UNIVERSITA' DEGLI STUDI DI VERONA

DEPARTMENT OF
Neurosciences, Biomedicine and Movement Sciences

GRADUATE SCHOOL OF
Translational Biomedical Sciences

DOCTORAL PROGRAM IN
Exercise Science and Human Movement

WITH THE FINANCIAL CONTRIBUTION OF
Università degli Studi di Brescia and ASST Spedali Civili di Brescia

Cycle / year: XXVIII / 2013

TITLE OF THE DOCTORAL THESIS

AUTONOMIC OUTPUT IN HEALTH AND DISEASE: CLOSED-LOOP DYNAMICS OF BAROREFLEX CHANGES

S.S.D. BIO/09 and M-EDF/02

Coordinator: Prof.ssa PAOLA ZAMPARO

Tutor: Prof. GUIDO FERRETTI

Doctoral Student: Dott. NAZZARENO FAGONI

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Autonomic output in health and disease: closed-loop dynamics of baroreflex changes

Nazzareno Fagoni

Doctoral Thesis

Verona, 11th May 2017

A Laura

Edoardo e Beatrice

L'amore, la vita, il sorriso

Acknowledgments

First, I HAVE to thank my family: Francesco, Mariangela, Franco e Lucia for their daily support. Hopefully I can reward their efforts!

I would like to express my sincere gratitude to Guido Ferretti, for his continuous support, for his patience, and immense knowledge. Since 2001 his guidance helped me: he has been my mentor for years.

Besides my advisor, I would like to thank Nicola Latronico for his insightful comments and encouragement, for his essential support I got during these years, in a stimulating work place.

My sincere thanks also go to Carsten Lundby, who provided me an opportunity to join his team in Zurich in 2016.

I am grateful to Frank Rasulo, Simone Piva, Massimiliano Gobbo, Alessandro Fanzani, Christian Moia, Aurélien Bringard, Timothée Matthieu Fontolliet, Alessandra Adami: without their precious support, it would not be possible to conduct these researches, in this field and in intensive care unit.

I thank my fellow lab for the stimulating discussions, for the intriguing debates we had together before deadlines, and for all the fun we have had in the last years: Anna Taboni, Giovanni Vinetti, Stefano Camelio, Andrea Sivieri.

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ABSTRACT

Introduction

Sympathetic–parasympathetic interaction plays a critical role in the evolution and outcome of many cardiovascular disorders. It is well established that the sympathetic activation has an arrhythmogenic potential, contrariwise the vagal activation has an opposite effect. These findings are summarised in the generic concept of the "autonomic balance", which generate the common perception that the loss of autonomic balance is a potentially proarrhythmic condition, and therapeutic strategies that aim at modulating the autonomic nervous system might increase the cardiac electrical stability. Several tools have been proposed to investigate the activity of the autonomic nervous system, and the analysis of the arterial baroreflex is considered an indirect measure of the cardiac vagal activity. In fact the spontaneous baroreflex sensitivity (BRS) is viewed as an index of the rise in the cardiac vagal efferent activity in response to an increase in arterial blood pressure. BRS has been assessed in a variety of conditions and with a variety of experimental techniques, focusing mostly on the cardiac-chronotropic efferent branch. Healthy subjects and several cardiovascular diseases have been extensively investigated by the analysis of baroreflexes with either a closed-loop and/or an open-loop approach. The latter allows computation of the characteristic parameters of the baroreflex curve, i.e. the centring point, the operating point, and the maximal gain. This approach can be applied only in steady state conditions, at rest and during exercise, since it make use of external factors (mechanical or pharmacological) to modify the operating range and to construct the responding range, in terms of heart rate (HR) or arterial blood pressure (BP) responses. Contrariwise, the closed-loop approach analyses the relationship between HR and BP to define the sensitivity of the baroreflex close to the operating point, which could be displaced toward the "threshold" of the baroreflex curves in some conditions, i.e. during exercise. In closed-loop condition, Bertinieri and colleagues (1988) proposed the so-called *sequence method* which they applied in steady state condition. In practice, they computed the mean slope of several BRS sequences, of at least three beats, in which the R-R interval (RRi) of the ECG varied consensually to BP, regardless of the direction. Recently, this method was applied also in unsteady state conditions (Adami et al., 2013, Bringard et al., 2017; Fagoni et al., 2015; Sivieri et al. 2015); the only a-priori assumption behind the sequence method is that each heart beat has a biunivocal effect on the following beat: no upper limit was imposed to the length of baroreflex sequences (minimum

three beats). Moreover, the BRS analysis was applied to estimate the prognosis in patients affected by cardiovascular diseases (Head, 1995; Korner et al., 1974; La Rovere et al., 1998, 2008, 2011). Autonomic output is different in health and disease and the BRS can be used to analyse these differences in several conditions. Thus, the purpose of this project was to perform a closed-loop baroreflex analysis, under different dynamic conditions (rest, exercise, apnoeas), in healthy subjects and in patients affected by mild arterial hypertension. The closed-loop approach was used to this aim, in order to deeply investigate the dynamics of the arterial baroreflex in the following unsteady state conditions: i) at exercise onset and ii) during apnoeas, in healthy volunteers; iii) during exercise, comparing healthy subjects and hypertensive patients. Commonly, the sequence method is computed starting from the R-R interval (RRi) of the ECG, and the systolic blood pressure (SAP). In literature, both HR and RRi are used to calculate BRS, even though RRi is the reciprocal of HR, and these two parameters provided two different information. To clarify this challenging point, a further detailed paper will be proposed to discuss this topic. In this thesis, we decided to use the relationship between HR and MAP to compute BRS. While HR has been an *a-priori* choice, the use of MAP was a consequence of the typology of experiments we carried out. The beginning of physical activity is accomplished by the sudden change in the total peripheral resistances (TPR), which predominantly acts on DAP; this modification affects more MAP than SAP, thus the former parameter was chosen to define the BRS.

First study: baroreflex at exercise onset

This first experiment analysed the dynamics of baroreflex resetting at exercise onset. Baroreflex resetting is generally studied at steady state, by means of open-loop procedures, and it was demonstrated that during exercise the operating point is displaced upward and rightward with respect to rest, and its maximal gain is invariant (Rowell et al. 1996; DiCarlo and Bishop 2001; Raven et al. 2002; Raven et al. 2006; Raven 2008; Fadel and Raven 2012; Mitchell 2013). Notwithstanding, the dynamics of baroreflex displacement from rest to exercise was never described so far. We aimed at investigating the temporal components of the mechanisms that intervene in determining baroreflex resetting during transient. Ten healthy volunteers took parts in the experiments. They performed three repetition of a 50 W exercise on a cycle ergometer, lasting seven minutes, in supine and upright position; the different posture was used to have an *a-priori* displacement the BRS operating point (Schwartz et al., 2013) even at rest. HR was

continuously recorded, on single beat basis, by electrocardiography. Arterial pressure was continuously recorded by a non-invasive finger pressure cuff. From pulse pressure profiles, we determined cardiac output (CO) by Modelflow, and we computed MAP; TPR was derived as the ratio between the former two parameters. We performed the closed-loop analysis of HR vs MAP relationship at rest before starting the exercise (BRS computed as the average of the mean slopes of all analysed sequences of each single subject, over one minute), during the transient (HR vs MAP relationship), and during exercise (BRS over one minute steady state recording). At exercise onset, HR was higher than in quiet rest. As exercise started, MAP fell to a minimum (MAPmin) of about 73 mmHg in both posture, while HR increased. The initial HR versus MAP relationship was linear, with flatter slope than resting baroreflex sensitivity, in both postures. TPR fell and CO increased. After MAPmin, both HR and MAP increased toward exercise steady state, with further CO increase. The sensitivity of baroreflex during steady state at exercise resulted lower than at rest, in both posture, and invariant compared to the beginning of exercise. These results suggest that, at exercise onset, the falling of MAP was corrected by a HR reduction along a baroreflex curve; the sensitivity of the baroreflex changed immediately during the transient, with lower sensitivity than at rest, and then BRS remained unchanged during the exercise steady state. After reaching MAPmin, the baroreflex resetting took place, yet with a delay after the beginning of exercise. Thus, the baroreflex resetting starts after the exercise onset, but the sensitivity of the baroreflex changes immediately, and this process is compatible with the central command hypothesis. However, the central command theory may not explain the resetting process, that lasted one minute upright, but not supine (it took more time), compatibly with a possible role of increasing sympathetic stimulation of the sinus node during exercise (Fagraeus and Linnarsson, 1976; Orizio et al., 1988).

Second study: baroreflex in apnoea.

The cardiovascular response to apnoea is characterised by three phases (Fagoni et al., 2015, 2017; Perini et al., 2008; Sivieri et al., 2015). The first dynamic phase (φ1) of the cardiovascular response to apnoea is characterised by a sudden drop in MAP, accompanied by an increase HR (Costalat et al, 2015; Fagoni et al., 2015; Perini et al, 2008, 2010; Sivieri et al., 2015). It was interpreted as a baroreflex attempt at correcting a MAP fall due to a reduction in venous return caused by an increase in intrathoracic pressure occurring at elevated lung volumes. The purpose was to perform the analysis of the HR vs MAP relationship during the φ1 of apnoeas performed

at lung volumes close to the total lung capacity, at rest and during exercise. Indeed, during exercise apnoeas, the characteristics of φ1 would be different than in resting apnoeas, because the BRS slope at exercise is lower than at rest, and the operating point of the baroreflex should be displaced. We calculated BRS in steady state condition before apnoeas, during phase II (φ2), and we analysed the HR vs MAP relationship during φ1, before and after attainment MAPmin, in resting and exercise apnoeas. Ten healthy divers performed resting and exercise (30 W) apnoeas. HR and MAP were recorded on a beat-by-beat basis by means of an electrocardiography and the Portapres®, respectively. The resulting slopes of the linear regression line of the HR versus MAP relationship, at rest, during steady φ2, before and after the attainment of MAPmin, were computed in both conditions. We also analysed the modification of the prevailing HR and MAP from the first part of φ1, before the MAPmin, and after MAPmin, to investigate if baroreflex resetting took place after attainment of MAPmin. Before the beginning of apnoeas, BRS was lower ($p<0.05$) during exercise than in resting apnoeas (-1.23 ± 0.23 and -0.87 ± 0.21 b min^{-1} $mmHg^{-1}$, respectively). This difference was also reported for the HR vs MAP relationship in all the investigated conditions. In either resting and exercise apnoeas, slopes were lower at the beginning of φ1 (-0.49 ± 0.20 and -0.31 ± 0.08 b min^{-1} $mmHg^{-1}$, resting and exercise, respectively), compared to rest, φ2 (-1.12 ± 0.33 and -0.82 ±0.27 b min^{-1} $mmHg^{-1}$, resting and exercise, respectively) and after MAPmin (-0.96 ± 0.24 and -0.70± 0.31 b min^{-1} $mmHg^{-1}$, resting and exercise, respectively). The prevailing HR and MAP at the beginning of apnoeas resulted different compared to after attainment of MAPmin, then both HR and MAP increased consensually to attain new levels: whereas at rest both HR and MAP increased, during exercise MAP was displaced upward and rightward, whilst the HR remained unchanged. The novelty of this study is that during the dynamic phase of apnoeas, the HR vs MAP relationship showed a baroreflex dynamic characterized by a sudden modification in the sensitivity compared to rest and to the steady phase II. After the attainment of MAPmin, a parallel rise in HR and MAP took place, which we interpreted as due to baroreflex resetting. Indeed, the prevailing HR and MAP resulted shifted upward and rightward during exercise compared to rest. During exercise, this process caused an increase in MAP after MAPmin, compared to before MAPmin, with an invariant HR: the prevailing sympathetic output during exercise might affects much more the vasomotor component of the cardiovascular responses compared to the cardiac one, resulting in higher TPR and lower HR values (Fagoni et al., 2015; Sivieri et al., 2015)

Third study: baroreflex in hypertensive patients.

The BRS in hypertensive patients is impaired (Bristow et al., 1969; Head, 1995; Korner et al., 1974; Mancia et al., 1978), and the modification in BRS is associated with worst outcome in cardiovascular patients (La Rovere et al., 1998, 2008, 2011; Osculati et al., 1990). Studies concerning the implantation of continuous baroreflex stimulators as a tool to diminish central sympathetic outflow (Mohaupt et al., 2007) and the introduction of catheter-based renal selective denervation for resistant hypertension show a significantly reduction in blood pressure (DiBona and Esler, 2010; Esler, 2011; Schlaich et al., 2009). These data suggest that the overall cardiovascular regulation in hypertensive patients may be different from normal, and the analysis of the dynamics of the baroreflex response to exercise might be different from healthy subjects. We aimed at investigating the steady-state and the dynamics of the HR vs MAP relationship in response to exercise in patients affected by essential hypertension compared to age-matched healthy controls, carried out in supine and upright postures, at two different workloads, 50 and 75W.

Ten patients affected by grade I or II of arterial hypertension were age-matched with ten healthy controls. HR and MAP were recorded on a beat-by-beat basis by means of an electrocardiography and the Portapres®, respectively. The resulting slopes of the linear regression line of the HR versus MAP relationship, at rest, during the transient and at steady state during exercise, were computed in supine and upright position. Data were compared between patients and healthy volunteers, between positions, and among the different phases before and during exercises. BRS resulted steeper in controls than in hypertensive patients (supine -1.43 ± 0.19 and -1.16 ± 0.33 b min^{-1} $mmHg^{-1}$ for controls and hypertensive patients, respectively; upright -1.22 ± 0.2 and -0.99 ± 0.19 b min^{-1} $mmHg^{-1}$ for controls and hypertensive patients, respectively), as well as the linear relationship between HR and MAP at the beginning of exercise at 50 W, in both positions, resulted higher in controls than in patients. In supine position controls showed higher slopes at rest than at the beginning and during exercise. In controls and hypertensive patients, at the beginning of exercise at 75 W the slopes were lower in upright than supine.

These data showed a trend characterised by a reduced baroreflex sensitivity in all conditions with sympathetic hyperactivity: hypertension versus control, exercise versus rest, and upright versus supine. Moreover, several slopes resulted lower at the beginning of exercise and during steady state exercise compare to rest, confirming previous findings. It is noteworthy that during

the transient at 75 W the baroreflex response was absent in several patients in supine position, probably due to sympathetic overactivity which limited the MAP fall demonstrated at the exercise onset because of the sudden drastic fall in TPR (Elstad et al., 2009; Faisal et al., 2010; Lador et al., 2006, 2008; Wieling et al., 1996).

Conclusion

The analysis of the relationship between HR and MAP by means of the closed-loop approach is a non-invasive method that can be easily applied in health and disease, and it can be used as an indirect measure of the autonomic nervous system activity. The reported results on the patterns of baroreflex changes in dynamic states suggested that the baroreflex resetting started after the beginning of exercise, but the modification of the sensitivity was almost immediate, as soon as the MAP fell and the baroreflex activity tried to counteract by increasing the HR. After the attainment of the MAPmin, which might be considered a trigger MAP value, the resetting phase took place. The change in slope at exercise onset might be attributed to the sudden vagal withdrawal, and compatibly more with the central command theory. Contrariwise, the resetting process may well be mediated by other neural mechanisms (Raven et al., 2006), and it is possible that the activation of the sympathetic efferent branch of the autonomic nervous system plays a role in the phase of the exercise transient after attainment MAPmin (Lador et al., 2006).

At the same time, apnoea provided interesting information about the baroreflex function, since the first phase is characterized by dynamic and deep modifications in MAP, sustained for several beats, counteracted by adjustments in HR. In exercise apnoeas BRS was lower than resting apnoeas, in all the investigated conditions. In φ1, rapid cardiovascular adjustments affect the baroreflex responses with different pattern before and after MAPmin, showing higher values of the HR vs MAP slopes after the attainment of MAPmin compared to the onset of φ1. The baroreflex sensitivity restored immediately after reaching the MAPmin in φ1, indeed BRS in φ2 was similar to the one computed at the beginning of apnoea. Finally, the prevailing HR and MAP points during exercise apnoeas were displaced rightward and upward compared to resting apnoeas. During φ2, HR decreased, and the TPR increased, thus a modification in the autonomic output can occur, with a dissociation between heart (characterised by predominant vagal activity) and vascular system (with predominant sympathetic activity), that may explain why these modifications did not affect the baroreflex sensitivity during φ2 apnoeas.

In the hypertension study, patients presented a reduced baroreflex gain, in agreement with previous findings (Bristow et al., 1969; Head, 1995; Korner et al., 1974; Mancia et al., 1978). The baroreflex sensitivity, in healthy and hypertensive subjects, changed immediately at the exercise onset, in both positions, and remained unchanged during the steady state of light-mild exercises: the baroreflex resetting acted in the same manner in healthy and hypertensive patients, but with a reduced gain in the latter compared to the former.

The closed-loop approach allows the analysis of the BRS in several conditions, such as rest, exercise, apnoea and in pathologies (hypertension, orthostatic intolerance, dysautonomic diseases). BRS could be a useful tool, i.e. to assess improvements after rehabilitation in neurological as well as in cardiorespiratory diseases, or after prolonged bed rest, in healthy volunteers and in patients after prolonged hospital stay. The application of this technique might be used to monitor the efficacy of the undertaken treatment, whether behavioural or pharmacological. Thus, the modification in BRS might be considered as a mirror of cardiovascular adjustments following a different stimulation of the two branches of the autonomic nervous system, in health and disease.

INTRODUCTION

Baroreflexes are considered as the main short-term control system of arterial blood pressure. Since their first description (Marey, 1863), baroreflexes had been widely investigated under a variety of conditions and with a variety of experimental techniques. Of the three effector functions of the arterial baroreflex system, cardiac – chronotropic, cardiac – inotropic, and vascular, only the first, and most easily accessible, has been extensively studied, whether with open-loop or with closed-loop methods. Most of the studies on baroreflexes are steady state studies, in which the carotid distending pressure is modified as the independent variable either by neck suction/pressure procedures or by administration of hypotensive (e.g. sodium nitroprusside) and hypertensive (e.g. phenylephrine) drugs at increasing doses, and the ensuing heart rate or blood pressure responses are looked at as the dependent variables (open-loop procedures) (Eckberg and Sleight, 1992). This has led to construct the classical arterial baroreflex curve (Figure A), which is often treated with a logistic model (Kent et al., 1972; Potts et al., 1993).

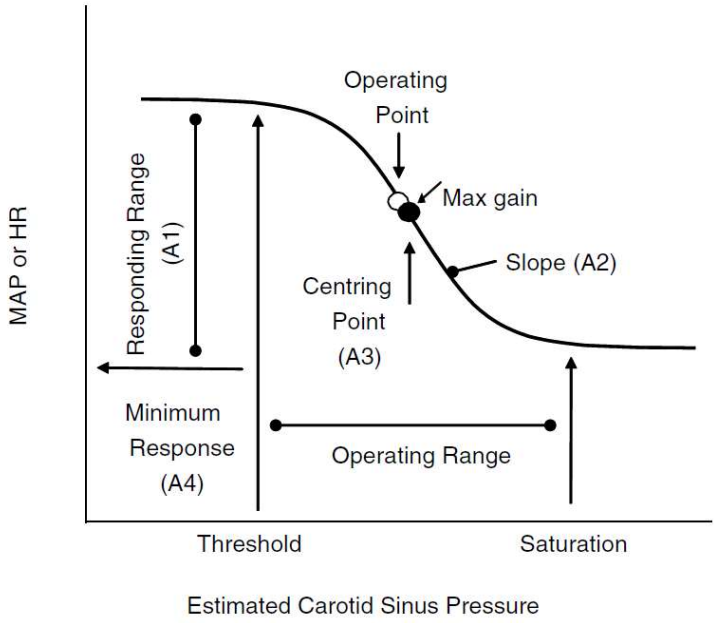

Figure A Schematic representation of the carotid baroreflex function curve and its operational parameters (Raven et al., 2006)

This model identified several parameters describing the baroreflex response: i) the centring point, which is the middle point of a baroreflex curve; ii) the operating point, defined as the prevailing mean arterial pressure before the introduction of an acute stimulus; and iii) the maximal baroreflex gain, defined as the gain value at the centring point of a baroreflex curve (Raven et al., 2006).

