-age (Howley et al. 1995).

GET was calculated using the *V*-slope method, which detects exchange threshold employing a regression analysis of the slope of $\dot{V}CO_2$ plotted as a function of $\dot{V}O_2$ (Beaver et al. 1986). During the CPT, participants familiarised with the equipment and the staff of the laboratory, thereby allowing habituation to the environment and the ergometer that was employed in the successive experimental session.

Sessions to study hemodynamics during exercise at GET intensity

After the CPT session (interval 4-7 days), each participant reported to the laboratory and performed a rectangular exercise session pedalling on the same cycle-ergometer utilised for the CPT. Before stating pedalling, the subjects sat on the cycle-ergometer for three minutes to collect data at rest. The exercise consisted in five minutes pedalling against a workload corresponding to that of the GET previously measured during the CPT. A recovery of six minutes was allowed.

Hemodynamic measurement.

Hemodynamics were measured using an echocardiographic system equipped with a hand-held 3.5-MHz adult ultrasound probe (Vivid iq, GE Healthcare, Fairefield, CT, USA). HR was assessed as the reciprocal of the electrocardiogram R-R interval provided by the echocardiograph. Two dimensional images and pulsed Doppler recording were acquired in the sitting position using the apical four-chamber view. Left ventricular ESV and EDV were calculated automatically by the software using the conventional formula $8A^2/3\pi L$, where A was the left ventricular area and L was ventricular longest length (Christie et al. 1987). The ventricular area was determined by tracing along the inner edge of the endocardium, and the length was considered as the distance from the ventricular apex to the midpoint of the mitral annulus. Left ventricular EF was considered as: (EDV-ESV/EDV) ·100.

SV was calculated as: EDV-ESV.

In the same beats utilised for ESV and EDV measurement, early (Evel) and atrial (Avel) transmitral filling peak velocities and their ratio (E/A) were measured with pulse wave Doppler (PWD), with a 5-mm PWD sample volume (3 mm) placed distal to the mitral anulus, between the mitral leaflets. The interrogation beam was aligned with mitral flow (Cohen et al. 1996).

Mitral valve motion velocity during early (Em) and late (Am) diastole was assessed with Doppler tissue imaging, with images captured from the apical four chamber view. The pulsed-wave sample volume was placed at the lateral mitral anulus. The ratio Evel/Em was considered as an estimate of left ventricular filling pressure (Choudhury et al. 2017, Correale et al. 2012). Systolic myocardial velocity (Sm) was also assessed to have a measure of longitudinal systolic function (Correale et al. 2012). Since tissue Doppler measures are highly dependent on the angle between scan beam and the vector of ventricular motion (Bassareo et al. 2010), particular care was employed during this kind of measures.

Aortic Doppler was also conducted from the four-chamber window to assess the pre-ejection period (PEP) and the ventricular ejection time (VET). In detail, PEP was measured as the time from the beginning of the QRS complex of the electrocardiogram and the opening of the aortic valve, and VET was measured as the total duration of ejection period in the Doppler trace. Also measured was diastolic time (DT), which was calculated subtracting the sum of PEP and VET from the total period of the cardiac cycle.

The ratio between SV and DT was calculated to obtain a measure of the mean rate of diastolic blood flow, i.e., the ventricular filling rate (VFR) (Gledhill et al. 1994, Sanna et al. 2017). Moreover, the SV/VET ratio was also calculated to obtain the mean systolic ejection rate (VER), which is directly related to myocardial performance (Gledhill et al. 1994, Sanna et al. 2017).

Echocardiography images were taken at rest and during the last (i.e., the fifth) minute of exercise by the same operator. When images were considered of good quality, a 6 s frame was recorded and then analysed offline. For each analysis, at least three beats were taken into consideration (range 3-6 beats) and data are reported as the average of the measures. All echocardiographic calculations were done by the same expert physician, with a 5-year experience in the field. The operator' coefficient of variation of measurements ranged from 8% (very good) to 12% (good).

A manual sphygmomanometer (Heine Gamma GP, Gilching, Germany) was placed in the non-dominant arm to assess systolic (SBP) and diastolic (DBP) blood pressure. Blood pressure measurement was performed by the same physician throughout all experiments. Mean arterial blood pressure (MAP) was calculated using a formula which takes into consideration changes in PEP, VET, and DT (Sainas et al. 2016). Systemic vascular resistances (SVR) were calculated as the

MAP/CO ratio. This quantity was then multiplied by 80, where 80 is a conversion factor to change units to standard resistance units.

Data Analysis

Data are presented as mean ± SD. The Kolmogorov-Smirnov test was utilised to verify variables' normality. Since all variables were normally distributed, parametric statistic was employed. Differences between groups in their anthropometric characteristics and in parameters gathered during the CPT test were found out using the *t* test for unpaired data. The differences between groups in variables gathered at rest and at the fifth minute of exercise at the GET intensity were tested with the two-way analysis of variance (ANOVA, factors: group and time), followed by Bonferroni post-hoc when appropriate. Furthermore, % change with respect to rest reached by each variable during the GET test was calculated, and comparison between groups was conducted using the *t* test for unpaired data. Statistical analysis was carried out with commercially available software (GraphPad Prism). A p value <0.05 was considered to determine statistical significance in all cases.

Second study

For the second investigation, twelve healthy Caucasian males aged 24–42 years [mean ± standard deviation (SD) of age 33.5 ± 4.8 years] were enrolled. All were physically active and were regularly involved in leisure-time sports activities such as amateur cycling and running at least 3 times/week. Their average ± SD of body mass and height were 72.5 ± 10.1 kg and 176.5 ± 3.9 cm respectively. All were non-smokers and none of them suffered from any known diseases or were on medication at the time of the experiment. They were asked to abstain from drinking alcohol or coffee for at least 24h before scheduled tests. All experiments were conducted in a room at controlled temperature and humidity (22 °C, relative humidity 50%).

Experimental protocol

The experimental protocol consisted in a preliminary screening test and in two experimental sessions in normoxia (test NORMO) and AH (test HYPO). Test NORMO and HYPO were randomly assigned. Randomization was obtained using an online random sequence generator (https://www.random.org/sequences/).

Preliminary test

All participants underwent a preliminary medical examination to assess their health status. After the medical examination, each participant underwent a cardiopulmonary exercise stress test (CPET) on an electromagnetically braked cycle-ergometer (CUSTO Med, Ottobrunn, Germany). During the CPET oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and V_E were assessed with a gas analyzer (Ultima CPX, MedGraphics St. Paul, MN, USA) calibrated immediately before each test accordingly to the manufacturer. The exercise consisted of a linear increase of workload (30

$W \cdot min^{-1}$), starting at 30 W, keeping a pedaling frequency of 60 rpm until exhaustion, which was considered as the point at which the subject was unable to maintain a pedaling rate of at least 50 rpm. Maximum workload (W_{max}), maximum oxygen uptake ($\dot{V}O_{2max}$), and maximum heart rate (HR_{max}) were collected. Moreover, anaerobic threshold (AT) was calculated using the *V*-slope method, which detects AT using a regression analysis of the slope of $\dot{V}CO_2$ plotted as a function of $\dot{V}O_2$ (Beaver et al. 1986). During this preliminary visit, participants familiarized with equipment and the staff of the laboratory, so allowing habituation to the environment and the ergometer that was employed in the successive experimental sessions.

Sessions to study hemodynamics during normoxia and hypoxia

After the preliminary test (interval 3-7 days), volunteers performed randomly assigned the NORMO and the HYPO sessions pedaling on the same cycle-ergometer utilized for the CPET. NORMO and HYPO tests were separated by at least three days (interval 3-7 days). During both sessions participants breathed through a mask connected to a hypoxic gas generator (Everest Summit II Generator, Hypoxico, New York, USA). This device utilizes a molecular sieve system with zeolites to separate nitrogen from oxygen and allows to have a gas mixture with a reduced oxygen content that can be regulated by an operator. A gas mixture with a FiO_2 of 21% and of 13.5% (corresponding to sea level and to an altitude of about 3500 m) was delivered during the NORMO and the HYPO respectively. The gas mixture was constantly checked by an operator by means of oxygen analyzer provided with the device (Maxtec, Handi+, Salt Lake City, UT, USA). Participants were blinded about the actual content of oxygen they were breathing. During NORMO and HYPO tests, the same cycle-ergometer used for the CPET was utilized. After wearing the mask connected to the hypoxic gas generator, participants sat on the cycle-ergometer for three minutes to collect data at rest. Then, they started pedaling for three minutes against a workload corresponding to the 30% of the W_{max} reached during the CPET. A recovery of six minutes was allowed after the exercise. A similar experimental approach was recently used to assess hemodynamics during metaboreflex stimulation during normobaric hypoxia (Mulliri et al. 2020).

Assessment of O_2 saturation

Peripheral blood O_2 saturation (SO_2) was continuously measured through finger pulse oxymetry (Nonin, SenSmart X-100, Plymouth, MN, USA) to confirm that the hypoxic stimulus was effective.

Hemodynamic measurement

An echocardiographic system (Vivid iq, GE Healthcare, Fairefield, CT, USA) equipped with a hand-held 3.5-MHz ultrasound probe was employed to assess cardiovascular functions. Heart rate (HR) was assessed as the reciprocal of the electrocardiogram R-R interval provided by the echocardiograph. Two dimensional and pulsed Doppler recording were acquired with participants in

the sitting position. Measures were obtained from the apical four-chamber view. End-systolic volume (ESV) and end diastolic volume (EDV) were calculated automatically by software using a conventional formula: $8A^2/3\pi L$, where A was the left ventricular area and L was ventricular longest length (Christie et al. 1987). The ventricular area was determined by tracing along the inner edge of the endocardial targets, and the length was obtained by measuring the distance from the left ventricular apex to the midpoint of the mitral annulus. Echocardiography images were taken at rest and during the recovery from strain (i.e., at the third minute of recovery). When images were considered to be of good quality, a 6 s frame was recorded and then analyzed offline by a skilled operator. For each analysis at least three beats were taken into consideration (range 3-6 beats) and data are reported as the average of the measures. Left ventricular ejection fraction (EF) was considered as: (EDV-ESV/EDV)·100, and SV as: EDV-ESV.

In the same beats utilized for left ventricular volumes assessment, early and atrial transmitral filling peak velocities (Evel and Avel respectively) and their ratio (E/A) were assessed using pulse wave Doppler (PWD) with a 5-mm PWD sample volume placed distal to the mitral anulus, between the mitral leaflets. The interrogation beam was aligned with mitral flow (Cohen et al. 1996, Gardin et al. 1986).

Mitral valve motion velocity during early (Em) and late (Am) diastole was determined by Doppler tissue imaging with the pulsed-wave sample volume placed at the lateral mitral anulus from the apical four chamber view. Septal early diastolic mitral anular velocities have been documented to detect impaired left ventricular diastolic functions independent of ventricular loading conditions (Nagueh et al. 1997). This technique has been already employed to analyze the effect of hypoxia on diastolic functions (Allemann et al. 2004). Moreover, systolic myocardial velocity (Sm) was determined in order to have a measure of longitudinal systolic function. This parameter has been found to correlate with measures of left ventricular EF and peak *dP/dt* (Correale et al. 2012). The ratio Evel/Em was used to estimate left ventricular filling pressure considering that an Evel/Em >10 is correlated with an elevated left ventricular diastolic pressure, whereas a value <8 indicates a normal pressure (Choudhury et al. 2017; Correale et al. 2012).

