# CARDIOVASCULAR REGULATION AND VASCULAR STRUCTURE IN PREHYPERTENSION AND CORONARY HEART DISEASE

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2009

This study was supported by grants from the Scientific Council of Halland, Göteborg Medical Society, Swedish Renal Fund and the Sahlgrenska University Hospital

*"Success is the ability to go from one failure to another, with no loss of enthusiasm"*

**Sir Winston Churchill**

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**Abstract**

Six years ago, a report of high blood pressure in the US defined individuals with a systolic blood pressure (SBP) of 120–139 mmHg or a diastolic blood pressure (DBP) of 80–89 mmHg as prehypertensives. With these definitions, about 60% of all adults in western world have hypertension or prehypertension.

The aim of this thesis was to characterize vascular structure and different aspects of cardiovascular regulation in otherwise healthy subjects with slightly elevated blood pressure and compare to healthy subjects with normotension and also investigate patients with established primary or secondary hypertension and coronary heart disease. Altered cardiac repolarization and arterial baroreflex function has been associated with adverse prognosis and increased risk for ventricular arrhythmias in patients with cardiovascular diseases.

We used the sequence method to measure the baroreflex sensitivity (BRS) and the baroreflex effectiveness index (BEI). The latter is an index of the numbers of times the arterial baroreflex is being active in controlling the heart rate. The myocardial repolarization was assessed using the QT variability index (QTVI), which is a non-invasive measurement of subtle beat to beat fluctuations of the QT interval. A novel very high frequency (55MHz) ultrasound technique was used to measure the vessel wall and separate the intima media (IMT) complex into measurements of intima and media thickness .

Increased lability of myocardial repolarization and impaired baroreflex function were seen in subjects with prehypertension and in otherwise healthy subjects with an attenuated reduction in blood pressure during night (non-dippers) compared to healthy subjects. Patients with renovascular hypertension and patients with coronary heart disease, who underwent coronary artery by-pass grafting (CABG) showed strikingly increased lability of myocardial repolarization. The alterations of myocardial repolarization after CABG improved during rehabilitation. Subjects with prehypertension showed increased radial artery intimal wall thickness compared to healthy subjects. Subjects who report low physical activity had increased intima thickness.

In conclusion, subjects with prehypertension show increased lability of myocardial repolarization, impaired baroreflex function and increased intimal wall thickness. Healthy individuals with a non-dipping blood pressure pattern had increased myocardial repolarization lability and impaired baroreflex function. The current findings may contribute to the increased risk for cardiovascular mortality and morbidity previously reported in the studied populations.

*Key words:* Prehypertension, repolarization, baroreflex, CABG, intima thickness, non-dippers

# List of original publications

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. **Myredal A**, Gao S, Friberg P, Jensen G, Larsson L, Johansson M

*Increased myocardial repolarization lability and reduced cardiac baroreflex sensitivity in individuals with high-normal blood pressure.*

J Hypertens. 2005 Sep;23(9):1751-6.

II. **Myredal A**, Gan LM, Osika W, Friberg P, Johansson M

*Increased intima thickness of the radial artery in individuals with prehypertension and hypertension*

Atherosclerosis, accepted for publication

III. **Myredal A**, Friberg P, Johansson M

*Elevated myocardial repolarisation lability and arterial baroreflex dysfunction in individuals with non-dipping blood pressure pattern*

Submitted

IV. **Myredal A**, Karlsson AK, Johansson M.

*Elevated temporal lability of myocardial repolarization after coronary artery bypass grafting.*

J Electrocardiol. 2008 Nov-Dec;41(6):698-702.

# Contents

# Abbreviations

| | |
|---|---|
| AMBP | ambulatory blood pressure |
| ANS | autonomic nervous system |
| BEI | baroreflex effectiveness index |
| BMI | body mass index |
| BRS | baroreflex sensitivity |
| CABG | coronary artery by-pass grafting |
| CHD | coronary heart disease |
| DBP | diastolic blood pressure |
| ECG | electrocardiogram |
| HR | heart rate |
| HS | healthy subjects |
| IMT | intima thickness |
| JNC VII | The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure |
| LVH | left ventricular hypertrophy |
| NDP | non-dipping blood pressure pattern |
| QTc | QT interval corrected for heart rate using Bazetts formula; $QT/\sqrt{RR}$ |
| QTVI | QT variability index |
| QTVN | variance of QT interval |
| SBP | systolic blood pressure |
| SCD | sudden cardiac death |

“To be conscious that you are ignorant is a great step to knowledge”