Open-loop studies have provided a remarkable body of knowledge on arterial baroreflex responses. In the context of this thesis, open-loop experiments have shown that the arterial baroreflex function, as described by Kent's model, is displaced at exercise (Potts et al., 1993; Papelier et al., 1994) and in patients affected by hypertension (Heusser et al., 2010; Mancia et al., 1978; Sleight et al., 1975), generally without changes in maximal baroreflex gain. By this, the authors of those studies mean that the entire heart rate versus arterial blood pressure function is displaced upward and rightward with respect to its position at rest, without changes in maximal baroreflex gain. It was later demonstrated that the "reset" baroreflex function curve is maintained up to exercise intensities corresponding to the maximum (Norton et al., 1999). The degree of baroreflex resetting, i.e. the size of the displacement of the baroreflex function curve (Figure B), is greater the higher is the exercise intensity (Fadel et al., 2001; Norton et al., 1999; Ogoh et al., 2005; Potts et al., 1993; Raven et al., 2006), and greater the higher the severity of hypertension (figure 3.1, Head, 1995; Korner et al., 1974).

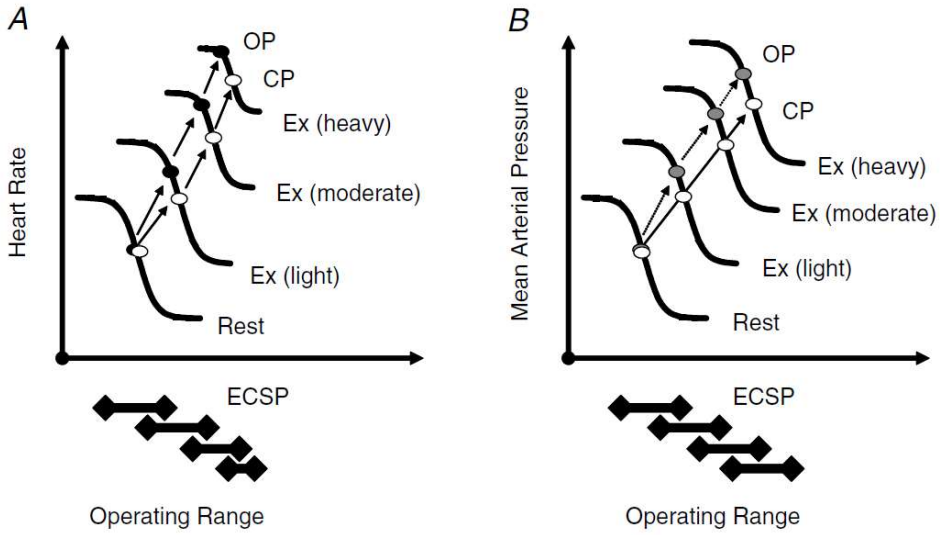

Figure B Representation of the carotid–cardiac (Panel A) and carotid–vasomotor (Panel B) resetting that occurs from rest to heavy exercise.

Moreover, it was observed that the baroreflex operating point moves along the baroreflex curve far from the centring point, toward the reflex threshold, implying that the reflex operates at a lower pressure and with a smaller gain (Raven et al., 2006).

Rowell and O'Leary (1990) formulated a mixed working hypothesis of baroreflex resetting during exercise, which has not been refuted by open-loop studies at steady state. According to these authors, two independent mechanisms may be involved in baroreflex resetting: a generic central command mechanism, and a peripheral muscle metaboreflex. Based on Kent's model, they suggested that central command is responsible for displacing the initial resting operating point to higher levels of arterial pressures, whence the horizontal shift of the baroreflex function curve, whereas the activation of the muscle metaboreflex increases heart rate, determining the vertical shift of the baroreflex function curve. As a consequence, the concerted actions of central command and muscle metaboreflex would generate the rightward and upward displacement of the baroreflex curve during exercise. This hypothesis underwent some changes in its formulation, with accent put more on one or on the other of these mechanisms (see e.g. Raven et al., 1997, 2006; Gallagher et al., 2001a; Gallagher et al., 2006; McIlveen et al., 2001; Mitchell, 2013; Ogoh et al., 2002; Querry et al., 2001; Smith et al., 2003), but the debate on baroreflex control during exercise is still centred around the concepts of central command and metaboreflex. These mechanisms were embedded in a comprehensive neurological theory of baroreflex resetting at exercise (Degtyarenko and Kaufman, 2006; Tsuchimochi et al., 2009).

An understanding of the possible interrelationship between these two hypothetical mechanisms requires a study of the kinetics of displacement of the baroreflex curve, or at least of its operating point, which was never carried out so far. However, the open-loop approach implies the introduction of an external perturbation that alters a stable equilibrium between heart rate and blood pressure. Therefore, it requires a strict steady state condition. This prevents from any application of open-loop methods to the study of dynamic states.

Contrariwise, closed-loop methods rely on a continuous beat-by-beat follow-up of the heart rate and blood pressure changes, thereby assuming the possibility of a counter-effect of the former on the latter. The most classical closed-loop method is the sequence method (Bertinieri et al., 1988), which was developed after prolonged steady state recordings. Application of the sequence method requires identification of sequences of at least three consecutive beats characterised by consensual variations of the RR-interval (RRi, the reciprocal of heart rate) and of blood pressure (opposite variations of heart rate and blood pressure). Within each sequence,

the RRi versus pressure relationship is treated as linear and the resulting slope is taken as representative of the spontaneous baroreflex sensitivity (BRS) around the baroreflex operating point.

The sequence method was applied to the study of BRS at rest and during exercise steady state, essentially in the context of heart rate (HR) and blood pressure (BP) variability studies (Hartwich et al., 2011; Kardos et al., 2001; La Rovere et al., 2008; Parati et al., 2001; Vallais et al., 2009). Notwithstanding, nothing prevents from using a closed-loop approach to the analysis of unsteady state conditions. Steady state is a prerequisite of HR variability studies (Malliani et al., 1986; Task Force, 1996), but it is not mentioned as a prerequisite of the sequence method for the computation of BRS (Bertinieri et al., 1988). The only *a-priori* assumption behind the sequence method, as nicely pointed out by Bertinieri et al. (1988), is that each heart beat has a biunivocal effect on the following beat. Bertinieri et al. (1988) did not impose any limit to the length of baroreflex sequences. In fact, admitting the possibility of a counter-effect of HR on BP in closed-loop analysis of arterial baroreflexes is tantamount to acknowledging the possibility of continuous control loops on single beat basis. On this basis, a closed-loop analysis of baroreflex dynamics in unsteady state, through the beat-by-beat analysis of the relationship between HR or RRi and BP (either SAP or MAP), was carried out during the re-ambulation procedure at the end of prolonged bed rest (Adami et al., 2013) and during the initial phase of dry apnoeas at rest and exercise in air (Sivieri et al., 2015) and in oxygen (Fagoni et al., 2015). This approach was never applied to the study of exercise transients yet, although we believe it could provide remarkable pertinent information on the dynamics of baroreflex resetting.

This sophisticated mechanism is essential to maintain the cardiovascular homeostasis of the system; its impairment may play an adverse role in several diseases (Bristow et al., 1969; Mancia et al., 1978, Mancia and Mark, 2011; Osculati et al., 1990); indeed, BRS was found to be reduced in patients affected by cardiovascular diseases, mostly in patients presenting hypertension or heart failure (Head, 1995; Korner et al., 1974; La Rovere et al., 1998, 2008, 2011). Autonomic activity in health and disease resulted different, and the analysis of BRS can be considered as a mirror of this fine and precise regulation. BRS is also reduced during prolonged bed rest (Ferretti et al., 2009).

The purpose of this project was to perform series of spontaneous baroreflex sensitivity analysis, in unsteady state conditions, by the closed-loop approach, to shed light on the possible

mechanisms involved in the dynamics of baroreflex resetting. Three different experimental conditions were investigated to this aim. First, a beat-by-beat analysis of the baroreflex resetting at exercise onset in healthy humans, to have an insight of the mechanisms which take place during the transient at exercise onset. The second investigation concerns the analysis of BRS during apnoeas, carried out in resting conditions and during exercise. The last study would deepen the BRS analysis during rest, exercise, and exercise transient in hypertensive patients. Before starting the presentations of the experiments, it is useful to provide a brief description of the data treatments carried out in this project.

Methodological considerations: the use of the systolic arterial pressure and/or the mean blood pressure to compute the spontaneous baroreflex sensitivity.

Arterial baroreceptors are stretch receptors connected to sensory nerve endings that increase their firing rate during systole (receptor stretching), and diminish it during diastole (receptor relaxation) (Figure C). They respond to pressure-induced deformation of the vessels' walls; indeed, direct application of catecholamines to the carotid sinus, or replacement of elastin with fibrin and collagen in the aortic wall (i.e with ageing) reduce their elongation and make them unable to sense changes in blood pressure (Heymans and Heuvel-Heymans, 1951; Victor, 2015).

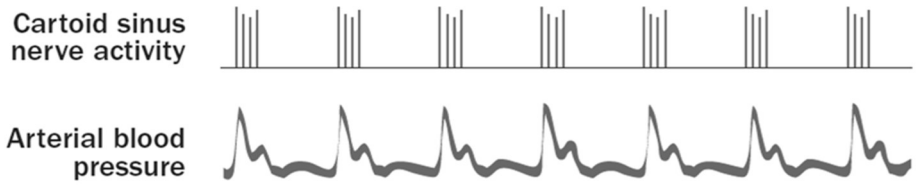

Figure C Schematic representation of single-fibre baroreceptor discharge in the carotid sinus nerve in relation to arterial blood pressure. (Bronk and Stella, 1935; Victor, 2015).

In the literature, the BRS analysis was performed using either systolic or mean arterial pressure. Many authors consider SAP as pressure input for baroreflex analysis (i.e.: Akimoto et al., 2011; Bertinieri et al., 1988; Bringard et al., 2017; Hartwich et al., 2011; Iellamo et al., 1997; Kardos et al. 2001; La Rovere et al., 1998, 2011; Parati et al., 2001; Vallais et al., 2009): this allowed identification of a single pressure value, corresponding to the peak pressure value within an arterial pressure cycle, and simultaneous to an R peak in an ECG recording. Contrariwise, MAP

was less used for BRS analysis (Akimoto et al., 2011; Bringard et al., 2017), and often data are presented comparing the analysis of BRS computed with SAP. Akimoto and colleagues (2011) analysed BRS during head up tilt to produce orthostatic stress in healthy subjects and found no differences in using SAP or MAP; contrariwise Bringard et al. (2017) found differences when using MAP in supine position at rest, whilst during exercise BRS computed by MAP or SAP were superimposable. Currently, there are scanty data comparing the BRS computed by means of SAP or MAP, and this argument deserves further investigations.

BRS analysis with SAP certainly has a physiological meaning in a steady state condition at rest, in which the total peripheral resistances (TPR) are high and invariant, and thus the changes in MAP are mostly the result of changes in SAP. However, the baroreceptor stimulation is a continuous phenomenon, which is more precisely represented by MAP than by SAP, since MAP corresponds to the mean baroreceptor stimulation pressure within a heart cycle. Moreover, MAP is more importantly affected than SAP by the changes in TPR occurring at the beginning of an exercise, since TPR acts on DAP, and thus on MAP, without affecting SAP. Notwithstanding, it is also noteworthy that SAP, but not MAP, contains information on dP/dt, which is also sensed by the baroreceptors (Eckberg and Sleight, 1992). These considerations explain why, in the studies described in this thesis, we analysed baroreflexes using MAP as pressure inputs.

Methodological considerations: the use of the R-R interval and/or the heart rate to compute spontaneous baroreflex sensitivity.

Conceptually, the time between two consecutive R peaks on an ECG trace (R-to-R interval, RRi) is the reciprocal of heart rate (HR). Both these variables can be, and indeed are used when arterial baroreflexes are analysed, whether with closed-loop or with open-loop methods. The closed-loop approach is extensively applied by using mostly the RRi versus blood pressure to compute BRS, although some authors proposed to analyse the relationship between HR and blood pressure, instead of RRi: the results did not differ between the two approaches (Akimoto et al., 2011). In fact, the most classical closed-loop method, i.e. the sequence method (Bertinieri et al, 1988), can be represented in both forms. If RRi is used as dependent variable, positive linear relationships between RRi and arterial pressure are described; *vice versa* negative linear relationships between HR and blood pressure are reported. The slope of these lines is taken as the BRS around the baroreflex operating point. On the other side, open-loop methods provide

the classical saturating negative HR versus pressure relationship (usually the mean arterial pressure, or the carotid distending pressure), which Potts et al. (1993) treated with a logistic model. The linear sequences of consecutive heart beats in which RRi and pressure vary consensually can be described as follows:

$$y = a\,x + b \qquad (1)$$

where the dependent variable *y* is RRi, the independent variable *x* is SAP or MAP, the constant *a* is the BRS (ms mmHg^{-1}), the constant *b* is the RRi for SAP/MAP = 0 mmHg. If we use HR instead, since it is the reciprocal of RRi ($y' = HR = \frac{1}{y}$), equation (1) becomes:

$$y'(x + \frac{b}{a}) = \frac{1}{a} \qquad (2)$$

thus defining an equilateral hyperbola of curvature 1/a, where y' is HR, x is SAP/MAP and the constants *a* and *b* are the same as in equation (1). This means that the BRS is not the negative slope of a HR versus arterial pressure line; it rather is the reciprocal of the curvature of the hyperbola described by a HR versus arterial pressure relationship.

Equations (1) and (2) demonstrate that the information conveyed by the slope of the former equation does not correspond to that conveyed by the latter equation. As a consequence, a choice must be made between the two BRS representations. In this thesis, coherently with the prevailing representation in open-loop studies, wherein BRS corresponds to the baroreflex gain around the operating point, the HR versus MAP representation was systematically used[1].

[1] In the published version of article 1, the RRi was used instead of HR upon request of a reviewer. Adhesion to this request occurred before the analysis leading to the development of Equations 1 and 2, and the consequent decision of systematically using the HR versus MAP representation in this thesis.

FIRST CHAPTER

FIRST STUDY: BAROREFLEX AT EXERCISE ONSET

1.1 Introduction

Exercise can be considered as a strong stimulus that introduces a perturbation of the cardiovascular system. The sudden fall in blood pressure at the beginning of exercise is considered a consequence of the massive muscle vasodilatation attributed to the action of a variety of vasoactive substances released following the first contraction (Saltin et al., 1998) and leads to a sudden increase in muscle blood flow (Chin et al., 2010; Clifford, 2007; DeLorey et al., 2003; Ferretti et al., 1995) and to a dramatic fall of total peripheral resistances (Elstad et al., 2009; Faisal et al., 2010; Lador et al., 2006, 2008; Wieling et al., 1996). As a consequence, the baroreflexes are involved in a wide process of re-organization. The huge amount of steady state studies on arterial baroreflexes demonstrated that the operating point is not fixed, but moves over a wide range of pressure and heart rate values, being determined by stimuli from the peripheral and central nervous systems.

At exercise, the operating point is displaced upward and rightward with respect to rest, normally without changes in maximal gain (DiCarlo and Bishop, 2001; Fadel and Raven, 2012; Mitchell, 2013; Raven et al., 2002, 2006; Raven, 2008; Rowell et al., 1996). This phenomenon is generally identified as *baroreflex resetting*. Several reviews have summarized the evolution of our understanding of the concept of baroreflex resetting at exercise (Di Carlo and Bishop, 2001; Fadel and Raven, 2012; Raven et al. 1997, 2002, 2006; Raven, 2008; Rowell et al. 1996). Central command and exercise pressor reflex were considered as possible mechanisms behind baroreflex resetting (Degtyarenko and Kaufman 2006; Gallagher et al., 2001a, 2006; McIlveen et al., 2001; Ogoh et al., 2002; Querry et al., 2001; Raven et al., 2006; Smith et al., 2003; Tsuchimochi et al., 2009).

Our comprehension of this fine resetting process, however, is still far from being established. Previous studies reported contradictory results on the relocation of the steady state carotid–cardiac reflex function curve after stimulation of the mechanoreceptors and/or the metaboreceptors of the exercise pressor reflex and after central command activation (see e.g. Fisher et al., 2008; Gallagher et al., 2001b, 2006; McIlveen et al., 2011; Ogoh et al., 2002; Papelier et al., 1997; Raven et al., 2006). These mechanisms are not mutually exclusive.

The ensemble of this data suggests possible interferences among mechanisms and differences in response characteristics to given stimuli that are not yet fully understood, indeed steady state data obtained before and after resetting did not concern the dynamics of the baroreflex resetting process itself, which occurs during the exercise transients.

The introduction of non-invasive techniques for the continuous beat-by-beat measurement of arterial blood pressure provided excellent opportunities for gaining insight into the exercise baroreflex resetting process through the study of the dynamics of the HR versus MAP relationship at the onset of light constant-load exercise. In spite of this, little attention was given to the dynamics of baroreflex resetting in humans so far. From the time course of the MAP and HR responses upon exercise onset, Lador et al. (2006, 2008) constructed contour plots of the dynamic evolution of the relationship between these two variables, without however inferring any interpretation of the described phenomenon. A study of the kinetics of displacement of the baroreflex curve, or at least of its operating point upon exercise onset, is needed to understand the possible interrelationship between the central command and the exercise pressor reflex hypotheses.

The aim of this study was to analyse the dynamic relationship between HR and MAP, in an attempt at underpinning some possible temporal components of the mechanisms that at various times intervene in determining baroreflex resetting in an exercise transient. In so doing we were driven by the hypothesis that the beginning of the resetting process does not necessarily coincide with the exercise start. We investigated the HR versus MAP relationship during exercise transients in a fully aerobic exercise domain, with reference to the central command and the exercise pressor reflex hypotheses. Exercise was performed in upright and supine position, to discriminate the effect of a change in the cardiac filling pressure induced by posture, on the hypothesis that the baroreflex operating point in supine posture is displaced with respect to the upright posture, even at rest (Schwartz et al., 2013).

1.2 Materials and methods

Subjects

Ten healthy, non-smoking, young volunteers (two men and eight women) participated in the experiments. They were 24 ± 6 years old, 167 ± 8 cm tall, and weighed 58 ± 9 kg. The volunteers

were instructed to take only light meals on the day of the experiments and to refrain from caffeine and other substances stimulating the autonomic nervous system. They also had to refrain from strenuous exercise the day before the tests. Women were admitted to the study independent of the phase of the menstrual cycle (Ogoh et al., 2007), since it was previously reported that the menstrual cycle does not influence indexes of baroreflex control of heart activity (Cooke et al., 2002; Minson et al., 2000).

All subjects were preliminarily informed about all procedures and risks associated with the experiments. Signed informed consent was obtained from each volunteer, who was aware of the right of withdrawing from the study at any time without jeopardy. The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the institutional ethical committee.

Measurements

HR was continuously measured by electrocardiography (Elmed ETM 2000, Heiligenhaus, Germany). Continuous monitoring of arterial pulse pressure profile was obtained at the middle phalange of a left-arm finger by means of a non-invasive cuff pressure recorder (Portapres®, TNO-TPD, Amsterdam, The Netherlands). Systolic and diastolic blood arterial pressures were identified at each beat. Beat-by-beat MAP was computed as the integral mean of each pressure profile, using the Beatscope™ software package (FMS, Amsterdam, The Netherlands).

