Stroke volume variations

Mild exercise and cardiovascular variability

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PhD Thesis

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Contents

1. Preface

The work that is presented below was carried out at the Institute of Basic Medical Sciences, Department of Physiology, University of Oslo during the period from I started as a first year medical student in 1998 to 2009. The experiments reported in paper III and IV were conducted at Massachusetts Institute of Technology, Cambridge.

I feel in great debt to my supervisor, Professor Lars Walløe who has been continuously supportive and encouraging. He has always been available for my questions. He has given me the freedom to pursue ideas, and has at the same time been straightforward and constructive in his criticism.

I am grateful to Professor Halfdan Ihlen and his colleagues who thaught me echocardiographic examination during their busy clinical schedule.

My warmest thanks go to dr.med. Karin Toska who has not only supervised parts of this work, she taught me how to measure variations in stroke volume, as well as providing me with excellent experimental data for my subsequent analysis. As a medical student she introduced me to research and was always patient when explaining difficult concepts in cardiovascular physiology.

I would like to thank all of my colleagues for making these past years so memorable and creating such a pleasant work atmosphere. Special thanks go to M.Sc. Inger Helene Nådland, who skilfully performed the femoral blood flow measurements in paper II, cand.med. Erling Bekkestad Rein for fruitful discussions, and Torun Flatebø for excellent technical assistance. I would also like to thank Alison J. Coulthard for her invalueable English corrections, and Håvard Tønnesen for his technical support. In addition, I would like to thank my former colleagues, dr.med. Signe Søvik, dr.med. Kristin Bergersen, and dr.scient. Jonny Hisdal, who created a comfortable environment for me during my first hesistant steps into research. Dr.med. Morten Eriksen has given valuable comments and generously answered questions regarding

his exceptional programs including the mathematical model. Professor Ki. H. Chon and M. Sc. Didrik Lilja have been superb collaborators, while dr.med. Jarlis Wesche, dr.med. Vegard Bruun Wyller and Associate Professor Don D. Sheriff have been interesting partners in discussions.

I would also like to thank all the subjects who participated in the study.

I am grateful to the Norwegian Health Association, which provided financial support for my PhD studies (2005-2009) and Carina Alm for sorting out practicalities to make my research run smoothly. I am also grateful for the funding from the Research Council of Norway as a medical student. In addition, I am grateful for grants from 'Nansenfondet og de dermed forbundne fond', 'Stud.med. Morten Dedekam Harboes legat', 'Legatutvalget ved Det medisinske fakultet, 13.Hjerte-/karsykdommer', 'Legatet til Henrik Homands Minde', 'Eva Hildebjørg f Reinskou og Gunnar Mørkveds legat til beste for medisinsk forskning', 'Nathalia og Knut Juul Christiansens stiftelse' and 'Dr. Fürst medisinske laboratoriums fond til klinisk kjemisk og klinisk fysiologisk forskning'.

My family and friends have been of uttermost support.

Yet, most of all, the care and encouragement from Endre and the smiles from Moa have made my day.

Oslo, July 2009

Maja Elstad

2. List of papers

PAPER I:

ELSTAD, M., TOSKA, K., & WALLOE, L. (2002). Model simulations of cardiovascular changes at the onset of moderate exercise in humans. *Journal of Physiology* **543**, 719-728.

PAPER II:

ELSTAD, M., NADLAND, I.H., TOSKA, K., & WALLOE, L. (2009). Stroke volume decreases during mild dynamic and static exercise in supine humans. *Acta Physiologica* **195**, 289-300.

PAPER III:

ELSTAD, M., TOSKA, K., CHON, K.H., RAEDER, E.A., & COHEN, R.J. (2001). Respiratory sinus arrhythmia: opposite effects on systolic and mean arterial pressure in supine humans. *Journal of Physiology* **536**, 251-259.

PAPER IV:

ELSTAD, M., WALLOE, L., CHON, K.H. & TOSKA, K. Low frequency fluctuations in heart rate, cardiac output and mean arterial pressure in humans: what are the physiological relationships? *Submitted*

3. List of abbreviations

ANS, autonomic nervous system;

BP, arterial blood pressure;

CO, cardiac output from left side of heart;

DP, diastolic blood pressure;

ECG, electrocardiogram;

FPR, femoral peripheral resistance;

FV, femoral beat volume;

HR, heart rate;

MAP, mean arterial blood pressure;

MBF, muscle blood flow (= FF, femoral blood flow in paper II, = Qmf in paper I);

PNS, parasympathetic nervous system;

RPR, remaining body peripheral resistance (excluding legs);

RR interval, interval between two successive R waves;

SNS, sympathetic nervous system:

SP, systolic blood pressure;

SV, left cardiac stroke volume;

TPR, total peripheral resistance

4. Introduction

4.1 Cardiovascular physiology

Cardiovascular physiology is a wide field ranging from gene and molecular level via cells and organs to the whole integrated system. The circulation carries oxygen and nutrients in the blood to the cells and transports waste products from the cells to elimination sites. The circulatory system includes the heart, the vasculature and various control mechanisms. In this thesis, I will focus on the short-term cardiovascular control of the human circulation. This short-term regulatory system has long-term health consequences, and several diseases are linked with impairment of circulatory control. Cardiovascular diseases kill millions of people each year. I believe that by improving our understanding of the optimal functioning of the circulation, we can provide a basis for new approaches to the prevention and treatment of cardiovascular disease. It is very important to study healthy humans in order to provide better healthcare for people with circulatory problems.

4.2 Human circulation and short-term regulation

The studies on which this thesis is based have looked at different aspects of integrated cardiovascular control in humans. Cardiovascular research in healthy humans is part of translational research to understand diseases and improve existing and introduce new therapies for different conditions (Sipido *et al.*, 2009). Knowledge of the integrated cardiovascular control is crucial in evaluating symptoms such as dizziness and syncope and on treating diseases such as heart failure and circulatory collapse in traumatised patients. A sound physiological understanding is also necessary to understand the causes of hypertension and to reduce the impact of risk factors on the cardiovascular system. But above all, the cardiovascular system with its control mechanisms is fascinating in itself, and has developed so that people can cope

with a wide range of situations from standing up after a good night's rest to diving in the sea. Rapid adaptations by the circulation (within seconds) are seldom recognised and appreciated until they fail.

Though this thesis focuses on healthy humans, I recognise that important research has also been conducted using animals. Control systems are remarkably similar in other mammals and also in a variety of other species, ranging from the nematode *C. elegans* used in baroreceptor studies to mice and dogs. A challenge in human research is to find investigation methods that do not present a hazard to the subject but still produce valid conclusions. Research in animals and in healthy humans can complement each other, contributing to greater knowledge of integrated cardiovascular physiology.

The autonomic nervous system (ANS) is responsible for important elements of the cardiovascular control mechanisms. The ANS and its role in circulatory control have been studied more intensively than other control mechanisms, and is also the main focus of this thesis. Though other control systems for the whole-body homeostasis are of fundamental importance, the ANS provides control within seconds. Humoral control and long-term adaptations of cells are not within the scope of this thesis.

A general introduction to the ANS is therefore justified. It consists of two separate divisions, the parasympathetic and sympathetic nervous systems (PNS and SNS respectively). Historically, it was believed that the PNS controls body functions at rest, while the SNS controls body functions during stress of different kinds. This generalisation is too simple to serve as explanation of the observed responses in the cardiovascular system (and other systems of the body) as the activation of sympathetic nerves to different target organs does not change in parallel (Malpas, 2002). Instead of discussing this controversy, I describe below how the two divisions of the ANS are thought to affect one important parameter, heart rate (HR), in healthy humans under various physiological conditions.

In all experiments we tried to ensure that subjects were as relaxed as possible. In general, HR is lowest when subjects are at rest and highest during whole-body exercise. The physiological range of HR in a healthy 20-year-old may be from ~30 to ~210 beats per min (bpm). This range of HR is reached (however not exclusively) by changes in firing activity in autonomic nerve fibres. A denervated heart has an intrinsic rhythm around 90-110 bpm (Levick, 2003). The effect of activity in parasympathetic nerves to the heart is to lower HR. Increases in HR up to 90-120 bpm are brought about by withdrawal of parasympathetic activity (Pickering & Davies, 1973;Ogoh *et al.*, 2005), while increases above this level are brought about by increases in sympathetic activity (transmitted by other nerves than the parasympathetic signals) to the heart (Levick, 2003). However there is a "grey" zone for a range of HR from about 50 bpm to 120 bpm in which the exact contribution by the two autonomic divisions is elusive (Fig. 1).

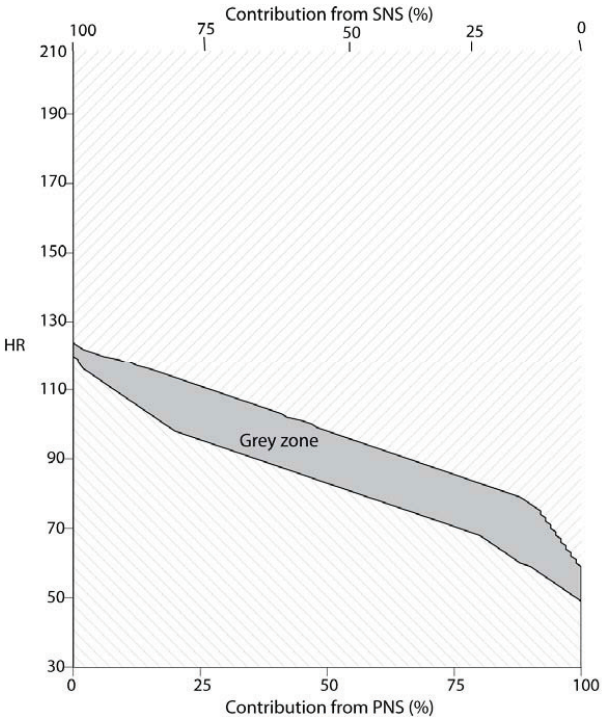

Figure 1. The relative influence of the parasympathetic (PNS) and sympathetic nervous systems (SNS) on HR.

The influence of PNS and SNS on HR may be seen as antagonistic, but they also appear to interact in a dynamic fashion to enhance the reflex-mediated effects (Malpas, 2002). Although reciprocal changes of sympathetic and parasympathetic cardiac nerve activities are triggered by the arterial baroreflex (Rea & Eckberg, 1987), the net effect of such neural changes on HR is not easily calculated (Cooke *et al.*, 1999). In dogs, the presence of any parasympathetic cardiac nerve traffic may actually cancel out sympathetic influences on HR (Samaan, 1935). Though this thesis does not systematically investigate the relative influence of PNS and SNS on HR, it seems likely that there is wide variation both among individuals and between species.

This thesis focuses on variations in left cardiac stroke volume (SV). In my opinion, SV is too seldom reported in cardiovascular studies. The thesis includes studies of the short-term regulation of the cardiovascular system during mild exercise and of cardiovascular variability. Regulation of exercise and cardiovascular variability are connected by changes in the cardiovascular reflexes and the ANS as well as changes in mechanical factors. Taken together these changes shed light on the circulatory control in humans.

4.3 Reflexes and physiological phenomena

The human cardiovascular system contains many distinct and overlapping reflexes to maintain homeostasis. There are three ways in which a reflex may operate to produce a response. Feedback uses the output of a system to continually alter its input (Karu, 1995). When a change in a variable triggers an inhibitory response that returns the variable towards its control value, the regulatory process is called negative feedback (Levick, 2003). A positive feedback mechanism is present if a change in a variable exaggerates the initial response. In contrast, a process is termed feedforward when the response is changed prior to the input variable.

For this thesis, I have studied various circulatory responses to some physiological phenomena and reflexes. In the short-term cardiovascular regulation the arterial baroreflex plays a central role.

4.3.1 Arterial baroreflex

The main role of the baroreflex is to buffer short-term (on the scale of a few minutes) fluctuations in arterial blood pressure (BP). I will here only give a brief overview of the arterial baroreflex (Levick, 2003).

The arterial baroreceptors are mechanoreceptors localised in the carotid sinus and the aortic arch. The receptors are stretch-sensitive and are stimulated by both the magnitude of the pressure and the rate of pressure change. The afferent signals are transported in cranial nerves IX and X to the nucleus tractus solitarius. Changes in autonomic nerve firing are elicited to restore BP. In addition, hormone release and other BP buffering mechanisms may be triggered by the arterial baroreflex, but this will not be discussed further. The effector organs are mainly the heart and the vasculature. A change in HR controlled by the PNS takes less than 0.5 s to initiate (Pickering & Davies, 1973), while a sympathetically controlled change of HR takes at least 2 s to initiate (Berger *et al.*, 1989). When HR is below ~120 bpm the chronotropic effect via arterial baroreflexes is regulated by the PNS (Pickering & Davies, 1973;Ogoh *et al.*, 2005).

The vasculature is largely under sympathetic control (Levick, 2003). The SNS can change the firing activity to the arterioles within 4-6 s (Wallin & Nerhed, 1982), and vasoconstriction develops within 10 s of the initial pressure change (Toska *et al.*, 1994). Venoconstriction, especially in venus plexa in the gastrointestinal region, may also be elicited by the SNS to relocate blood to the central veins. This is of great importance for the filling of the heart during changes of posture (Levick, 2003).

The main function of the arterial baroreflex is to prevent hypo- and hypertensive episodes. A common way to describe how the baroreflex operates is illustrated in

Figure 2. The set point is the pressure that the reflex attempts to maintain and the baroreflex gain is the maximum slope of the stimulus-response curve. The set point may be changed either centrally as discussed below (Baroreflex resetting) or peripherally, for instance as a result of elevated pressure in hypertension causing stiffness in the arterial wall (Aars, 1969).