**Benjamin Disraeli (1804-1881)**

# 1 Introductory remarks

Ischemic heart disease is still the leading cause of death in the western societies, with an increasing incidence in low income countries.[1] Elevated blood pressure is an important and treatable risk factor for cardiovascular diseases. Already in the 1970:s, Sir George Pickering pointed out that hypertension is defined by an arbitrary division in the continuous blood pressure distribution. [2] More recently, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC-VII), suggested that individuals with a systolic blood pressure (SBP) of 120–139 mmHg or a diastolic blood pressure (DBP) of 80–89 mmHg should be considered as pre-hypertensive. [3] With these new definitions, about 60% of a western adult population is classified as having either pre- or primary hypertension. [4] The rationale for this recommendation was that the risk for cardiovascular diseases increases linearly with increasing blood pressures, beginning at 115/75 mmHg. [5] A novel study suggests that a tight control of blood pressure (SBP below 130 mmHg), is beneficial regarding cardiovascular events and left ventricular hypertrophy (LVH) compared to conventional control (SBP <140 mmHg). [6] Recently, the global burden of disease attributed to elevated blood pressure was estimated to 7,6 million of premature deaths, in which half of this burden occurred in people without manifest hypertension (that is, in individuals with SBP between 115 mmHg and 140 mmHg). [7] Given the increased risk of cardiovascular disease in individuals with elevated blood pressure, there is a demand for characterization of the pathophysiological mechanisms behind end-organ damage occuring in some individuals at an early stage of the disease. The aim of the current thesis was to explore the early consequences of slightly elevated blood pressure on cardiovascular regulation and vascular structure, compared to individuals with established primary and secondary high blood pressure and to investigate individuals with manifest coronary heart disease (CHD).

# 2 Background

## 2.1 Historical context

Although the etiology of primary hypertension is unknown, the research of Björn Folkow and coworkers at the Depatment of Physiology, Gothenburg University has been invaluable for the understanding of the physiological aspects of primary hypertension and a prerequisite for this thesis. [8] Dr Folkow formulated a theory on the development of hypertension, where the genetic predisposition in adjunct to environmental factors, such as psychoemotional influences and salt-intake habits cause adjustments in renal and cardiovascular structure and function. These adjustments are of importance for both the initiation and maintenance of hypertension. [8, 9] The importance of environmental factors such as lifestyle is supported by previous studies in different populations. For example, no development of high blood pressure is seen in a population of modern-day hunter-gatherers. [10] Furthermore, in the middle of the 1960´s a longitudinal study comprehending nuns in a secluded order showed that their blood pressure did not increase during 30 year follow-up, whereas the blood pressure of a matched control group of women living in the society increased and the nuns also had fewer cardiovascular events. [11, 12]

The 2003 JNC-VII [3] suggested a new definition of slightly elevated blood pressure, prehypertension, and recommended health-promoting lifestyle modifications to prevent cardiovascular disease in prehypertension. Furthermore, the 2003 European Society of Hypertension Guidelines for the Management of Arterial Hypertension defined blood pressures of 130–139/85–89 mmHg in healthy individuals as being high-normal. [13] Prehypertension has been linked to heart failure and cardiovascular diseases by several studies and the higher range (130-139/84-89) of prehypertension has been linked to cardiovascular mortality. [14-17] The interest in early stages of hypertension is not new. The term borderline hypertension has been used during several decades, suggested to be manifested by a hyperkinetic circulation with elevated heart rate, blood pressure and cardiac output. With time, a transition from the early hyperkinetic state of borderline hypertension to a high-resistance state of established hypertension is thought to occur. [18, 19] The elevated blood pressure induces vascular hypertrophy, which in turn leads to increased vascular resistance.

## 2.2 Regulation and diurnal variation of blood pressure

The autonomic nervous system (ANS) plays an important role in the regulation of cardiovascular function and could induce rapid changes of the arterial blood pressure by changing the peripheral resistance and cardiac output, which are the two main regulators of blood pressure. [20] In addition to ANS, long-term blood pressure is controlled by a variety of systems such as circulating catecholamines, the renin-angiotensin system, endothelium derived factors and the renal control of body fluid balance. [20] The ANS adjusts the blood pressure in response to various physiological demands. The parasympathetic and sympathetic nervous systems act in general reciprocally, although co-activation of the two divisions may occur. [21] The sympathetic and parasympathetic nervous outflow is often differentiated and hence, a physiological stimulus may increase the nervous activity to one organ whereas the nervous activity to another is decreased. Cardiac parasympathetic nervous activity is mediated through the vagal nerve which innervates the sinoatrial node, the atrioventricular conducting pathways and the atrial myocytes. One important regulator of the ANS and the short time variation of blood pressure are the baroreceptors and the baroreflex arc. Denervation of the baroreceptors in animal models is followed by increased blood pressure variability. [22] Baroreceptors are nerve endings lying in the arterial walls. They are stimulated when stretched and are abundant in the wall of the internal carotid sinuses and in the wall of the aortic arch. Signals are transmitted from the Herring´s nerve to the glossopharyngeal nerve and then to the solitary tract in the medullary area of the brain stem. Signals from the arch of aorta are transmitted through the vagus nerve into the same area of the medulla. [20]. The net effects are vasodilation of the veins and arterioles and decreased heart rate and strength of heart contraction and hence, the stimulation of the baroreceptors reflexely reduces the arterial blood pressure. Reduced arterial blood pressure increases the efferent sympathetic nervous activity, whereas the efferent cardiac parasympathetic nervous activity decreases. [20]