Single beat stroke volume (SV) was determined by means of the Modelflow method (Wesseling et al., 1993), applied off-line to the pulse pressure profiles, using the Beatscope™ software package. Beat-by-beat cardiac output (CO) was computed as the product of single beat SV times the corresponding single beat HR. The data were then corrected for method's inaccuracy, as described elsewhere (Azabji Kenfack et al., 2004; Lador et al., 2006; Tam et al., 2004), using steady-state CO values obtained by means of the open circuit acetylene method (Barker et al. 1999) on each subject, after determination of individual partition coefficients for acetylene (Meyer and Scheid, 1980). The mean calibration factors were the same at rest (1.01 ± 0.55) as at exercise (1.04 ± 0.36, $p = 0.79$), so that corrected CO values could be and thus were used for beat-by-beat analysis during exercise transients (van Lieshout et al., 2003).

All the signals were digitalized in parallel by a 16-channel A/D converter (MP150, Biopac Systems, Goleta CA, USA) and stored on a computer. The acquisition rate was 200 Hz.

Protocol

In both postures, exercise was performed on an electrically-braked cycle ergometer (Ergoselect 400, Ergoline GmbH, Bitz, Germany). After performance of the open circuit acetylene CO determination at rest, and after further three minutes of quiet resting recordings, the subject performed a series of three exercises at 50 W, the first lasting seven minutes, the others five minutes. The steady state CO values by the open circuit acetylene method were obtained during the first exercise, after the end of the fifth minute of exercise. Subsequent exercises were separated by a six minutes recovery. The protocol was performed twice, once in upright and once in supine position, administered in random order. The subjects pedalled at a spontaneously selected frequency comprised between 60 and 70 rpm; they maintained their own selected frequency throughout the study.

Caution was taken in order to avoid pre-exercise stress. After rest monitoring, participants received the following communication "We are ready: you can start whenever you want". If the participant delayed the exercise onset by more than ten seconds after communication, the procedure was interrupted and started again after further two min at rest. During off-line data processing, the exact time of exercise start was detected from the pedalling frequency recording.

Data treatment

Individual beat-by-beat values of HR, MAP and CO from the three repetitions were time aligned, by setting the time of exercise onset as analysis time zero. Then they were linearly interpolated on a 0.1 s basis (10 Hz) and averaged to obtain a single superimposed time series for each parameter, using Matlab (version 7.9.0, MathWorks, Natick, MA, USA). This analysis was not performed beyond exercise fifth minute. The individual TPR was calculated from these averaged time series, by dividing each MAP value by the corresponding CO value, on the assumption that the pressure in the right atrium can be neglected as a determinant of peripheral resistance in healthy individuals (Faisal et al., 2010; Lador et al., 2006).

Rest and exercise values at steady state for each investigated variable were calculated as the mean during the last minute of rest (from -60 to 0 s) and during the last minute of exercise (from 240 to 300 s of exercise), respectively.

At rest and exercise steady states, we also computed the spontaneous baroreflex gain (BRS) by means of the sequence method (Bertinieri et al., 1988), using MAP as independent variable. Briefly, sequences of three or more consecutive beats in which MAP and HR changed in

opposite direction, were identified. A phase shift of one beat was introduced between the MAP and the HR values of each sequence, as in previous studies (Bertinieri et al., 1988). Within each individual sequence, the HR versus MAP relationship was analysed by linear regression, to compute the slope and the corresponding coefficient of determination (R^2). Only slopes showing R^2 values higher than 0.85 were retained (Iellamo et al., 1994, 1997). For each subject, the mean slope of the HR versus MAP relationships was then computed and taken as a measure of individual BRS, at rest and at exercise, respectively. Baroreflex sequences were searched during the two minutes of quiet rest (from -120 to -10 s); at exercise, baroreflex sequences were identified during exercise min 3-to-5. In each subject and condition, the number of analysed sequences ranged between 1 and 17.

Assuming closed-loop approach, the relationship between HR and MAP in the early phase of exercise was constructed. On this relationship, the minimum of MAP (MAPmin) was identified. The segment of this relationship between the exercise onset and the attainment of MAPmin was linear and had a negative slope, being characterised by opposite variations of MAP and HR. The slope of this segment, which consisted of 8-to-13 consecutive beats, was computed by linear regression. The resulting slopes were taken as representative of the spontaneous baroreflex gain in the early phase of exercise, and were compared with the BRS obtained at rest and at exercise steady state by means of the sequence method.

Statistics

Data are given as mean and standard deviation (SD) of the values obtained for each variable from the average superimposed files of each subject, in order to account for inter-individual variability. The cardiovascular values at the time instant of exercise onset (t = 0 s) and at the instant of MAPmin attainment were compared with the corresponding mean values at rest and at exercise steady state, using two-way ANOVA for repeated measures, for time (four conditions) and posture (upright versus supine), with Tukey post-hoc test. The slopes of the linear segment of the HR versus MAP relationships at the beginning of exercise were compared with MAP-based BRS values, respectively, at rest and exercise, using two-way ANOVA for repeated measures, for condition (BRS at rest, slope of the linear segment after exercise start, and BRS at exercise steady state) and posture (upright versus supine), with Tukey post-hoc test. The BRS at the beginning of exercise was compared using one-way ANOVA for repeated

measures, for posture (upright versus supine), with Tukey post-hoc test. The differences were considered significant if $p < 0.05$.

The linear regression parameters were calculated by the least square method, using a function implemented under Matlab (version 7.9.0, MathWorks, Natick, MA, USA). Linear regression was performed using a robust weighting algorithm (fitlm Matlab function with robust option) that uses iteratively reweighted least squares with bi-square weighting function. Since conventional least-square linear regression models are based on the assumption of a normal distribution of error, the robust weighting algorithm was originally proposed as being poorly influenced by outliers, compared to the conventional least-square linear regression fit. The R^2 of the linear regressions were obtained by the fitlm Matlab function.

1.3 Results

The values obtained at steady state, both at rest and at exercise, are reported in Table 1.1. HR, SV and CO were higher at exercise than at rest in all conditions. SV was higher in supine than in upright posture, both at rest and at exercise steady-state. HR was lower in supine than in upright posture at rest, but not at exercise steady-state. As a consequence, resting CO did not differ significantly between postures, despite a tendency to be higher supine than upright; conversely, at exercise, CO turned out to be higher supine than upright. In both postures, steady state MAP was higher and TPR was lower at exercise than at rest. Resting TPR was lower supine than upright. No differences either in MAP or in TPR were observed between postures at exercise steady-state.

Using MAP as independent variable, the BRS in supine posture was -2.22 ± 0.70 b min^{-1} $mmHg^{-1}$ at rest, and -1.07 ± 0.37 b min^{-1} $mmHg^{-1}$ at exercise steady state ($p < 0.05$ with respect to rest); in upright posture, we obtained -1.33 ± 0.33 b min^{-1} $mmHg^{-1}$ at rest and -0.98 ± 0.27 b min^{-1} $mmHg^{-1}$ at exercise steady state.

UPRIGHT				
	Rest	**t = 0 s**	**MAP_{min}**	**Exercise**
HR (b min^{-1})	88.6 ± 15.5	94.3 ± 12.7*	108.8 ± 10.9 *#	116.1 ± 17.5 *#°
MAP (mmHg)	86.8 ± 8.5	89.7 ± 12.0	73.8 ± 9.5*#	93.1 ± 13.1°
SV (ml)	57.9 ± 14.0	57.2 ± 12.5	66.9 ± 14.5 *#	80.8 ± 15.4 *#°
CO (l min^{-1})	5.03 ± 1.14	5.35 ± 1.22	7.25 ± 1.67 *#	9.17 ± 1.12 *#°
TPR (mmHg min l^{-1})	18.27 ± 4.29	17.79 ± 4.80	10.64 ± 2.00*#	10.34 ± 1.93*#
SUPINE				
	Rest	**t = 0 s**	**MAP_{min}**	**Exercise**
HR (b min^{-1})	73.3 ± 12.8	80.2 ± 11.3*	92.4 ± 10.3*#	112.2 ± 15.0*#°
MAP (mmHg)	83.2 ± 6.8	84.8 ± 7.3	73.9 ± 6.5*#	92.0 ± 8.9*#°
SV (ml)	74.9 ± 15.1	72.7 ± 14.0	77.1 ± 16.6	91.1 ± 17.4*#°
CO (l min^{-1})	5.40 ± 1.19	5.73 ± 0.95	7.05 ± 1.46*#	10.11 ± 1.89*#°
TPR (mmHg min l^{-1})	16.11 ± 3.42	15.23 ± 2.63	10.87 ± 2.04*#	9.39 ± 1.87*#

Table 1.1 Steady state values of cardiopulmonary parameters at rest (Rest), at exercise onset (t = 0 s), at the moment of minimum of MAP (MAPmin) and at exercise steady state (Exercise), in upright and supine posture. Data are means ± SD, N = 10. * = significantly different from the value at rest in the same posture. # = significantly different from the value at t = 0 s in the same posture. ° = significantly different from the value at MAPmin in the same posture

An example of the time courses of the recorded and calculated variables upon exercise onset is shown in Figure 1.1. At the time instant of exercise start (t = 0 s), in both postures, the single beat MAP was equal to the respective mean resting steady state values (+ 2.9 ± 4.7 mmHg upright, and + 1.7 ± 3.8 mmHg supine for MAP). In spite of this, the single beat HR value at exercise start was higher than the corresponding mean value at quiet rest in both postures (+ 5.7 ± 6.7 b min^{-1} upright and + 7.0 ± 5.8 b min^{-1} supine). As exercise started, HR, SV and CO increased to reach a new steady state within 60-to-120 seconds. MAP was characterized by an initial rapid drop, which led to MAP_{min} within 10.6 ± 5.6 s upright and 12.3 ± 7.3 s supine (NS). TPR decreased rapidly at exercise onset, toward a new steady state value. In both postures, MAP_{min} was significantly lower than the MAP and SAP values at t = 0 s, as was the corresponding TPR value. Subsequently, MAP increased slowly and progressively toward a new steady state. This increase in MAP was consensual with the corresponding increase in HR. These patterns were followed by all volunteers, whether in supine or in upright posture.

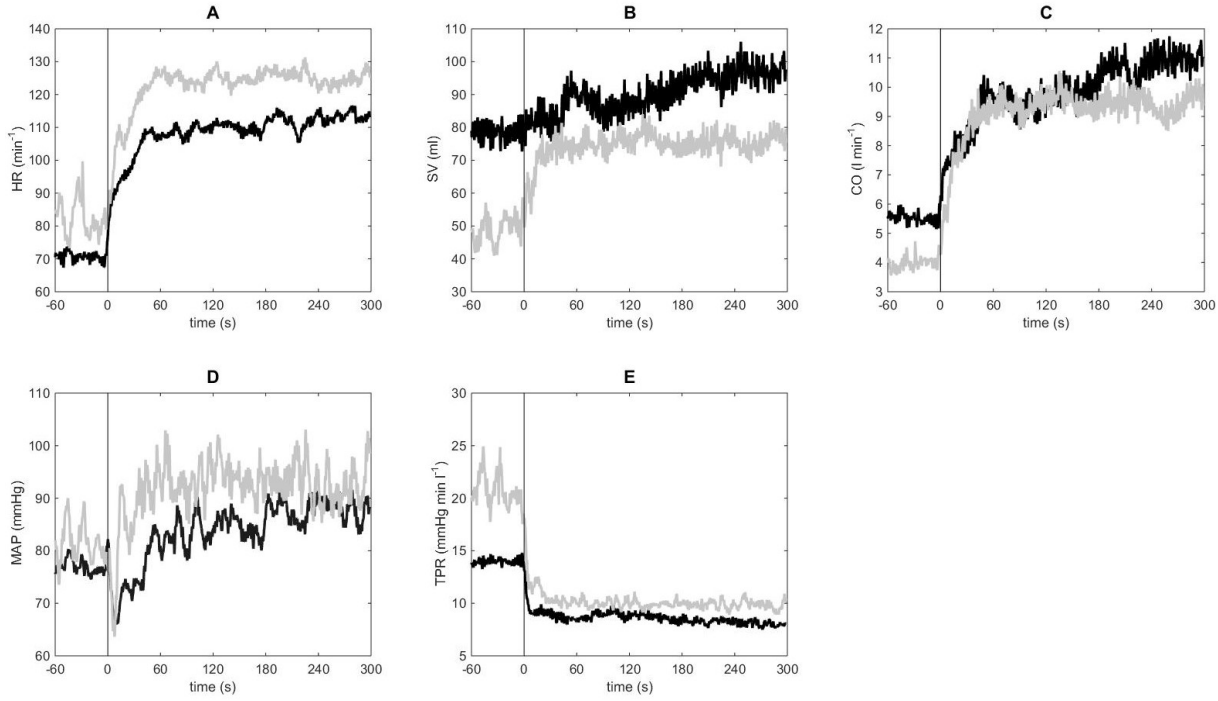

Figure 1.1 Representative example of beat-by-beat HR (panel A), SV (panel B), CO (panel C), MAP (panel D) and TPR (panel E) responses upon the onset of exercise, for upright (grey line) and supine (black line) positions. Vertical line identifies the exercise onset (Bringard et al., 2017).

A contour plot showing the evolution of beat-by-beat HR as a function of beat-by-beat MAP over the last ten seconds of rest and the first minute of exercise is presented in Figure 1.2. For any given MAP value following exercise onset, HR was lower supine than upright. The portion of the relationship between HR and MAP comprised between the exercise onset and the attainment of MAPmin had a negative slope, equal to -0.66 ± 0.16 b min^{-1} $mmHg^{-1}$ upright and -1.04 ± 0.43 b min^{-1} $mmHg^{-1}$ supine ($p < 0.05$ with respect to rest; NS with respect to exercise steady state in both postures). Subsequently, MAP increased slowly and progressively toward a new steady state, consensually with the corresponding increase in HR, until a new steady state condition was achieved. Some differences between postures yet appear, as long as in upright posture the pattern followed a large loop, whereas in supine posture the way to steady state was more straightforward.

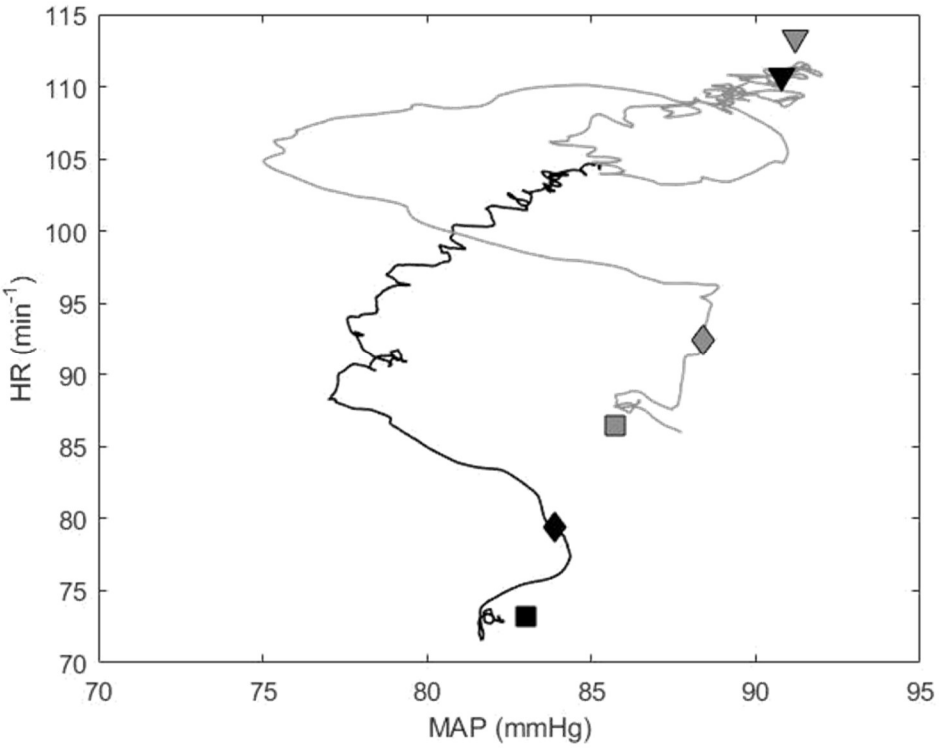

Figure 1.2 Beat-by-beat HR vs MAP relationship upon the onset of exercise for upright (grey line) and supine (black line) positions. Curves represent the group averaged data for the last 10 s of rest and the first min of exercise. Diamonds indicate the exercise onset. Squares and triangles are respectively the average rest and exercise steady state values as reported in Table 1.

An analogous contour plot showing the evolution of beat-by-beat MAP as a function of beat-by-beat CO over the last ten seconds of rest and the first minute of exercise is presented in Figure 1.3. This relationship also showed two distinct phases. In both postures, the portion comprised between the exercise onset and the attainment of MAPmin was negative, implying a decrease of MAP with increasing CO during the first seconds of exercise and a remarkable decrease in TPR. After the attainment of MAPmin, in supine posture, CO and MAP increased consensually, pointing to the new steady state: the pattern implied a tendency ($p = 0.096$) toward a further decrease in TPR. In upright posture, MAP increased much more than CO, which was already close to its steady state, so that MAPmin and MAP at exercise steady state were on the same TPR isopleth.

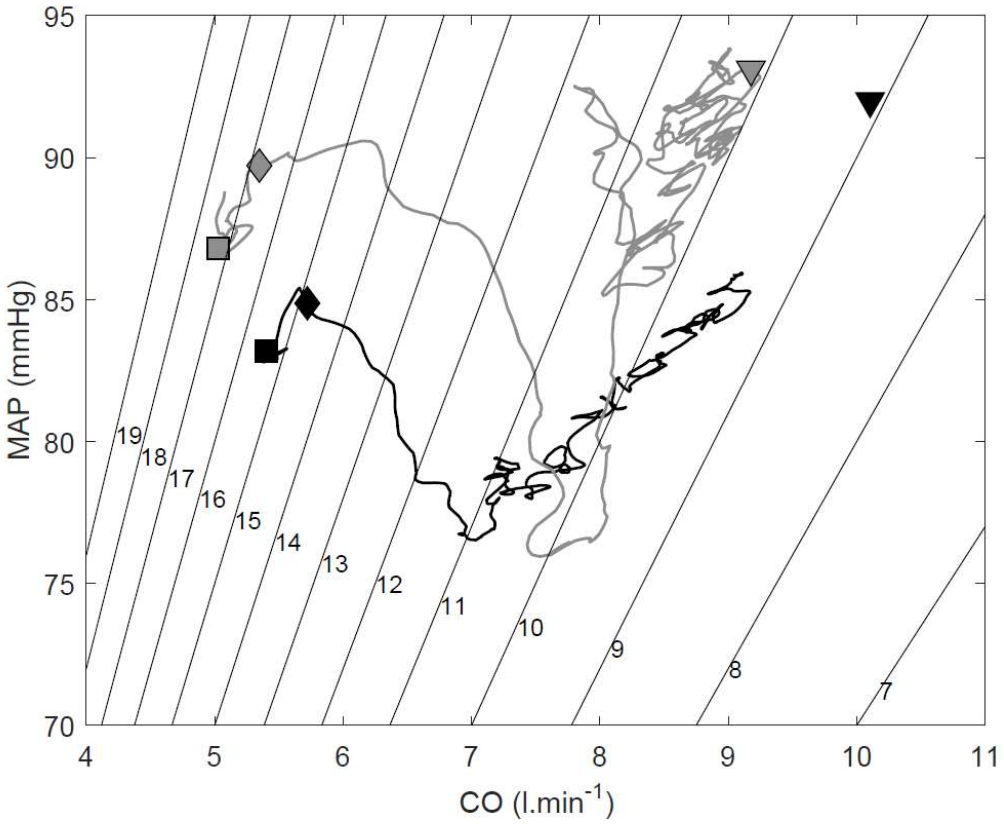

Figure 1.3 Contour plot describing the relationship between mean arterial pressure (MAP) and cardiac output (CO) during the exercise transient, for the upright (grey line) and supine (black line) positions. The continuous lines represent the group averaged data for the last 10 s of rest and the first min of exercise. Diamonds represent the combination of MAP and CO values at the exercise onset. Squares and triangles represent the combination of MAP and CO mean values during quiet rest and at exercise steady-state, respectively, as from Table 1. The dotted lines are isopleths for TPR, with the corresponding TPR values as labels (Bringard et al., 2017).