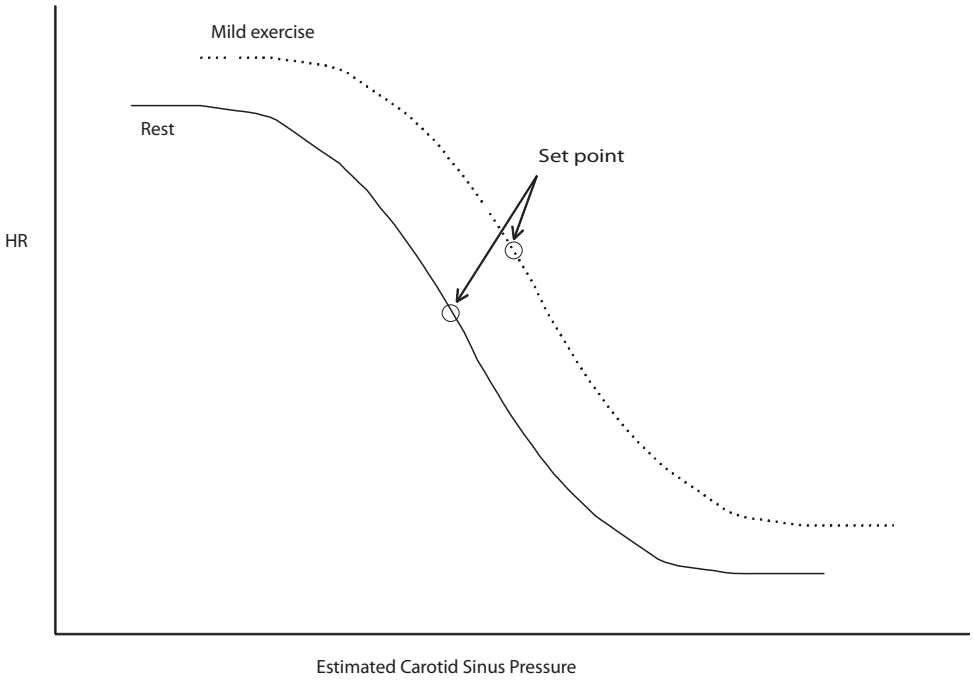

Figure 2. A schematic illustration of the carotid baroreflex function curve at rest (continuous line) and during mild exercise (dotted line). When the estimated carotid sinus pressure increases, HR decreases, and the relationship produces a sigmoid curve. During mild exercise there is a shift in set point (open circles) towards higher BP, but the maximal gain (the maximal slope of the curves) is conserved. This figure was introduced by Koch in 1931 and similar figures are more recently presented by others (Potts *et al.*, 1993;Ogoh *et al.*, 2003;Raven *et al.*, 2006).

It should be noted that due to the methods often used to elicit the response, often only the carotid part of the arterial baroreflex is elicited. Another complicating factor in

obtaining these stimulus-response curves for the baroreflex is that the maximal baroreflex gain probably varies even over short periods (Eckberg, 2008). These complicating factors are seldom taken into account in studies of the arterial baroreflex. In the studies described in this thesis, we elicited changes in BP mostly by altering physiological conditions. This affects the whole circulation and not only the carotid part, thereby circumventing the problem.

To study a response in a system, a known (or measurable) stimulus is introduced. There are several ways of experimentally stimulating arterial baroreflexes in healthy humans. One elegant method is to use thigh cuffs to exclude the legs from the circulation within one heartbeat and raise peripheral resistance accordingly (Toska *et al.*, 1994). Other methods include neck pressure and neck suction (Eckberg *et al.*, 1975;Ogoh *et al.*, 2002), passive tilting (Cooke *et al.*, 1999), lower body negative pressure (Hisdal *et al.*, 2001) and medication-induced change in BP (Smyth *et al.*, 1969). In the first studies described in this thesis, we used mild exercise to elicit responses from the arterial baroreflexes.

4.3.2 Mild exercise

Major cardiovascular changes take place at the onset of, during and after the cessation of exercise. The current view of cardiovascular control in exercise is that three main mechanisms are involved; central command activation, arterial baroreflex resetting and the exercise pressor reflex. None of these mechanisms is thought to operate alone, but because there is so much redundancy in the cardiovascular control systems, the circulatory responses to exercise can be achieved when one mechanism is attenuated or even lacking. This redundancy makes it difficult to investigate how each of the mechanisms contributes to cardiovascular changes. Nevertheless, researchers do attempt to investigate their separate contributions, bearing this difficulty in mind. Later in this introduction, I also look briefly at local vasodilation, and more closely at SV during exercise. At the end of this section, I compare the circulatory changes during dynamic and static exercise.

First of all, it is important to realise that exercise is not a precisely defined concept. To prevent movement at the measurement sites, the exercise bouts in the studies described here were of light intensity only. During mild-intensity exercise where HR < 90 bpm, both PNS and SNS are important (Fig. 1). Exercise in these studies was performed in the supine position, and I will return to this at the end of this section.

Central command activation

The central command hypothesis was originally introduced in the late 19th century. This was thought of as a mechanism that causes interaction between the motor flow from cerebral cortex and the centrally regulated cardiovascular response to exercise (Zuntz & Geppert, 1886;Johansson, 1895;Rowell, 1993). Krogh and Lindhard (1913) were the first to test this concept, and claimed that the rises in ventilation and HR during exercise were "not produced reflexly but by irradiation of impulses from the motor cortex". Later, Godwin *et al*. (1972) found that the cardiovascular and respiratory responses depended on the level of central command activation.

Currently, central command activation is defined as a feedforward mechanism with *simultaneous or parallel activation* of both motor and cardiovascular centres. This definition makes it doubtful whether the anticipatory increases in MAP and HR which we observed in paper II should be classed as central command activation. An anticipitatory effect has previously been described in the literature as an increase in HR prior to isometric exercise (Ebert, 1986). However, central command may also be described as an effort-induced modulation of autonomic function (Williamson *et al.*, 2006). Using this definition, central command can act as a feedback system based on the individual's sense of effort. An investigation in which central motor command was uncoupled from central cardiovascular command showed that central command and anticipation may actually be the same phenomenon. This study showed that when hypnotised subjects performed an imaginary hand grip the same cardiovascular responses were produced as during actual hand grip (Williamson, 2002). This study also localised the central cardiovascular command to the insular and anterior cingulate cortexes (Williamson *et al.*, 2002). Williamson *et al.* (2006) also described

a network of structures that are involved in centrally mediated cardiovascular activation and that do not require parallel motor activation to exert their influence. They hypothesised that the same brain regions that alter cardiovascular responses during non-exercise conditions could also be activated during exercise (Williamson *et al.*, 2006). The debate on central command activation will probably continue for many years. However, I believe that it should be discussed in the light of baroreflex resetting.

Baroreflex resetting

There has been a prolonged debate on whether or not the arterial baroreflex is reset during exercise, which was reviewed recently (Joyner, 2006). The current understanding is that the baroreflex is reset by a shift of the arterial baroreflex set point during exercise towards higher BP and away from the centering point towards the threshold (Raven *et al.*, 2006) (Fig. 2). The arterial baroreflex continues to function during exercise to buffer short-term fluctuations in BP (Toska *et al.*, 1994). Complete restoration of BP towards the reset value is thought to require infinite reflex sensitivity (Rowell, 2004). I will return to this in the general discussion.

In paper II, we tried to elicit central command selectively during countdown to exercise. We believed that as the subjects had been familiarised with the exercise protocol, an increase in MAP and HR prior to the onset of exercise could be regarded as central command activation. In paper I, we simulated central command activation as an exponential increase in baroreflex setpoint from countdown which continued throughout the onset of exercise. Thus, we conclude that the central command is responsible for the resetting of the baroreflex set point during exercise.

For the preliminary studies prior to paper I, we split the central command activation of baroreflex resetting into two parts, “anticipation” (prior to onset of exercise) and “onset of exercise”, but this added little to the understanding of the cardiovascular changes. We wished to keep the mathematical model as simple as possible, and this redundancy was therefore removed. In both papers I and II, we regarded the

anticipatory response to exercise and the cardiovascular response at the onset of exercise as part of central cardiovascular command activation.

In paper I, we simulated the shift of set point towards higher BP, but as the sensitivity of the baroreflex was kept constant, we assumed that the baroreflex was still operating in the linear section of the stimulus-response curve (Fig. 2). This assumption should be valid as the exercise was mild (HR < 90 bpm). In paper I, we ignored the sympathetic effect on chronotropy as this added little to our understanding, since the PNS mainly controls HR as HR < 90 bpm (Fig. 1). After the onset of exercise other mechanisms may modulate the initial central command output.

The exercise pressor reflex

The exercise pressor reflex is a peripheral neural drive that originates in skeletal muscle and acts as a modulator of cardiovascular changes (Smith *et al.*, 2006). The afferent branch consists of mechanically and chemically sensitive signals arising from skeletal muscle that regulate circulatory responses to exercise by providing feedback to cardiovascular centres within the brainstem (Gallagher *et al.*, 2006). The efferent branch makes cardiovascular adjustments to exercise through increases in sympathetic nerve activity and withdrawal of parasympathetic activity (Smith *et al.*, 2006).

Rowell and O'Leary (1990) suggested that HR and CO were controlled by central command, while the exercise pressor reflex controlled vascular resistance. This was based on the view that there is a time lag of at least 30-60 s before the sympathetic nerve activity increases (Mark *et al.*, 1985). However, muscle sympathetic nerve activity has been shown to increase within 4-6 s of the onset of exercise (Wallin & Nerhed, 1982;Herr *et al.*, 1999), although, the response is greater after ~50 s (Herr *et al.*, 1999). Again, there must be some overlap between these systems, which is yet to be clarified.

The only attempt we made to include the exercise pressor reflex in our studies was in paper II. Muscle contractions in the protocol we used lasted for two minutes, giving

time for the exercise pressor reflex to be activated. Chemosensitive fibres in the exercise pressor reflex respond with a latency of 15-30 s (Gallagher *et al.*, 2006). This means that the exercise pressor reflex is probably active for a few seconds after the cessation of exercise, while the central command activation is turned off immediately at the cessation of mild exercise (Rowell, 1993). In paper II, we reported that MAP and SV returned to baseline immediately after the cessation of dynamic leg exercise (Fig. 2, paper II), while HR, CO and total peripheral resistance (TPR) returned to baseline within 20 seconds after exercise. If central command activation is switched off immediately when exercise ceases, this is not in agreement with Rowell and O'Leary's hypothesis mentioned above (Rowell & O'Leary, 1990). If central command activation is not immediately switched off when exercise ceases, this means that many results regarding the exercise pressor reflex should be reconsidered. For paper I, we only simulated the first 30 seconds of the exercise. I suggest that our mathematical model might be more suitable for simulating exercise after the initial 30 seconds if we included the exercise pressor reflex.

The three mechanisms that are believed to control circulatory changes during exercise do not operate in isolation from each other. Central command and the exercise pressor reflex interact in such a way that signals from one input facilitate signals from the other, resulting in accentuated resetting of the baroreflex during exercise (Gallagher *et al.*, 2006). There is also functional and anatomical evidence of interaction between the exercise pressor reflex, central command and arterial baroreflex (Gallagher *et al.*, 2006).

The exercise pressor reflex is thought to play an important role in changes in cardiovascular response to exercise in heart failure patients (Sinoway & Li, 2005). The exercise pressor reflex is mainly thought to develop from skeletal muscle in the limbs, but some suggest that the respiratory muscles (which are also skeletal muscles) may significantly evoke this reflex as well (St Croix *et al.*, 2000;Kaufman & Hayes, 2002).

Local vasodilation in exercising muscle

There is general agreement that muscle blood flow (MBF) increases during exercise, as discussed in the thorough review by Rowell (2004). At least three mechanisms may work together to raise MBF: these include the effects of metabolites from contracting muscle cells, the muscle pump, and conducted vasodilation triggered by signals from the endothelium and deoxygenation of red blood cells (Ellsworth *et al.*, 2009). In the papers for this thesis, we did not distinguish between these mechanisms, and I will therefore limit the discussion about this interesting topic. However, I would like to emphasise that the muscle pump both increases muscle perfusion (Nådland *et al.*, 2009) and ensures that blood returns to the heart at sufficient speed to maintain cardiac output (Rowell, 2004). The respiratory and abdominal pumps make a so far unknown contribution to the translocation of blood from the lower body to the thorax (Rowell, 2004).

MBF may reach a peak flow of ~250 ml/100g/min (Walløe & Wesche, 1988), and as the body may contain 20-30 kg of muscle, the implication is that skeletal MBF must be under tonic vasoconstrictor constraint to avert hypotension (Rowell, 2004). During exercise there is predominantly local control over the exercising muscle vasculature, but the systemic BP is well maintained (Wray *et al.*, 2004). The conflict between adequate muscle perfusion and maintenance of BP is prevented by different combinations of cardiac output (CO) and total peripheral resistance (TPR).

In paper I, we simulated the unknown increase in MBF by assuming an exponential increase in MBF with a time delay (~1.8 s) and a simulated time constant (Fig. 3). This may have increased the number of degrees of freedom in the model, and our direct motive for conducting the studies reported in paper II was to reduce the number of degrees of freedom. The assumption was that the recorded MBF would fit well with the mathematical model used in paper I. This is confirmed by a visual comparison of the recorded MBF from paper II with one combination of time delay, time constant and increase in MBF used in paper I (Fig. 3).

In paper I, we found the maximal simulated increase in MBF to be 2.1 l/min (Wilcoxon median calculated from table 1, paper I), whereas the increase we reported in paper II was about half of this. It is not possible to make an exact comparison of these results as both the subjects and the work load differed between the two studies.