Aortic Doppler was also conducted from the four-chamber window to assess the pre-ejection period (PEP), which was measured as the time from the beginning of the QRS complex of the electrocardiogram and the opening of the aortic valve, and the ventricular ejection time (VET), which was assessed as the total duration of ejection period in the Doppler trace. Diastolic time (DT) was calculated by subtracting the sum of PEP and VET from the total period of the cardiac cycle (Sainas et al. 2016). We used PEP variations to have an estimate of sympathetic activity towards the left ventricle. Actually, when there is a more rapid development of intraventricular pressure, PEP

shortens. Furthermore, the influence of parasympathetic activity on PEP is negligible as ventricles are not innervated by the parasympathetic nervous system. Yet, PEP is not substantially altered by changes in HR (Michael et al. 2017).

A manual sphygmomanometer was placed in the non-dominant arm and systolic (SBP) and diastolic (DBP) blood pressure were measured by the same physician throughout all protocol sessions. Mean arterial blood pressure (MAP) was calculated using a formula which takes into consideration changes in PEP, VET, and DT due to tachycardia (Sainas et al. 2016).

Data Analysis

Data are presented as mean ± SD. The Kolmogorov-Smirnov test was employed to assess distribution normality for each variable. Since all variables were normally distributed, parametric tests were used. Paired *t* test was employed to find out differences between the NORMO and the HYPO test at rest and at recovery. Statistical analysis was performed using commercially available software (GraphPad Prism). A p value <0.05 was considered to determine statistical significance.

RESULTS

First study

The study protocol was completed by all the participants. Table 1 shows the anthropometric data and the results of the preliminary CPT. Subjects in the YG were taller than those in the EG, but no difference between groups was found in body mass and in BMI. HR_{max}, W_{max}, $\dot{V}O_{2max}$ (expressed in terms of absolute value as well as indexed by body mass), $\dot{V}CO_{2max,}$ and V_{Emax} were higher in the YG compared to the EG, whereas maximum RER was not significantly different. HR, workload, $\dot{V}O_2$, and $\dot{V}CO_2$ at GET was significantly higher in the YG than in the EG, while RER and V_E were similar. GET occurred at the 51.76 ± 8.76 and at 73.91 ± 13.18 % of W_{max} in the YG and in the EG respectively ($p< 0.0001$).

Figures 1-6 exhibit values of variables collected at rest and at the fifth minute of the exercise test conducted at GET intensity. Figures also show the % changes with respect to rest reached by each cardiovascular parameter.

Figure 1 demonstrates that HR increased during the GET test with respect to rest, with the YG reaching higher HR levels as compared to the EG (panel A). HR was 144.1 ± 13.6 vs. 126.7 ± 16.9 bpm for the YG and the EG, respectively. It is worthy to note that these values were close to those reached at the GET workload during the preliminary CPT (see Table 1). Panel B of Figure 1 also illustrates that there was no difference between groups in terms of HR % increment with respect to rest level. The YG showed higher values of SV during the GET test in comparison with the EG (Figure 1, panel C). This parameter was 72.5 ± 16.7 vs. 52.4 ± 8.4 ml for the YG and the EG, respectively. The SV % increment with respect to rest was also more elevated in the YG than in the EG, reaching statistical significance ($p= 0.0498$, panel D). Resulting from the HR and the SV increments, the YG achieved a higher CO level in comparison with the EG (panel E), although the % increment with respect to baseline was not different between groups (panel F).

Figure 2 (panel A) shows that PEP was shorter in the YG than in the EG both at rest (94.5 ± 17.0 vs. 148.0 ± 20.9 ms for the YG and the EG, respectively) and during exercise (78.4 ± 24.3 vs. 134.1 ± 17.3 ms). However, the capacity to shorten PEP was no different between groups, as testified by the % decrement with respect to rest (panel B). In both groups, VET was on average lower during exercise with respect to rest, but there was no difference between groups neither in absolute values of VET (panel C) nor in its % change from rest (panel D). Groups significantly affects DT (panel E), which was on average shorter in the YG as compared to the EG. However, post-hoc comparison between columns did not find out any significant difference at rest as well as during exercise. Panel F demonstrates that % DT shortening due to exercise was similar between groups.

Figure 3 shows that the YG had a higher capacity to increase VFR in response to exercise as

compared to the EG (463.90 ± 162.80 vs. 265.92 ± 81.63 $ml \cdot s^{-1}$, panel A), although no difference between groups was found in the capacity to % increase this parameter from rest (panel B). Similarly, group significantly affected the capacity to increase VER, which reached a value of 424.55 ± 110.33 vs. 316.14 ± 121.33 $ml \cdot s^{-1}$ in the YG and in the EG, respectively (panel C). No difference was found between groups in the % VER increment from rest (panel D). Ageing did not influence the MAP level both at rest and during exercise (panel E), nor its % increment from rest (panel F). Differently, SVR was significantly higher in the EG as compared to the YG both at rest (1798.76 ± 458.13 vs. 1277.41 ± 345.11 $dynes \cdot s^{-1} \cdot cm^{-5}$ for the EG and the YG, respectively) and during exercise (1299.08 ± 369.88 vs. 771.30 ± 147.77 $dynes \cdot s^{-1} \cdot cm^{-5}$, panel G). The capacity to % decrease SVR during exercise was not different between groups (panel H).

Panel A of Figure 4 shows that EDV was similar between groups at rest. However, during exercise, EDV was significantly different as this parameter was higher in the YG than in the EG (99.56 ± 15.80 vs. 80.47 ± 15.66 ml). Moreover, the capacity to increase EDV in response to exercise was greater in the YG as compared to the EG (panel B). No difference was discovered between groups neither in the ESV level, nor in its % increment from rest (panel C and D respectively). EF (panel E) was on average lower in the EG than in the YG both at rest (59.60 ± 7.95 vs. 67.64 ± 8.11 for the EG and the YG, respectively) and during effort (65.53 ± 8.21 vs. 72.79 ± 5.22). In both groups, EF increased to a similar extent with respect to rest (panel F).

In both groups, exercise significantly increased Evel with respect to rest (Figure 5, panel A), without any detectable difference in groups capacity to % increase this parameter with respect to rest (panel B). Avel (panel C) was also increased during exercise as compared to rest, with the YG showing a higher capacity to increase this parameter than the EG (112.02 ± 24.55 vs. 85.54 ± 20.70 $cm \cdot s^{-1}$). Furthermore, the YG exhibited a greater capacity to % enhance Avel in response to exercise than the EG (panel D). E/A was unaffected by group (panel E), but YG decreased this parameter in response to exercise, while the EG did not.

The last figure (Figure 6) is related to variables gathered with tissue Doppler. Although close to significance, on average Em was not affected by exercise, as it was unchanged in the YG while it increased in the EG (panel A). There was, however, no significant difference between groups, although the EG showed a higher % increment with respect to rest than the YG (panel B). In both groups, Am increased with respect to rest in response to exercise (panel C), although the capacity to % increase this parameter was higher in the YG as compared to the EG (panel D). Em/Am was more elevated during the rest period in the YG than in the EG (panel E), but this difference was abolished during exercise. The % change from rest in this parameter was significantly different between groups, as it decreased in the YG while it increased in the EY subjects (panel F). Sm was,

on average, higher in the YG than in the EG, although post-hoc analysis did not yield any significance in the direct columns' comparison. Moreover, in both groups this parameter significantly increased in response to exercise (panel G). There was not significant difference between groups int the Sm % change from rest (panel H). Time and group did not affect Evel/Em (panel I), nor any difference was found between groups in the % change from rest (panel L).

Second study

Concerning the second study, results of the CPET are reported in Table 2, while Table 3 shows the values of variables collected during the third minute of rest preceding the NORMO and the HYPO test. Statistics found out that the HYPO test induced a significant increase in Evel and in E/A ratio, whereas Avel was reduced. Moreover, DT was significantly longer during the HYPO test.

Figure 7 exhibits values of variables collected during recovery from the HYPO and the NORMO test. Panel A shows that SO_2 was significantly reduced during the HYPO test (97.81 ± 1.20 vs. 91.97 ± 2.23% for the NORMO and the HYPO test, respectively, $p< 0.001$), while HR (99.08 ± 17.58 vs. 98.00 ± 12.01 bpm, $p= 0.8628$), and MAP (90.25 ± 4.41 vs. 89.75 ± 4.49 mmHg, $p= 0.5412$), while HR and MAP were unaffected by conditions (panels B and C respectively). Panel D demonstrates that PEP was shorter during the HYPO test than during the NORMO test (124.96 ± 15.30 vs. 112.21 ± 11.00 ms for the NORMO and the HYPO test, respectively, $p= 0.0178$), whereas VET (218.14 ± 18.41 vs. 214.60 ± 14.33 ms, $p= 0.6040$), and DT (266.40 ± 74.97 vs. 282.59 ± 51.08 ms, $p= 0.4321$) were unaffected by condition (panels E and F).

Figure 8 illustrates that EDV, SEV, EF, and SV were not significantly different between conditions (panels A, B, C, and D). In detail, ESV was 37.88 ± 17.73 vs. 38.56 ± 12.04 ml for the NORMO and the HYPO test, respectively ($p= 0.7993$), EDV was 119.77 ± 24.81 vs. 123.30 ± 17.88 ml ($p= 0.3943$), EF was 69.70 ± 9.93 vs. 68.99 ± 7.94% ($p= 0.6219$), and SV was 81.88 ± 117.79 vs. 84.73 ± 13.40 ml ($p= 0.2312$). HYPO test increased Evel and E/A with respect to the NORMO test (75.41 ± 14.01 vs. 67.41 ± 10.69 $cm \cdot s^{-1}$, $p= 0.0478$, Figure 9, panel A), while Avel and Em (panels B and D) were not different between conditions. Similarly, E/A was also increased by the HYPO with respect to the NORMO test (1.17 ± 0.30 vs. 0.93 ± 0.26, $p= 0.0315$, Figure 3, panel C), while Avel and Em (panels B and D, respectively) were not different between conditions (75.27 ± 15.60 vs. 67.13 ± 12.95 $cm \cdot s^{-1}$, $p= 0.1603$, and 9.55 ± 1.94 vs. 10.44 ± 3.15 $cm \cdot s^{-1}$, $p= 0.3343$ for Avel and Em during the NORMO and the HYPO test, respectively). Finally, Figure 10 shows that Sm (panel C) was higher during the HYPO than during the NORMO test (14.30 ± 1.49 vs. 12.72 ± 2.445 $cm \cdot s^{-1}$, $p= 0.0431$), whereas Am (panel A), Em/Am (panel B), and Evel/Em were not different between conditions (9.55 ± 2.75 vs. 9.30 ± 2.05 $cm \cdot s^{-1}$, $p= 0.7387$; 1.10 ± 0.40 vs. 1.19 ± 0.41, p=

0.4623; and 7.29 ± 1.23 vs. 7.71 ± 1.77, p= 0.4875 for Am, Em/Am, and Evel/Em during the NORMO and the HYPO test).

DISCUSSION

First study

The aim of the first study was to compare hemodynamics of healthy elderly and young subjects during an exercise bout conducted at the same relative submaximal workload, i.e., at the GET intensity. Exercise is a powerful tool to indagate how the cardiovascular apparatus responds to stress and to assess its functional reserves. The importance of assessing the adjustments to exercise of the cardiovascular system as a marker of cardiovascular health is well acknowledged (Moreira et al. 2021). Moreover, information about expected values may help establishing targets and providing parameters to evaluate the impact of training interventions. This is particularly true in the elderly, where functional reserves often deteriorate and cardiovascular, metabolic, and respiratory diseases often develop.