1.4 Discussion

This study reports the first description of the kinetics of arterial baroreflex resetting using a beat-by-beat investigation of the HR versus MAP relationship at exercise onset. The results indicated that i) no displacement of arterial baroreflex operating point (baroreflex resetting) was evident at the very exercise start; ii) in the first seconds of exercise, there was a linear negative segment of the HR versus MAP relationship, which corresponded conceptually – yet including a bigger number of single beat values – to a sequence of heart beats, as defined by Bertinieri et al. (1988) in resting steady state; iii) the slopes of these segments did not differ from the corresponding BRS at exercise, but significant differences were observed with respect to rest, suggesting that the change in baroreflex sensitivity occurred since the beginning of exercise; v)

that linear segment was suddenly interrupted upon the attainment of a minimum value of MAP; vi) the subsequent part of the HR versus MAP relationship was characterised by an essential progressive increase in HR, associated with a progressive increase in blood pressure, toward the exercise steady state, suggesting that a resetting of the baroreflex operating point, without further changes in baroreflex gain, occurred after the attainment of MAPmin.

The HR, SV and CO data at rest conformed to previous results in upright and supine posture (Leyk et al., 1994; Loeppky et al., 1981; Nishiyasu et al., 1998; Spaak et al., 2005). Resting BRS showed similar values to those found in previous studies (Kardos et al., 2001) in both postures. Previous studies showed that in the upright versus supine position there is a reduction of vagal (Cooke et al., 1999) and increase of sympathetic (Burke et al., 1977; Ray et al., 1993) activities at rest. In fact, coherently with previous observations (Schwartz et al., 2013), the BRS supine was greater than the BRS upright, whether for MAP-based BRS, or for SAP-based BRS. Since TPR acts on DAP, and thus on MAP, without affecting SAP, the fact that TPR at rest was higher upright than supine, due to peripheral vasoconstriction in the former posture, may explain the different behaviour of MAP-based and SAP-based BRS.

At the single beat corresponding to exercise start, while MAP kept the same value as in quiet rest, HR was higher both supine and upright, being lower in the former than in the latter posture. This finding indicates that the increase in HR preceded the exercise start, compatibly more with the central command theory (Fisher et al., 2015; Legramante et al., 1999; Raven et al., 1997) than with the exercise pressor reflex theory. Within that theory, the lack of changes in MAP, CO and TPR suggests that a selective stimulation of the sinus node is the distal component of the feed forward mechanism. The lack of changes in SV, however, implied that either the intensity of the hypothesized feed forward stimulation was not strong enough to determine visible inotropic effects, or the increase in HR was a consequence of withdrawal of vagal stimulation of the heart.

The BP fall at the beginning of exercise is considered a consequence of the massive muscle vasodilatation that is attributed to the action of a variety of vaso-active substances liberated following the first contraction (Saltin et al., 1998) and leads to a sudden increase in muscle blood flow (Chin et al., 2010; Clifford, 2007; DeLorey et al., 2003; Ferretti et al., 1995) and to a dramatic fall of TPR (Elstad et al., 2009; Faisal et al., 2010; Lador et al., 2006, 2008; Wieling et al., 1996). The concomitant sudden increase in HR indicates a baroreflex attempt at correcting the MAP fall. This explains why, in the early phase of exercise, at least between the

exercise start and MAPmin, there was a linear negative segment of the HR versus MAP relationship. The slope of this segment corresponds conceptually – yet including a bigger number of single beat values – to the BRS computed from a baroreflex sequence. The observation, over a series of consecutive beats, of linear HR versus MAP relationships in the early phase of exercise reveals that the point defining the instantaneous combination of MAP (or SAP) values was displaced upward and leftward along a baroreflex curve, indicating an attempt at correcting a fall in MAP by means of an increase in HR. The slope of that baroreflex curve (gain) was lower than the BRS at rest, but equal to the BRS at exercise steady state, suggesting that the BRS decrease during exercise appears since the exercise start, compatibly with the central command hypothesis. However, if we define baroreflex resetting as the displacement of the prevailing baroreflex function, with consequent shift of the baroreflex operating point (Gallagher et al., 2006; Raven et al., 2006), we would have to accept that the initial linear segment of the HR versus MAP relationship is not representative of baroreflex resetting, which therefore does not occur immediately as exercise starts.

In this study, the fall of MAP continued for about 10 s since the beginning of exercise, until the attainment of MAPmin (~73 mmHg in both postures in the present study). That time corresponded to the duration of the rapid phase (phase I) of the cardiovascular response to exercise (Lador et al., 2006). At MAPmin, HR had increased by an amount corresponding well to the phase I amplitude of the HR response to exercise (Lador et al., 2006). The MAPmin values of the present study were similar to the threshold MAP values observed during the standing-up procedure at the end of prolonged bed rest (Adami et al., 2013), and to the minimum MAP values observed in the early phase of the cardiovascular response to apnoea (Sivieri et al., 2015). The sudden pattern change in the HR versus MAP relationship (Figure 1.2) after attainment of MAPmin led us to propose that baroreflex resetting, implying a positive HR versus MAP relationship, started only after the achievement of MAPmin. If indeed resetting starts only after the attainment of MAPmin, then i) MAPmin might represent a threshold value triggering exercise baroreflex resetting; ii) the central command theory may not explain the resetting process, even if it may explain the sudden change in baroreflex gain at the beginning of exercise; iii) the resetting process would not imply further changes in baroreflex gain, as witnessed by the equality of the slope of the HR versus MAP segments at exercise start and of the BRS at exercise steady state. This last statement is in agreement with the concept that the operating point is displaced with respect to rest but without changes in maximal gain (DiCarlo

and Bishop, 2001; Fadel and Raven, 2012; Mitchell et al., 2013; Raven et al., 2002, 2006; Raven, 2008; Rowell et al., 1996).

Notwithstanding the relationship between HR and MAP changed drastically after attainment of MAPmin, compatibly with the resetting hypothesis, the consensual variations of HR and MAP followed complex wandering pathways, which are hard to disentangle, and thus to represent by means of neat mathematical functions in absence of an a-priori model. A new steady state was achieved within one minute upright, but not supine. This indicates, on one side, faster cardiovascular adaptation during exercise in the former than in the latter posture, as already reported (Leyk et al., 1994; Loeppky et al., 1981), and on the other side, that the postulated resetting process may not be complete yet at the end of the first min of exercise (Figure 1.2), compatibly with a possible role of increasing sympathetic stimulation of the sinus node during exercise (Fagraeus and Linnarsson, 1976; Orizio et al., 1988). This hypothesis could be nicely tested under selective blockade of β1-adrenergic receptors.

At exercise steady state, the single-beat HR and MAP values clustered around new stable mean values, defining the new location of the operating point (Figure 1.2). This location is compatible with the predictions of baroreflex open-loop steady state studies (Raven et al., 2006). SV remained lower upright than supine, although in the former posture a larger amount of blood, located in the lower limbs, was made available for sudden SV increase upon exercise onset (Lador et al., 2006; Sheriff et al., 1993). These changes in SV were not fully compensated for by an equivalent increase in HR, so that lower CO values at exercise were obtained upright than supine. The present results at exercise steady state in upright and supine posture are in good agreement with the results of previous studies (Nishiyasu et al., 1998).

In conclusion, our results show that the patterns of the dynamics of baroreflex resetting upon exercise onset is characterised by two distinct phases, independently of the posture. A first phase goes from the beginning of the exercise till the attainment of MAPmin: in this phase, a fall in MAP was corrected by an increase in HR along a baroreflex curve, which had lower sensitivity than that at rest but equal to that at exercise steady state, and which was likely operating in the same range of HR and MAP values as at rest. A second phase was characterised by a positive HR versus MAP relationship toward the exercise steady state prevailing HR and MAP values. This second phase started after the attainment of MAPmin.

To sum up, a resetting of the baroreflex operating point and a fall in baroreflex gain both take place in the exercise transient, being temporally dissociated phenomena. The latter is

immediate, whereas baroreflex resetting does not begin at exercise start. The central command hypothesis may still explain the reductions of baroreflex gain that were observed at exercise start: so, it cannot be refuted as a hypothesis at least partially explaining the baroreflex changes in the exercise transients.

SECOND CHAPTER

SECOND STUDY: BAROREFLEX IN APNOEA

2.1 Introduction

During the first dynamic phase (or phase I, φ1) of the cardiovascular response to apnoea, there is a drastic drop in MAP, accompanied by an increase in HR (Costalat et al., 2015; Fagoni et al., 2015; Sivieri et al., 2015). The HR pattern was viewed as a baroreflex response counteracting a sudden fall in arterial pressure (Fagoni et al., 2015; Sivieri et al., 2015). In all these studies, apnoeas were performed at a lung volume close to the individual total lung capacity. On this basis, Fagoni et al. (2015) interpreted the MAP fall as due to a reduction in venous return caused by an increase in intrathoracic pressure occurring at elevated lung volume. Since φ1 is an unsteady state condition, only closed-loop methods can conveniently be used for the analysis of arterial baroreflexes, as demonstrated by studies on the exercise transients (Bringard et al., 2017) and postural changes at the end of prolonged head-down tilt bed-rest (Adami et al., 2013).

As stated in the previous chapter, the closed-loop approach to baroreflexes allows a functionally significant analysis of the HR versus MAP relationship: sequences of consecutive beats in which HR and MAP vary in opposite directions can be identified, defining linear segments of the HR versus MAP relationship (Bringard et al., 2017), the slope of which can be compared with the closed-loop baroreflex sensitivity calculated at steady state by means of the sequence method (Bertinieri et al., 1985, 1988; Parati et al., 1988). Similar approaches were applied not only to study healthy subjects, but also in hypertensive patients (Palmero et al., 1981; Parati et al., 1988), and resting patients with spinal cord injuries (Grimm et al., 1998; Houtman et al., 1999), by means of the analyses of the phase IV of Valsalva Manoeuvre (VM), during which MAP increases and HR decreases.

Whilst during φ1 a baroreflex response has been detected before the attainment of the minimum of MAP (MAPmin), the BRS during φ2, the steady phase of the cardiovascular response to apnoea, has never been analysed so far.

The aim of this study was to perform a closed-loop analysis of the cardiac-chronotropic component of arterial baroreflexes during the φ1 of apnoeas performed at lung volumes close to the total lung capacity, at rest and during exercise. We hypothesised that, during exercise apnoeas, the characteristics of φ1 would be different than in resting apnoeas, because the BRS slope at exercise is lower than at rest, and the operating point of the baroreflex is displaced upward and rightward with respect to rest. We compared BRS computed in steady state condition before apnoeas, during φ1, and φ2, in resting and exercise apnoeas. The apnoeas were carried out in air and in oxygen, because the duration of φ2 was found to be longer in oxygen than in air, due to the higher oxygen stores in the latter compare to the former (Fagoni et al., 2017b). In oxygen apnoeas, it should be easier to recognize more sequences due to the longer φ2 in oxygen than in air.

2.2 Materials and methods

Subjects

Ten professional male divers volunteered for this study. They were 37 ± 6 years old, 79 ± 7 kg heavy and 176 ± + 5 cm tall. All divers were healthy and non-smokers. None had previous history of cardiovascular, pulmonary or neurological diseases, or was taking medications at the time of the study. All gave their informed consent after having received a detailed description of the methods and experimental procedures of the study. The study conformed to the Declaration of Helsinki and was approved by the local ethical committee.

Experimental procedure

Experiments were carried out in Lindos, Greece, in an air-conditioned room at 23-24°C, with relative humidity between 60 and 65%. Subjects came to the laboratory on two occasions. On the first day, upon arrival in the laboratory and after instrumentation, the subject took the supine posture. Five minutes were allowed to achieve steady state conditions; then, ten minutes of measurements were obtained during quiet spontaneous breathing, and subsequently the subject was asked to perform one maximal apnoea. After the maximal apnoea, the subject recovery at least three minutes. Then the diver performed six apnoeas longer than φ1, at least 30 seconds, to detect more sequences during φ1 (i.e. six apnoeas, six sequences, since during φ1 it was

possible to recognize just one BRS sequence), separated by a recovery intervals of two minutes between the apnoeas. At the end of the last apnoea in air, after further two minutes of recovery, the subject changed position and was asked to sit on a cycle-ergometer. After five minutes pedalling at 30 W, the subject performed one maximal apnoea, during exercise, in air. Technical conditions allowed us to perform no more than one maximal apnoea during exercise. The second day, the same procedure was carried out while breathing pure oxygen.

Both in air and in oxygen, subjects undertook their pre-dive breathing routine before breath-holding, generally consisting of a couple of deep respiratory acts. This procedure was ended by a deep inspiration, so that the lung volume at which the apnoeas started was close to the subject's total lung capacity.

Measurements and data treatment

Arterial blood pressure profiles (Portapres®, TNO-TPD, Amsterdam, The Netherlands) were continuously recorded throughout the experiments. Peripheral blood O_2 saturation (SpO_2) was continuously monitored by infrared spectroscopy (BioPac System Inc., Goleta, CA, USA) at an earlobe. HR was continuously measured on a beat-by-beat basis by electrocardiography (ECG100C module, BioPac System Inc., Goleta, CA, USA). The signals were sampled at 100 Hz by using a 16-bit A/D converter (MP100 VS, BioPac System Inc., Goleta, CA, USA) and stored on a personal computer for subsequent off-line analysis. The breath-by-breath recording of inspiratory and expiratory flows was performed by an ultrasonic flowmeter (Spiroson, Ecomedics, Duernten, Switzerland) calibrated with a three litres syringe, and the time with flat flow signals provided the duration of apnoeas.

Arterial pressure profiles were analysed off line, to obtain beat-by-beat values of systolic, diastolic and mean arterial pressure, using the Beatscope™ software (FMS, Amsterdam, The Netherlands).

The beat-by-beat data of maximal apnoeas were analysed off-line to identify the three phases of apnoeas, both in air as in oxygen, while the other apnoeas were analysed to identify φ1 and the beginning of φ2. An automated procedure implemented under Matlab (version 7.6.0.324, MathWorks, Natick, MA, USA) was used to this aim (Fagoni et al.; 2015, 2017a, 2017b; Sivieri et al., 2015). The procedure was based on linear regression analysis, allowing detection of changes in slope between successive phases.

Before and after the attainment of the MAPmin, we computed the slope of the linear regression between HR and MAP, in air and oxygen, during both resting and exercise apnoeas. BRS was computed during the two minutes recording at rest, before starting apnoeas, and during the entire φ2 in both air and oxygen maximal resting apnoeas, using the sequence method (Bertinieri et al., 1988). A phase shift of one beat was introduced between the MAP and the HR values of each sequence, as in previous studies. Each slope was retained if the R^2 was higher than 0.85. For each subject, the mean slope of the HR versus MAP relationships during steady state (rest or φ2) was then computed and taken as a measure of individual BRS. The operating point for each sequence was computed as the average of HR and MAP values of the series. The mean operating points were computed before and after the attainment of MAPmin, during resting and exercise apnoeas.

Statistical analysis

Data are presented as mean and standard deviation (SD). The Student's T test was performed to locate differences in BRS between oxygen and air apnoeas, and between resting and exercise apnoeas. One way ANOVA was used to compare BRS at rest before starting apnoea, during φ1 (before and after the attainment of MAPmin), and φ2, in both resting and exercise apnoeas, and Tukey post-hoc test was used to locate differences. Differences were considered significant when $p < 0.05$, otherwise they were considered non-significant (NS). The Stata 10.0 statistical software (StataCorp, College Station, TX, USA) was used to this aim.

2.3 Results

All subjects presented resting maximal apnoeas, in air and oxygen, characterised by three phases, thus, nobody was excluded from analysis; φ1 was identified in all the interrupted apnoeas. Table 2.1 shows the durations of the phases identified in oxygen and air apnoeas, in resting condition and during exercise. φ2 was absent during light exercise apnoeas in air, as previously described (Sivieri et al., 2015), thus it was not possible to compute BRS; contrariwise, during exercise apnoeas in oxygen, it was possible to detect a short φ2.

φ1 duration was unaffected by administration of oxygen; it was shorter during exercise than in resting apnoeas. In resting apnoeas, φ2 and φ3 were systematically longer in oxygen than in air.

During exercise apnoeas, φ3 duration was similar between air and oxygen; the presence of φ2 in oxygen apnoeas and its absence in air exercise apnoeas provided longer exercise apnoeas in oxygen than in air.

At the beginning of all the investigated apnoeas, during φ1, MAP dropped immediately for several beats, until reaching of the minimum of blood pressure. After the attainment of MAPmin, blood pressure restored to the level before starting apnoea, and then the steady phase, φ2, took place in resting oxygen, resting air apnoeas, and exercise oxygen apnoeas. φ2 was absent in exercise air apnoeas. Conversely, the HR increased at the beginning of apnoeas, and then decreased to reach a stable value at the beginning of φ2.

Duration (s)	**Resting Apnoeas**		**Exercise Apnoeas**	
	Air	**Oxygen**	**Air**	**Oxygen**
Total	214 ± 36	418 ±141*	44 ± 17$^{\#}$	74 ± 28*$^{\#}$
Phase I (φ1)	17 ± 5	17 ± 4	11 ± 1$^{\#}$	11 ± 1$^{\#}$
Phase II (φ2) **[n. of BRS sequences]**	102 ± 48 [87]	202 ± 112* [187]	ABSENT	26 ± 7*$^{\#}$ [34]
Phase III (φ3)	95 ± 33	199 ± 68*	32 ± 17$^{\#}$	37 ± 23$^{\#}$

Table 2.1 Durations of the single phases for air and oxygen maximal apnoeas performed in both resting and exercise, and related BRS number of sequences identified for each phase. During phase I it was possible to identify just two HR vs MAP sequences, the first before attainment of the minimum of MAP (MAPmin), the second after MAPmin. * $p < 0.05$ compared to air apnoeas. $^{\#}$ $p < 0.05$ compared to resting apnoeas

BRS was computed for each single diver. In oxygen, more BRS sequences were identified compared to air apnoeas during φ2 (Table 2.1), as a consequence of prolonged φ2 in the former compare to the latter case. The values of the slopes provided in each condition were not different between air and oxygen apnoeas, in all the investigated time-frame of apnoea, thus all the data obtained had been analysed together.

Table 2.2 shows all the slopes of the HR vs MAP relationship detected for each subject, in resting and exercise apnoeas. The BRS were computed in steady state condition (REST) and φ2; in φ1, before and after MAPmin, the slopes of the HR vs MAP were calculated. Concerning

the φ2, since it was not possible to detect the φ2 in air apnoeas during exercise, BRS data are reported only for oxygen apnoeas.