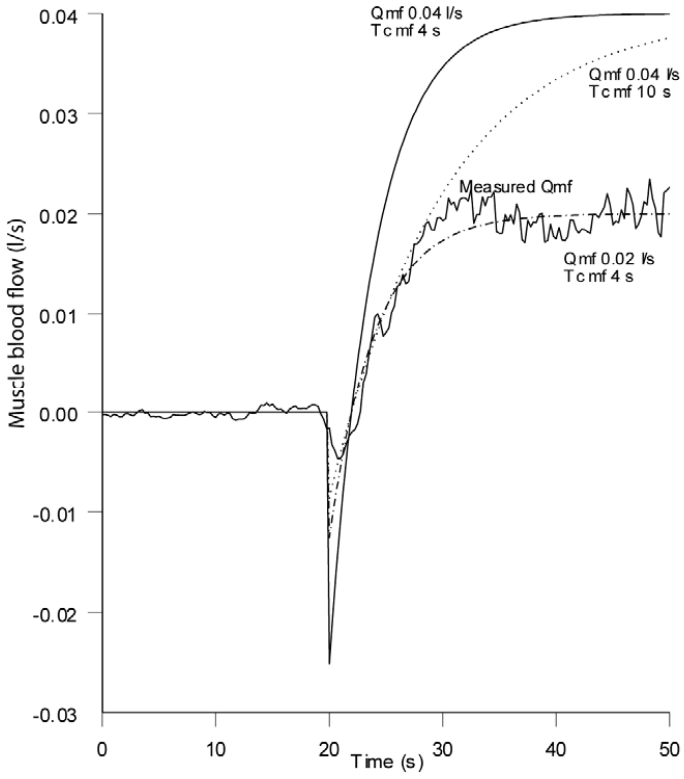

Figure 3: Increase in muscle blood flow (MBF, Qmf) at onset of dynamic leg exercise. The continuous, dotted and dashed smooth curves show different theoretical simulations of the increase in MBF (Fig. 2B, paper I, Reprinted, by permission from Wiley-Blackwell, Oxford, UK.), while the continuous wavy line curve shows the median increase in MBF described in paper II.

In paper I, the simulated time constants (Tc mf in Fig.3) of the increase in MBF varied individually between 0.9 and 13.2 seconds. The time constant in this mathematical model indicates how long it takes for the increase to reach 2/3 of its maximal value. Once again, there may be too many degrees of freedom in the

mathematical model, and there is still room for improvement of the model. The varying overshoot in MBF at the onset of exercise (Nådland *et al.*, 2009) is not accounted for in the model.

Stroke volume during exercise

Physiology text books generally states that SV increases during exercise (Levick, 2003). However, the factors that are known to produce a change in SV during exercise can both increase and reduce it. According to Starling's law of the heart, an increase in venous return (increase in preload/end-diastolic volume) brought about by the venous muscle pump and venoconstriction increases SV. In addition, the increase in sympathetic nerve firing, which leads to greater contractility, increases SV by decreasing the end-systolic volume. On the other hand, a reduction in filling time as a result of an increase in HR may decrease SV. However, this effect is not apparent until HR approaches its maximum in healthy subjects (Levick, 2003). Last but not least, increased afterload reduces SV. An increase in afterload is mostly due to elevated MAP, but chamber radius and wall thickness may also play a part (Levick, 2003). In animal models and isolated heart models, each factor and its effects can be tested separately. This is usually not possible with human subjects, but our exercise protocol using mild supine exercise enabled us to investigate the effects of changes in some of these factors on SV.

In physiology text books, a temporary fall in SV due to an increase in BP is described as being rapidly compensated by the Frank-Starling mechanism, as the reduction in the ejection volume leads to accumulation of blood within the ventricle (Levick, 2003). In paper II, we describe the restoration of SV shortly after the onset of exercise, which may be an indication of this effect. However, this tendency was short lasting, and we found the afterload effect to be -0.4 ml/mm Hg during both dynamic and static exercise. This is comparable to -0.3 ml/mm Hg in dogs (Janicki *et al.*, 1996). The inverse relationship is shown below (Fig. 4).

In paper I, where diastolic pressure (DP) was used to determine the afterload effect, we found the simulated afterload effect to be -0.8 ml/mm Hg. The afterload is proportional to the pressure in the proximal aorta prior to systolic ejection. Therefore, it would have been more correct to use DP for paper II as well, but our measurements were too uncertain (see Methods).

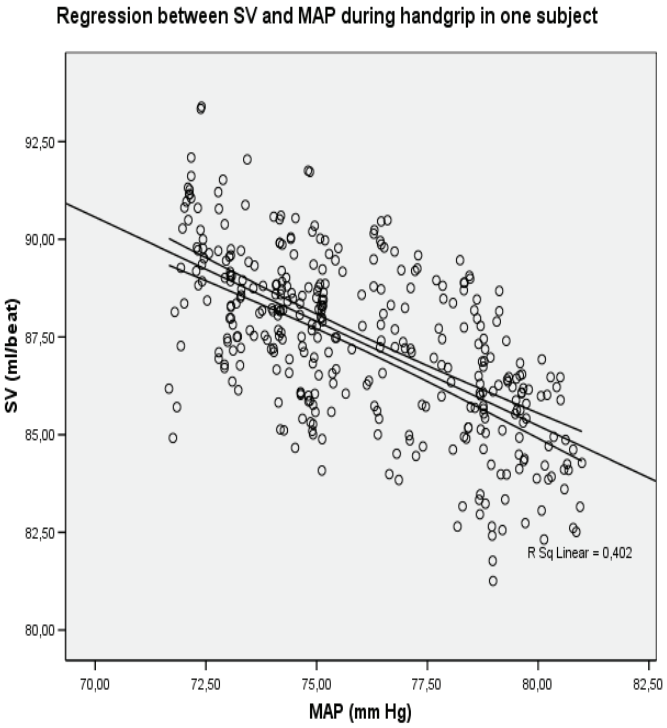

Figure 4. The regression line between SV and MAP during hand grip in one subject. There is a clear tendency for SV to decrease as MAP increases. The Wilcoxon median linear regression coefficient between SV and MAP for all subjects was -0.42 ml/mm Hg (95% Wilcoxon confidence interval: -0.81, -0.04) during dynamic exercise and -0.43 ml/mm Hg (-0.65, -0.24) during static exercise. (See methods for details.)

We also analysed the relationship between SV and HR to evaluate the effect of changes in filling time of the heart, which showed no significant trend in paper II. In

paper I, we only found that filling time had an effect on SV in four of the ten subjects. At rest (HR ~60 bpm), the filling time of the heart (the diastole) is ~0.6 s, while during mild exercise (HR ~90 bpm) it is ~0.4 s (Levick, 2003). Most of the reduction in filling time is in the diastase (period of diastole with minimal ventricular filling). The effects of contractility and relaxation are probably not activated when HR < 90 bpm. In the light of this we would not expect this level of HR to compromise SV in healthy subjects.

In paper II, we reasoned that as HR was well below 90 bpm, sympathetically induced changes in contractility would be negligible. In paper I, it was necessary to include the sympathetically induced change of contractility in order to simulate SV changes, but the afterload effect was also greater, another example of redundancies in the cardiovascular system. Even during dynamic leg exercise, the increase in venous return and the supine position, which is thought to maximise cardiac filling (Ogoh *et al.*, 2002), were not sufficient to prevent SV from decreasing due to the afterload effect. However, I must emphasise that the decrease was by no means dramatic (~5-8%), and HR and to some extent CO did increase. This finding may be limited to supine exercise, as CO depends on HR during supine exercise, while during upright exercise it depends more on end-diastolic volume (Warburton *et al.*, 2002).

In an early version of the model, we included increase in venous return as one of the mechanisms that changed at the onset of exercise. However, this added barely anything to the understanding of the circulatory changes, as the heart in the mathematical model is simulated to pump out the amount of blood received without limitations on end-diastolic volume and ejection fraction. This simplification may only be valid during mild exercise bouts. In paper II, we showed that SV actually decreases (though only to a limited extent) even during dynamic exercise. In paper I, we described a short lasting decrease in SV at the start of countdown and continuing for the first seconds after the onset of exercise (Fig. 2 & 3, paper I). However, the simulation run only included the first 30 seconds of exercise, so any later effects on SV during mild supine exercise were not observed.

My point is that SV is a parameter that may change even in situations where we do not expect it to do so, and that it should therefore be measured whenever possible. CO is one of the most important factors determining the circulatory state in both healthy subjects and patients, and CO is determined by HR and SV. In my opinion, it is natural to include SV when circulatory changes are assessed, even in situations where HR appears to describe the cardiovascular changes observed. CO measurements are valuable in diagnosis, risk stratification and treatment choices for patients who are critically ill or suffering from cardiovascular disease (Moshkovitz *et al.*, 2004). However, more knowledge is needed about SV changes in humans in response to different stimuli.

Dynamic leg exercise vs static hand grip exercise

Some investigators believe that static and dynamic exercise are regulated by such diverse mechanisms that it is inappropriate to compare them. Some authors have discussed whether cardiovascular changes during arm and leg exercise are differently regulated (Volianitis *et al.*, 2004;Tschakovsky *et al.*, 2006) while others have discussed how the cardiovascular response varies with muscle mass (Williams, 1991;Iellamo *et al.*, 1999). This makes it clear that there are many pitfalls in discussions of "exercise", one of which is the risk of discussing differences in methods rather than actual physiological differences.

Next, I would like to point out a few of the similarities and differences between dynamic and static exercise. Though there is great redundancy in the cardiovascular control during exercise, there are a few key mechanisms that may operate in concert, making different contributions to produce the diverse responses.

During both dynamic and static exercise MAP increases, but the mechanisms behind this increase are very different (Fig. 5). During dynamic exercise, CO increases as a result of a rise in HR, and there is vasodilation in exercising muscle (large decrease in femoral peripheral resistance (FPR)) and some vasoconstriction (increase in remaining body peripheral resistance (RPR)). The net effect is vasodilation (decrease

in TPR). An important point here is that these results were obtained during mild dynamic exercise (HR ~80 bpm). It is generally believed that vasoconstriction in the non-exercising body does not appear until HR is above 90 bpm (Rowell, 2004). This disagrees with the finding that the increase in MBF is greater than the increase in CO, indicating that redistribution of blood must have taken place (Eriksen *et al.*, 1990). The latter finding is in accordance with our results in paper II.

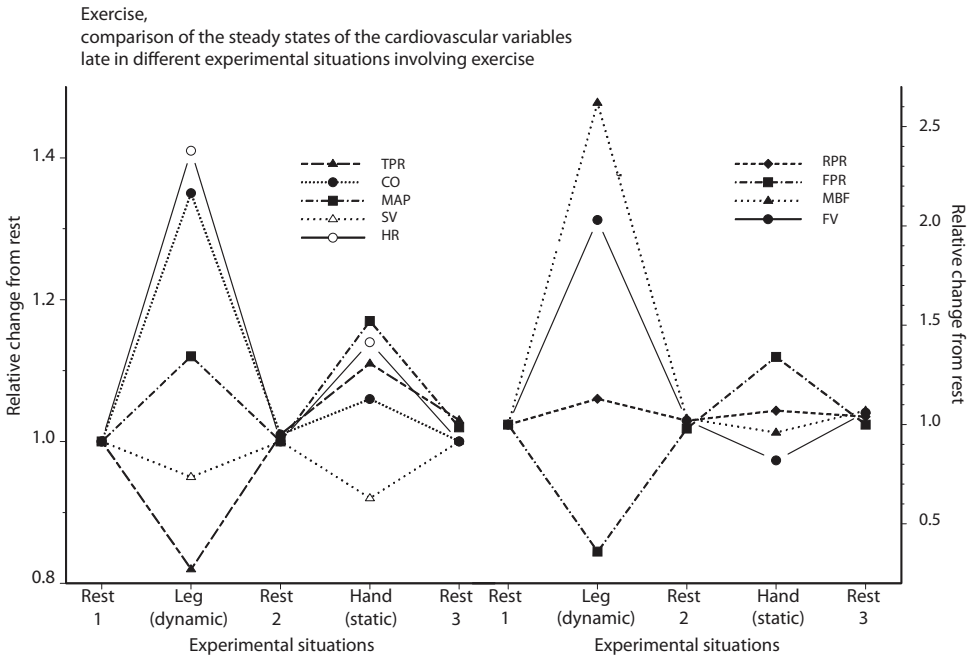

Figure 5: Point estimates of relative changes. Rest 1, Leg (dynamic leg exercise), Rest 2, Hand (static hand grip) and Rest 3 represent different time periods (Table 1, paper II). The panel to the left summarises changes in TPR, CO, MAP, SV and HR, while the panel to the right summarises RPR, FPR, MBF and femoral beat volume (FV). The calculation of RPR is explained in the methods section. Note the two y-axes are not of the same scale. See text for further explanation.

During static exercise, the situation is somewhat different. The increase in MAP is caused by vasoconstriction alone, as TPR increases and CO is more or less

unchanged. Vasoconstriction appears mainly in the legs, probably because these non-exercising muscles have a large blood flow reserve. During dynamic leg exercise, a greater share of the SV supplied the legs, while there was a significant decrease during static hand grip, indicating active redistribution. I suggest that the increase in HR during static exercise is adjusted to keep CO or regional blood flow at an acceptable level, because very little of this increase in flow is passed on to the exercising muscle (Barcroft & Millen, 1939;Asmussen, 1981).

In paper II, we showed that various combinations of CO and TPR may be found at similar MAP levels, and this redundancy in the cardiovascular regulation is very important when considering the mechanisms behind changes in the circulation. Our findings are in accordance with the current view that CO raises MAP during dynamic exercise, while TPR raises MAP during static exercise (Kaufman & Hayes, 2002). However, the debate on whether CO or TPR dominates the increase in MAP during exercise is still not settled. Shoemaker *et al.* (2007) claim that CO is the most important factor behind increases in MAP, even during static hand grip. Nevertheless, it is plausible that changes in CO may take on greater importance if the ability to alter vascular resistance is blocked (Fisher *et al.*, 2006).

4.3.3 Cardiovascular variability

Cardiovascular variability was first observed by Stephen Hales in 1733 as fluctuations in BP (Cohen & Taylor, 2002). Traube in 1865 and Hering in 1869 observed oscillations in BP that coincided with respiration (Cohen & Taylor, 2002), while Mayer observed BP waves that were independent of respiration (Mayer, 1876). Later, when oscillations in HR were found at the same frequencies, the search for causal relationsships between fluctuations in BP and HR started. (There is also significant cardiovascular variability at longer timescales (>20 s), but I barely touch on this here.)