This investigation was conducted to compare hemodynamics of healthy elderly and young individuals during an effort conducted at the same relative submaximal workload, i.e., at the GET intensity. This workload allows discriminating the transition from moderate to heavy exercise and identifies a workload that can be sustained for long periods (Poole et al. 2021). To the best of our knowledge, very few information exists about the hemodynamic differences between young and elderly individuals during exercise at the GET intensity.

Our main hypothesis was that, during effort conducted at the GET intensity, elderly individuals showed a reduced capacity to increase SV and CO because of their reduced diastolic and systolic functions as compared to the young ones. Results confirmed this hypothesis, since elderly showed a reduced capacity to increase SV in response to exercise in comparison with young subjects. This phenomenon has been already described in investigations with incremental exercise tests up to exhaustion (Farinatti et al. 2018, Fleg et al. 2012), but, to the best of our knowledge, the present is the first one to compare hemodynamics during submaximal rectangular exercise at the same relative intensity. Moreover, results found that not only the EG had reduced SV value, but their capacity to increase SV with respect to rest was blunted in comparison with the YG (see Figure 1, panel D), thereby reinforcing our hypothesis of an impaired capacity to properly increase SV during effort in this group.

The reduced SV, in combination with the lower HR, resulted in a lower CO level in the EG than in the YG, thereby explaining at least in part the lower workloads achieved by EG at GET. It is to be noticed that in the EG, although the HR in absolute terms was lower, the chronotropic response was increased to the same relative extent as in the YG, as testified by the similar % increase with respect to rest (Figure 1, panel B). Furthermore, in the EG, HR was on average 126 bpm, which was about

the 86% of the maximum HR reached in the preliminary CPT (see Table 1). Similarly, at the GET, the YG reached a HR of 144 bpm, which was on average about the 81% of maximum HR achieved during the preliminary CPT. Overall, these findings suggest that, in term of chronotropic reserve, the two groups exercised at a similar relative intensity.

Concerning SV, in our interpretation, the impaired capacity to enhance this parameter resulted from the convergence of several concomitant phenomena: a reduced capacity to increase cardiac pre-load, a reduced capacity to augment cardiac contractility, and an increase in cardiac afterload.

The impaired capacity to increase pre-load appears from the EDV behaviour. At rest, this parameter was similar between groups, but it increased in the YG in response to exercise, whilst it did not in the EG. The reduced capacity of the EG to increase EDV also appears looking at Figure 4 (pane B), where the % increment from rest is shown. Thus, results suggest that ageing was accompanied by an impairment in the capacity to enhance pre-load in response to exercise, and this is line with the concept that diastolic function deteriorates with age, probably ought to an increase in cardiac stiffness and in ventricular relaxation (Arbab-Zadeh et al. 2004, Carrick-Ranson et al. 2012, De Souza 2002, Lakatta 2002, Milia et al. 2015). Moreover, VFR, e measure of diastolic flux, was more elevate in the YG than in the EG during exercise, thereby reinforcing the hypothesis that elderly people can not properly enhance cardiac flux during diastole. In this regard, it should be noticed that DT was on average longer in the EG as compared to the YG, and this would have allowed a more efficient cardiac filling in the EG; however, this was not the case. Collectively, data of EDV and VFR indicate that in the EG the preload reserve can not be recruited to the same extent as in younger subjects during submaximal exercise.

The second phenomenon which could be responsible for the blunted SV response of the EG was myocardial contractility, which was impaired in this group. Data indicate that EF was different between groups already at rest, with the YG showing higher EF values. This difference remained also during exercise. However, the contractility reserve was similarly recruited in the two groups, as EF increased to the same relative extent (see Figure 4, panel F). It has been already observed that the EF response is reduced in the elderly, and it was speculated that the impaired myocardial performance is due to a combination of an increased arterial afterload (i.e., increased SVR), a decreased effectiveness of the autonomic nervous system to modulate myocardial contractility, and an impaired cardiomyocytes contractility function (Farinatti et al. 2018, Fleg et al. 2012, Moreira et al. 2021).

It is worthy to note that PEP was longer in the EG than in the YG, and this reinforced the hypothesis that the myocardial contractility was reduced in the elderly group since this parameter is inversely related to the development of intraventricular pressure and it responds to sympathetic stimulation

(Michael et al. 2017). However, it is to be highlighted that PEP decreased to a similar extent between groups, and this suggests that the sympathetic stimulation could still effectively recruit the contractility reserve in the EG, as also supported by the reduction in EF already discussed. To further support our hypothesis there is Sm, a parameter gathered with tissue Doppler, which is relate to myocardial contractility. This parameter is correlated with ventricular peak dP/dt, and it is considered an index of inotropism (Correale et al. 2012, Höglund et al. 1988). Moreover, the systolic excursion of mitral anulus has been found reduced in patients with ventricular dysfunction (Berg et al. 2020, Grue et al. 2018). In the present study, Sm showed a behaviour similar to that of EF and PEP, i.e., it was on average lower in the EG than in the YG, but it increased to a similar extent between groups in response to exercise. The concept that sympathetic stimulation still effectively recruited contractility in the EG also appears looking at VET. This variable was quite similar between groups at rest, and it decreased to a similar extent in response to exercise. Moreover, both groups exhibited similar capacity to reduce ESV during exercise, thereby reinforcing the hypothesis that the aged group had still the capacity to recruit the contractility reserve.

An alternative explanation for the reduced EF may be the higher SVR level of the EG. In this regard, it is useful to consider the VER behaviour of the EG, who did not properly enhance this variable during exercise as compared to the YG, notwithstanding the two groups had the same VET. This could be the consequence of the higher level of SVR shown by the EG, which potentially limited the ventricular emptying in comparison with YG. This result is in line with the concept that older individuals have higher levels of SVR both at rest and during exercise, thus suggesting that cardiac afterload is increased by ageing, and this fact may at least in part explain their reduced EF and ventricular emptying. It should be considered that healthy aging is associated with an elevation in vascular tone both at rest and during exercise (Barrett-O'Keefe et al. 2014, Proctor et al. 1998). Inasmuch as adequate muscle perfusion is vital to meet the metabolic demand of the tissue, this implies profound functional consequence in terms of limitations of the capacity for physical activity. Thus, the reduced workload found in the EG found in the present investigation may be also the consequence of a reduced muscle perfusion.

Results of the present study suggest that ageing did not significantly impact on the capacity to increase MAP during exercise, and this occurrence suggests that mechanisms controlling blood pressure during exercise were well preserved in ageing.

Concerning parameters gathered by transmitral and tissue Doppler, Evel was not influenced by age neither at rest nor during exercise, moreover, the capacity to increase Evel in response to effort was well preserved in the EG, as testified by panel B of Figure 5. Similar results were obtained for Em,

although it appears that, in the EG subjects, for this parameter the increment with respect to baseline was more pronounced than in the YG. Taken together, these findings are not consistent with the concept that healthy aging is associated with an increase in cardiac stiffness and/or in ventricular relaxation capable of impairing early ventricular filling (Arbab-Zadeh et al. 2004, Lakatta 2002), although it is to be considered that our population was composed by physically active elderly subjects, and this may explain the outcome of our study. These findings are instead consistent with previous investigations reporting that Evel and Em did not show any relationship with exercise SV in healthy young individuals (Carrick-Ranson et al. 2012, Rowland et al. 2009), thereby suggesting that faster early filling velocity is not the primary mechanism to improve ventricular filling in young individuals during submaximal workloads as those employed in the present investigation.

However, to fully understand the complexity of diastolic filling, it should be considered also the atrial component. In this regard, a particular behaviour was shown by Avel, as this parameter increased in both groups in response to exercise, but the increment was more evident in the YG than in the EG. This difference between groups was demonstrated by both absolute Avel values and by the Avel % increment with respect to rest. This phenomenon appears to suggest that YG relied more on the capacity to increase the atrial contribution to ventricular filling than the EG. This hypothesis is supported also by Am, whose increment with respect to rest was more elevated in the YG as compared to the EG. The different Avel and Am behaviour between groups can be explained by an increased ventricular stiffness and/or an impaired atrial contraction in the EG as compared to the YG. With the present experimental set-up, we can not solve the question of whether ventricular stiffness and/or impaired atrial contraction were both present in the EG. However, whatever the cause, it appears as though the young subjects could rely more on the atrial reserve than the elderly ones to fill the ventricle during exercise.

Since in the EG the Evel and Avel increment was similar in terms of % from rest, whereas in the YG the Avel increment was steeper than Evel, in the YG the E/A ratio decreased more than in the EG. This occurrence further supports the hypothesis that in the YG the possibility to enhance ventricular filling during submaximal exercise relies more on the recruitment of the atrial contribution than on an increase in early filling. This is in line with recent findings demonstrating that during effort, when cardiac cycle shortens due to tachycardia, the atrial contraction becomes determinant in left ventricular filling (Gabrielli et al. 2018). In this regard, it should be considered that previous research reported that the E/A gradually decreased with increasing exercise intensity in young individuals, and this has been interpreted as a more prominent increase in atrial vs. early ventricular filling during exercise (Sato et al. 1999). This result is also in line with another investigation reporting a deeper decrement in E/A in young vs. old subjects during exercise

(Carrick-Ranson et al. 2012). To further support this hypothesis there was Em/Am, which in our experiment showed a behaviour similar to that of E/A. Moreover, in older male athletes it was observed that SV is not related to faster filling during early or late ventricular filling (Carrick-Ranson et al. 2012).

Regarding Evel/Em, this parameter is the ratio between the maximum velocity of early rapid filling and the maximum velocity of mitral valve anulus during early filling and is considered a load-independent estimate of end diastolic pressure (Chung et al. 2015). It did not differ between groups, and this suggests that ventricular filling pressure is unaffected by aging during effort at the GET intensity.

One potential limitation of the investigation should be honestly acknowledged. Specifically, the present study was conducted in active healthy individuals, thus its results can not be applicable for non-active elderly people or for those suffering from any cardiovascular, metabolic, or respiratory disease, which are however more common than healthy active ageing (Carta et al. 2021). Further research in groups of sedentary aged people and/or suffering from age-related diseases is warranted to gather a clearer picture of the hemodynamic consequences of aging in these sub-groups.

In conclusion, taken together our data suggest that normal ageing is characterised by several changes in hemodynamics during submaximal exercise at the GET intensity with respect to young people. The well-known reduction in chronotropism is accompanied by a reduced capacity to increase SV. This is in turn the result of several phenomena, namely the impaired filling rate, the reduced capacity to increase EDV, the impaired inotropic response, and the elevated systemic vascular resistance. In short, all the main hemodynamic modulators, i.e., chronotropism, inotropism, cardiac pre-load, and afterload, are significantly affected by normal ageing. One new finding of the present investigation is the role played by the atrial contraction on ventricular filling, as it appears that the atrial reserve can be recruited by young individuals to increase EDV during submaximal exercise, while the elderly ones could not. Further research is warranted to better clarify this latter point.