	Slopes Resting Apnoeas (b min^{-1} mmHg^{-1})				Slopes 30 WExercise Apnoeas (b min^{-1} mmHg^{-1})			
	REST STEADY STATE before apnoeas	φ1 Before MAP min	φ1 After MAP min	φ2	REST STEADY STATE before apnoeas	φ1 Before MAP min	φ1 After MAP min	φ2 only O_2
1AV	-1.03 (28)	-0.34	-0.67	-0.91 (89)	-0.74 (24)	-0.26	-0.40	-0.58 (5)
2CD	-0.9 (19)	-0.25	-0.75	-0.87 (15)	-0.74 (21)	-0.43	-0.73	-0.73 (3)
3KV	-0.89 (26)	-0.34	-0.64	-0.91 (15)	-0.66 (16)	-0.23	-0.38	-0.31 (1)
4KP	-1.30 (21)	-0.38	-1.03	-1.90 (6)	-0.81 (65)	-0.23	-0.54	-1.00 (5)
5AK	-1.32 (13)	-0.42	-1.23	-1.08 (25)	-1.25 (22)	-0.32	-1.13	-0.96 (4)
6VT	-1.22 (18)	-0.65	-0.84	-0.98 (42)	-0.89 (48)	-0.24		-1.12 (4)
7KS	-1.60 (26)	-0.49	-0.88	-0.95 (15)	-0.91 (44)	-0.30	-0.61	-1.09 (3)
8SA	-1.33 (25)	-0.50	-1.15	-0.88 (18)	-0.97 (41)	-0.46	-0.61	-1.11 (2)
9AM	-1.33 (41)	-0.93	-1.21	-1.37 (13)	-1.13 (46)	-0.26	-1.28	-0.67 (2)
10MC	-1.43 (32)	-0.57	-1.25	-1.39 (36)	-0.59 (26)	-0.34	-0.65	-0.69 (5)

Table 2.2 Baroreflex sensitivity slopes computed for each subject in resting and exercise apnoeas. BRS is reported for steady state condition before the beginning of apnoea and during phase II (φ2). The slopes of HR vs MAP relationship during phase I (φ1), before and after the attainment of minimum of mean arterial pressure (MAPmin), were also reported. The number of sequences identified for each diver is reported into brackets.

The mean slopes computed are shown in Table 2.3. This relationship was lower ($p < 0.05$) in all the investigated conditions during exercise apnoeas compared to resting apnoeas, showing a reduced baroreflex gain during physical activity compared to rest.

Slopes of HR vs MAP relationship (b min^{-1} mmHg^{-1})	REST STEADY STATE before apnoeas	Phase I Before MAPmin	Phase I After MAPmin	Phase II
Resting Apnoeas	**-1.23** (0.23)$^{\#}$	**-0.49** (0.20)	**-0.96** (0.24)$^{\#}$	**-1.12** (0.33)$^{\#}$
30 W Exercise Apnoeas	**-0.87** (0.21)*$^{\#}$	**-0.31** (0.08)*	**-0.70** (0.31)*$^{\#}$	**-0.82** (0.27)*$^{\#}$

Table 2.3 Mean slopes obtained during resting and exercise apnoeas. The slopes were computed in steady state conditions before apnoeas, before and after the attainment of the minimum of mean arterial pressure (MAPmin), and during φ2. * $p < 0.05$ compared to resting apnoeas. $^{\#}$ $p < 0.01$ compared to "Phase I Before MAPmin".

Moreover, data show a flatter ($p < 0.01$) slope at the beginning of apnoea, during the falling in MAP before the attainment of MAPmin, compared to all the other investigated conditions (REST, phase I after MAPmin, and phase II). The recovery after MAPmin shows steeper ($p < 0.01$) slopes compared to the onset of φ1.

BRS was similar during φ2 compared to steady state before the beginning of apnoea, in resting and in exercise apnoeas.

The mean MAP and mean HR, representing the prevailing values around which slopes were calculated during φ1, are reported in Table 2.4, and represented in Figure 2.2.

	Resting Apnoeas		**30 W Exercise Apnoeas**	
	Before MAPmin	**After MAPmin**	**Before MAPmin**	**After MAPmin**
MAP (mmHg)	76 ± 15	88 ± 13 $^{\#}$	84 ± 20 *	108 ± 17 *$^{\#}$
HR (b min^{-1})	89 ± 17	94 ± 16 $^{\#}$	103 ± 13 *	102 ± 13 *

Table 2.4 Prevailing values of the mean arterial pressure (MAP) and heart rate (HR) of the linear parts of phase I response to apnoea, for both resting and exercise apnoeas, before and after the attainment of minimum of MAP (MAPmin). * $p < 0.05$ compared to resting apnoeas. $^{\#}$ $p < 0.05$ compared to "Before MAPmin".

The exercise apnoea provided higher ($p < 0.05$) MAP and HR mean values compared to resting apnoeas in all the investigated conditions during φ1. The prevailing MAP was higher ($p < 0.001$) after the attainment of MAPmin than before MAPmin in both resting and exercise apnoeas, whilst the prevailing HR was higher ($p < 0.05$) after the attainment of MAPmin compared to before MAPmin only in resting apnoeas; during exercise apnoeas the HR was the same after and before MAPmin.

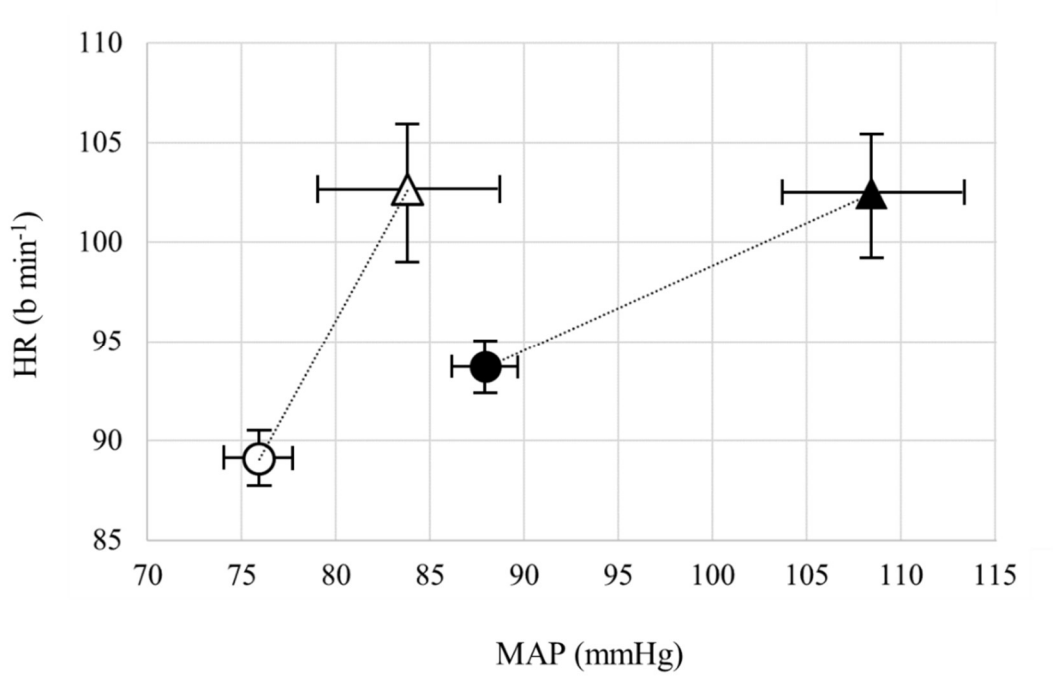

Figure 2.2 Graphical representation of the prevailing heart rate (HR) and mean arterial pressure (MAP) values and related standard errors (bars) in the four investigated conditions. Dotted lines refer to the displacement of these points upward and rightward in exercise apnoea compared to resting apnoeas. White circle: mean values for resting apnoea before the attainment of the minimum of mean arterial pressure (MAPmin). White triangle: mean values for exercise apnoea before the attainment of MAPmin. Black circle: mean values for resting apnoea after the attainment of MAPmin. Black triangle: mean values for exercise apnoea after the attainment of MAPmin.

2.4 Discussion

The analysis of baroreflex during resting and exercise apnoeas suggests that i) during exercise the overall regulation of the baroreflex is different, as indicated by the lower BRS values during exercise compared to resting apnoeas, in all the investigated conditions; ii) in φ1, rapid cardiovascular adjustments affect the baroreflex responses with different pattern before and after the attainment of minimum of pressure, showing higher values of the HR vs MAP slopes after the attainment of MAP min compared to the onset of φ1; iii) the baroreflex sensitivity restored immediately after reaching the MAPmin in φ1, indeed BRS was similar in steady state before the beginning of apnoea and the φ2; iv) the prevailing HR and MAP points during exercise apnoeas are displaced rightward and upward compared to resting apnoeas.

Data concerning the durations of the different phases between air and oxygen, and between resting and exercise apnoeas confirms previous findings (Fagoni et al., 2015, 2017b; Perini et al., 2008, 2010; Sivieri et al., 2015). In resting apnoeas, φ2 resulted shorter in air than in oxygen,

as described elsewhere (Fagoni et al., 2015), and was absent during exercise apnoeas in air (Sivieri et al., 2015). During exercise apnoeas it was possible to identify the φ2: probably the higher oxygen stores in oxygen than in air allowed increasing the total duration of apnoea. In this context φ3 was unaffected by oxygen administration, being similar in air and in oxygen; the appearance of φ2 during exercise apnoeas in oxygen provided longer apnoeas compared to exercise apnoeas in air.

The analysis of baroreflex during exercise apnoeas confirmed lower baroreflex gain compared to resting apnoeas. This is probably due to a different organization of the autonomic output during exercise (Raven et al., 2006) and is in line with the results of study 1. These results confirmed previous finding concerning the BRS under sympathetic stimulation, characterised by lower variability in BRS (Bruno et al., 2012; Chapleau et al., 1995), notwithstanding no studies explored the modifications of BRS during apnoeas so far**.** Despite the effort during exercise apnoeas was mild, 30 W, it was enough to have a different baroreflex sensitivity during the entire apnoea. The slopes provided during resting apnoeas were steeper than during exercise apnoeas, meaning that at rest there is a greater response in HR for the same MAP variation than during exercise.

The beginning of apnoea is characterised by a sudden drop in MAP counteracted by a baroreflex response. Notwithstanding, during this period the BRS showed a different sensitivity compared to steady conditions, rest and φ2. After the attainment of the minimum of MAP, in both rest and exercise apnoeas, the MAP versus HR relationship showed a higher sensitivity, thus this relationship would have a different origin than in the earlier φ1. Although we postulate that in the late φ1, after the MAPmin, a sort of baroreflex resetting took place (Figure 2.3), revealing a plausible sympathetic activation (Bringard et al., 2017), the slopes resulted steeper compared to before the MAPmin. This concept would appear in contrast with previous observations, however the two phenomena describe two completely different conditions: before the attainment of MAPmin the HR increases as a consequence of decreasing in MAP, and the vagal withdrawal takes place; contrariwise after MAPmin the HR decreases and in this phase a vagal response is provided to counteract the increasing MAP compared to the former response.

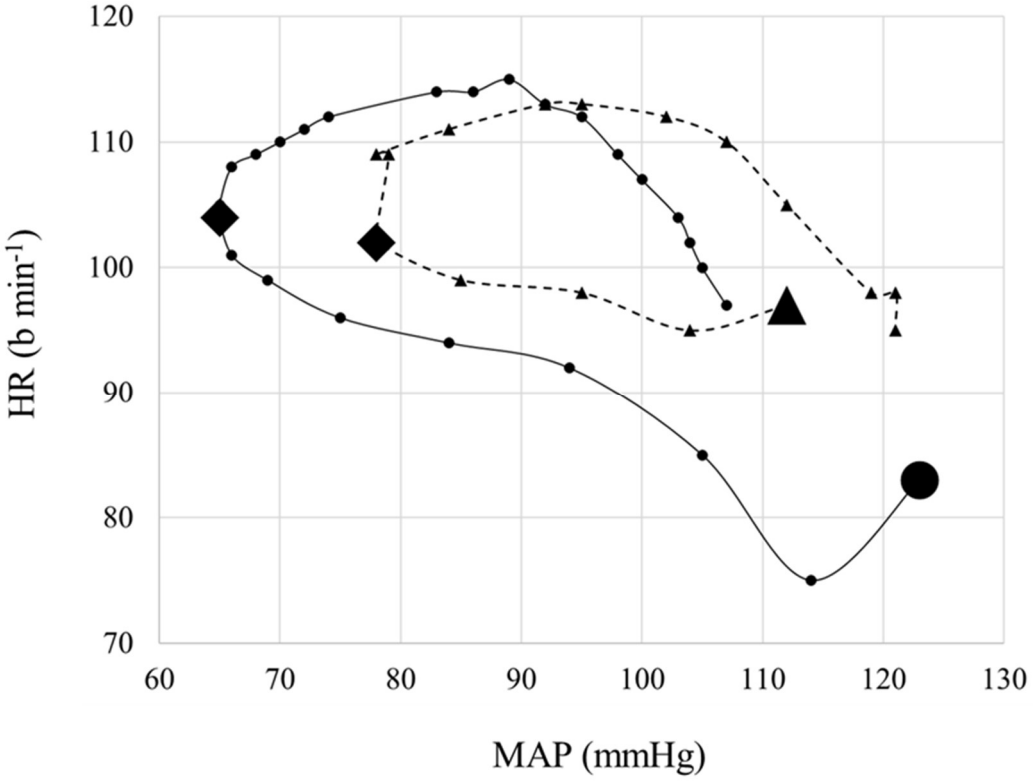

Figure 2.3 Closed-loop contour plot of the relationship between heart rate (HR) and mean arterial pressure (MAP) during φ1 of resting (continuous line and dots) and exercise (dashed line and triangles) apnoeas. Data obtained from a representative subject, showing the presence of a baroreflex responses before the attainment of minimum of mean arterial pressure (MAPmin), and after the MAPmin. The resetting phases start from the diamond points; during these phases both HR and MAP rise toward a new operating level. Big circle and tringle: beginning of apnoeas. Diamond: minimum of mean arterial pressure in φ1.

The prevailing HR and MAP during exercise are shifted upward and rightward compared to resting apnoeas, as expected, both before and after MAPmin (Figure 2.2). This result agrees with previous findings (Raven et al., 2006), although we cannot construct the entire response range of the baroreflex curve that is the prerogative of the open-loop approach. The values obtained after MAPmin are displaced on the right compared to the HR and MAP points found before the attainment of MAPmin. This is in agreement with the concept that after attainment of MAPmin there is a sort of resetting of the baroreflex, probably as a consequence of sympathetic activation. During exercise, this process caused an increased MAP after MAPmin compared to the data obtained before MAPmin, with an invariant HR; we hypothesized that, during exercise apnoeas, the prevailing sympathetic output affects much more the vasomotor component of the cardiovascular responses compared to the cardiac one.

Our speculation is that in φ1, arterial baroreflexes attempt at controlling blood pressure, in resting and in exercise apnoeas. After attainment of MAPmin, a sort of baroreflex resetting took place, that might be considered as a trigger point, likewise to what stated by other authors concerning the baroreflex resetting at exercise onset (Bringard et al., 2017). Moreover this is the first study investigating the sensitivity of the baroreflex response in resting and exercise apnoeas; previous studies made use of the Manoeuvre of Valsalva (VM) in resting condition to analyse the BRS (Palmero et al., 1981; Grimm et al., 1998; Houtman et al., 1999), but they analysed only of the fourth part of the VM, during which MAP increases, and HR decreases, thus the analysis was performed after the attainment of MAPmin, and probably after the resetting of baroreflex. We speculate that φ1 could be characterised by two different conditions which reflect two different phenomena: i) before the attainment MAPmin the parasympathetic activity could prevail, whereas ii) after MAPmin, a sort of baroreflex resetting took place, modifying the sympathetic and parasympathetic output.

Concerning the baroreflex sensitivity in resting apnoeas during φ2, we did not find any differences in the BRS values compared to steady condition. In φ2 several modifications take place (Perini et al., 2008; Fagoni et al., 2015, 2017b; Sivieri et al., 2015): HR decreases, and the total peripheral resistances (TPR) increases. We supposed that a modification in the autonomic output can occur, with a dissociation between heart (characterised by predominant vagal activity) and vascular system (with predominant sympathetic activity), that is probably why these modifications do not affect the baroreflex sensitivity.

THIRD CHAPTER

THIRD STUDY: BAROREFLEX IN HYPERTENSIVE PATIENTS

3.1 Introduction

Hypertension is one of the most important preventable causes of morbidity and mortality in the world, with a remarkable health impact on our societies. High blood pressure is responsible for 13% of deaths globally (WHO, 2009). Hypertension remains the most common and reversible risk factor for myocardial infarction, stroke, heart failure, atrial fibrillation, aortic dissection, and peripheral arterial disease (Mancia et al., 2013). The risk for cardiovascular diseases is associated with a 7% increased risk of mortality from ischaemic heart disease and 10% increased risk of mortality from stroke with each 2 mmHg rise in systolic blood pressure (Lewington et al., 2002; NICE guidelines, update in November 2016).

Several forms of secondary hypertension were identified, notwithstanding more than 90% of the cases of hypertension are still of unknown origin and thus classified as essential. Current behavioural measures and pharmacological treatments have remarkably improved the clinical control of essential hypertension, although the causes of hypertension remain still unclear in most cases. Treatment for hypertension typically requires combination therapy with two or three medications with different mechanisms of action and, therefore, undertreatment of hypertension occurs frequently (Fontil et al., 2014; Khanna et al., 2012), and the percentage of patients affected by essential hypertension with controlled arterial pressure is only between 30 and 50%. Shedding light on the physiopathology of this disease will add much to our understanding of the causes of the disease and to the measures that can be taken.

A genetic component in essential hypertension is highly probable, although the genetics of hypertension is likely very complex and hard to disentangle yet. Currently, the molecular aspects of hypertension and the renin-angiotensin-aldosterone system are the main investigated fields. Contrariwise, there are scanty data on the neural mechanisms of cardiovascular control in humans, although it has been demonstrated that increased renal sympathetic outflow is often present in essential hypertension, and that the introduction of catheter-based renal selective denervation for resistant hypertension significantly reduced blood pressure (DiBona and Esler, 2010; Esler, 2011; Schlaich et al., 2009). These results re-emphasised the role of neural

mechanisms in the genesis of essential hypertension: some authors have even proposed to implant continuous baroreflex stimulators as a tool to diminish central sympathetic outflow (Mohaupt et al., 2007).

Moreover, it was demonstrated that in hypertensive patients the baroreflex sensitivity (BRS) computed by open-loop procedures is impaired (Bristow et al., 1969; Head, 1995; Korner et al., 1974; Mancia et al., 1978) (Figure 3.1) and that modification in BRS was associated with worst outcome in patients affected by myocardial infarction and heart failure (La Rovere et al., 1998, 2008, 2011; Osculati et al., 1990). These studies clearly support the notion that the overall cardiovascular regulation in hypertensive patients may be different from normal, and the BRS can be considered as a mirror of autonomic dysregulation in patients with an increased cardiovascular risk.

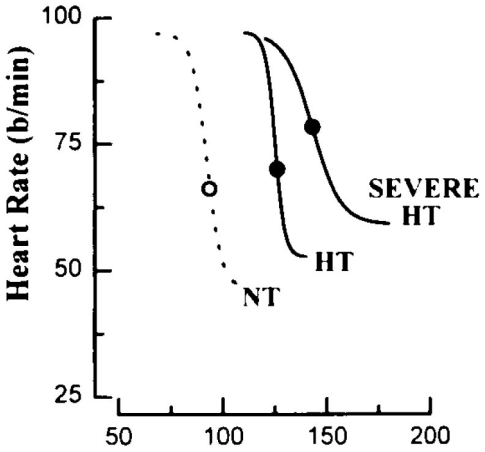

Figure 3.1: Relationship between blood pressure and heart rate in hypertensive patients (HT) and normotensive subjects (NT). (Head, 1995; Korner et al., 1974).

These differences inevitably affect the dynamics of the cardiovascular response to exercise onset and the cardiovascular steady state during exercise in patients affected by essential hypertension. Current evidences refer to steady state condition, regardless the application of open-loop or closed-loop approach. The application of closed-loop approach might be useful to investigate the cardiovascular dynamics at exercise onset; the study of the baroreflex response to exercise would provide important information on the subject, because a re-organisation of the neural control system of cardiovascular function would translate into differences in the BRS at rest, during the exercise transients, and during exercise in steady state condition.