The oscillations in HR and BP are the most commonly studied form of cardiovascular variability. When fluctuations at the same frequency were found in HR and BP, it

was natural to attribute them to the arterial baroreflex because of its ability to elicit fast changes in HR and BP. However, as I explain below, there is no consensus on the role the baroreflex plays in cardiovascular variability. Moreover, there is controversy regarding whether cardiovascular variability can be used as an index of autonomic control of the circulation (Parati *et al.*, 2006;Malliani *et al.*, 2006). The idea that certain frequencies of cardiovascular variability may indicate either sympathetic or parasympathetic tone led to great interest in finding a diagnostic tool for autonomic tone (Malpas, 2002), but as explained earlier in the introduction, the relative contribution from PNS and SNS is still elusive (Fig. 1).

Physiological understanding of cardiovascular variability is still evolving, but clinicians have already investigated various patient populations. Clinically, cardiovascular variability predicts the outcome of cardiovascular diseases (Bigger *et al.*, 1993;Frenneaux, 2004). This observation highlights the fact that a more complete physiological understanding is essential. The question is of course whether cardiovascular variability is an irrelevant by-product of time delays in control mechanisms, or whether the fluctuations are functional. This is highly relevant for patients: is it possible to control cardiovascular variability with selective therapy, or does a change in cardiovascular variability only reveal that something else has changed? Another intriguing question is whether cardiovascular variability has a protective role in certain diseases, or whether these oscillations only indicate health status. The type of therapy selected is also influenced by the underlying cause of the oscillations. Cardiovascular variability is coupled to respiration, but there are also connections to mental states and psychiatric conditions (Gorman & Sloan, 2000). This is why suggested therapies for improving patterns of cardiovascular oscillations have included meditation using special breathing patterns (Cysarz & Bussing, 2005), recitation of the rosary prayer (‘Ave Maria’) or yoga mantras (Bernardi *et al.*, 2001), listen to music (Bernardi *et al.*, 2009), as well as medication (Frenneaux, 2004).

Emerging evidence from clinical and experimental trials suggests that cardiovascular variability may be relevant in the development of pathology, and not only as a

response to disease itself. Hypertension and subclinical end-organ damage correlates with increases in BP variations independent of mean BP levels, and the increased BP variations probably appear early in the development of hypertension (Parati & Lantelme, 2002;Tatasciore *et al.*, 2007). Increases in spontaneous BP fluctuations together with decreased HR fluctuations have been shown to be of both clinical and prognostic relevance in hypertension and heart failure (Bigger *et al.*, 1993;Pinna, 2007).

Returning to the physiological perspective, I should emphasise the diversity of cardiovascular variability – both which variables oscillate, and the frequencies at which they oscillate. Most of the physiological parameters oscillate in healthy humans, and one well known fluctuation is the diurnal pattern in BP. Activities such as exercise also alters the cardiovascular variables, which are subsequently returned to baseline. The variability I will discuss in this part of the thesis is restricted to short-term variations in resting subjects at steady state. It is doubtful whether it is appropriate to evaluate cardiovascular variability in non-resting subject. I will return to this in the general discussion.

Respiratory sinus arrhythmia

Respiratory sinus arrhythmia is a cardiorespiratory phenomenon characterised in mammals by HR fluctuations that are in phase with respiration (Grossman & Taylor, 2007). Central, neural, humoral and mechanical mechanisms act together to generate respiratory sinus arrhythmia, which is a phenomenon that results from the interaction between the cardiovascular and respiratory systems (Grossman & Taylor, 2007). The main mechanism involved is inhibition of parasympathetic (vagal) nerve firing during inspiration, which causes an increase in HR, and removal of this inhibition during expiration.

Several feedforward, feedback and intrinsic mechanisms may play a role (Saul & Cohen, 1994). Briefly, the feedforward mechanism is a central coupling between the respiratory and the cardiovascular centres, where increased firing in the phrenic nerve

elicits an inhibitory signal to the vagal nerve. The feedback mechanisms include the arterial baroreflex, lung stretch receptors, and perhaps the Bainbridge reflex (a reflex increase in HR due to increased venous return). An intrinsic "reflex" has also been described, which causes a minimal change in HR due to sinoatrial node stretch (Saul & Cohen, 1994). However, the question of whether respiratory sinus arrhythmia is mainly of central or peripheral origin is far from resolved (Eckberg, 2009;Karemaker, 2009;Julien *et al.*, 2009).

One fact remains uncontroversial; there are redundancies in the mechanisms that are capable of generating respiratory sinus arrhythmia. One intriguing question is why cardiorespiratory coupling is found not only in mammals but also in fish, reptiles, amphibians and birds (Grossman & Taylor, 2007)? Either this inherited mechanism is still important, or it was important to our ancestors and eliminating it has later given no evolutionary advantage. Respiratory sinus arrhythmia is hypothesised to enhance pulmonary gas exchange (Hayano *et al.*, 1996;Sasano *et al.*, 2002;Hayano & Yasuma, 2003). Furthermore, it is hypothesised that cardiovascular and respiratory systems save energy by removing unnecessary heartbeats and thus pulmonary blood flow during expiration, when alveolar gas volume is reduced (Grossman & Taylor, 2007). This still remains to be established in humans (Tzeng *et al.*, 2009). On teleological grounds, I would argue that the fact that several mechanisms underlie respiratory sinus arrhythmia in humans makes it more probable that this form of arrhythmia indeed has a regulatory role in the cardiovascular system, one which has yet to be clarified.

Besides the effects of respiratory sinus arrhythmia, respiration itself affects the cardiovascular variables. The most striking example is SV, which changes by at least 10-15 % during a respiratory cycle at supine rest (Fig. 6). Inspiration reduces SV from the left side of the heart, while expiration restores SV (Guz *et al.*, 1987;Toska & Eriksen, 1993). We confirmed this in paper III. It has not yet been resolved whether these fluctuations are caused by respiratory fluctuations in venous return only, or whether other factors modulate, dampen or augment respiratory SV fluctuations.

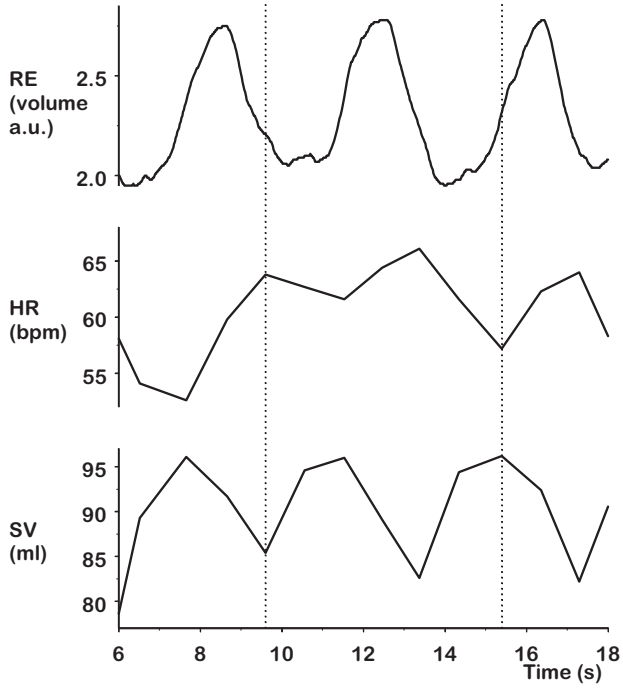

Figure 6: Respiratory variations in HR and SV. Upward slope indicates inspiration (upper panel). The dotted lines illustrate the inverse relationship between HR (middle panel) and SV (lower panel). RE, respiration (volume in a.u., arbritrary units).

Possible mechanisms for reduction of SV during inspiration are many and disputed (Guz *et al.*, 1987). The respiratory variation in filling time due to respiratory sinus arrhythmia is a major cause of the inspiratory decline in SV (Olsen *et al.*, 1985). Variations in filling and/or emptying of the left ventricle due to variations in output from the right ventricle (Harrison *et al.*, 1963), pooling of blood in the lungs (Hoffman *et al.*, 1965), increased afterload (Karam *et al.*, 1984), reduced effective left ventricular ejection pressure (Olsen *et al.*, 1985) and interventricular dependence (Peters *et al.*, 1989) have all been suggested as explanations.

There is controversy about what effect respiratory sinus arrhythmia has on BP. An inspiratory decrease in SV may lead to a decrease in CO, but HR increases simultaneously, and the combined impact on CO is not clear. Systolic pressure (SP) and pulse pressure may rely more on SV (Karemaker & Wesseling, 2008), ejection time, arterial stiffness and wave reflection, while CO is the main influence on MAP at respiratory frequency. To investigate the effect of respiratory sinus arrhythmia on BP, we therefore need to record all the parameters involved.

In paper III, we contributed to the debate on whether variations in BP are buffered or augmented by respiratory sinus arrhythmia (Toska & Eriksen, 1993;Taylor & Eckberg, 1996). We found that conflicting conclusions could actually depend on whether SP or MAP was measured. We also included SV in this study. This is important because although the effect of respiratory sinus arrhythmia on BP is often described, SV is seldom included in the argument (Toska & Eriksen, 1993).

In newborns, positive pressure ventilation increases respiratory-induced SV variations, and this may precede a decrease in CO (Nelson & Janerot-Sjoberg, 2001). Such observations are of great importance in treating critically ill patients when mechanical ventilation and other supportive therapy need to be optimised. Along with the evolving non-invasive techniques for SV measurements, I forecast that respiratory SV variations will be integrated into clinical evaluations of patients in the future.

Mayer waves

In humans, the ~0.1 Hz oscillations in BP are called Mayer waves. Mayer waves have fascinated investigators for more than a century (Mayer, 1876;Malpas, 2002;Julien, 2006). These ~10 second oscillations are intriguing as the arterial baroreflex could be part both of their cause and of the effect. I will try to explain this paradox, but first stress that even though Mayer waves were first described in BP, oscillations at ~0.1 Hz exist in many circulatory parameters, although they may be imperceptible on visual assessment of the recorded traces (Fig. 7). This means that frequency analysis is an appropriate technique.

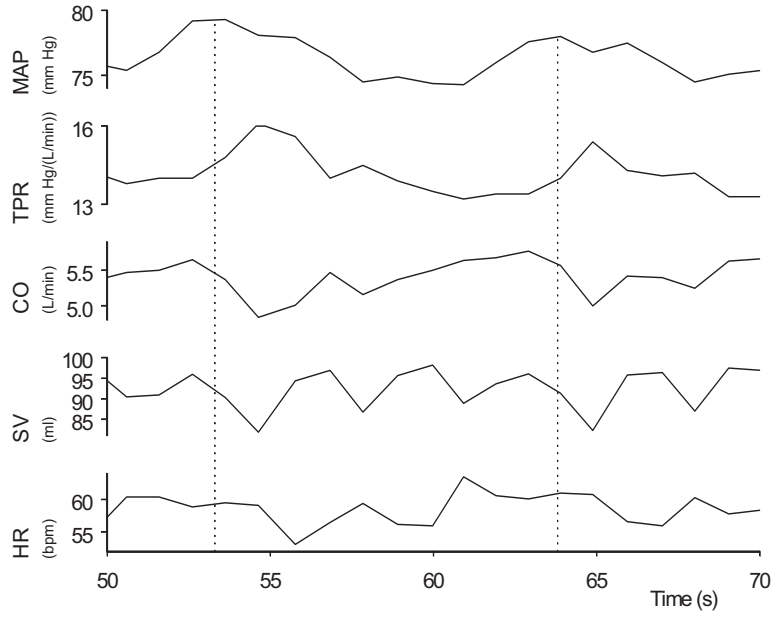

Figure 7: Cardiovascular variables in one subject at rest, illustrating Mayer waves in MAP (peaks at 53 s and 64 s).

It has been proposed that oscillations at ~0.1 Hz in BP are the product of either central oscillator or baroreflex gain and time delays (Malpas, 2002). Figure 8 provides an overview of the relative contributions of various factors to the variations in the cardiovascular variables for each of the test situations described in paper IV, and in particular which factors contribute to the fluctuations at ~0.1 Hz in MAP. Figure 8a shows the situation for supine control with mainly parasympathetic cardiac control. MAP is determined by CO and TPR. TPR in turn is influenced by the SNS, temperature regulatory output via arteriovenous anastomoses (Lossius *et al.*, 1993) and local factors.

CO is determined by SV and HR, but these two factors are not independent: a change in the filling time of the heart may change preload and hence SV. HR variations at ~0.1 Hz are mainly caused by the PNS, as shown by the fact that atropine administration greatly reduces variation in HR (Fig. 3d, paper IV)(Jokkel *et al.*, 1995). More importantly, this shows that oscillations in HR at ~0.1 Hz are not an

index of SNS alone, as most of the oscillations are of parasympathetic origin and thus not suitable as a diagnostic tool for sympathetic tone (Malpas, 2002). SV is dependent on both mechanical factors and ventricular contractility and relaxation mediated by the SNS, and MAP influences SV through the afterload effect. SNS and PNS receive input from the arterial baroreflexes, but other parts of the central nervous system also modulate firing in these nerves, as exemplified by the central oscillator in Figure 8. Central thermoregulation may be one example of a central oscillator. PNS mainly influences HR, while SNS influences HR, SV and TPR. The fluctuations in MAP are sensed by the baroreflex, which completes the closed loop.

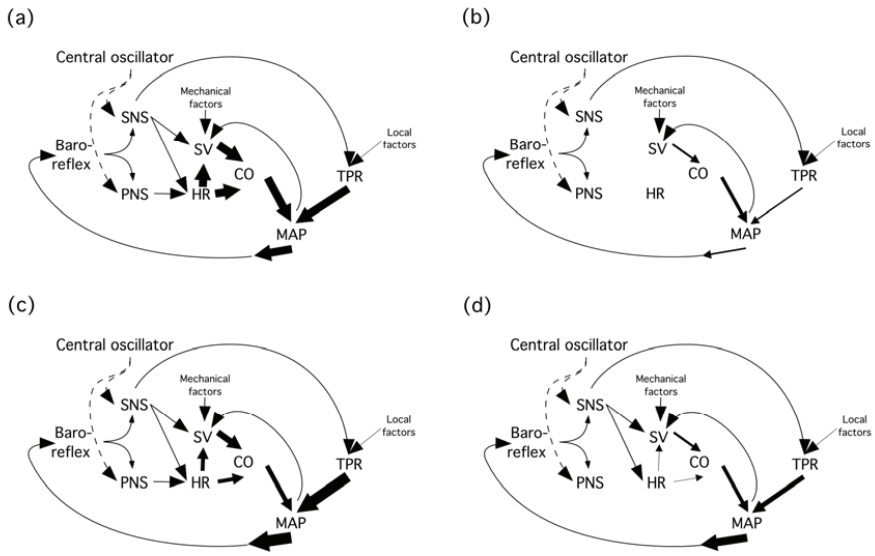

Figure 8: An overview of the parts of the cardiovascular system that influence fluctuations in MAP at ~0.1 Hz. The size of the arrows is derived from Table 1 and Fig. 3, paper IV. See text for more details. Figure 8a illustrates supine control, 8b supine total autonomic cardiac blockade, 8c head-up tilt control and 8d head-up tilt with parasympathetic blockade.