Second study

In the second study, a group of healthy physically active male subjects performed a brief, mild exercise bout during acute hypoxia to study the cardiovascular response during the following recovery. The fact that our experimental approach was capable of effectively inducing hypoxemia was testified by SO_2, which substantially dropped during the HYPO session, as shown by Figure 7 (panel A). The main purpose of the present investigation was to verify whether classical echocardiographic measures of ventricular volume confirmed or rejected our previous hypothesis of

a reduced pre-load during the recovery from mild efforts conducted in AH. Another aim was to verify whether Doppler and tissue Doppler measures confirmed or rejected the hypothesis that, during the recovery from exercise in AH, systolic and diastolic functions increased due to sympathetic activation. Based on results, we must reject the first hypothesis. Indeed, EDV was similar between conditions, thereby indicating that there were not reductions in cardiac pre-load in participants. Moreover, ESV, EF, and SV were not different between the NORMO and the HYPO tests. These findings suggest that a brief bout of mild exercise in AH could not cause any modification in cardiac variables related to heart volumes. This finding appears to contradict our recent investigation, where, in healthy humans, a reduction in the capacity to increase SV due to impairment in ventricular filling rate was found in response to the muscle metaboreflex activation after exercise in AH (Mulliri et al. 2020). In this paper, it was hypothesised that the reduced ventricular filling rate was the consequence of an increase in the production of metabolite-mediated venodilation, such as NO, adenosine, and prostaglandin derived factors, which exerted vasodilatory activity in the venous bed (Marshall 2015, Dinenno 2016) and prevented the recruitment of the Frank-Starling mechanism. It should however be considered that, in the quoted study, hemodynamics was studied during the metaboreflex stimulation, which causes a substantial sympathetic activation. Thus, the present and the former studies are quite different in the experimental approach, and their results can be only partially comparable. Moreover, in our previous investigation, we could not conduct any echocardiography assessment. Thus, our hypothesis was speculative. Further study using echocardiography, during the metaboreflex, should be conducted in order to verify whether metaboreflex-induced sympathetic activation after exercise in AH reveals any impairment in EDV and in the capacity to vasoconstrict the venous bed in healthy humans.

Another result of the present research was that, during the recovery of the HYPO test, PEP significantly shortened in comparison with the NORMO test (Figure 7, panel D). This indicated that, in this setting, there was an increase in myocardial contractility, as this parameter is inversely related to the development of intraventricular pressure. Concerning the influence of autonomic activity, it is to be highlighted that PEP responds only to sympathetic stimulation, since the influence of parasympathetic tone is negligible on ventricles. Moreover, PEP does not depend on changes in HR (Michael et al. 2017). Thus, the PEP shortening could be the consequence of an increase in sympathetic tone, although other phenomena may have taken part in the myocardial contractility enhancement (see the following part of Discussion). The fact that after the exercise bout in AH an increase in contractility took place is also confirmed by the Sm velocity gathered by tissue Doppler, which was faster during the HYPO as compared to the NORMO test (Figure 10,

panel C). Sm velocity at the lateral mitral anulus is correlated with ventricular peak dP/dt and it can be considered an index of inotropism (Correale et al. 2012). Actually, ventricular systole pulls down the atrio-ventricular plane, and it seems reasonable to assume that the displacement of this plane is an expression of the myocardial contractility (Höglund et al. 1988). In support to the notion that Sm is related to myocardial performance there are findings that mitral anulus systolic excursion is reduced in patients with ventricular dysfunction (Grue et al. 2018, Berg et al. 2020).

It is to be noticed that, during the rest period of the HYPO session, neither PEP nor Sm was affected by the administration of the hypoxic gas mixture (see Table 3). This result suggests that, rather than hypoxia per se, the exercise bout in AH was the real responsible for changes in both parameters related to myocardial contractility. It is also possible that sympathetic activation was not the only responsible for the enhanced myocardial contractility. The concept that several substances produced during exercise in AH can enhance inotropism independently from sympathetic activity has been the subject of active research in the last years.

Specifically, apart from sympathetic tone, during AH some metabolic products, such as apelin, may exert positive inotropic effect (Calbet et al. 2009), thus explaining why we noticed an increase in Sm only after exercise in AH and not at rest. For instance, recent findings demonstrated that left ventricular twist mechanic is not impaired by acute hypoxia and that endocardial dysfunction did not occur during AH (Williams et al. 2019). Moreover, it should be mentioned that during exercise in ischemic conditions several metabolites are produced, and these metabolites can trigger the phenomenon termed ischemic preconditioning, which confers cardioprotection and favourable hemodynamics effects (i.e., increase in myocardial performance and vasodilation) within few minutes (Marongiu and Crisafulli, 2014). It is then conceivable to hypothesise that exercise in AH leads to a similar metabolites production as during ischemia. To the best of our knowledge, this possibility has never investigated before, and it may represent an intriguing field of research in a physiological and clinical perspective.

Whatever the cause responsible for the increased myocardial performance, results of the present investigation confirm that contractile function is preserved after mild exercise bouts in AH and that SV is well preserved by mechanisms, which are only partially known. While a decrease in SV, during acclimatisation at high altitude has been several times reported, this phenomenon is usually not observed during AH (Stembridge et al. 2016).

Another result of the present investigation was that diastolic function was significantly modified by AH both at rest and after the exercise bout. Regarding results at rest, it appears that the hypoxic gas administration shifted ventricular filling from the late to the early phase. This can be at least partially explained by the longer DT during the HYPO in comparison with the NORMO test. This

was the result of the slight reduction in HR occurring during the rest period of the HYPO test, which, although insignificant with respect to the NORMO test, nonetheless led to a longer cardiac cycle with respect to the NORMO test. Considering that PEP and VET were quite similar between conditions, then it followed that DT was longer in the HYPO test. We cannot however rule out that the hypoxic condition could improve early diastolic function by any unknown mechanism able to enhance ventricular relaxation. To the best of our knowledge, there are no studies focusing on the potential effect of AH on the myocardial early diastolic proprieties, and further research is warranted in this area. It should however be acknowledged that the presence of any hypoxic-mediated mechanism was unlikely in our setting at rest as the hypoxic stimulus did not significantly reduce SO_2, so indicating that the hypoxic stress was mild.

A different diastolic behaviour between tests was present also during the recovery phase. Indeed, Evel and E/A were significantly higher after AH. An increase in Evel, during exercise in AH, has been already reported in the scientific literature (Yan et al. 2007), but the phenomenon has been never replied by other groups to date. Authors of the quoted paper suggested that acute hypoxic exercise increased diastolic function, although no explanation for the phenomenon was provided. Our results seem to confirm these previous findings. Moreover, our results suggest that the increased contractility could be at least in part responsible for it. Both PEP and Sm indicated that, during the HYPO test, myocardial contractility was more elevated with respect to the NORMO test. It was observed that the energy generated during systole is stored in the extracellular collagen matrix and then released during diastole, thereby supporting ventricular filling (Notomi et al., 2008). Furthermore, it was also proposed that the atrio-ventricular plane acts like a piston driven by ventricular contraction and that its movement during systole pulls blood from the venous tree to the atria (Arutunyan, 2015). In short, when the ventricles contract, the A-V plane descends towards the apex, while the pulmonary veins remain fixed in the mediastinum. The descent of the A-V plane aspirates blood from the pulmonary circulation and generates one of the forces able to fill the atria (Chung et al. 2015). In humans, it has been demonstrated that up to 70% of atrial filling occurs during ventricular emptying and is driven by ventricular longitudinal contraction (Steding-Ehrenborg et al. 2013).

Then, the increased myocardial performance during the HYPO test may have enhanced early diastolic filling with at least two different phenomena: (a) an increase in the energy generated during systole and recoiled during diastole and (b) a more efficient A-V displacement, which allowed a more effective atrial filling. A third phenomenon that could theoretically affect diastolic filling could be the increase in left ventricular filling pressure due to hypoxia-induced pulmonary vasoconstriction (Naeije and Dedobbeleer 2013, Stembridge et al. 2016). We employed Evel/Em to

estimate the left ventricular filling pressure, but we did not find out any significant difference between the HYPO and the NORMO test. It can be then concluded that a brief bout of exercise in AH cannot significantly affect left ventricular filling pressure.

Limitations of the study

Some limitations of the present investigation should be honestly acknowledged.

In detail, echocardiographic measures were conducted only during recovery and not during exercise. This because in a pilot study we could not collect good images during cycling mainly because of chest movements due to respiration. Probably, the best position in this kind of research is the recumbent one. However, this position is not very natural as normally individuals exercise standing or sitting, as in the present investigation. Moreover, the recumbent position increases venous return, thereby affecting EDV and diastolic functions.

It should be pointed out that diastolic measures obtained with tissue Doppler yielded different results with respect to trans-mitral Doppler. Specifically, while Evel was significantly increased by the HYPO test, Em was not affected by this condition. One explanation for this different outcome could be that tissue Doppler measures are highly dependent on the angle between scan beam and the vector of ventricular motion, which should be parallel (Bassareo et al. 2010). It is then possible that chest movements due respiration after effort may have rendered problematic tissue Doppler measures in our experimental setting, thus affecting assessment precision.

Another limit could be that we did not directly assess myocardial inotropism. Instead, indirect measures were used, i.e., PEP and Sm. However, direct assessment of myocardial inotropism is problematic in humans as it requires the use of invasive technologies which are not advisable in study such the present one.

Finally, the present study was conducted in healthy male individuals, thus its results can not be applicable for elderly people, for females, or for patients suffering from any disease. Further research in different groups of individuals is needed to have a clearer picture of the hemodynamic consequences of hypoxia during exercise in these sub-groups.

Overall, the results of the second study support the hypothesis that a brief exercise bout of mild intensity in acute normobaric hypoxia does not impair systolic or diastolic functions. Rather, it appears that SV is well preserved thanks to an improvement in inotropism and in early diastolic function. It remains to be ascertained whether the described improvement in diastolic function is a direct consequence of the enhanced systolic activity or it is due to an unknown metabolic process triggered by exercise in hypoxia. Taking into consideration that exercise in hypoxia has been proposed as a useful tool for training as well as for therapeutic purposes, its effects should be further investigated to better understand its hemodynamics and its capacity to product regulating

metabolites. Lastly, it could be interesting study the effects of hypoxia in healthy ederly subjects respect to young subjects already studied.

GENERAL CONCLUSIONS

The general aim of both studies was to apply Doppler-Echocardiography tissue Doppler measures to investigate on systolic and diastolic functions during acute stressors in healthy populations. To this aim, submaximal exercise (study 1) and hypoxia (study 2) were applied.

The results of study 1 support the hypothesis that normal individuals of different age show a distinct hemodynamic change during an exercise bout conducted at submaximal intensity. Specifically, elderly subject showed a reduced capacity to increase SV and CO because of their reduced diastolic and systolic functions as compared to the young ones. Echocardiography and its related methods were able to detect these differences. In detail, results found that not only the elderly had reduced SV value, but their capacity to increase SV with respect to rest was blunted in comparison with young individuals. Moreover, Echocardiography was able to detect subtle signs of the impaired filling rate and inotropic response. One new finding was that the role played by the atrial contraction on ventricular filling, as it appears that the atrial reserve can be recruited by young individuals to increase EDV during submaximal exercise, while the elderly ones could not.

The results of study 2 support the use of echocardiography as a good tool to investigate on hemodynamics during exercise in hypoxia. In particular, we found that a brief exercise bout of mild intensity in acute normobaric hypoxia did not impair systolic or diastolic functions. Rather, SV was well preserved thanks to an improvement in inotropism and in early diastolic function.

Taking together, both studies support the use of Echocardiography imaging to detect hemodynamic changes during manoeuvre able to stress the cardiovascular apparatus in healthy individuals. Investigations in patients suffering from cardiovascular diseases during acute stressor, such as those employed in our two investigations, are needed to extend our knowledge of the cardiovascular response in the normal and in the failing circulation.