Thus, the main purpose of this study was to investigate the steady-state and the dynamics of the baroreflex response to exercise in patients affected by essential hypertension compared to age-matched healthy controls. Supine and upright postures were investigated in order to modify the sympathetic outflow even in resting condition. Two different workloads, 50 and 75W, were applied and subsequently analysed. A closed-loop technique was applied to address these purposes.

3.2 Materials and methods

Subjects

We matched ten hypertensive male patients (HP) with ten healthy male controls (CTRL) (Table 3.1). HP were 29 ± 8 years old, 81 ± 9 kg heavy and 178 ± 5 cm tall. CTRL were 27 ± 7 years old, 77 ± 9 kg heavy and 179 ± 7 cm tall.

Inclusion criteria were: age ≥ 18 y and ≤ 40 y, presence of essential hypertension of grades I and II, absence of anti-hypertensive treatment. Hypertension was defined by a sustained office blood pressure ≥ 140/90 mmHg or daytime ambulatory blood pressure monitoring values ≥ 135/85 mmHg. Essential hypertension was defined by high blood pressure values with no evident cause of primary renal damage, no renovascular hypertension (renal IRM or Renal US Doppler disclosing any renal artery stenosis) and no endocrinologic cause. Primary aldosteronism was excluded by plasma renin activity and aldosterone levels and adrenal computed tomography scans. Pheochromocytoma or paraganglioma were excluded by plasma and/or urine meta-normetanephrines levels. Cushing syndrome was ruled out by 24h urine cortisol evaluation, or by night time salivary cortisol dosage. A sleep apnoea syndrome was checked. Drugs ingestion was searched: oral contraceptives, anti-inflammatory drugs, and others. In case of patients with essential hypertension of grade I, the possibility of interrupting treatment for one week prior to the performance of tests was be considered. Exclusion criteria included: heart failure of any stage, renal failure (eGFR ≤ 60ml/min/1.73 m^2), severe exercise asthma, history of myocardial infarct, stroke or any condition that the investigator estimated incompatible with the protocol.

Control subjects were recruited *in primis* among the medical students of the Faculty of Medicine and the staff of the University Hospital of Geneva, by means of leaflets and advertisements,

and they were age-matched with hypertensive patients. They should be in apparent good health, had normal blood pressure values, and not be taking any drugs or oral contraceptives.

All subjects were preliminarily informed about all procedures and risks associated with the experiments. Signed informed consent was obtained from each volunteer, who was aware of the right of withdrawing from the study at any time without jeopardy. The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the institutional ethical committee.

	Subjects	Weight (Kg)	Height (cm)	Age (years)	BMI (Kg m^2)
Controls	**AF**	79.5	177	20	25.4
	CB	69	181	23	21.1
	DB	97	184	41	28.7
	FB	65	179	22	20.3
	FR	68.5	175	38	22.4
	MD	73.1	185	28	21.4
	NE	75	175	25	24.5
	NF	84	192	35	22.8
	TF	73	182	31	22.0
	YD	82.5	181	25	25.2
	Mean ± SD	**77 ± 9**	**180 ± 7**	**27 ± 7**	**23.4 ± 2.5**
Hypertensive Patients	**AGM**	94.4	172	36	31.9
	AR	93.5	168	43	33.1
	BA	82	183	33	24.5
	CG	79	179	34	24.7
	DA	77	175	23	25.1
	GG	75.5	178	21	23.8
	NM	67.7	174	22	22.4
	RB	82	177	23	26.2
	TB	68.5	188	34	19.4
	TM	88.2	184	19	26.1
	Mean ± SD	**81 ± 9**	**178 ± 5**	**29 ± 8**	**25.7 ± 4.1**

Table 3.1 demographic characteristics of the investigated population. No differences were observed between controls and hypertensive patients. BMI: body max index.

Measurements

HR was continuously measured by electrocardiography (Polar, Finland, included in the metabolic cart). Continuous monitoring of arterial pulse pressure profile was obtained at the middle phalange of a left-arm finger by means of a non-invasive cuff pressure recorder

(Portapres®, TNO-TPD, Amsterdam, The Netherlands). Systolic and diastolic blood arterial pressures were identified at each beat. Beat-by-beat MAP was computed as the integral mean of each pressure profile, using the Beatscope™ software package (FMS, Amsterdam, The Netherlands).

All the signals were digitalized in parallel by a 16-channel A/D converter (MP150, Biopac Systems, Goleta CA, USA) and stored on a computer. The acquisition rate was 100 Hz.

Protocol

Experiments were carried out in Geneva, Switzerland, in an air-conditioned room at 23-24°C, with relative humidity between 60 and 65%.

In both postures, exercise was performed on an electrically-braked cycle ergometer (Ergoselect 400, Ergoline GmbH, Bitz, Germany). The subjects pedalled at a spontaneously selected frequency comprised between 60 and 70 rpm; they maintained their own selected frequency throughout the study. Caution was taken in order to avoid pre-exercise stress. After rest monitoring, participants received the following communication "We are ready: you can start whenever you want". If the participant delayed the exercise onset by more than ten seconds after communication, the procedure was interrupted and started again after further two min at rest. During off-line data processing, the exact time of exercise start was detected from the pedalling frequency recording.

In each session, the investigated parameters were determined at rest and during steady state of submaximal dynamic leg exercises. Investigated workloads were 50 and 75 W, administered in two different test, in random order. The duration of each workload was 8-to-10 min, recordings were continued during the first six minutes of recovery.

The protocol was performed twice, once in upright and once in supine position, administered in random order.

Data treatment

At rest and exercise steady states, we computed the spontaneous baroreflex gain (BRS) by means of the sequence method (Bertinieri et al., 1988), using MAP as independent variable. Briefly, sequences of three or more consecutive beats in which MAP and HR changed in the opposite direction, were identified. A phase shift of one beat was introduced between the MAP and the HR values of each sequence, as in previous studies (Bertinieri et al., 1988). Within each

individual sequence, the HR versus MAP relationship was analysed by linear regression, to compute the slope and the corresponding coefficient of determination. Only slopes showing R^2 values higher than 0.85 were retained (Iellamo et al., 1994, 1997). For each subject, the mean slope of the HR versus MAP relationships was then computed and taken as a measure of individual BRS, at rest and at exercise, respectively. Baroreflex sequences were searched during the 100 seconds of quiet rest (from -110 to -10 s); at exercise, baroreflex sequences were identified during the last 100 seconds of exercise. In each subject and condition, the number of analysed sequences ranged between 0 and 44.

Assuming closed-loop approach, the relationship between HR and MAP in the early phase of exercise was constructed. The segment of this relationship between the exercise onset and the attainment of MAPmin was linear and had a negative slope, being characterised by opposite variations of MAP and HR. The resulting slopes were compared between at rest and at exercise steady state by means of the sequence method.

Statistics

Data are given as mean and standard deviation (SD). One way ANOVA was performed between the BRS values in steady state conditions (rest, 50 and 75 W); the slopes of the linear segment of the HR vs MAP relationships at the beginning of exercise were compared with BRS, respectively, at rest and exercise, using two-way ANOVA for repeated measures, for condition (BRS at rest, slope of the linear segment after exercise start, and BRS at exercise steady state) and posture (upright versus supine), with Tukey post-hoc test. The BRS at the beginning of exercise was compared using one-way ANOVA for repeated measures, for posture (upright versus supine), with Tukey post-hoc test. The differences were considered significant if $p < 0.05$. The Stata 10.0 statistical software (StataCorp, College Station, TX, USA) was used to this aim.

3.3 Results

No differences were found between the two groups concerning age, weight, height, and body max index (Table 3.1).

BRS b min^{-1} mmHg^{-1}	CONTROLS																			
	REST				START 50W				START 75W				EXERCISE 50W				EXERCISE 75W			
	Supine		Upright		Supine		Upright		Supine		Upright		Supine		Upright		Supine		Upright	
	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.
AF	-1.23 ± 0.52	4	-1.06 ± 0.51	9			-0.53		-0.60		-0.40		-0.53 ± 0.19	2	-0.81 ± 0.44	11	-0.81 ± 0.46	10	-1.43 ± 0.08	4
CB	-1.64 ± 0.86	2	-1.67 ± 0.38	23	-1.29		-0.84				-1.00		-1.38 ± 0.42	10	-1.14 ± 0.61	13	-0.97 ± 0.46	12	-0.9 ± 0.41	20
DB	-1.1 ± 0.98	4	-0.93 ± 0.36	16	-0.50		-0.72		-1.50		-0.71		-1.31 ± 0.77	5	-1.39 ± 0.58	11	-1.24 ± 0.47	11	-1.09 ± 0.39	24
FB	-1.26 ± 0.46	10	-1.09 ± 0.59	11	-0.67		-0.64		-0.86		-0.57		-1.26 ± 0.48	6	-0.88 ± 0.45	10	-1.07 ± 0.39	12	-1 ± 0.6	10
FR	-1.48 ± 0.48	10	-1.36 ± 0.62	17			-0.79		-1.61		-0.38		-1.28 ± 0.36	18	-1.55 ± 0.49	18	-1.35 ± 0.47	10	-0.85 ± 0.35	17
MD	-1.59 ± 0.62	4	-1.22 ± 0.46	44	-1.25				-0.93				-0.72 ± 0.37	15	-0.51 ± 0.3	9	-0.79 ± 0.34	11	-0.68 ± 0.34	6
NE	-1.38 ± 0.47	17	-1.22 ± 0.52	32	-1.29		-0.71		-0.61		-0.52		-1.38 ± 0.42	15	-1.05 ± 0.49	17	-0.97 ± 0.5	16	-0.9 ± 0.37	8
NF	-1.62 ± 0.47	20	-1.22 ± 0.51	27			-0.80				-0.71		-1.24 ± 0.45	16	-1.04 ± 0.45	27	-1.09 ± 0.35	15	-0.77 ± 0.33	27
TF	-1.43 ± 0.58	6	-1.1 ± 0.54	26	-0.76				-1.37				-1.72 ± 0.57	7	-0.98 ± 0.49	11	-1 ± 0.38	4	-0.89 ± 0.34	9
YD	-1.57 ± 0	1	-1.27 ± 0.68	25			-1.20		-1.93		-1.10		-1.39 ± 0.36	6	-1.4 ± 0.38	20	-0.78 ± 0.41	19	-1.01 ± 0.57	14
MEAN ± SD	**-1.43 ± 0.19**	**78**	**-1.22 ± 0.2**	**230**	**-0.96 ± 0.35**		**-0.78 ± 0.2**		**-1.17 ± 0.49**		**-0.67 ± 0.26**		**-1.22 ± 0.34**	**100**	**-1.07 ± 0.31**	**147**	**-1.01 ± 0.19**	**120**	**-0.95 ± 0.21**	**139**
	HYPERTENSIVE PATIENTS																			
BRS b min^{-1} mmHg^{-1}	REST				START 50W				START 75W				EXERCISE 50W				EXERCISE 75W			
	Supine		Upright		Supine		Upright		Supine		Upright		Supine		Upright		Supine		Upright	
	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.	BRS	obs.
AGM	-1.06 ± 0.49	6	-1.78 ± 0.48	11	-0.67		-0.50		-1.50				-1.47 ± 0.51	12					-1.3 ± 0.43	7
AR	-0.52 ± 0	1	-0.7 ± 0.25	22	-0.36		-0.28				-0.37		-0.93 ± 0	1	-1.09 ± 0.66	5	-0.86 ± 0.19	3	-0.71 ± 0.38	7
BA	-1.39 ± 0.38	23	-0.8 ± 0.29	34			-0.38				-0.39		-0.49 ± 0.18	11	-0.41 ± 0.13	9	-0.62 ± 0.14	3	-0.47 ± 0.44	11
CG	-1.36 ± 0.58	19	-1.03 ± 0.53	23	-0.64						-0.33		-0.51 ± 0.19	7	-0.56 ± 0.17	13	-0.81 ± 0.44	14	-0.77 ± 0.58	14
DA	-0.85 ± 0.44	11	-1.28 ± 0.47	30	-0.50		-0.80				-0.67		-1.02 ± 0.44	17	-1.07 ± 0.47	18	-0.97 ± 0.44	12	-0.52 ± 0.17	9
GG	-1.38 ± 0.09	4	-1.04 ± 0.42	23			-0.32				-0.45		-0.94 ± 0.36	6	-0.72 ± 0.42	24	-1.12 ± 0.35	5	-0.64 ± 0.36	16
NM	-1.14 ± 0.36	22	-0.85 ± 0.42	21	-0.64		-0.64				-1.00		-1.2 ± 0.67	6	-0.57 ± 0.31	15	-0.68 ± 0.39	8	-0.66 ± 0.2	5
RB					-0.67		-0.57		-0.75		-0.75		-1.43 ± 0.54	11	-1.21 ± 0.52	17	-1.53 ± 0.44	23		
TB	-1.6 ± 0.46	7	-1.1 ± 0.39	22			-0.57				-0.28		-0.9 ± 0.47	12	-0.74 ± 0.45	10	-0.72 ± 0.35	9	-0.94 ± 0.48	18
TM	-1.1 ± 0.39	27	-1.1 ± 0.58	37	-0.68		-0.47		-0.69		-0.56		-0.93 ± 0.37	19	-1.09 ± 0.5	26	-1.07 ± 0.54	18	-0.81 ± 0.4	26
MEAN ± SD	**-1.16 ± 0.33**	**120**	**-0.99 ± 0.19**	**223**	**-0.77 ± 0.51**		**-0.5 ± 0.16**		**-0.98 ± 0.45**		**-0.53 ± 0.23**		**-0.98 ± 0.33**	**102**	**-0.91 ± 0.36**	**137**	**-0.91 ± 0.22**	**95**	**-0.81 ± 0.29**	**113**

Table 3.2 Heart rate (HR) vs mean arterial pressure (MAP) relationship for controls and hypertensive patients. Data are presented as means ± SD. Obs.: number of individual sequences detected and from which the regression was calculated and used to compute the individual slope.

The computed mean slopes for each subject are provided in table 3.2. The table reports all the values in upright and supine position, before the onset of exercise during steady state (REST), during the transients at 50 and 75 W (START 50 and 75 W), and during steady state at exercise (EXERCISE 50 and 75 W). During transients, it was possible to identify just one sequence for each subject, or in some cases no one. The total number of sequences found in the two groups was 844 for controls and 818 for patients. Table 3.3 reports the averages and the differences among the investigated conditions.

Slopes (b min^{-1} mmHg^{-1})		**CONTROLS**		**PATIENTS**		
		MEAN ± SD	***Supine Vs Upright***	**MEAN ± SD**	***Supine Vs Upright***	***CTRL Vs PTS***
REST	**Sup**	-1.43 ± 0.19	***p = 0.001***	-1.16 ± 0.33	*p > 0.05*	***p = 0.04***
	Up	-1.22 ± 0.2		-0.99 ± 0.19		***p = 0.03***
START 50W	**Sup**	-0.96 ± 0.35*	*p > 0.05*	-0.77 ± 0.51	*p > 0.05*	***p = 0.03***
	Up	-0.78 ± 0.2*		-0.5 ± 0.16*$^{\#}$		***p = 0.01***
START 75W	**Sup**	-1.17 ± 0.49	***p = 0.024***	-0.98 ± 0.45	***p = 0.044***	*p > 0.05*
	Up	-0.67 ± 0.26*		-0.53 ± 0.23*$^{\#}$		*p > 0.05*
EXERCISE 50W	**Sup**	-1.22 ± 0.34	*p > 0.05*	-0.98 ± 0.33	*p > 0.05*	*p > 0.05*
	Up	-1.07 ± 0.31*		-0.91 ± 0.36		*p > 0.05*
EXERCISE 75W	**Sup**	-1.01 ± 0.19*	*p > 0.05*	-0.91 ± 0.22	*p > 0.05*	*p > 0.05*
	Up	-0.95 ± 0.21		-0.81 ± 0.29		*p > 0.05*

Table 3.3 Average of slopes before the exercise onset (REST), at the beginning of 50 W and 75 W exercises (START 50W, and START 75W), after resetting during steady state at 50 W and 75 W exercises (EXERCISE 50W, and EXERCISE 75W).The p values between position (supine versus upright) and between controls and patients are presented. Sup: supine. Up: upright. * $p < 0.05$ compared to REST. $^{\#}$ $p < 0.05$ compared to EXERCISE 50W.

HP versus CTRL. It is noteworthy that there is a trend showing flatter slopes in hypertensive patients than in controls. BRS were lower ($p<0.05$) in resting condition before the exercise onset, for either supine, -1.43 ± 0.19 and -1.16 ± 0.33 b min^{-1} mmHg^{-1} (for CTRL and HP, respectively) and upright positions, -1.22 ± 0.20 and -0.99 ± 0.19 b min^{-1} mmHg^{-1} (for CTRL and HP, respectively). The HR vs MAP relationship during the 50 W transient resulted different between patients and controls, in both positions (supine, -0.96 ± 0.35 and -0.77 ± 0.51 b min^{-1} mmHg^{-1} for CTRL and HP, respectively; and upright, -0.78 ± 0.20 and -0.50 ± 0.16 b min^{-1} mmHg^{-1}, for CTRL and HP, respectively). In steady state during exercises, there was a trend, although not significant, with lower BRS for patients compared to healthy volunteers.

Supine versus upright. In HP, the slopes did not differ between positions, although there was a tendency with lower values in upright than in supine position (NS). In CRTL, the slopes were lower ($p < 0.01$) at REST in upright than supine, -1.43 ± 0.19 b min^{-1} $mmHg^{-1}$ and -1.22 ± 0.2 b min^{-1} $mmHg^{-1}$, respectively. Also during the 75 W transient the slopes were lower ($p < 0.05$) in upright than supine, -1.17 ± 0.49 b min^{-1} $mmHg^{-1}$ and -0.67 ± 0.26 b min^{-1} $mmHg^{-1}$, respectively.

Within the two groups, ANOVA was used to check variations between REST, transitions and steady state during exercises.
The slopes in upright position resulted steeper at REST ($p < 0.05$) compared to transients at 50 W and 75 W, in both CTRL and HP.
In supine position, no significant differences were find within the HP group. In CTRL, BRS was higher ($p < 0.05$) at rest than during the steady state at 75 W (-1.43 ± 0.19 b min^{-1} $mmHg^{-1}$ and -1.01 ± 0.19 b min^{-1} $mmHg^{-1}$, respectively), and during the 50 W transient the provided slope was lower ($p < 0.05$) compared to REST.

Between the two workloads, it was possible to notice a minimal decrement, however not significant, in the BRS during steady state at 75 W exercise, compared to 50 W exercise. The slopes during transients were unaffected by workloads, with a trend characterised by lower values in supine than upright position (NS).

3.4 Discussion

The analysis of the baroreflex sensitivity in hypertensive patients and in healthy controls, showed in Figure 3.2, demonstrates that: i) patients had a lower baroreflex gain than controls at rest, and flatter HR vs MAP slopes at the beginning of light exercise; ii) between position the differences in slopes are more pronounced in controls than in patients; iii) during exercise the baroreflex is lower than at rest in healthy controls and no differences were found in patients; iv) in supine position during the transient at 75 W the baroreflex response was absent in the majority of HP.