From this overview it is clear that both changes in MAP and phenomena in the CNS may induce the oscillations in MAP at ~0.1 Hz. This complexity may be the reason

why there has been so much debate on HR oscillations at ~0.1 Hz and their capacity to buffer MAP fluctuations.

The peripheral response to signals in the SNS takes about 6 seconds to develop (Wallin & Nerhed, 1982), but the whole loop from a change in BP sensed by baroreceptors to signalling in sympathetic nerves and finally a change in BP, takes about ten seconds to complete (DeBoer *et al.*, 1987;Toska & Eriksen, 1994). The cardiovascular system can produce waves of change every ten seconds that weaken for every circuit of the feedback loop. But as long as events that change MAP occur several times a minute, new disturbances are introduced into the system, and the oscillations at ~0.1 Hz continue to recur. A possible mechanism for the fluctuations in TPR at ~0.1 Hz is that there are slow fluctuations in sympathetic activity to the skin or other organs that are attenuated or reinforced by reflexes, thus forming a resonance loop (Akselrod *et al.*, 1985;DeBoer *et al.*, 1987;Lossius *et al.*, 1993;Toska *et al.*, 1996).

I suggest in accordance with Lossius *et al.* (1993) that one possible origin of the Mayer waves is a connection between skin vascular conductance and MAP fluctuations in a thermoregulatory process. Spontaneous fluctuations in blood flow through the acral skin have a significant impact on variations in BP and HR (Lossius *et al.*, 1993). Cutaneous vasoconstrictions occur two to three times a minute, and are probably caused by simultaneous activation of the peripheral vascular and cardiac efferent branches of the ANS (Lossius *et al.*, 1993). We concluded in paper IV that the oscillations in TPR produce oscillations in MAP, and they could therefore be the input for oscillations in cardiovascular variables at ~0.1 Hz. A similar unifying theory was proposed by Malpas, although he did not point to central thermoregulation as the central oscillator (Malpas, 2002). Another hypothesis is that slower TPR oscillations are produced by autoregulatory myogenic response while Mayer waves are produced by baroreflex resonance (Julien *et al.*, 2008).

Oscillations in HR at ~0.1 Hz are probably of baroreflex origin (Bernardi et al., 1994;Grasso et al., 1997;Fazan, Jr. et al., 2005). HR oscillations produced by the

arterial baroreflexes supposedly buffer BP changes. But the ~0.1 Hz oscillations in HR do not buffer BP variations effectively, at least not in the supine position (Taylor & Eckberg, 1996;Cooke *et al.*, 1999). We confirm these results in our paper IV. However, in paper IV, we also found that an analysis of CO fluctuations, which directly influence BP oscillations, makes an alternative conclusion possible: CO fluctuations at ~0.1 Hz mildly dampen MAP oscillations at ~0.1 Hz. The latter are produced by fluctuations in TPR (Cevese *et al.*, 2001). Our finding was made possible by the fact that we studied SV oscillations and thereby fluctuations in CO, and did not base our results on HR and BP measurements only. Once again we conclude that SV variations actively contribute to cardiovascular changes.

The overall effect of the arterial baroreflexes is to buffer variations in BP. However, arterial baroreflexes may paradoxically also promote ~0.1 Hz BP oscillations through a resonance phenomenon in the baroreflex loop (Julien *et al.*, 2008). There is a possibility that exposing subjects to greater physiological challenges than we did would change the baroreflex to give a clearer buffering effect at ~0.1 Hz (Taylor & Eckberg, 1996). Posture affects time delays in the arterial baroreflex (Gulli *et al.*, 2005), as confirmed in paper IV, and in my opinion this suggests that the arterial baroreflexes adjust to buffer BP oscillations.

BP fluctuations are limited by cardiovascular reflexes. In an areflexic preparation (ganglionic blockade in rat), BP was highly unstable due to TPR fluctuations, and BP changes were usually initiated by sharp decreases in SV and CO (Letienne *et al.*, 1998). The BP changes per se may also elicit TPR changes via a myogenic response (Letienne *et al.*, 1998). Lossius *et al.* (1993) reported a similar finding, but they studied arteriovenous anastomoses and attributed the changes to them (Lossius *et al.*, 1993). In another paper, Eriksen and Lossius (1995) proposed that a central oscillator that opens and shuts arteriovenous anastomoses is causally connected with respiration during normal quiet breathing. This could link very low frequency oscillations (>20 s) not only with Mayer oscillations, but also with oscillations at respiratory frequency.

In addition, if the baroreflex affects SP and MAP differently (as we showed in paper III) many earlier conclusions may be revised. As mentioned previously, the input to the baroreflex is stretch in the arterial wall. BP may at best be only a marker of this stretch and SV may in fact be a better indicator of input to the baroreflexes (Eckberg, 2008).

Oscillations in BP at ~0.1 Hz in dogs have an antihypertensive effect by stimulating nitric oxide liberation and reducing renin activation, and thus linking BP oscillations to renal function (Nafz *et al.*, 2000). I suggest that both too much and too little variability in BP indicate an unhealthy cardiovascular system. To obtain the appropriate balance, a mix of feedforward and feedback mechanisms may be necessary (Legramante *et al.*, 1999). The interactions in the cardiovascular system that result in oscillations at ~0.1 Hz may improve overall cardiovascular performance (Nafz & Persson, 2001). The baroreflex may be one control mechanism that replaces the unwanted variations in BP with better tolerated or even favourable HR variations (Parati & Lantelme, 2002;Karemaker & Wesseling, 2008). However, further studies need to be conducted to explore possible functions of Mayer waves, and to establish their place in clinical practice.

5. Aims

The aim of the study was to investigate the circulatory changes that occur during mild exercise and in connection with spontaneous cardiovascular oscillations. More specifically, I focused on the role of SV variations in short-term regulation of the circulation. The specific aims of each paper were as follows:

I) Are baroreflex resetting and local vasodilation sufficient to produce the cardiovascular adaptations observed at the onset of exercise? (Paper I)

II) Does SV change during mild supine exercise? Is there a redistribution of blood flow even when HR < 90 bpm? (Paper II)

III) Does respiratory sinus arrhythmia buffer BP oscillations? (Paper III)

IV) Does oscillation in HR and CO at ~0.1 Hz buffer Mayer waves? (Paper IV)

6. Discussion of methods

6.1 Subjects

In all 30 healthy subjects, including both men and women were studied for papers I-IV. Ten participated in the experiments performed by Karin Toska at MIT in 1995-96 (papers III & IV), and another 10 in her experiments in Oslo (Toska & Eriksen, 1994) (paper I). We recruited 13 subjects for our exercise study (paper II), but as a result of technical difficulties only 10 were used in the analysis.

6.2 Experimental methods

6.2.1 ECG: R-detection and the beat-by-beat recording method

In all experiments, the subjects were monitored by a three-lead ECG (lead II) (SD-50, SD-100 or CFM-750, Vingmed Sound A/S, Horten Norway). The recording program used makes it essential to record the RR interval precisely, as many of the other recorded parameters are processed in relation to HR. The R detection of the program is very robust (Toska, 1995). Most of the cardiovascular variables are logically reported beat-by-beat, i.e. MAP, SP, SV, CO and sometimes TPR. We have attributed them all to the next QRS complex (Fig. 9). In most of our studies this is unimportant, but it may be of significance in studies of phase relationship.

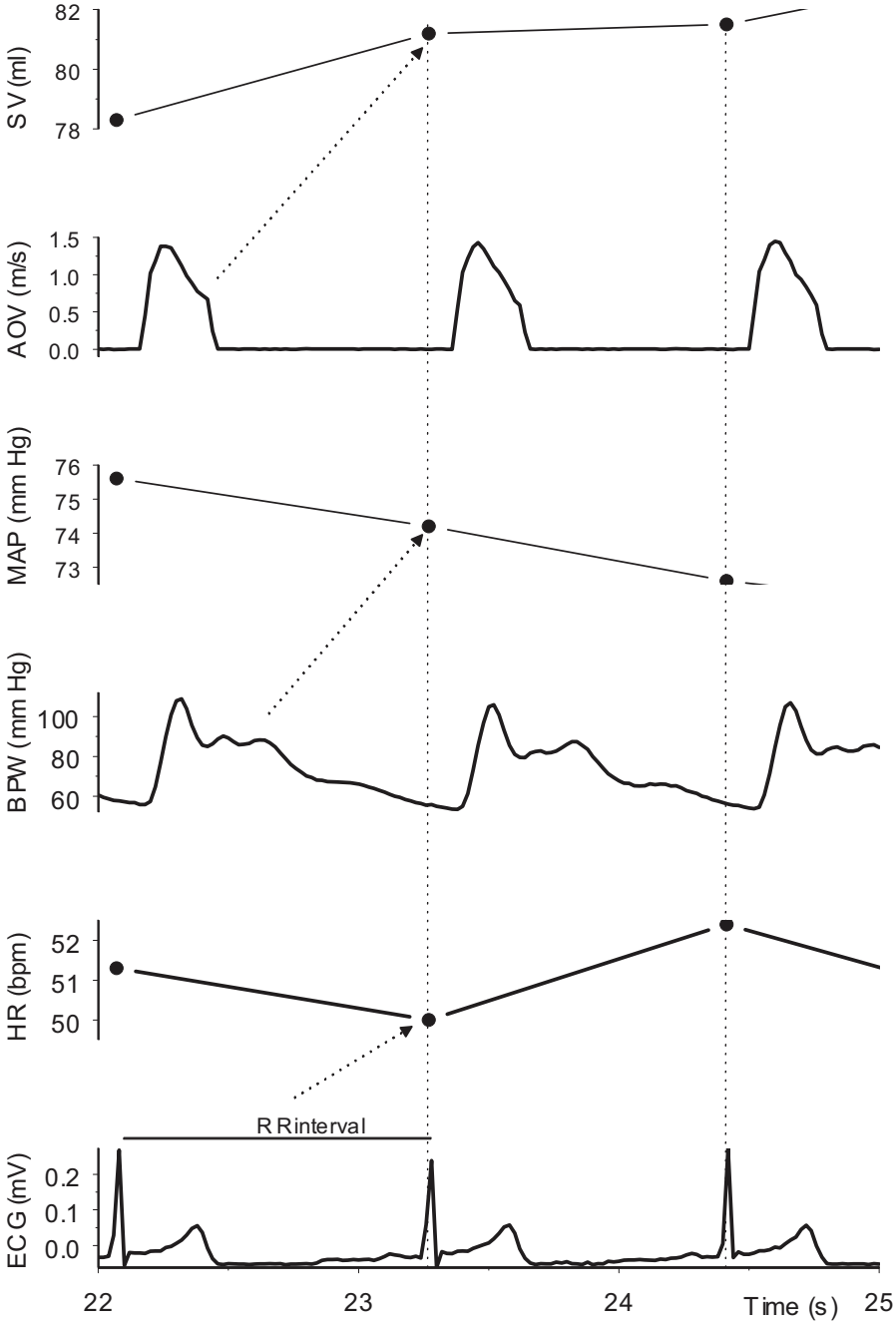

Figure 9. RR interval from ECG is used to calculate HR, blood pressure wave (BPW) to calculate MAP, and aortic velocity (AOV) to calculate SV as described in methods.

6.2.2 Stroke volume using ultrasound Doppler

We used an improved method to measure SV (Eriksen & Walløe, 1990;Eriksen, 1991). The important features of this method are 1) the velocity profile is rectangular through the aortic valve, 2) this velocity is conserved as the maximal velocity for a few cm into the ascending aorta, and 3) the sample volume is large enough to include the site for the maximal velocity at all times. We used SD-50, SD-100 or CFM-750 (GE Vingmed, Horten, Norway), with a stand-alone bidirectional pulsed 2 MHz Doppler probe hand-held at the suprasternal notch, and assumed an angle of 20 degrees between the sound beam and the direction of the bloodstream in the ascending aorta. To remove vessel wall and valve motion artefacts, together with any recorded diastolic movement of blood, the built-in high pass filter was set to remove signals originating from velocities of < 0.25-0.3 m/s. The maximal velocity signal was transferred to the recording computer and the aortic velocity integral was calculated (program for real-time data acquisition (version REGIST3), Morten Eriksen, Oslo, Norway). The integral was multiplied by the area of the orifice (described below) to give beat-by-beat SV (Fig. 9).

This method involves certain assumptions, for instance that the aortic valve area is almost circular. In paper II, we measured the diameter of the aortic ring as 'trailing-to-leading edge' (Skjaerpe *et al.*, 1985) (Vivid 7, GE Vingmed, Horten, Norway) while in papers I, III and IV, the diameter was measured as 'leading-to-leading edge' (CFM-750) (Eriksen *et al.*, 1990). The former method may underestimate the flow area compared to the latter, but there will be only a small difference. In addition, the absolute value of SV was not important as we were looking at transient changes.