Tables and Figures

Figure 1. **Exemples of measures obtained from TDI**

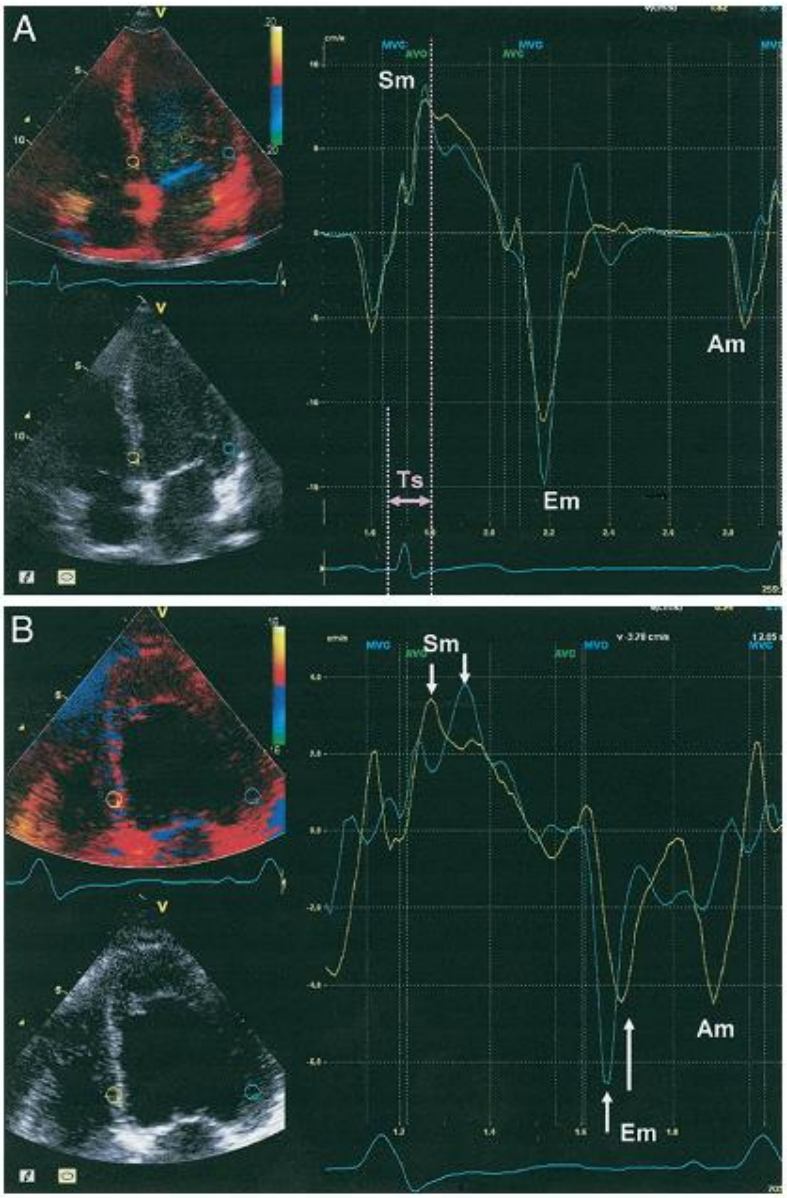


Table 1. Anthropometric characteristics of groups together with results of the cardiopulmonary test of the study 1. EG= elderly group (n= 11, 7 females), YG= young group (n= 13, 7 females). GET= gas exchange threshold. Values are mean ± SD.

	EG	YG	*p* value
Age (years)	65.18±4.71	29.84±4.06	<0.0001
Height (cm)	161.00±8.64	169.38±8.41	0.0252
Body mass (kg)	61.37±7.45	64.82±9.77	0.3485
Body mass index ($kg \cdot m^2$)	23.70±2.42	22.53±2.46	0.2547
Maximum heart rate (bpm)	145.81±18.21	177.92±9.21	<0.0001
Maximum workload (W)	115.45±45.68	206.92±51.25	<0.0001
Maximum O_2 uptake ($mL \cdot min^{-1}$)	1419.61±558.9642	2191.59+648.0	0.0053
Maximum O_2 uptake/kg ($mL \cdot min^{-1} \cdot kg^{-1}$)	22.68±6.60	32.98±5.61	0.0004
Maximum CO_2 production ($mL \cdot min^{-1}$)	1794.78±661.8642	2894.14±919.21	0.0032
Maximum respiratory exchange ratio	1.27±0.13	1.31±0.09	0.3843
Maximum pulmonary ventilation ($L \cdot min^{-1}$)	54.46±22.46	83.31±27.58	0.0111
Heart rate at GET (bpm)	126.64±13.29	140.69±9.24	0.0036
Workload at GET (W)	81.82±23.58	105.00±23.97	0.0265
O_2 uptake at GET ($mL \cdot min^{-1}$)	1089.95±338.783	1368.88±274.40	0.0363
CO_2 production at GET ($mL \cdot min^{-1}$)	1093.46±343.49	1375.53±310.17	0.0461
Respiratory exchange ratio at GET	1.01±0.03	1.00±0.07	0.7006
Pulmonary ventilation at GET ($L \cdot min^{-1}$)	33.62±10.45	29.23±8.67	0.2717

Figure 2. Hemodynamic data at rest and during exercise conducted at the gas exchange threshold intensity in the elderly (EG, n=11) and in young (EG, n= 13) groups. Panels A, C, and E show absolute values; panels B, D, and F show % variation from rest level. HR= heart rate; SV= stroke volume; CO= cardiac output. Values are mean ± SD. *= p<0.05 between groups at the same timepoint.

Figure 2

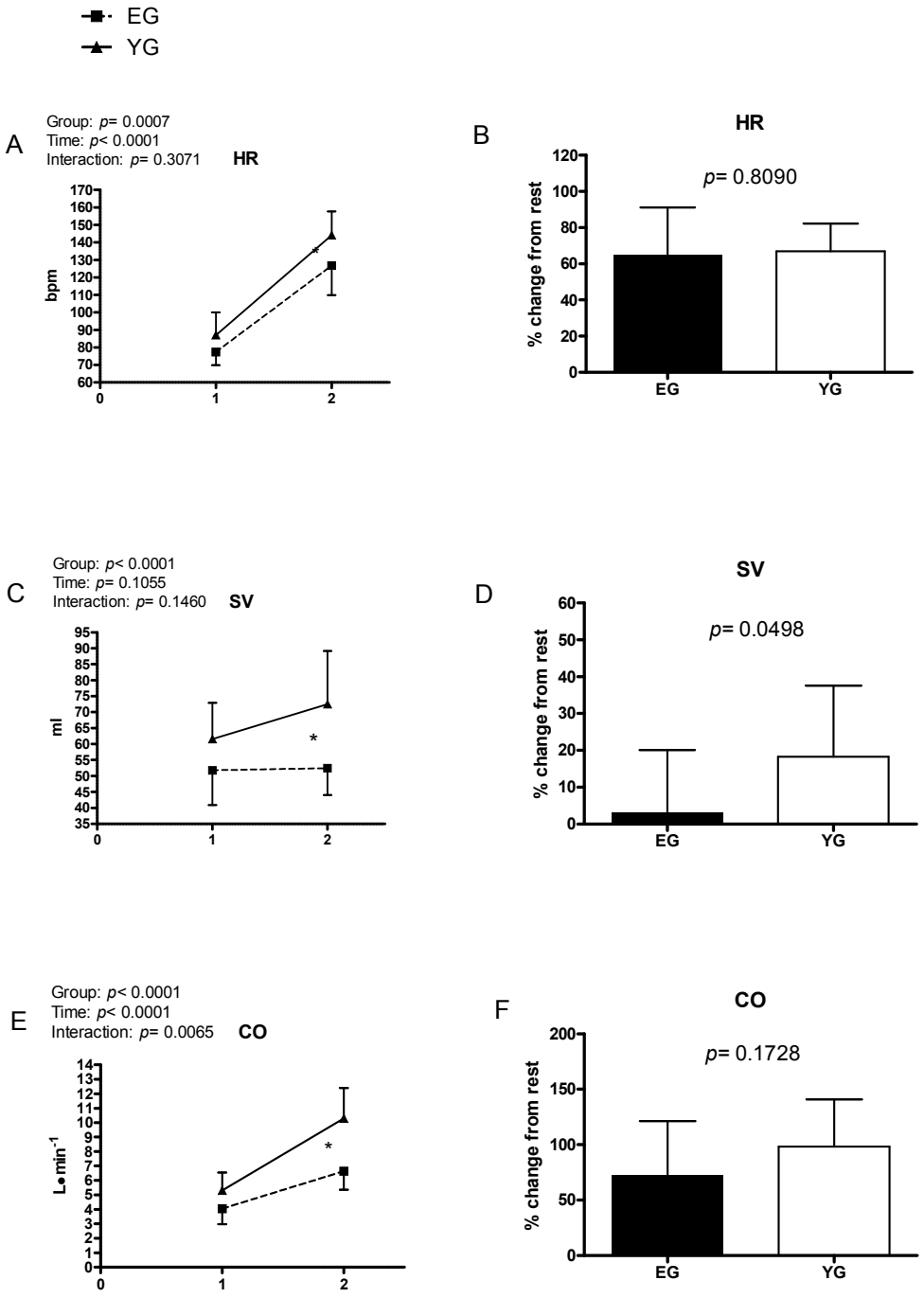


Figure 3. Hemodynamic data at rest and during exercise conducted at the gas exchange threshold intensity in the elderly (EG, n=11) and in young (EG, n= 13) groups. Panels A, C, and E show absolute values; panels B, D, and F show % variation from rest level. PEP= pre-ejection period; VET= ventricular ejection time; DT= diastolic time. Values are mean ± SD. *= p<0.05 between groups at the same timepoint.