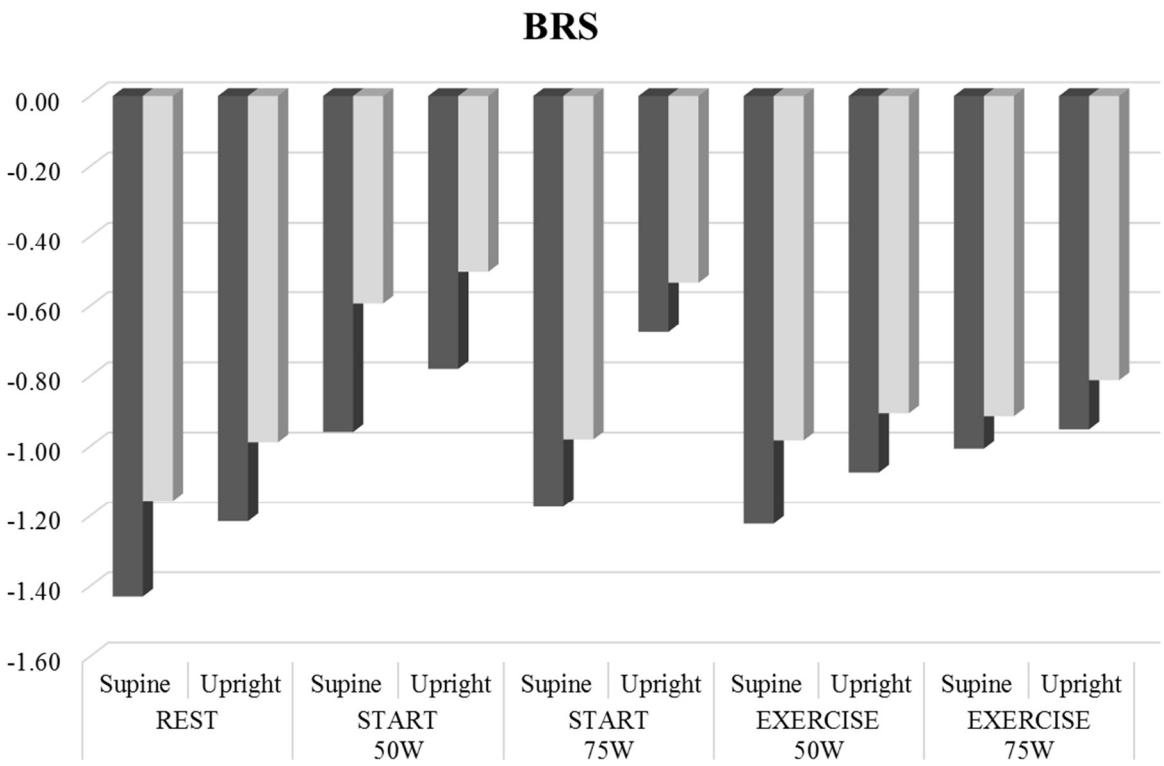

Figure 3.2 Slopes of the heart rate versus mean arterial pressure relationship (in b min^{-1} $mmHg^{-1}$) in controls (dark grey) and hypertensive patients (light grey), before beginning the exercise (REST), during the transient (START 50 and 75 W), and at 50 and 75 W steady state (EXERCISE 50 and 75 W)

These data show a trend characterised by a reduced baroreflex sensitivity in all conditions with sympathetic hyperactivity: hypertension versus control, exercise versus rest, and upright versus supine. In hypertension, the baroreceptors reset to counteract the higher level of blood pressure (Mancia and Mark, 2011). These results confirm previous findings. First, we found flatter slopes during exercise than at rest in controls, as already shown by Bringard et al. (2017), with a difference between postures (Bringard et al., 2017; Kardos et al., 2001; Schwartz et al., 2013). This is not so in hypertensive patients, in whom it was not possible to demonstrate differences between positions, probably due to a sympathetic overactivity which affects the cardiovascular responses: the higher sympathetic outflow is able to modify the cardiovascular response in supine position, which resulted greater than in controls (lower BRS values). Moreover, flatter HR vs MAP slopes were found in the 50 W transient compared to rest in controls in supine posture (as already stated by Bringard et al., 2017) and this difference was absent in hypertensive patients. Finally, it is interesting to note that only three hypertensive patients showed the characteristic baroreflex response found in almost all the controls at the beginning of 75 W exercise: this can be considered as another sign of sympathetic overactivity which

preclude the normal drop in MAP that is demonstrated at the beginning of exercise as a consequence of the sudden drastic fall in TPR (Elstad et al., 2009; Faisal et al., 2010; Lador et al., 2006, 2008; Wieling et al., 1996).

Mancia et al. (1978) applied the open-loop approach in the early mild stages of hypertension and demonstrated that arterial baroreflex control of parasympathetic outflow and heart rate were impaired, whereas arterial baroreflex control of sympathetic vasoconstrictor outflow to the peripheral circulation, and thus blood pressure, seemed to be preserved. Our results showed an impairment of the baroreflex responses in HP, characterised by a reduced response in HR following blood pressure modifications, compared to CTRL.

It was proposed that the resetting of baroreflex in mild hypertension might be the result of different components: i) stiff vessels reducing the mechanical stimulation of baroreceptor nerve endings; ii) altered central processing; or iii) impaired efferent parasympathetic and sympathetic pathways (Victor, 2015). Regardless the causes of impairment in arterial baroreflex, patients affected by hypertension are characterised by altered HR response to variation in arterial blood pressure (Figure 3.3).

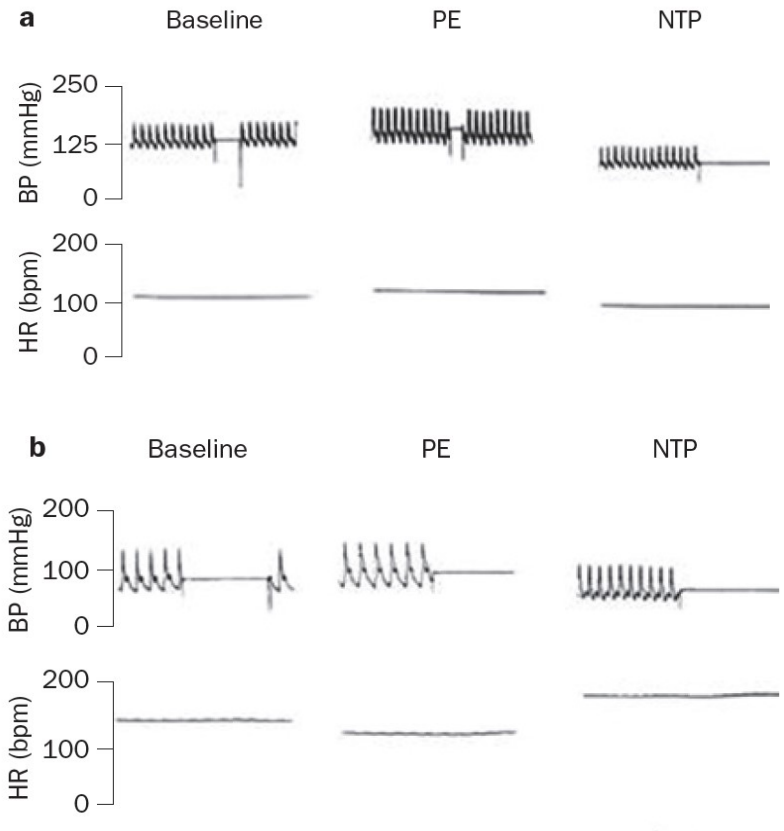

Figure 3.3 Arterial baroreflex failure. Patient with baroreflex failure (panel a): increasing or decreasing arterial blood pressure with intravenous phenylephrine or nitroprusside had no effect on heart rate (HR). Panel b shows normal baroreflex responses in a healthy control individual. (Aksamit et al., 1987).

The reduction of stiffness of the vessels, and thus the lower mechanical baroreceptor stimulation, might be one of the mechanism underpinning the baroreflex impairment. To overcome this dysfunction, the electrical baroreceptor pacing has been proposed as an opportunity for resistant arterial hypertension to bypass the possible mechanical–electrical coupling by driving the baroreceptor axons directly with an electrical pacemaker. Heusser and colleagues (2010) enrolled 11 patients affected by treatment-resistant arterial hypertension and implanted a carotid stimulator. As soon as the stimulators was switched on, an immediate sharp decrease in muscle sympathetic activity took place, followed by a decrease in the arterial blood pressure (Figure 3.4), even though they did not find any differences in the BRS, computed by the sequence technique, during the stimulation of carotid sinus compared to before.

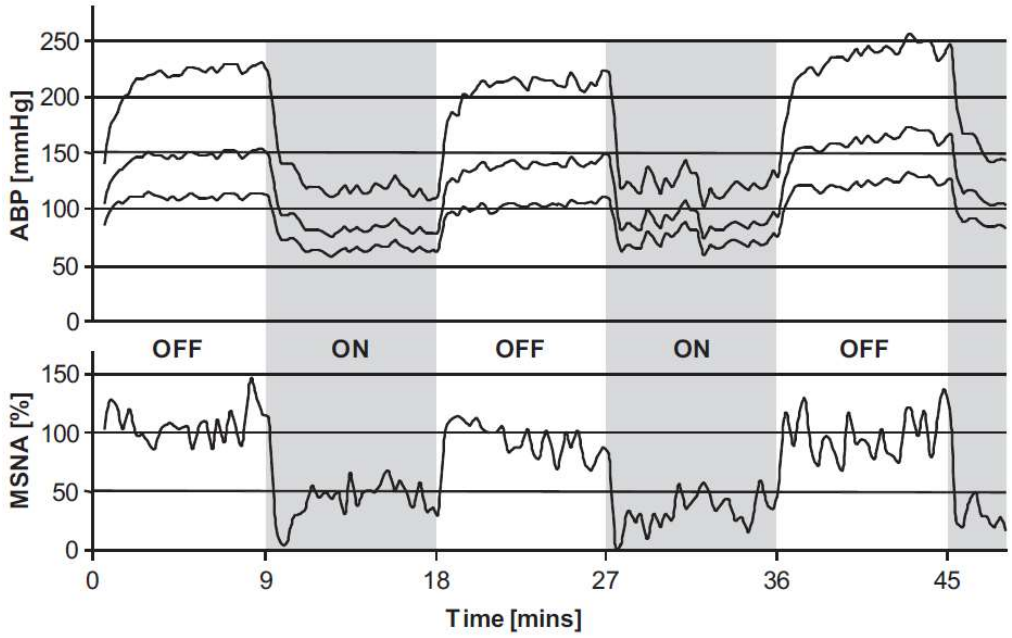

Figure 3.4 Time course of arterial blood pressure (ABP) and MSNA (Muscular Sympathetic Nerve Activity) during stimulation of carotid sinus. Each time the stimulator was switched on, ABP and MSNA decreased acutely and remained suppressed throughout the stimulation period.

Notwithstanding, technical difficulties with electrode implantation, adverse effects related to nerve injury, and the amelioration in antihypertensive drug precluded the development and implementation of these devices into daily practice. We cannot exclude that this baroreflex resetting in hypertensive patients is responsible of the modification of the baroreflex responses described in this study.

Baroreflex sensitivity can be considered as a mirror of the autonomic output: knowledge of a patient's autonomic status improves the discrimination of the cardiovascular risk. La Rovere et al. (1998, 2013) have demonstrated that a low BRS implies a less favourable prognosis, e.g. after myocardial infarction and in heart failure, and BRS measurement may be clinically useful to assess the efficacy of interventions that are meant to increase BRS (e.g., exercise training of heart failure patients). These authors computed BRS by means of the relationship between RR interval and SAP, showing correlation between lower variability in HR and higher mortality risk. In our report, we found lower BRS in patients affected by mild hypertension, at rest and during exercise. During the exercise transients, the HR vs MAP slope is reduced also in healthy subject (Bringard et al., 2017), nevertheless in HP at 50 W was even lower. These data support the notion that HP are affected by baroreflex impairment at rest and also during exercise, confirming the imbalance of the autonomic system even for the mild form of hypertension.

Several limitations deserve discussion. Closed-loop approach provide BRS for few beats, frequently triplets, usually fewer beats compared to the open-loop technique, since it is not possible to construct the entire baroreflex curve during steady-state with the former approach. The BRS is computed around the operating point, which is, at rest, close to the centring point of the baroreflex cure, point where there is the maximal gain of the baroreflex function. During exercise the upward and rightward shift of this curve displaces the operating point toward the flat part of the baroreflex curve (Raven et al., 2006), close to the baroreflex threshold for the severe exercise domain. Thus, these data should be interpreted with caution. However, it is noteworthy that there is a trend characterised by lower BRS values for hypertensive patients compared to healthy subjects in all the investigated conditions, and the absence of statistical significance in some condition might be the consequence of the low number of subject enrolled. Moreover, we recruited patients with a low grade of hypertension, which probably present a mild impairment of the autonomic nervous system function, compared to the more severe degrees of hypertension.

CONCLUSIONS

The two branches of the autonomic nervous system have been extensively investigated, and the spontaneous baroreflex analysis is one of the methods commonly used as a measure of the autonomic functions. Since their discovery, baroreflexes aroused considerable interest, and speculation on their dysfunction was suggested in the genesis of hypertension and in other cardiovascular diseases.

Despite the importance of this topic, there are scanty and conflicting data concerning the baroreflex activity and the cardiovascular regulation at exercise onset in health and disease.

The resulting data aim at shedding light on some possible mechanisms underpinning the baroreflex resetting and the MAP recovery at exercise onset in healthy volunteers and the baroreflex activity in hypertensive patients. The two main theories concerning the trigger point of the baroreflex resetting, the central command theory and the metaboreflex one, have been discussed since long time. Few elements discussed in this thesis can hardly be accommodated in the central command theory. First (see discussion in chapter 1), the increased HR at the first beat at the beginning of exercise, since the other cardiovascular parameters stayed invariant, might be more compatibly with the central command theory than with the exercise pressor reflex theory. At the same time, at the exercise onset a baroreflex response counteract a sudden drop in MAP; during this transient the relationship between HR and MAP resulted superimposable to the BRS at exercise and lower compared to BRS in resting condition before exercise: this change in slope might be attributed to the sudden vagal withdrawal, and compatibly more with the central command theory. This theory may explain the sudden change in baroreflex gain at the beginning of exercise, but it does not explain the resetting process, which takes place only after the attainment of MAPmin, thus after few seconds from the beginning of exercise.

Yet baroreflex resetting may well be mediated by neural mechanisms (Raven et al., 2006), and it is possible that activation of the sympathetic efferent branch of the autonomic nervous system plays a role in the phase of the exercise transient after attainment MAPmin (Lador et al., 2006), thereby explaining the further HR and SV increases after phase I and the ensuing recovery of MAP.

Moreover, the resetting process was achieved within one minute upright, but not supine. This indicate i) faster cardiovascular adaptation during exercise in upright than supine, as already

reported (Leyk et al., 1994; Loeppky et al., 1981), and ii) the resetting process could take more than one minute of exercise, compatibly with a possible role of sympathetic stimulation of the sinus node during exercise (Fagraeus and Linnarsson, 1976; Orizio et al. 1988).

During apnoeas (see chapter 2) the baroreflex sensitivity was characterised by lower slopes at the beginning of breath-hold compared to before starting apnoeas. BRS restored during the second steady phase. The HR vs MAP slopes reported in φ1 resulted similar to the BRS computed during steady state at exercise. This can be attributable to a possible vagal withdrawal at the beginning of apnoea, and then to a sympathetic activation, which can be responsible of the resetting of the operating point revealed during φ1. This resetting is similar to what occur during exercise, when the operating points shift upward and rightward after the exercise onset; nevertheless the phenomena is quite different since the new operating points during exercises are reached in at least one minute, whereas during apnoeas these dynamic changes occurred during φ1, thus in the first 30 seconds after starting an apnoeas. At the end of φ1, BRS returned to the previous values, being similar to the values computed at rest before starting the apnoea.

It would be a further hypothesis to investigate the cardiovascular responses in hypertensive patients during apnoeas, to verify if the dynamic phase at the beginning of apnoeas is still present in this group of subjects or if their cardiovascular responses are modified.

The last study confirms a reduced baroreflex gain in hypertensive patients compared to healthy subject, at rest and during the transient at 50 W, in agreement with previous findings (Bristow et al., 1969; Head, 1995; Korner et al., 1974; Mancia et al., 1978). It is noteworthy that the baroreflex sensitivity, in both healthy and hypertensive subjects, changed immediately at the exercise onset (confirming the results in chapter 1), in both positions, and remained unchanged during the steady state, either at 50 and 75 W. It is possible to state that the baroreflex resetting acted in the same manner in healthy and in the hypertensive patients, but with a reduced gain in the latter compared to the former.

The application of the closed-loop approach as a tool to analyse BRS in several conditions, such as rest, exercise, apnoea and also in pathologies (hypertension, orthostatic intolerance, dysautonomic diseases…), can be safely performed. It is a non-invasive technique that can provide interesting information about the autonomic nervous system activity. Further investigations about BRS analysis could be a useful tool to assess improvements after rehabilitation in neurological as well as in cardiorespiratory diseases, or after prolonged bed rest, in healthy volunteers and in patients after prolonged hospital stay. The application of this

technique might be used to monitor the efficacy of the undertaken treatment, whether behavioural or pharmacological. The modification in BRS might be considered as a mirror of the modification of the output of the autonomic system, in health and disease.

BIBLIOGRAPHY

Adami A, Pizzinelli P, Bringard A, Capelli C, Malacarne M, Lucini D., Simunič B, Pišot R, Ferretti G (2013). Cardiovascular re-adjustments and baroreflex response during clinical reambulation procedure at the end of 35-day bed rest in humans. Appl Physiol Nutr Metab. 38: 673-680.

Aksamit TR, Floras JS, Victor RG, Aylward PE (1987). Paroxysmal hypertension due to sinoaortic baroreceptor denervation in humans. Hypertension. 9: 309-314.

Azabji Kenfack M, Lador F, Licker M, Moia C, Tam E, Capelli C, Morel D, Ferretti G (2004). Cardiac output by Modelflow method from intra-arterial and fingertip pulse pressure profiles. Clin Sci Lond. 106: 365-369.

Barker RC, Hopkins SR, Kellogg N, Olfert IM, Brutsaert TD, Gavin TP, Entin PL, Rice AJ, Wagner PD (1999). Measurement of cardiac output during exercise by open-circuit acetylene uptake. J Appl Physiol. 87: 1506-1512.

Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G (1988). Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. Am J Physiol Heart Circ Physiol. 254: H377-H383.

Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G (1985). A new approach to analysis of the arterial baroreflex. J Hypertens. Suppl. 3: S79-S81.

Bringard A, Adami A, Fagoni N, Lador F, Moia C, Tam E, Ferretti G (2017). Dynamics of the RR-interval versus blood pressure relationship at exercise onset in humans. Eur J Appl Physiol. Epub. doi: 10.1007/s00421-017-3564-6.

Bristow JD, Honour AJ, Pickering, GW, Sleight P, Smyth HS (1969). Diminished baroreflex sensitivity in high blood pressure. Circulation. 39: 48-54.

Bronk DW, Stella G (1935). The response to steady pressures of single end organs in the isolated carotid sinus. Am J Physiol. 110: 708-714.

Bruno RM, Ghiadoni L, Seravalle G, Dell'oro R, Taddei S, Grassi G (2012). Sympathetic regulation of vascular function in health and disease. Front Physiol. 3: 284.

Burke D, Sundlöf G, Wallin G (1977). Postural effects on muscle nerve sympathetic activity in man. J Physiol. 272: 399-414.

Chapleau MW, Cunningham JT, Sullivan MJ, Wachtel RE, Abboud FM(1995). Structural versus functional modulation of the arterial baroreflex. Hypertension. 26, 341-347.

Chin LMK, Heigenhauser GJF, Paterson DH, Kowalchuk JM (2010) Pulmonary O_2 uptake and leg blood flow kinetics during moderate exercise are slowed by hyperventilation-induced hypocapnic alkalosis. J Appl Physiol. 108: 1641-1650.

Clifford PS (2007). Skeletal muscle vasodilatation at the onset of exercise. J Physiol. 583: 825-833.