6.2.3 Femoral blood flow by ultrasound Doppler

The femoral blood flow (FF=MBF) measurements for paper II were performed by Inger Helene Nådland (Wesche, 1986;Nådland *et al.*, 2009). For this method, it is important to ensure that the sample volume includes the whole diameter of the artery, as the method uses the spatial average velocity. We used SD-100, with a stand-alone

bidirectional pulsed 3 MHz Doppler probe, and aimed at an angle of 45 degrees between the sound beam and the direction of the bloodstream in the femoral artery. The cross-sectional intensity-weighted mean velocity signal was transferred to the recording computer and the velocity integral was calculated (program for real-time data acquisition (version REGIST3), Morten Eriksen, Oslo, Norway). The integral was multiplied by the area of the artery to give beat-by-beat FV. The diameter of the femoral artery at the recording site was measured by CFM-750.

6.2.4 Continuous finger arterial blood pressure

Continuous finger arterial BP was recorded by a Finapres (2300 Finapres BP monitor; Ohmeda, Madison, WI, USA). In every experiment a finger cuff was placed around one finger, and the finger was kept at heart level at all times. This device uses photoplethysmographic method to ensure constant blood volume in the finger throughout the cardiac cycle using a clamp, and converts the pressure of the clamp into finger arterial BP (Bogert & van Lieshout, 2005). This non-invasive technique measures both MAP and DP as reliably as intra-arterial devices (Imholz *et al.*, 1998). However, as the pressure is measured in small arteries at some distance from the heart, wave reflection will tend to produce a larger pulse pressure than it would closer to the heart. The reduction in DP and increase in SP will be cancelled out in the calculation of MAP. In all four papers, MAP was calculated by integration of the BP wave and divided by the RR interval (Fig. 9). DP in our recordings will be the lowest BP value within the heartbeat, and not the 'actual' DP. I have therefore disregarded the DP measurements.

In paper II, the calibration signals from the Finapres were filtered by a specially designed program in MATLAB. The calibration signal was recognised and interpolation was made between the last and first successful measurement. In addition, each filtration was visually checked. In papers I, III and IV, the calibration process was turned off during the experiments.

6.2.5 Respiratory chest volume changes

In paper II, an index of lung volume was measured by one belt around the chest (Respiration and Body Position Amplifier, Scan-Med a/s, Drammen, Norway). In paper III (and IV) respiration was measured by two-belt chest-abdomen inductance plethysmography (Respitrace System, Ambulatory Monitory Systems, Ardsley, NY, USA). For these studies, the equipment was calibrated for volume changes before each recording.

6.2.6 Autonomic cardiac blockade

In papers III and IV, we obtained a pharmacological autonomic cardiac blockade by administering atropine and propranolol. Propranolol is a non-selective β_1- and β_2-adrenergic blocker. Atropine is a muscarinic acetylcholine blocker and penetrates the blood-brain barrier (Rang *et al.*, 1995). The intention of the pharmacological autonomic cardiac blockade was to remove the nervous regulation of HR and thereby the fluctuations in HR. In the terminology of system physiology this blockade opens the closed loops in the cardiovascular reflexes with efferent effects in the PNS and SNS. For future studies, a combination of a peripheral parasympathetic blocker (glycopyrrolate) and β_1-blockade might be a more specific cardiac autonomic blockade without an increase in BP as observed in our study (papers III and IV) (Ogoh *et al.*, 2005).

6.3 Mathematical simulation models

Mathematical simulation models are often introduced to shed light on how different processes interact. The mathematical model used in this thesis was initially developed by Morten Eriksen and Karin Toska to investigate circulatory changes during sudden changes in peripheral resistance (induced by thigh cuff inflations) (Toska *et al.*, 1996). My contribution was to modify the model to simulate the onset of dynamic leg exercise. Most of the programming structure was set in advance. The special feature

of this mathematical model is that it compares recorded and simulated data (Toska *et al.*, 1996). We chose to make the model as simple as possible, leaving out mechanisms we thought were less important even though they are important in providing redundancy in the cardiovascular control mechanisms. However, even with only two important changes between rest and exercise, the mathematical model has many degrees of freedom. The simulated sensitivities, time constants and delays should therefore be verified by conducting experiments in humans or animals. The simulated afterload effect was -0.8 ml/mm Hg, whereas we calculated it to be -0.4 ml/mm Hg in paper II. The parasympathetic sensitivity was ~35 ms/mm Hg (with HR 60 this means -1.6 bpm/mm Hg). This is four times recently reported values (Ogoh *et al.*, 2005). At the mild level of exercise performed by the subjects (HR < 90 bpm), we found that the sympathetic cardiac branch added little to the HR changes and omitted this from the model. This is in agreement with findings that HR is dominantly controlled by vagal firing until 100-120 bpm (Ogoh *et al.*, 2005). But as I pointed out in the introduction, the PNS and SNS act simultaneously over a wide range of the HR response (Fig. 1).

"The beauty of modeling is that it puts physiological insight to the test: does my interpretation of experimental findings fulfill all the requirements: can it describe what has been measured,....?" (Karemaker, 2009). With this quotation I leave the topic of mathematical modeling of cardiovascular responses.

6.4 Mathematical calculations

Mathematical tools are very important in evaluating physiological processes but also have their limitations, so that the results must often be interpreted with some caution.

6.4.1 Calculations of derived variables

CO was calculated beat by beat from corresponding HR and SV values. TPR was calculated by dividing MAP by CO beat by beat, and FPR by dividing MAP by MBF

beat by beat. We had no measure of central venous pressure and calculated resistance by assuming central venous pressure and femoral venous pressure to be zero. The former does not change during mild supine exercise (Toska & Eriksen, 1994), whereas the latter probably at least shows rhythmic changes during dynamic leg exercise.

$$CO = HR \bullet SV$$

$$TPR = \frac{MAP}{CO} \text{ and } FPR = \frac{MAP}{MBF}$$

In order to calculate RPR we took advantage of the fact that the regional conductances can be summed to obtain the total vascular conductance in the body and the fact that conductance (C) is the inverse of the resistance (R). Thus, total peripheral conductance (TPC) equals the conductance in the right and left leg (FPC) added to the remaining conductance (RPC):

$$TPC = rightFPC + leftFPC + RPC \text{ and } rightFPC = leftFPC$$

$$TPC = 2 \bullet FPC + RPC \text{ and } C = \frac{1}{R}$$

$$\frac{1}{TPR} = \frac{2}{FPR} + \frac{1}{RPR} \text{ giving } \frac{1}{RPR} = \frac{1}{TPR} - \frac{2}{FPR}, \text{ which equals}$$

$$RPR = \frac{1}{(\frac{1}{TPR} - \frac{2}{FPR})}$$

At rest (rest1, paper II), RPR was 19.9 mm Hg/(L/min).

6.4.2 Spectral analysis

Much of the analysis of cardiovascular variability is done in the frequency domain. Analyses in the frequency domain include both variability analyses at different frequencies that are thought to be of different origins and analyses of their

relationship at these frequencies. There has been a growing number of publications on cardiovascular variability after Penaz introduced frequency domain research in 1978 (Peñáz, 1978;Cohen & Taylor, 2002).

We analysed variations in the cardiovascular recordings obtained from a five-minute recording. The consensus is that in order to draw conclusions from one recording, it must contain at least ten times the wavelength of the oscillation in question (Peñáz, 1978;Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology, 1996). The limit of resolution of a five-minute recording is oscillations every 30 s or less frequently. I have focused on cardiovascular variability at respiratory frequencies (~0.25 Hz) and at intervals of approximately ten seconds (~0.1 Hz).

We selected the frequency intervals to be analysed on the basis of the individual's respiratory frequency and used an interval of bandwidth 0.15 Hz. The Task Force recommends choosing a fixed interval of 0.15-0.40 Hz (Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology, 1996). During spontaneous breathing, the respiratory frequency of each individual is quite stable, but in the ten subjects studied in paper III, peak respiratory frequency varied between 0.16 Hz and 0.37 Hz. If we had followed the Task Force's recommendation, we should have used a fixed breathing frequency of 0.25 Hz. This would probably not have changed our results as paced breathing does not affect respiratory sinus arrhythmia differently from spontaneous breathing (Grossman & Taylor, 2007). In paper IV, we analysed the frequency interval 0.04-0.15 Hz

Caution should be shown in switching between the frequency and time domain. The duration of the heart period (converted to HR), MAP during the heartbeat, the DP just after the R-wave that started off the beat and the SP within that beat are all given the same sequence number (Fig. 9) (Karemaker, 2009). When only BP and HR are available, it is impossible to measure time delays within the heartbeat (Karemaker, 2009). This is important when evaluating phase delays. "Any correlation that is highest within the same beat will show up as a phase delay of zero, or rather a

number within the boundaries of that beat." (Karemaker, 2009). In papers III and IV, I used the mathematical technique fast Fourier transform to calculate power spectra and cross-spectra (Toska, 1995).

The cross-spectral transfer function is a noncausal correlation technique that does not have directionality (Julien *et al.*, 2008). In a closed loop, the results from cross-spectral analysis are therefore open to interpretation. If we change the configuration to an open loop (for instance by autonomic blockade), the use of cross-spectral analysis is more straightforward. However, redundancies in the control systems include both neural and mechanical factors, as I mention in the general discussion, and this may be of some significance in analyses of mechanisms in the cardiovascular system. As pointed out in paper IV, a decrease in coherences between cardiovascular variables (physiologically connected) after autonomic blockade indicate that the relationship is neurally regulated. Low coherence values obtained from cross-spectral analysis in a closed loop set-up may imply that the variations are connected by both neurally and non-neurally mediated mechanisms (Julien *et al.*, 2008).

Coherence is an important concept in cross-spectral analysis. In this technique, two independent signals are compared to provide phase angles and transfer functions between them at different frequencies and calculate the coherence value. If a transfer function (for instance a lateral shift of one of the curves (adding of a phase angle)) makes the two curves identical, their coherence is exactly 1. This is the case for HR and RR interval (which are dependent variables). Coherence is a number between 0 and 1, and the coherence of two signals indicates how well correlated the signals are as quantified by the cross-spectral analysis. Coherency can be compared to the correlation coefficient in regular statistics; only that it is calculated independently for each frequency and for the best phase shift. When the coherence function exceeds 0.5 at any frequency, the phase function provides a statistically reliable estimate of the time relations between the two signals (Taylor & Eckberg, 1996). We averaged the phase angles by weighting the phase angles with their squared coherence (papers III & IV) with the same result as choosing a cut-off at coherence value of 0.5.

6.5 Statistical methods

The statistical tools I have used are estimation by arithmetic mean, median and non-parametric Wilcoxon median. My first chronological article (paper III) contains nonparametric paired Wilcoxon signed rank sum tests against a one-sided alternative. Paper I reports mainly on individual parameters. Papers II and IV use estimation by Wilcoxon median and the nonparametric paired Wilcoxon signed rank sum test against a two-sided alternative (Hollander & Wolfe, 1999). I no longer assume that the cardiovascular variables are normally distributed.

6.5.1 Coherent averaging and estimation by Wilcoxon method

In papers I and II, we studied transient changes at the onset (and cessation) of mild exercise. Many researchers do not use coherent averaging to report transient changes in the recorded parameters at the time resolution we use, and small changes over short periods may therefore be overlooked. The method we use to report transient changes is called coherent averaging (Toska *et al.*, 1994). It may be necessary to average response dynamics from multiple trials to provide better characterisation of the physiological responses for a given individual (Tschakovsky *et al.*, 2006). The technique of coherent averaging reduces the noise introduced by the measuring techniques and also from irrelevant oscillations in the cardiovascular parameters, for instance those produced by respiration. Calculating the median of the responses in several identical experiments on one subject (Fig. 10) makes it easier to detect the transients. The purpose of this technique is to highlight similar responses to a stimulus and to reduce the noise in the recordings. We also used coherent averaging to present averaged group responses, where the ten individual responses were coherently averaged (Fig. 11). In paper II, we used the Wilcoxon median in the coherent averaging process, while in paper I, the arithmetic mean of the responses provided the basis for comparison with the model (Toska & Eriksen, 1994).

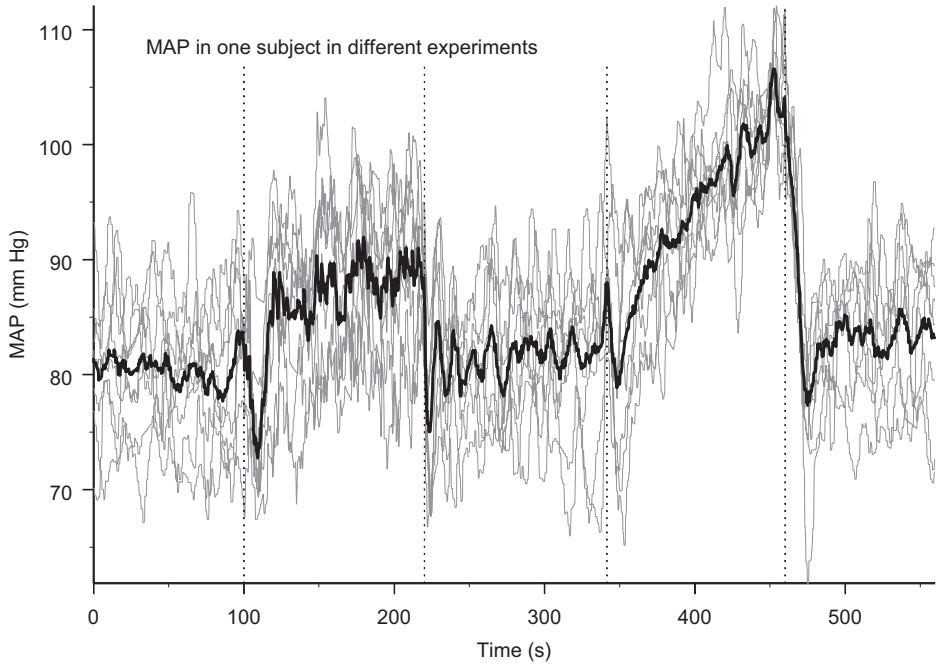

Figure 10: Coherent averaging for one individual. Grey lines indicate the eight identical experimental runs, while the solid black line indicates the estimated Wilcoxon median. Dotted vertical lines indicate onset and cessation of dynamic leg exercise and static hand grip.