Figure 3

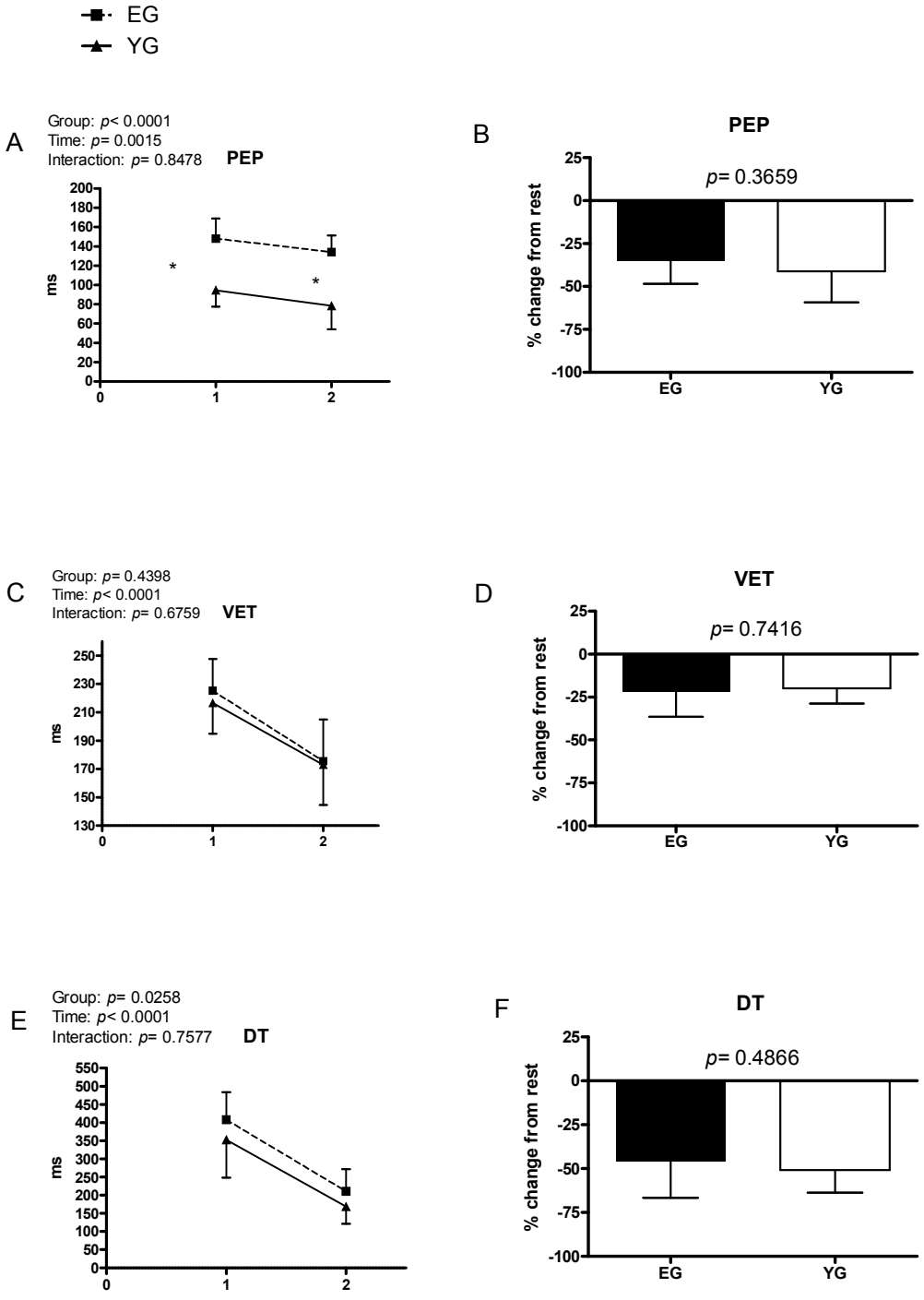


Figure 4. Hemodynamic data at rest and during exercise conducted at the gas exchange threshold intensity in the elderly (EG, n=11) and in young (EG, n= 13) groups. Panels A, C, E, and G show absolute values; panels B, D, F, and H show % variation from rest level. VFR= ventricular filling rate; VER= ventricular emptying rate; MAP= mean arterial pressure; SVR= systemic vascular resistance. Values are mean ± SD. *= $p<0.05$ between groups at the same timepoint.

Figure 4

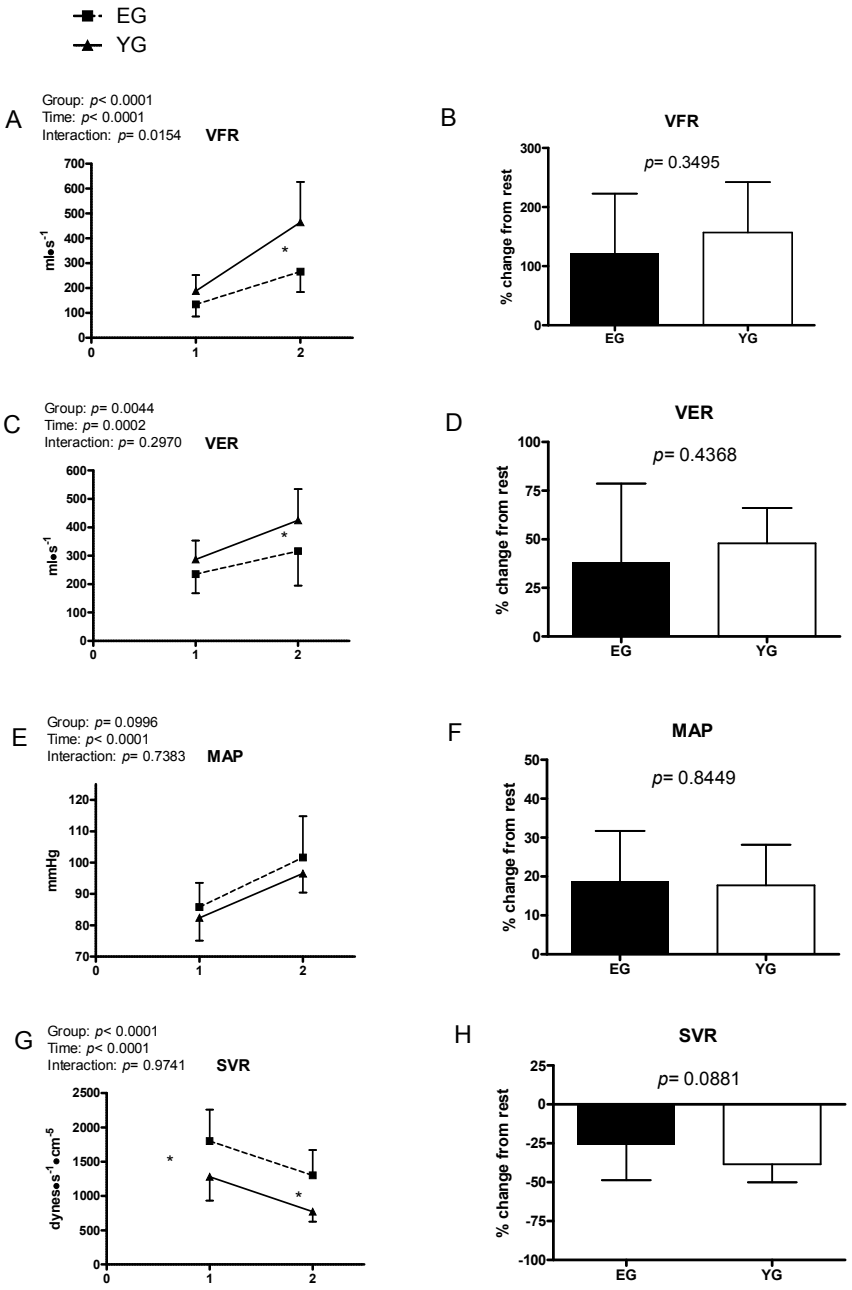


Figure 5. Hemodynamic data at rest and during exercise conducted at the gas exchange threshold intensity in the elderly (EG, n=11) and in young (EG, n= 13) groups. Panels A, C, and E show absolute values; panels B, D, and F show % variation from rest level. EDV= end diastolic volume; ESV= end systolic volume; EF= ejection fraction. Values are mean ± SD. *= $p<0.05$ between groups at the same timepoint.

Figure 5

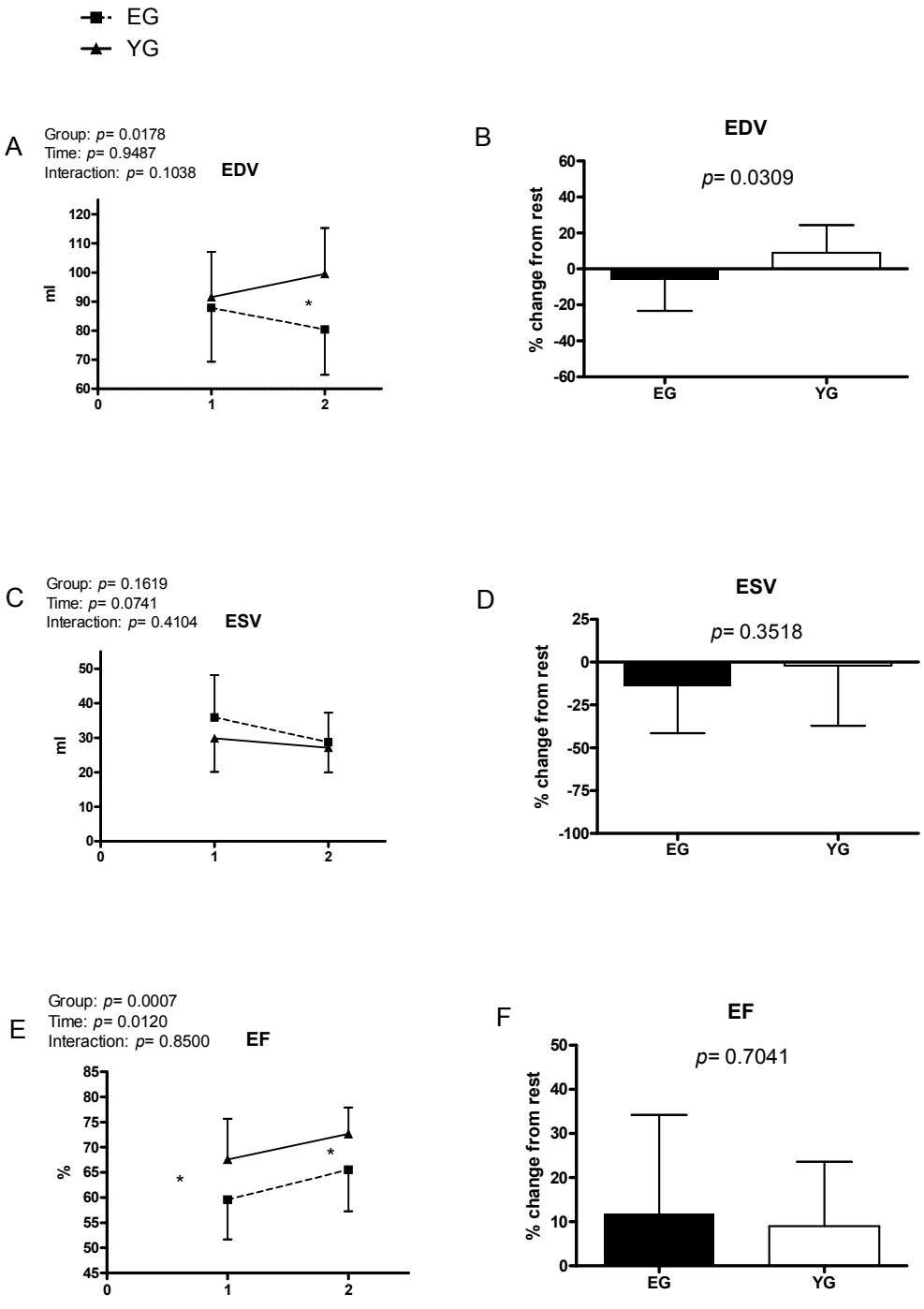


Figure 6. Hemodynamic data at rest and during exercise conducted at the gas exchange threshold intensity in the elderly (EG, n=11) and in young (EG, n= 13) groups. Panels A, C, and E show absolute values; panels B, D, and F show % variation from rest level. Evel= transmitral filling peak velocity during early diastole; Avel= transmitral filling peak velocity during atrial contraction. Values are mean ± SD. *= p<0.05 between groups at the same timepoint.