Cooke WH, Hoag JB, Crossman AA, Kuusela TA, Tahvanainen KU, Eckberg DL (1999). Human responses to upright tilt: a window on central autonomic integration. J Physiol. 517: 617-628.

Cooke WH, Ludwig DA, Hogg PS, Eckberg DL, Convertino VA (2002). Does the menstrual cycle influence the sensitivity of vagally mediated baroreflexes? Clin Sci Lond. 102: 639-644.

Costalat G, Pichon A, Joulia F, Lemaître F (2015). Modeling the diving bradycardia: Toward an "oxygen-conserving breaking point"? Eur J Appl Physiol. 115: 1475-1484.

Degtyarenko AM, Kaufman MP (2006). Barosensory cells in the nucleus tractus solitarius receive convergent input from group III muscle afferents and central command. Neuroscience. 140: 1041-1050.

DeLorey DS, Kowalchuk JM, Paterson DH (2003). Relationship between pulmonary O_2 uptake kinetics and muscle deoxygenation during moderate-intensity exercise. J Appl Physiol. 95: 113-120.

DiBona GF, Esler M (2010). Translational medicine: the antihypertensive effect of renal denervation. Am J Physiol. 298: R245-R253.

DiCarlo SE, Bishop VS (2001) Central baroreflex resetting as a means of increasing and decreasing sympathetic outflow and arterial pressure. Ann N Y Acad Sci. 940: 324-337.

Eckberg DL, Sleight P (1992). Human Baroreflexes in Health and Disease. Oxford: Clarendon Press

Elstad M, Nådland IH, Toska K, Walløe L (2009). Stroke volume decreases during mild dynamic and static exercise in supine humans. Acta Physiol Oxf. 195: 289-300.

Esler M (2011). The sympathetic nervous system through the ages: from Thomas Willis to resistant hypertension. Exp Physiol. 96: 611-622.

Fadel PJ, Ogoh S, Watenpaugh DE, Wasmund W, Olivencia-Yurvati A, Smith ML, Raven PB (2001). Carotid baroreflex regulation of sympathetic nerve activity during dynamic exercise in humans. Am J Physiol Heart Circ Physiol. 280: H1383–H1390.

Fadel PJ, Raven PB (2012). Human investigations into the arterial and cardiopulmonary baroreflexes during exercise. Exp Physiol. 97: 39-50.

Fagoni N, Breenfeldt Andersen A, Oberholzer L, Haider T, Meinild Lundby AK, Lundby C (2017a). Reliability and validity of non-invasive determined haemoglobin mass and blood volumes. Clin Physiol Funct Imaging. Epub. doi: 10.1111/cpf.12406.

Fagoni N, Sivieri A, Antonutto G, Moia C, Taboni A, Bringard A, Ferretti G (2015). Cardiovascular responses to dry resting apnoeas in elite divers while breathing pure oxygen. Respir Physiol Neurobiol. 219: 1-8.

Fagoni N, Taboni A, Vinetti G, Bottarelli S, Moia C, Bringard A, Ferretti G (2017b). Alveolar gas composition during maximal and interrupted apnoeas in ambient air and pure oxygen. Respir Physiol Neurobiol. 235: 45-51.

Fagraeus L, Linnarsson D (1976) Autonomic origin of heart rate fluctuations at the onset of muscular exercise. J Appl Physiol 40:679-682.

Faisal A, Beavers KR, Hughson RL (2010). O_2 uptake and blood pressure regulation at the onset of exercise: interaction of circadian rhythm and priming exercise. Am J Physiol Heart Circ Physiol. 299: H1832-H1842.

Ferretti G, Binzoni T, Hulo N, Kayser B, Thomet JM, Cerretelli P (1995). Kinetics of oxygen consumption during maximal exercise at different muscle temperatures. Respir Physiol. 102: 261-268.

Ferretti G, Iellamo F, Pizzinelli P, Azabji Kenfac, M, Lador F, Lucini D, Porta A, Narkiewicz K, Pagani M (2009). Prolonged head down bed rest-induced inactivity impairs tonic autonomic regulation while sparing oscillatory cardiovascular rhythms in healthy humans. J Hypertension. 27: 551-561.

Fisher JP, Young CN, Fadel PJ (2008). Effect of muscle metaboreflex activation on carotid-cardiac baroreflex function in humans. Am J Physiol Heart Circ Physiol. 294: H2296-H2304.

Fisher JP, Young CN, Fadel PJ (2015). Autonomic adjustments to exercise in humans. Compr Physiol. 5: 475-512.

Fontil V, Pletcher MJ, Khanna R, Guzman D, Victor R, Bibbins-Domingo K (2014). Physician underutilization of effective medications for resistant hypertension at office visits in the United States: NAMCS 2006-2010. J Gen Intern Med. 29: 468-476.

Gallagher KM, Fadel PJ, Smith SA, Strømstad M, Ide K, Secher NH, Raven PB (2006). The interaction of central command and the exercise pressor reflex in mediating baroreflex resetting during exercise in humans. Exp Physiol. 91: 79-87.

Gallagher KM, Fadel PJ, Strømstad M, Ide K, Smith SA, Querry RG, Raven PB, Secher NH (2001a). Effects of exercise pressor reflex activation on carotid baroreflex function during exercise in humans. J Physiol. 533: 871-880.

Gallagher KM, Fadel PJ, Strømstad M, Ide K, Smith SA, Querry RG, Raven PB, Secher NH (2001b). Effects of partial neuromuscular blockade on carotid baroreflex function during exercise in humans. J Physiol. 533: 861-870.

Grimm DR, Almenoff PL, Bauman WA, De Meersman RE (1998). Baroreceptor sensitivity response to phase IV of the Valsalva manoeuvre in spinal cord injury. Clin Auton Res. 8: 111-118.

Hartwich D, Dear WE, Waterfall JL, Fisher JP (2011). Effect of muscle metaboreflex activation on spontaneous cardiac baroreflex sensitivity during exercise in humans. J Physiol. 589: 6157-6171.

Head GA (1995). Baroreflexes and cardiovascular regulation in hypertension. J Cardiovasc Pharmacol. 2: S7-S16.

Heusser K, Tank J, Engeli S, Diedrich A, Menne J, Eckert S, Peters T, Sweep FC, Haller H, Pichlmaier AM, Luft FC, Jordan J (2010). Carotid baroreceptor stimulation, sympathetic activity, baroreflex function, and blood pressure in hypertensive patients. Hypertension. 55: 619-626.

Heymans C, van den Heuvel-Heymans G (1951). New aspects of blood pressure regulation. Circulation. 4: 581-586.

Houtman S, Oeseburg B, Hopman MT (1999). Non-invasive assessment of autonomic nervous system integrity in able-bodied and spinal cord-injured individuals. Clin Auton Res. 9: 115-122.

Iellamo F, Hughson RL, Castrucci F, Legramante JM, Raimondi G, Peruzzi G, Tallarida G (1994). Evaluation of spontaneous baroreflex modulation of sinus node during isometric exercise in healthy humans. Am J Physiol Heart Circ Physiol. 267: H994-H1001.

Iellamo F, Legramante JM, Raimondi G, Peruzzi G (1997). Baroreflex control of sinus node during dynamic exercise in humans: effects of central command and muscle reflexes. Am J Physiol Heart Circ Physiol. 272: H1157-H1164.

Kardos A, Watterich G, de Menezes R, Csanady M, Casadei B, Rudas L (2001). Determinants of spontaneous baroreflex sensitivity in a healthy working population. Hypertension. 37: 911-916.

Kent BB, Drane JW, Blumenstein B, Manning JW (1972). A mathematical model to assess changes in the baroreceptor reflex. Cardiology. 57: 295-310.

Khanna RR, Victor RG, Bibbins-Domingo K, Shapiro MF, Pletcher MJ (2012). Missed opportunities for treatment of uncontrolled hypertension at physician office visits in the United States, 2005-2009. Arch Intern Med. 172: 1344-1345.

Korner PI, West MJ, Shaw J, Uther JB (1974). "Steady-state" properties of the baroreceptor-heart rate reflex in essential hypertension in man. Clin Exp Pharmacol Physiol. 1: 65-76.

La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ (1998). Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. Lancet. 351: 478-484.

La Rovere MT, Maestri R, Robbi E, Caporotondi A, Guazzotti G, Febo O, Pinna GD (2011). Comparison of the prognostic values of invasive and noninvasive assessments of baroreflex sensitivity in heart failure. J Hypertens. 29: 1546-1552.

La Rovere MT, Pinna GD, Raczak G (2008). Baroreflex sensitivity: measurement and clinical implications. Ann Noninvasive Electrocardiol. 13: 191-207.

Lador F, Azabji Kenfack M, Moia C, Cautero M, Morel DR, Capelli C, Ferretti G (2006). Simultaneous determination of the kinetics of cardiac output, systemic O_2 delivery, and lung O_2 uptake at exercise onset in men. Am J Physiol Regul Integr Comp Physiol. 290: R1071-R1079.

Lador F, Tam E, Azabji Kenfack M, Cautero M, Moia C, Morel DR, Capelli C, Ferretti G (2008). Phase I dynamics of cardiac output, systemic O_2 delivery, and lung O_2 uptake at exercise onset in men in acute normobaric hypoxia. Am J Physiol Regul Integr Comp Physiol. 295: R624-R632.

Legramante JM, Raimondi G, Massaro M, Cassarino S, Peruzzi G, Iellamo F (1999). Investigating feed-forward neural regulation of circulation from analysis of spontaneous arterial pressure and heart rate fluctuations. Circulation. 99: 1760-1766.

Lewington S, Clarke R, Qizilbash N, Peto R, Collins R (2002). Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 360: 1903-1913.

Leyk D, Essfeld D, Hoffmann U, Wunderlich HG, Baum K, Stegemann J (1994). Postural effect on cardiac output, oxygen uptake and lactate during cycle exercise of varying intensity. Eur J Appl Physiol. 68: 30-35.

Loeppky JA, Greene ER, Hoekenga DE, Caprihan A, Luft UC (1981). Beat-by-beat stroke volume assessment by pulsed Doppler in upright and supine exercise. J Appl Physiol. 50: 1173-1182.

Mancia G, Ludbrook J, Ferrari A, Gregorini L, Zanchetti A (1978). Baroreceptor reflexes in human hypertension. Circ. Res. 43: 170-177.

Mancia G, Mark AL (2011). Arterial baroreflexes in humans. Supplement 8: Handbook of Physiology, The Cardiovascular System, Peripheral Circulation and Organ Blood Flow. Comprehensive Physiology.

Mancia G. et al. (2013). ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. 34: 2159-2219.

Marey EJ (1863). Physiologie Médicale de la Circulation du Sang. Delahaye, Paris.

McIlveen SA, Hayes SG, Kaufman MP (2001). Both central command and exercise pressor reflex reset carotid sinus baroreflex. Am J Physiol Heart Circ Physiol. 280: H1454-H1463.

Meyer M, Scheid P (1980). Solubility of acetylene in human blood determined by mass spectrometry. J Appl Physiol. 48: 1035-1037.

Minson CT, Halliwill JR, Young TM, Joyner MJ (2000). Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. Circulation. 101: 862-868.

Mitchell JH (2013). Neural circulatory control during exercise: early insights. Exp Physiol. 98: 867-878.

Mohaupt MG, Schmidli J, Luft FC (2007). Management of uncontrollable hypertension with a carotid sinus stimulation device. Hypertension. 50: 825-828.

NCGC. National Clinical Guideline Centre (2016). Update of clinical guidelines 18 and 34. Hypertension, the clinical management of primary hypertension in adults. Commissioned by the National Institute for Health and Clinical Excellence.

Nishiyasu T, Nagashima K, Nadel ER, Mack GW (1998). Effects of posture on cardiovascular responses to lower body positive pressure at rest and during dynamic exercise. J Appl Physiol. 85: 160-167.

Norton KH, Boushel R, Strange S, Saltin B, Raven PB (1999) Resetting of the carotid arterial baroreflex during dynamic exercise in humans. J Appl Physiol. 87: 332-338.

O'Leary DS (1996). Heart rate control during exercise by baroreceptors and skeletal muscle afferents. Med Sci Sports Exerc. 28: 210-217.

Ogoh S, Fisher JP, Dawson EA, White MJ, Secher NH, Raven PB (2005). Autonomic nervous system influence on arterial baroreflex control of heart rate during exercise in humans. J Physiol. 566: 599-611.

Ogoh S, Fisher JP, Raven PB, Fadel PJ (2007). Arterial baroreflex control of muscle sympathetic nerve activity in the transition from rest to steady-state dynamic exercise in humans. Am J Physiol Heart Circ Physiol. 293: H2202-H2209.

Ogoh S, Wasmund WL, Keller DM, O-Yurvati A, Gallagher KM, Mitchell JH, Raven PB (2002). Role of central command in carotid baroreflex resetting in humans during static exercise. J Physiol. 543: 349-364.

Okazaki K, Iwasaki K, Prasad A, Palmer MD, Martini ER, Fu Q, Arbab-Zadeh A, Zhang R, Levine BD (2005). Dose-response relationship of endurance training for autonomic circulatory control in healthy seniors. J Appl Physiol. 99: 1041-1049.

Orizio C, Perini R, Comandè A, Castellano M, Beschi M, Veicsteinas A (1988). Plasma catecholamines and heart rate at the beginning of muscular exercise in man. Eur J Appl Physiol. 57: 644-651.

Osculati, G., G. Grassi, C. Giannattasio, Seravalle G, Valagussa F, Zanchetti A, Mancia G (1990). Early alterations of the baroreceptor control of heart rate in patients with acute myocardial infarction. Circulation. 81: 939-948.

Palmero HA, Caeiro TF, Iosa DJ, Bas J (1981). Baroreceptor reflex sensitivity index derived from phase 4 of the Valsalva maneuver. Hypertension. 3: 134-137.

Papelier Y, Escourrou P, Helloco F, Rowell LB (1997). Muscle chemoreflex alters carotid sinus baroreflex response in humans. J Appl Physiol. 82: 577-583.

Parati G, Di Rienzo M, Mancia G (2001). Dynamic modulation of baroreflex sensitivity in health and disease. Ann N Y Acad Sci. 940: 469-487.

Perini R, Gheza A, Moia C, Sponsiello N, Ferretti G (2010). Cardiovascular time courses during prolonged immersed static apnoea. Eur J Appl Physiol. 110: 277-283.

Perini R, Tironi A, Gheza A, Butti F, Moia C, Ferretti G (2008). Heart rate and blood pressure time courses during prolonged dry apnoea in breath-hold divers. Eur J Appl Physiol. 104: 1-7.

Potts JT, Shi XR, Raven PB (1993). Carotid baroreflex responsiveness during dynamic exercise in humans. Am J Physiol. 265: H1928-H1938.

Querry RG, Smith SA, Strømstad M, Ide K, Raven PB, Secher NH (2001). Neural blockade during exercise augments central command's contribution to carotid baroreflex resetting. Am J Physiol Heart Circ Physiol. 280: H1635-H1644.

Raven PB (2008). Recent advances in baroreflex control of blood pressure during exercise in humans: an overview. Med Sci Sports Exerc. 40: 2033-2036.

Raven PB, Fadel PJ, Ogoh S (2006). Arterial baroreflex resetting during exercise: a current perspective. Exp Physiol. 91: 37-49.

Raven PB, Fadel PJ, Smith SA (2002). The influence of central command on baroreflex resetting during exercise. Exerc Sport Sci Rev. 30: 39-44.

Raven PB, Potts JT, Shi X (1997). Baroreflex regulation of blood pressure during dynamic exercise. Exerc Sport Sci Rev. 25: 365-389.

Ray CA, Rea RF, Clary MP, Mark AL (1993). Muscle sympathetic nerve responses to dynamic one-legged exercise: effect of body posture. Am J Physiol Heart Circ Physiol. 264: H1-H7.

Rowell LB, O'Leary DS (1990). Reflex control of the circulation during exercise: chemoreflexes and mechanoreflexes. J Appl Physiol. 69: 407-418.

Rowell LB, O'Leary DS, Kellogg DL (1996). Integration of Cardiovascular Control Systems in Dynamic Exercise. In: Handbook of physiology. Exercise: regulation integration of multiple systems. American Physiological Society, Bethesda, MD, pp 770-838.

Saltin B, Rådegran G, Koskolou MD, Roach RC (1998). Skeletal muscle blood flow in humans and its regulation during exercise. Acta Physiol Scand. 162: 421-436.

Schlaich MP, Sobotka PA, Krum H, Lambert E, Esler MD (2009). Renal sympathetic-nerve ablation for uncontrolled hypertension. N Engl J Med 361: 932-934.

Schwartz CE, Medow MS, Messer Z, Stewart JM (2013). Spontaneous fluctuation indices of the cardiovagal baroreflex accurately measure the baroreflex sensitivity at the operating point during upright tilt. Am J Physiol Regul Integr Comp Physiol. 304: R1107-R1113.

Sheriff DD, Rowell LB, Scher AM (1993). Is rapid rise in vascular conductance at onset of dynamic exercise due to muscle pump? Am J Physiol Heart Circ Physiol. 265: H1227-H1234.

Sivieri A, Fagoni N, Bringard A, Capogrosso M, Perini R, Ferretti G (2015). A beat-by-beat analysis of cardiovascular responses to dry resting and exercise apnoeas in elite divers. Eur J Appl Physiol. 115: 119-128.

Sleight P, Robinson SL, Brooks DE, Rees PM (1975). Carotid baroreceptor resetting in the hypertensive dog. Clin Sci Mol Med. 48: 261s-263s.

Smith SA, Querry RG, Fadel PJ, Gallagher KM, Stromstad M, Ide K, Raven PB, Secher NH (2003). Partial blockade of skeletal muscle somatosensory afferents attenuates baroreflex resetting during exercise in humans. J Physiol. 551: 1013-1021.

Spaak J, Montmerle S, Sundblad P, Linnarsson D (2005). Long-term bed rest-induced reductions in stroke volume during rest and exercise: cardiac dysfunction vs. volume depletion. J Appl Physiol Bethesda Md 1985 98: 648-654.

Tam E, Azabji Kenfack M, Cautero M, Lador F, Antonutto G, di Prampero PE, Ferretti G, Capelli C (2004). Correction of cardiac output obtained by Modelflow from finger pulse pressure profiles with a respiratory method in humans. Clin Sci Lond. 106: 371-376.

Tank J, Baevski RM, Fender A, Baevski AR, Graves KF, Ploewka K, Weck, M (2000). Reference values of indices of spontaneous baroreceptor reflex sensitivity. Am J Hypertens. 13: 268-275.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Eur Heart J 17: 354-381.

Tsuchimochi H, Hayes SG, McCord JL, Kaufman MP (2009). Both central command and exercise pressor reflex activate cardiac sympathetic nerve activity in decerebrate cats. Am J Physiol Heart Circ Physiol. 296: H1157-H1163.

Vallais F, Baselli G, Lucini D, Pagani M, Porta A (2009). Spontaneous baroreflex sensitivity estimates during graded bicycle exercise: a comparative study. Physiol Meas. 30: 201-213.

van Lieshout JJ, Toska K, van Lieshout EJ, Eriksen M, Walloe L, Wesseling KH (2003). Beat-to-beat noninvasive stroke volume from arterial pressure and Doppler ultrasound. Eur J Appl Physiol. 90: 131-137.

Victor RG (2015). Carotid baroreflex activation therapy for resistant hypertension. Nat Rev Cardiol. 12: 451-463.

Wesseling KH, Jansen JR, Settels JJ, Schreuder JJ (1993). Computation of aortic flow from pressure in humans using a nonlinear, three-element model. J Appl Physiol. 74: 2566-2573.

Wieling W, Harms MP, ten Harkel AD, van Lieshout JJ, Sprangers RL (1996). Circulatory response evoked by a 3 s bout of dynamic leg exercise in humans. J Physiol. 494: 601-611.