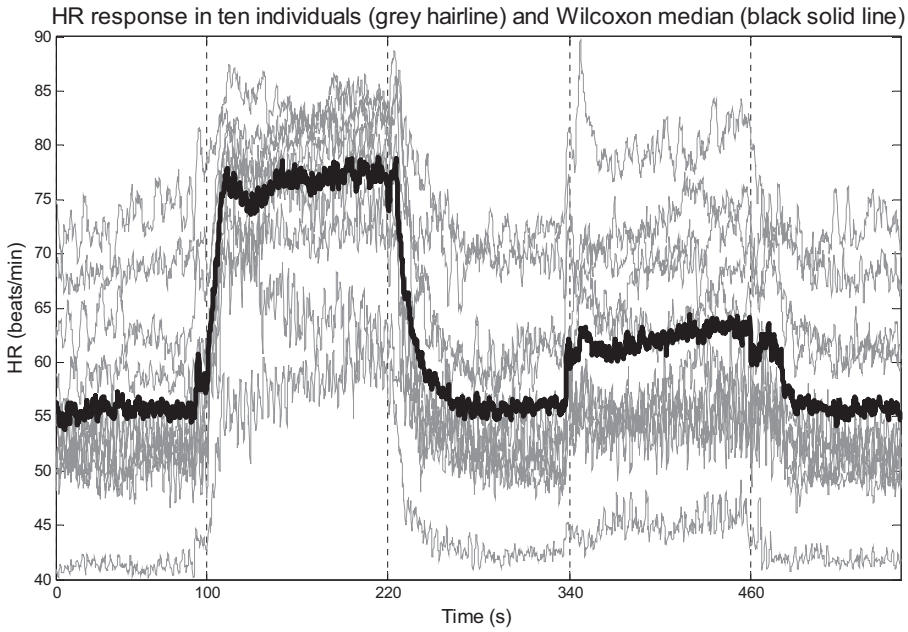

Figure 11: Coherent averaging for the group: hairlines (ten different subjects, eight experimental runs for each subject) and solid black line (Wilcoxon median of the HR response in the ten subjects). Dashed vertical lines indicate onset and cessation of dynamic leg exercise and static hand grip.

This technique was extremely important for the studies of SV during mild exercise in paper II. SV is never a static value as there are large respiratory variations. For each individual, the respiratory SV variations in each experimental run were larger than the response to the stimuli (here mild dynamic and static exercise) (Fig. 12). A small change in SV could therefore be camouflaged by respiration; however, by coherent averaging we could detect a tendency and were able to perform statistical tests on the change.

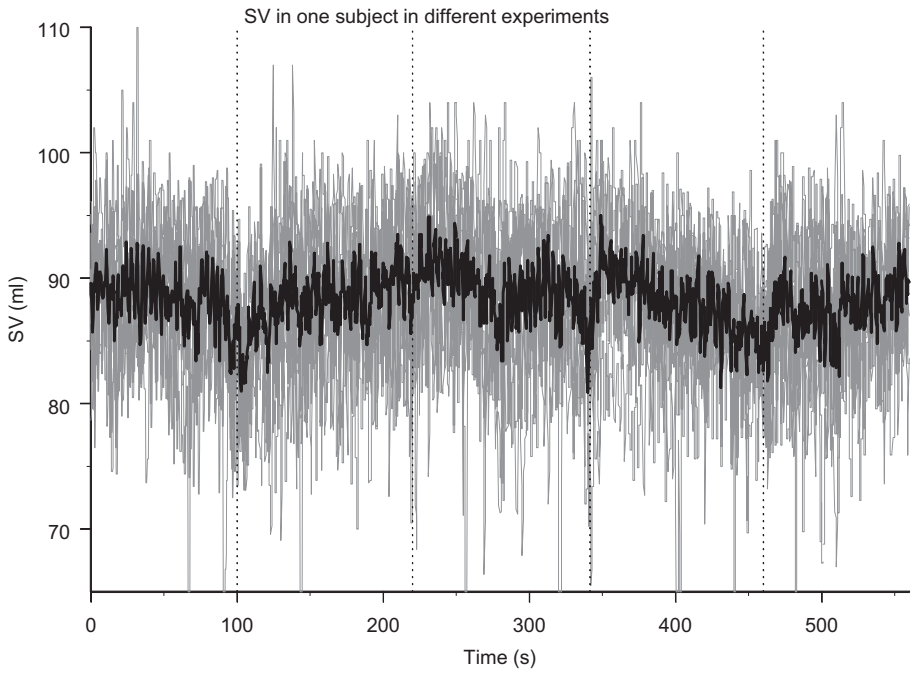

Figure 12: SV from eight similar experimental runs (grey lines) in one subject, and the coherently averaged response (black line, Wilcoxon median). Dashed vertical lines indicate onset and cessation of dynamic leg exercise and static hand grip.

When we estimated the afterload effect (paper II), we calculated the linear regression coefficient during the last 90 s of exercise between SV (dependent variable) and MAP in the Wilcoxon median response for each subject. To overcome the internal dependence between the successive values of SV and MAP, we used only every tenth point in the regression analysis supported by the autocorrelation functions (Papoulis, 1965). Each subject had two regression line coefficients, one from dynamic leg exercise and one from static hand grip. We estimated the Wilcoxon median and 95% confidence interval for the regression line coefficients.

7. Summary of papers

7.1 Model simulations of cardiovascular changes at the onset of moderate exercise in humans

In paper I, we used a modified mathematical model to simulate the cardiovascular changes at the onset of mild exercise. We hypothesised that two mechanisms were sufficient to elicit the observed changes: baroreflex resetting and vasodilation in exercising muscles. The simulated responses of MAP, RR interval and SV were compared with experimental data, and the parameters were adjusted individually to optimise the curve fit. This simple model was able to explain almost all of the cardiovascular changes at the onset of exercise. However, even this model had redundancies and the many degrees of freedom in the mathematical model are a potential drawback, although we did try to reduce the number.

7.2 Stroke volume decreases during mild dynamic and static exercise in supine humans

In paper II, we studied the cardiovascular response to mild supine exercise. We hypothesised that SV would remain unchanged during both dynamic and static exercise, and that FV would remain unchanged during static hand grip. We observed that SV decreased during both dynamic and static mild supine exercise due to the afterload effect. The increase in MAP accounted for ~80 % of the decrease in SV. FV decreased during static hand grip, but an increase in HR counteracted the changes in MBF. This indicates that there is active redistribution of blood flow even during mild exercise. In addition, anticipatory responses were apparent during countdown to exercise.

7.3 Respiratory sinus arrhythmia: opposite effects on systolic and mean arterial pressure in supine humans

In paper III, we investigated and discussed whether respiratory sinus arrhythmia buffers or reinforces BP variations. Previous studies had reached conflicting conclusions, and we identified measurement of different aspects of BP as the potential cause of this. Removal of HR variations by pharmacological autonomic cardiac blockade decreased SV variations in both supine and tilted position. In supine position, removal of HR variations led to an increase in MAP variations, while SP variations decreased. This was probably the cause of the conflicting results reported in the literature, and we concluded that respiratory sinus arrhythmia buffers fluctuations in MAP. In the tilted position this was more pronounced, as removal of HR variations increased variations in CO, MAP and SP. Thus, respiratory sinus arrhythmia buffers MAP regardless of posture, while SP variations are only buffered during orthostatic stress.

In this paper, I should have acknowledged Lars Walløe's contributions.

7.4 Low frequency fluctuations in heart rate, cardiac output and mean arterial pressure in humans: what are the physiological relationships?

In paper IV, we used a pharmacological autonomic cardiac blockade to investigate Mayer waves (~10 s oscillations in BP). We hypothesised that variations in CO (produced by variations in HR and SV) buffered MAP variations at ~0.1 Hz. In the supine position, removal of HR oscillations reduced all cardiovascular variability at ~0.1 Hz. Tilting the subject reduced HR and SV variations (both non-significantly), while CO variations were significantly reduced. Variations in both MAP and TPR increased non-significantly. Reduction of HR variations in tilted position by parasympathetic blockade reduced SV variations, but did not change CO variations significantly from the tilted position. With intact autonomic control, variations in CO and TPR were inversely related, as were variations in HR and SV. In addition,

variations in HR were in phase with CO variations, and MAP variations were in phase with TPR variations. In conclusion, variations in MAP at ~0.1 Hz are produced by variations in TPR, and CO variations mildly dampen fluctuations in MAP. As HR variations produce these CO variations, variations in HR mildly dampen fluctuations in MAP at ~0.1 Hz. Most of the HR variations at ~0.1 Hz are produced by the parasympathetic activity, and are therefore not a good indicator of sympathetic tone.

8. General discussion and conclusions

In all four papers in this thesis, the arterial baroreflex is discussed as a central explanatory mechanism. This reflects its importance in the short-term regulation of the circulation. I conclude that the arterial baroreflex plays a very important role both during mild exercise and in controlling cardiovascular variability. This is not an entirely new observation, but below I discuss some of the findings from our studies that broaden our understanding of variations in SV.

8.1 Cardiovascular variability during mild exercise

As the papers for this thesis cover two main topics, mild exercise (papers I and II) and cardiovascular variability (papers III and IV), I wanted to combine the two topics by studying respiratory variations in cardiovascular variables during exercise. My general hypothesis was that reflex-mediated neural cardiovascular variability buffers unwanted mechanically-imposed variations. During light exercise, where the PNS still controls HR (Fig. 1), respiratory sinus arrhythmia is apparent. I therefore hypothesised that during light exercise, respiratory sinus arrhythmia would decrease (due to the decrease in parasympathetic activity) (Grossman & Taylor, 2007), and variations in SV would increase because they would not be as effectively counteracted by HR variations. This has proved to be an over-simplification. As I have mentioned a number of times, there are various control mechanisms and a great deal of redundancy in the cardiovascular system. In addition, the relationship between SV and respiratory sinus arrhythmia may be more complex than simply that variations in HR and SV are in inverse phase. Nevertheless, I reanalysed the data collected for paper II at rest and during dynamic leg exercise and calculated respiratory variations in HR and SV by spectral analysis (Table 1).

HR increases while SV is unchanged from rest to exercise (Table 1). In paper II, SV decreased in the same subjects during the last 30-40 s of exercise, so I emphasise that

in the present analysis I included the last 90 s of the 120 s dynamic leg exercise. The length of the respiratory cycle decreased from 3.6 s at rest to 3.1 s during mild exercise. The time lag between changes in HR and SV was unchanged. The essence of the spectral analysis was that light intensity exercise reduced respiratory variations in HR (non-significantly), while in contrast to what I expected, variations in SV at respiratory frequency declined. Similarly, in papers III and IV we found that variations in SV decreased after removal of HR variations by autonomic cardiac blockade. Perhaps variations in filling time are more important than we concluded in paper II (Olsen *et al.*, 1985).

Table 1. Estimates of Wilcoxon medians (95 % confidence interval) at supine rest and during supine dynamic leg exercise

	Supine rest	Supine dynamic leg exercise
HR (bpm)	53 (48, 61)	75* (67, 81)
SV (ml)	81 (67, 101)	80 (64, 101)
Respiratory frequency (Hz)	0.28 (0.25, 0.30)	0.32 * (0.28, 0.37)
HR variations (bpm^2)	2.8 (1.0, 5.9)	1.8 (0.7, 3.9)
SV variations (ml^2)	12.1 (9.4, 15.6)	8.6 * (7.4, 9.7)
Time lag between HR and SV (s)	1.56 (1.42, 1.71)	1.52 (1.33, 1.89)

This analysis was similar to the one in paper III. From each of the nine subjects recordings of 80-90 s at rest or during dynamic leg exercise were analysed. Each recording was without loss of signal and each subject had 3-8 recordings from each condition.
* Significantly different tested by one-sample test for individual difference (n=9)

This investigation did not record tidal volume. The magnitude of respiratory sinus arrhythmia under steady-state conditions is inversely related to respiration rate and directly related to tidal volume (Grossman & Taylor, 2007). Venous return and thus SV may also be related to tidal volume (Guz *et al.*, 1987).

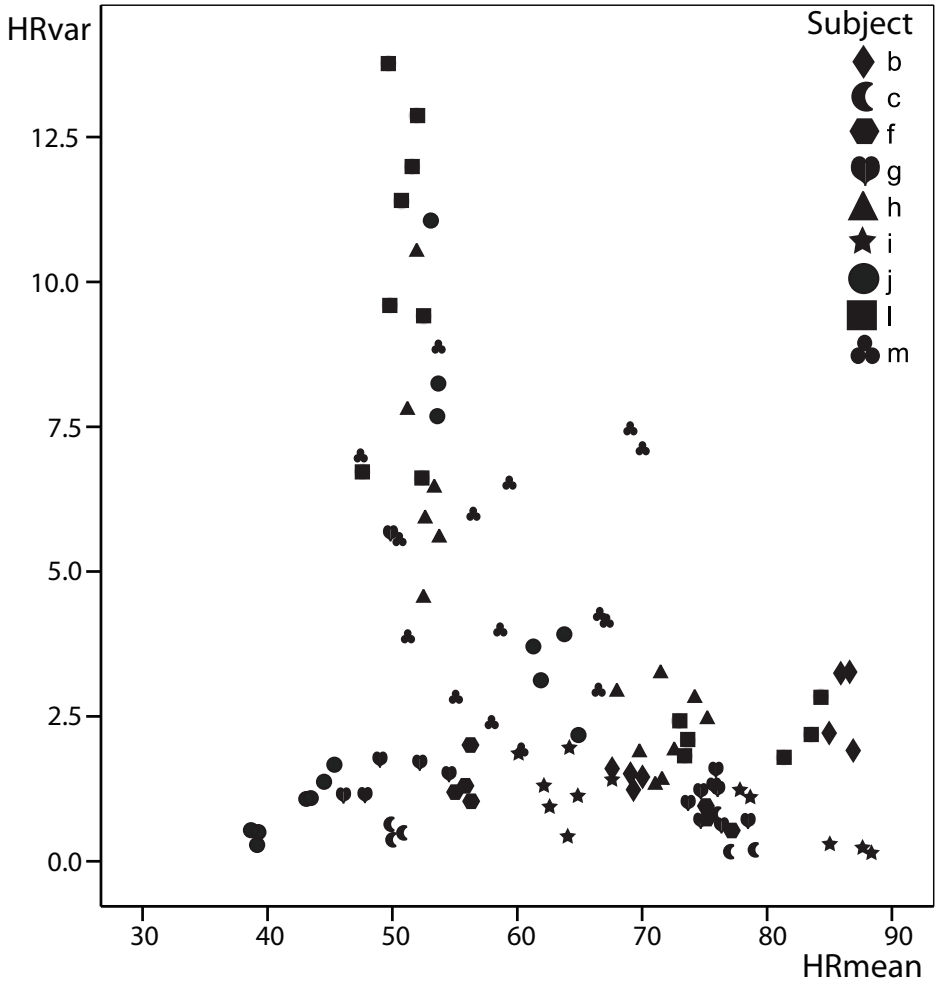

Figure 13: There is no obvious linear connection between HRmean (an indication of parasympathetic firing activity) and HRvar (variation in HR at respiratory frequency). Small variations during high parasympathetic tone (low HR) indicate that the relationship between the oscillations in firing in PNS and its mean firing activity is not linear.