Figure 6

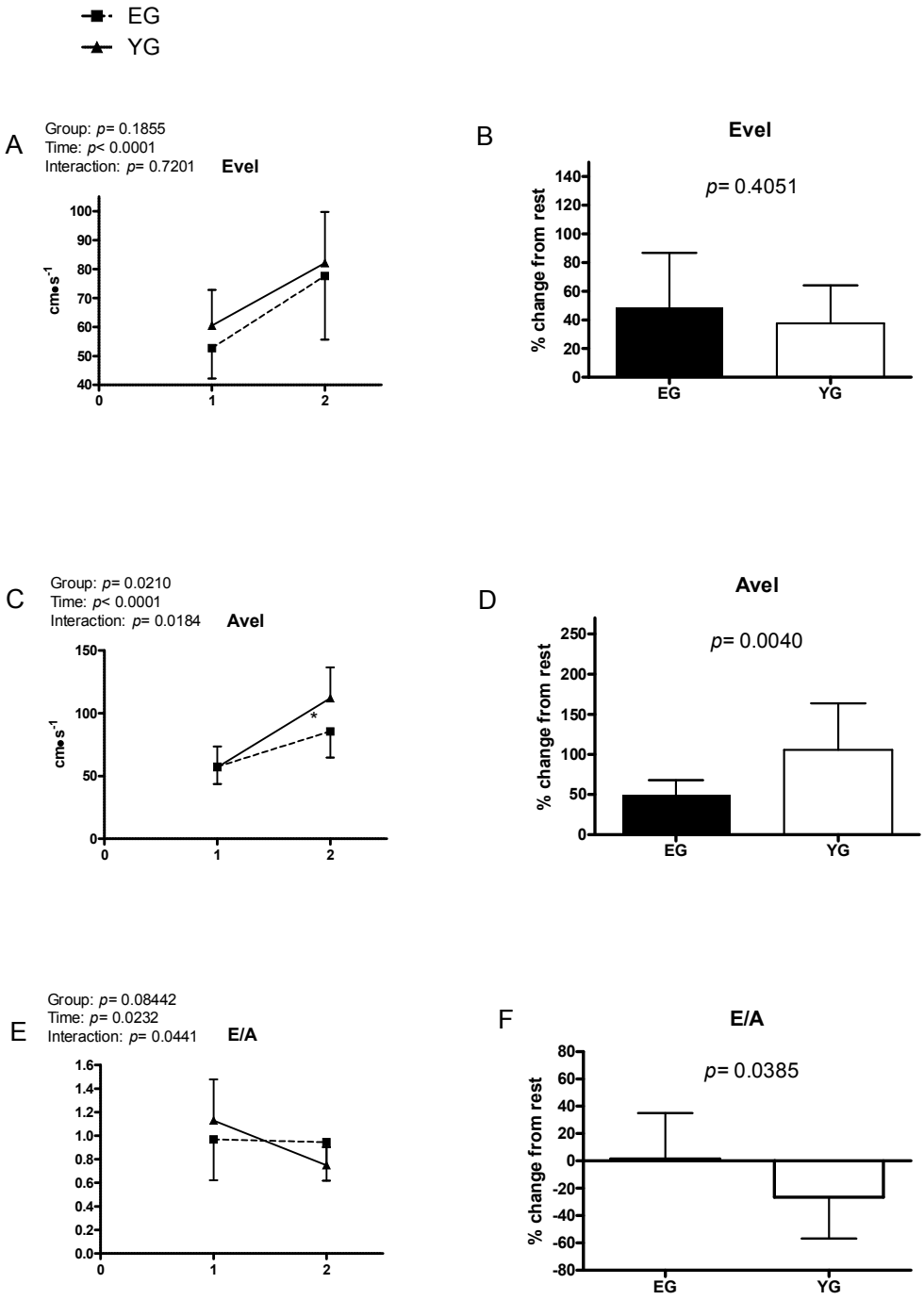


Figure 7. Hemodynamic data at rest and during exercise conducted at the gas exchange threshold intensity in the elderly (EG, n=11) and in young (EG, n= 13) groups. Panels A, C, E, G, and I show absolute values; panels B, D, F, H, and L show % variation from rest level. Em= mitral valve motion velocity during early transmitral filling; Am= mitral valve motion velocity during atrial contraction; Sm= systolic myocardial tissue velocity at mitral anulus. Values are mean ± SD. *= p<0.05 between groups at the same timepoint.

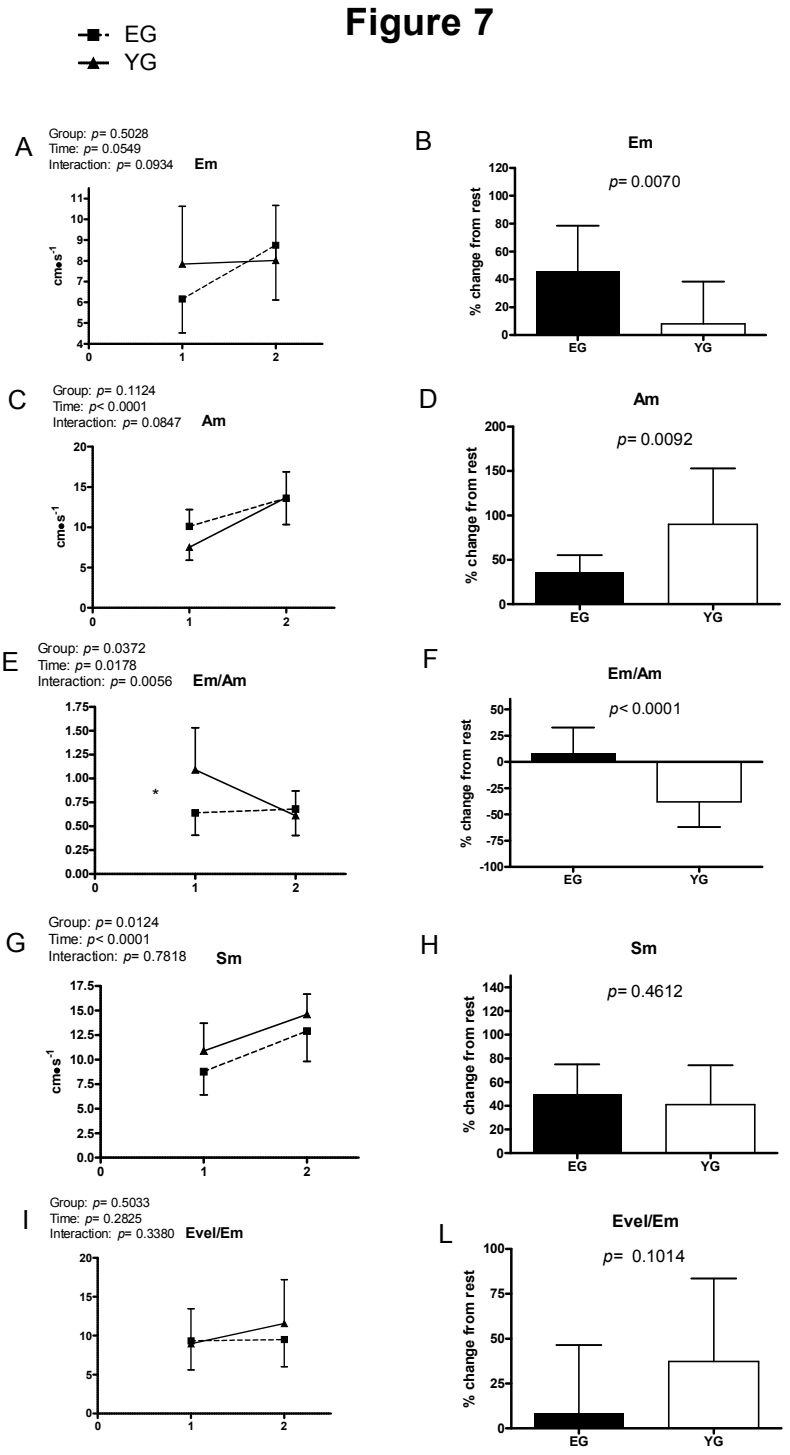


Table 2. Mean values ± SD of metabolic data at the anaerobic threshold (AT) and at maximum workload (W_{max}) collected during the cardiopulmonary test of the study 2. N= 12.

	AT	W_{max}
Workload (W)	165.80 ± 31.68	244.27 ± 38.40
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	25.61 ± 3.18	38.45 ± 4.10
$\dot{V}O_2$ ($ml \cdot min^{-1}$)	2008 ± 350	2780 ± 528
$\dot{V}CO_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	1771 ± 440	3920 ± 688
RER	1.08 ± 0.10	1.41 ± 0.05
VE ($l \cdot min^{-1}$)	42.75 ± 10.91	98.34 ± 18.06
HR (bpm)	146.15 ± 8.65	182.50 ± 10.50

Table 3. Hemodynamic values during the third minute of rest the test in normoxia (NORMO) and in hypoxia with FiO_2 at 13.5% (HYPO) of the study 2. N= 12.

	NORMO	HYPO	*p* value
SO_2 (%)	97.33 ± 1.36	98.04 ± 1.25	0.1840
HR (bpm)	88.92 ± 14.77	80.58 ± 9.98	0.0855
MAP (mmHg)	89.75 ± 4.55	85.83 ± 4.91	0.2106
PEP (ms)	137.90 ± 19.27	135.50 ± 24.16	0.7176
VET (ms)	238.02 ± 25.17	237.85 ± 38.04	0.9876
DT (ms)	261.53 ± 55.57	347.99 ± 82.60	0.0148
ESV (ml)	37.66 ± 12.83	39.27 ± 15.88	0.9828
EDV (ml)	120.81 ± 17.89	120.19± 21.82	0.9135
EF (%)	68.87 ± 9.14	67.85 ± 9.06	0.5927
SV (ml)	83.13 ± 15.08	80.91 ± 13.85	0.5244
Evel ($cm \cdot s^{-1}$)	54.47 ± 10.69	62.81 ± 14.54	0.0325
Avel ($cm \cdot s^{-1}$)	62.05 ± 13.41	50.53± 7.87	0.0036
E/A	0.91 ± 0.23	1.28 ± 0.38	0.0014
Em ($cm \cdot s^{-1}$)	8.44 ± 2.36	9.14 ± 3.25	0.5695
Am ($cm \cdot s^{-1}$)	8.22 ± 2.33	7.06 ± 1.46	0.1959
Em/Am	1.09 ± 0.40	1.37 ± 0.54	0.0767
Sm ($cm \cdot s^{-1}$)	10.19 ± 2.07	9.86 ± 1.52	0.6189
Evel/Em	6.82 ± 1.60	7.48 ± 2.15	0.3926

Figure 8. Scatter plot graphs of levels of blood O2saturation (SO2, panel A), heart rate (HR, panel B), mean arterial pressure (MAP, panel C), pre-ejection period (PEP, panel D), ventricular ejection time (VET, panel E), and diastolic time (DT, panel F) during the recovery from sessions of exercise in normoxia (NORMO) and in normobaric hypoxia with a FiO2of 13.5% (HYPO). N = 12. *p < 0.05 vs. NORMO test.

Figure 8

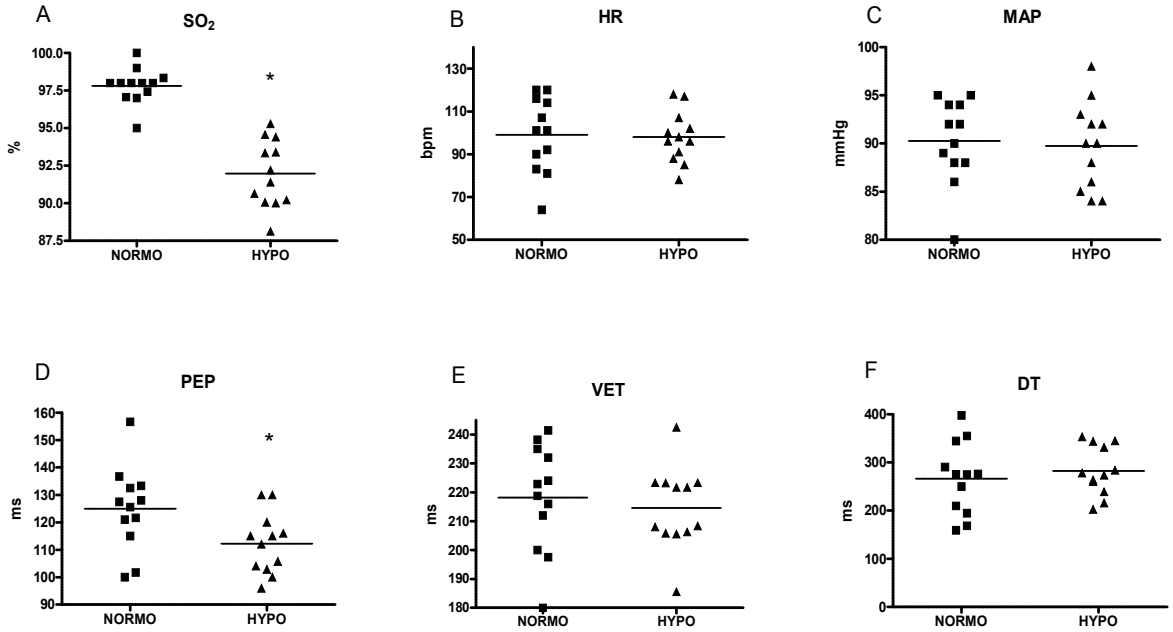


Figure 9. Scatter plot graphs of levels of end-systolic volume (ESV, panel A), end-diastolic volume (EDV, panel B), ejection fraction (EF, panel C), and stroke volume (SV, panel D) during the recovery from sessions of exercise in normoxia (NORMO) and in normobaric hypoxia with a FiO2of 13.5% (HYPO). N = 12.