As there is no linear relationship between respiratory sinus arrhythmia (variations in HR) and parasympathetic tone (mean of HR) (Fig. 13), it is not a good indicator of parasympathetic tone (Malpas, 2002). The relationship between respiratory sinus arrhythmia and vagal tone is quadratic across the entire range of vagally mediated HR change (Grossman & Taylor, 2007). This may be linked to saturation of the vagal effects across the respiratory cycle, loss of phasic respiratory changes in the vagal nerve discharge, or a simple floor effect in which minimal HR has no room left to oscillate (Grossman & Taylor, 2007).

In this study, I ignored MAP and its oscillations, thus disregarding a central part of the circulatory control mechanisms, as I discuss in the next section. So this part of the investigation did not result in any significant new knowledge as I do not have proper explanation for the decrease in variations in SV in this study.

I would now like to return to the question of whether it is possible to evaluate cardiovascular variability in a non-resting subject. It could be argued that a subject in the head-up tilted position or engaged in mild exercise, though not resting, may reach a new steady state as defined by Penaz (1978). I propose that cardiovascular variability can be evaluated in this new condition, and that this may reveal important changes in neural regulation mechanisms. However, I reason that this depends on what the variability reflects. If variability is a by-product of time delays without any physiological impact, it will be of less importance to measure the variations, and we should look for a better indicator of autonomic activity.

During my investigations, I have become more and more convinced that any answers regarding the relationship between autonomic activity and cardiovascular oscillations will depend on an integrated analysis of both the cardiovascular and the respiratory systems. Even though the studies described here have not provided a tool for evaluating oscillations during mild exercise, it may be possible to develop one in the future. I conclude that SV is a highly changeable variable that is influenced by a variety of factors. My studies show that SV is not a constant value and that the product of HR and SV also varies. SV changes from one beat to the next. I hope that

in future we will be able to gain further insight into the cardiovascular system and its interactions with the respiratory system on the basis of a better understanding of SV variability.

8.2 The afterload effect and the arterial baroreflex

In this last section, I will take the opportunity to comment on a central aspect of the short-term regulation of SV and CO in comparison with the regulation of BP. The afterload effect and the arterial baroreflex may work in concert to counteract changes in MAP. Previously it has been concluded that SV does not contribute to the cardiac part of the baroreflex (Ogoh *et al.*, 2002;Ogoh *et al.*, 2003). At least theoretically, the arterial baroreflex may elicit a sympathetic change of the contractility (inotropic effect) and perhaps of relaxation (lusitropic effect) of the heart that reflexly may change SV (Katz, 1990;Levick, 2003;Alipov *et al.*, 2003). But, although SV may not be changed by the arterial baroreflex, the afterload effect produce SV changes directly related to BP alterations (Janicki *et al.*, 1996). Left ventricular afterload sensitivity may change during disease; for example, dogs with heart failure were found to have greater reciprocal changes in SV probably due to increased afterload sensitivity (Sala-Mercado *et al.*, 2008).

However, the afterload effect is seldom taken into account in studies of the arterial baroreflex. Studies using neck suction and pressure elicit changes in wall stretch at the level of the carotid arterial baroreflexes, but do not elicit actual changes in BP initially (Ogoh *et al.*, 2002), and thus do not elicit the afterload effect. Since the afterload effect is a mechanical component of the cardiovascular system, actual changes in BP must be elicited to study the combined impact of arterial baroreflex and afterload effect on CO and BP.

As the baroreflex does not operate in isolation, studies of the integrated cardiovascular responses should include the afterload effect in the argument. I elaborate on this below. Ogoh et al (2005) found the maximal gain of the baroreflex

to be -0.4 bpm/mm Hg in situations where the baroreflex alone counteracted virtual BP changes. In paper II, we found the afterload sensitivity (with an intact baroreflex with unchanged gain) to be -0.4 ml/mm Hg. In an intact body, these two mechanisms work in concert, so I calculated their combined effects in a theoretical situation, assuming that the MAP set point was 90 mm Hg (Table 2).

Table 2. Theoretical example of cahnges in CO due to MAP variations

Change in MAP (mm Hg)	MAP (mm Hg)	HR (bpm)	SV (ml)	CO (L/min)	New MAP (mm Hg)
-10	80	64	87	5.6	100
-5	85	62	85	5.3	95
0	90	60	83	5.0	90
5	95	58	81	4.7	85
10	100	56	79	4.4	80

TPR assumed to be constant, 18 (mm Hg/(L/min))

In this example, a sudden drop of 10 mm Hg in MAP elicits an increase in HR of 4 bpm if the baroreflex is operating at full effect, and CO increases by 0.3 L/min (Fig. 14). The afterload sensitivity increases SV by 4 ml/beat, and without the baroreflex effect, this increases CO by 0.2 L/min (Fig. 14). If the two mechanisms act together, the resulting increase in CO is 0.6 L/min (Fig. 14). If TPR does not change, the rise in MAP is 20 mm Hg. Table 2 shows the calculations using estimates from human physiological studies as our paper II and Ogoh *et al.* (2005).

This results in an unstable situation with very labile BP. Such conditions are seldom observed, and the theoretical calculations above, if correct, indicate that the redundancy in the control mechanisms in a healthy subject means that they do not need to operate at maximal effect. Although regulation is much stronger when the

afterload effect and the baroreflex operate at maximal effect, this is generally unnecessary, as one of the control systems can provide the necessary level of control alone. This may be at least a partial explanation for the observed variability in baroreflex gain (Eckberg, 2008). The situation is probably very different if BP regulation is under more stress.

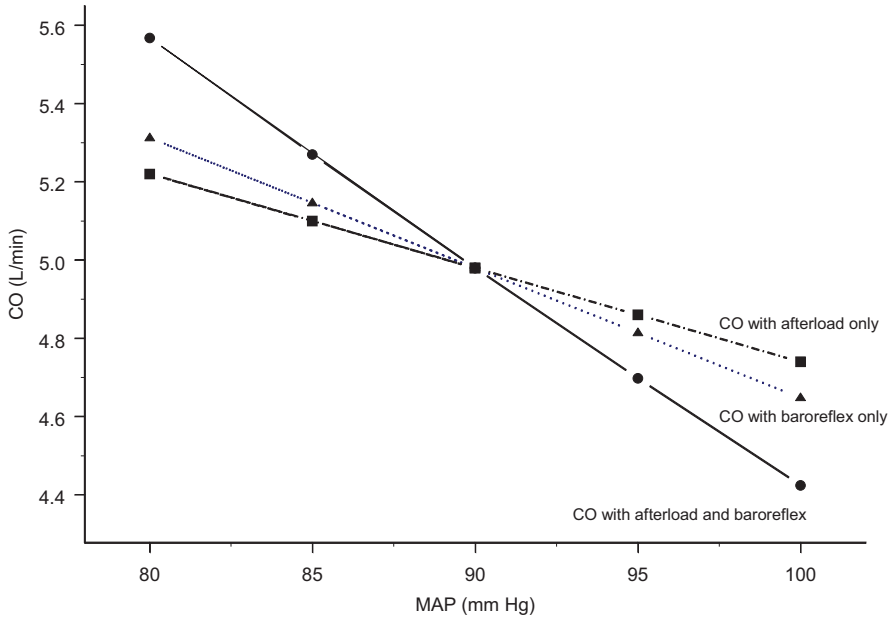

Figure 14: Theoretical example with baroreflex sensitivity -0.4 bpm/mm Hg and afterload sensitivity -0.4 ml/mm Hg. Circles indicate CO changes when both afterload effect and baroreflex are operating, triangles CO changes produced by the baroreflex alone, and rectangles CO changes produced by the afterload effect alone.

An overcorrection in BP via CO may be downregulated by vasodilation, either reflexly or through another vascular response to stretch. The assumption that changes in TPR can be ignored in this example relies upon the fact that neurally transmitted changes in TPR take ~10 s to evolve (Toska & Eriksen, 1994). Fig. 14 thus shows the immediate effects on CO of changes in MAP. This example shows that BP may be

buffered both neurally and mechanically by changes in CO. Both the mechanical afterload effect and the arterial baroreflex may elicit instant changes (within the next heartbeat). In this example I have not included either the fact that a decrease in filling time may counteract the increase in SV or the fact that the full effect of the baroreflex may take two to three heartbeats to develop (Rea & Eckberg, 1987). In addition, due to the Frank-Starling mechanism, an increase in SV produced by the afterload effect will be short lasting as a decrease in end-diastolic volume will eventually reduce SV again. This may be overcome by a change in contractility and/or relaxation.

I suggest that the control mechanisms of the cardiovascular system probably seldom operate at full effect. If more than one mechanism operate in concert complete restoration of BP do not require infinite reflex sensitivity as proposed by Rowell (2004). Recent evidence suggests that baroreflex gain is not a constant value, even during short periods (Eckberg, 2008). This implies that the arterial baroreflex has the ability to adapt to new conditions, even in supposed steady-state. The baroreflex is influenced by other incoming signals from chemoreceptors and the central nervous system (Karemaker & Wesseling, 2008). Nevertheless, the baroreflex is able to maintain BP and organ blood flow within acceptable limits, in spite of noise in the system (Karemaker & Wesseling, 2008); this noise may actually be prerequisite for a sensory system to work at all (Stein *et al.*, 2005;Karemaker & Wesseling, 2008).

At this point, I would like to raise one question, to which I do not have a complete answer at present. What if the baroreflex is actually a flow controller rather than a pressure controller? Researchers measure what we believe to be the important input to the system, but sometimes we may be misled by the fact that even though one variable (such as BP) is easy to measure, it is not the one that is regulated. At this point, I can only indicate why this possibility should be considered. The baroreceptors do not react to pressure changes per se but to stretch of the arterial wall. Stretch may be more closely related to SV than to BP (Eckberg, 2008). The effect of the baroreflex is to ensure that any decrease in stretch of the arterial wall (due to a decrease in SV or BP), reduces parasympathetic firing and thus increases HR. This

counteracts a decrease in CO and restores CO (and eventually BP). But in dogs, only half of the baroreflex-induced HR changes result in CO changes (Sala-Mercado *et al.*, 2008). If the baroreflex mainly controls BP, why do not all HR changes alter CO, which is the only way the heart can influence BP? In addition, in paper III variations in CO increased when HR variations were reduced, indicating that the arterial baroreflex buffers CO variations. Evidence indicates that sympathetic activity to the muscles elicited by the carotid arterial baroreflex is close to its threshold in healthy subjects at rest, and has a smaller operating range than the cardiac branch of the carotid arterial baroreflex (Rea & Eckberg, 1987). This suggests that removal of vasoconstriction is not important during hypertensive episodes at rest, but vasoconstriction becomes increasingly important during hypotensive episodes. However, some consider this to be the most important function of the baroreflex (Karemaker & Wesseling, 2008).

Another argument supporting the baroreflex as a flow controller is that if SV is the input to the baroreflex, the continued firing during head-up tilt can easily be explained (Eckberg, 2008), as the drop in SV during tilt is not restored until the subject is supine again (Toska & Walloe, 2002;Van Lieshout *et al.*, 2003), in contrast to BP that is restored or increased during tilt (Cooke *et al.*, 1999;Eckberg, 2008). But the most important point is that the body with its organs and cells is entirely dependent on blood flow, and thus blood flow is the important variable to regulate (Karemaker & Wesseling, 2008). In this setting, resistance (vasoconstriction) may just be a tool to direct blood to where it is needed.

At the moment, this discussion is speculative, but it could lead to exciting new research that challenges the commonly held view of baroreflex control. If BP is a confounding factor, the real responses of the baroreflex may be obscured. Of course there may be differences between the aortic and carotid baroreceptors, as the aortic baroreceptors are well located to register SV, while at the carotid level BP may be the important input. From this viewpoint it is also interesting that synchronised signals from a sensory system to the central nervous system accentuates a response (Stein *et*

al., 2005). If there are redundancies in the control mechanisms, there is also a possibility for redundancy in the input to the controller. The idea of the baroreflex as a flow controller is at least worth considering, because if pressure per se was important in an evolutionary perspective, why have the receptors not developed a method of measuring pressure? And why are there discrepancies between the results of investigations on SP and MAP? Perhaps researchers have focused too much on what can be measured and overlooked the obvious – that the flow out of the heart may be the regulated variable. There is a similar debate on whether the peripheral circulation during exercise is flow- or pressure-regulated (Rowell, 2004). For the moment, these are my personal opinions, and more research is needed to find the answer to the questions I have raised here. This could lead to valuable new insights that can be used in treating patients.

8.3 Conclusions

I conclude that SV is an important variable which should be measured whenever possible. SV is highly sensitive to afterload changes. The phase relationship between HR and SV suggests that they counteract each other to reduce fluctuations in CO. In this thesis, I have tried to shed new light on SV variations, and explain why they should be further investigated. Studying variations in SV may be one of the few approaches that allow us to study mechanical and neural mechanisms in the cardiovascular system simultaneously.

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10. Papers