Figure 9

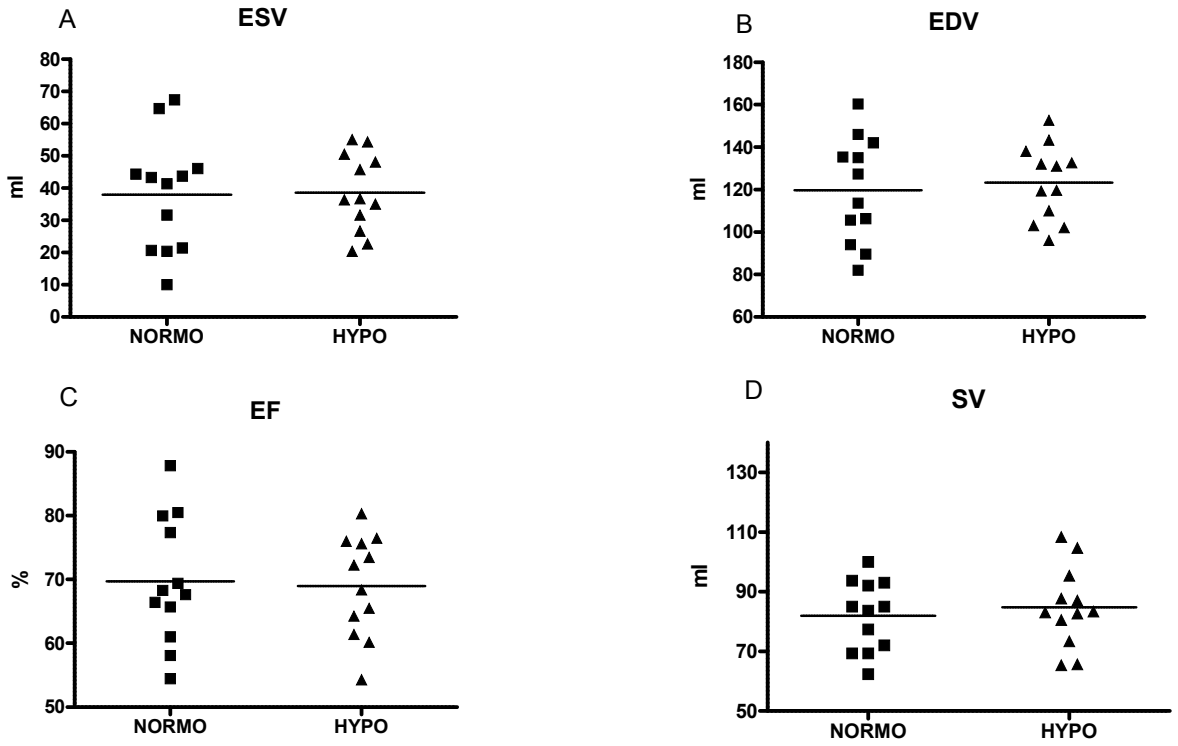


Figure 10. Scatter plot graphs of levels of early transmitral filling peak velocity (Evel, panel A), late transmitral filling peak velocity (Avel, panel B), their ratio (E/A, panel C), and early diastolic mitral valve motion velocity (Em, panel D) during the recovery from sessions of exercise in normoxia (NORMO) and in normobaric hypoxia with a FiO2of 13.5% (HYPO). N = 12. *p < 0.05 vs. NORMO test

Figure 10

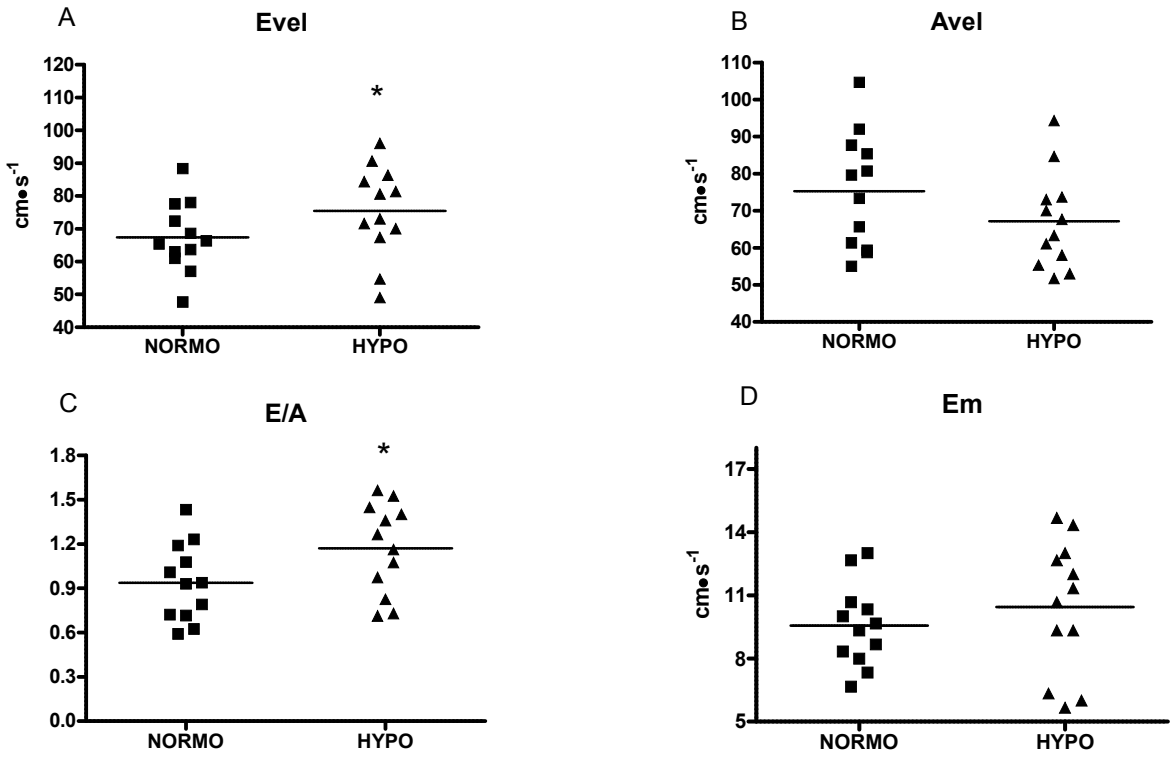
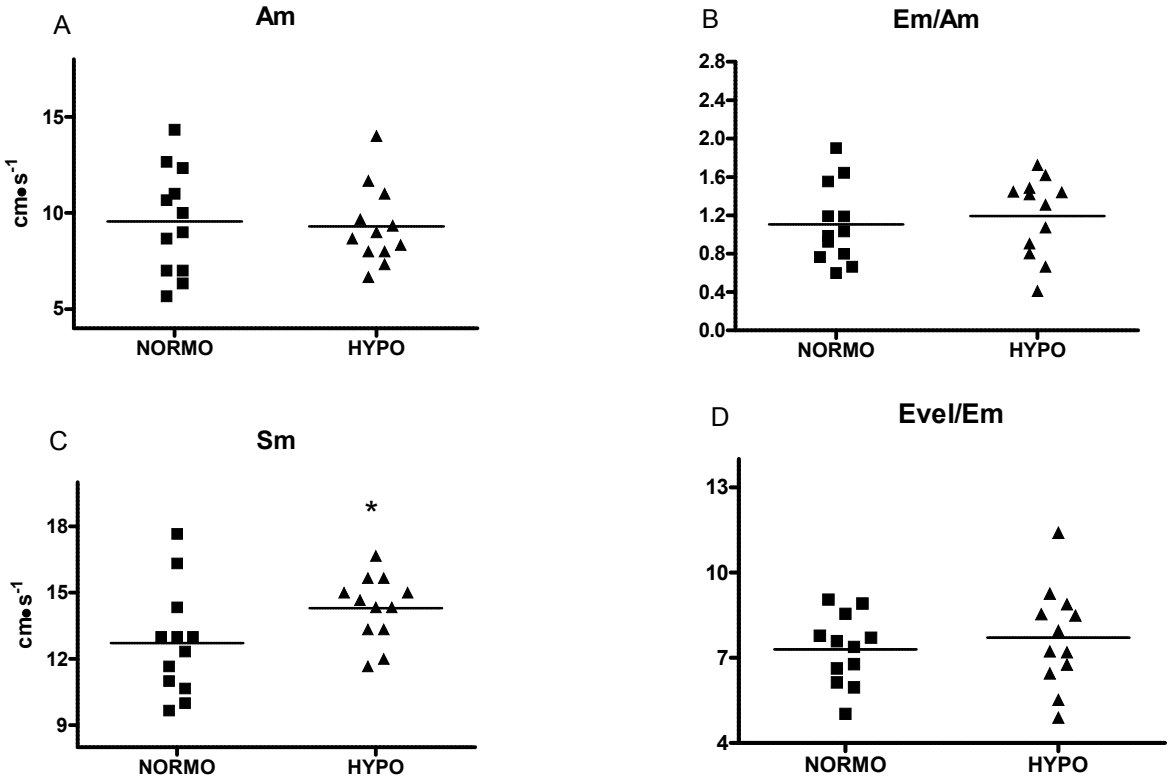


Figure 11. Scatter plot graphs of levels of late diastolic mitral valve motion velocity (Am, panel A), ratio between early and late diastolic mitral valve motion velocities (Em/Am, panel B), systolic myocardial velocity (Sm, panel C), and ratio between early transmitral filling peak and early mitral valve diastolic velocities (Evel/Em, panel D) during the recovery from sessions of exercise in normoxia (NORMO) and in normobaric hypoxia with a FiO2of 13.5% (HYPO). N = 12. *p < 0.05 vs. NORMO test

Figure 11



List of acronyms

AT Anaerobic Threshold

AV Atrio-ventricular

BMI Body Mass Index

BP Blood pressure

CO Cardiac Output

CPET Cardiopulmonary Test

DBP Diastolic blood pressure

DT Deceleration time

DT Diastolic time

EDV End Diastolic Volume

ESV End Systolic Volume

EF Ejection Fraction

EG Elderly group

GET Gas Exchange Threshold

HRmax Maximum heart rate

HR Heart Rate

LA Left Atrium

LV Left Ventricular

MAP Mean Blood Pressure

PEP Pre ejection period

PW Pulsed Wave

PWD Power Doppler Wave

SBP Systolic blood pressure

SD Standard Deviation

SO_2 Peripheral Blood O_2 Saturation

SVR Systemic Vascular Resistance

SV Stroke Volume

TDI Tissue Doppler Imaging

VCO_2 Carbon Dioxide Production

VET Left ejection time

VER Mean systolic ejection rate

VFR Ventricular filling rate

VO_2 Oxygen Uptake

VO2max Maximal oxygen uptake

Wmax Maximum workload

YG Young group

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