In hypertension, a reduced sensitivity of arterial baroreceptors has been reported both in animal models and in human studies. [23-26] There are a variety of possible mechanisms to reduced arterial baroreflex sensitivity in hypertension since the arterial baroreflex could be affected at different levels. Decreased large arterial distensibility, reduced sensitivity of the baroreceptor itself, reduced function of the afferent or efferent nerves and effects on the central nervous system are possible mechanisms behind reduced baroreflex sensitivity in hypertension. Furthermore, reduced function of the sinus node in the heart could affect the baroreflex function. Previously,

the arterial baroreflex was considered to have minor effects on the long-time regulation of blood pressure, partly due to resetting mechanisms.[27] Resetting of the baroreceptors have a rapid time course, starting within minutes of a rise in blood pressure and being complete within days or even hours. [28, 29] Today there is a growing interest of the long-time effects of the baroreflex control on blood pressure. Recent observations in dogs have suggested that the baroreflex do not completely reset and that the afferent nervous activity from the baroreceptors remain chronically elevated in hypertension, suggesting that the baroreflex activation could have long-term influences on blood pressure. [30, 31] Furthermore, a prospective study of normotensive individuals demonstrated that a lower baroreflex sensitivity predicted the rise in blood pressure during 5 years follow-up, which further supports a baroreceptor effect on the long-term regulation of blood pressure. [32]

During the last decade, a number of studies have established the superiority of ambulatory blood pressure measurements (AMBP) in predicting the future risk of target organ damage in hypertension compared to office measurements. [33-35] AMBP with automatic blood pressure measurements provides a large number of blood pressure readings. The resulting avarage values are more stable compared to office pressures obtained by sphygmomanometer. AMBP is devoid of errors related to the examination such as white coat hypertension (elevated office blood pressure but normal AMBP) and masked hypertension (normal office blood pressure but elevated AMBP). Furthermore, AMBP provides information on blood pressure variations over day and night. The normal pattern of blood pressure is a nocturnal systolic and diastolic blood pressure fall. Several earlier studies have demonstrated that a non-dipping blood pressure pattern (NDP) with less than 10% blood pressure fall during night is associated with target organ damage in patients with primary hypertension. [35] Previous studies report increased sympathetic nervous activity and decreased parasympathetic nervous activity among individuals with NDP in conjunction to diabetes, obesity, end stage renal disease and hypertension. [36-41] Increased incidence of cardiovascular mortality comprehending stroke, myocardial infarction and sudden cardiac death has been linked to NDP. [41] In subjects with primary hypertension, previous data suggest that NDP independently predicts the frequency and complexity of ventricular arrhythmias. [42] In a study of type 2 diabetic patients, NDP was associated to mortality and autonomic neuropathy. [43] Dysfunction of cardiac repolarization and prolongation of the QT interval are linked to cardiac autonomic dysfunction, which may explain some of the increased mortality observed in NDP.

## 2.3 Elevated blood pressure and the vasculature

The arterial circulatory system, which should provide adequate blood supply to body tissues, is regulated by humoral factors, the ANS, endothelium derived factors and other local factors throughout the body.

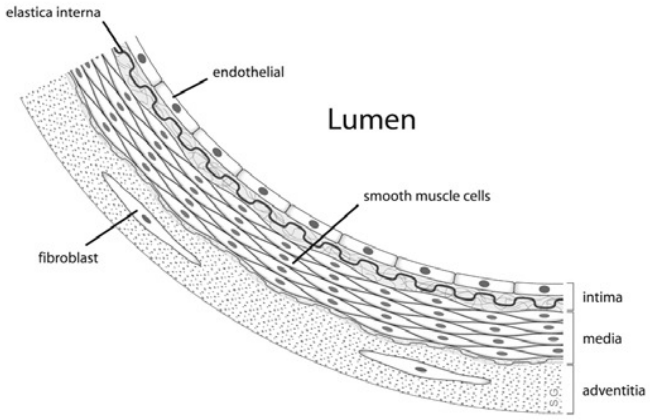


**Figure 1** *The arterial vessel wall*

The arterial vessel wall consists of three concentric layers: the intima, media and adventitia (Figure 1). The intima, adjacent to the blood vessel lumen, is composed of a monolayer of endothelial cells with underlying connective tissue. An early sign of atherosclerosis is intimal thickening due to lipoprotein and lipid retention and inflammatory changes within the intima layer. [44] Atherosclerosis starts early during childhood and adolescence and is accelerated by hypertension. [45] Endothelial cells have pivotal roles in the atherosclerotic process by providing a non-thrombotic surface, maintaining vascular tone by releasing nitric oxide, prostacyklin and endothelin and by providing a surface that is non-adherent for leukocytes. [46] Hence, normal endothelial function prevents the atherosclerotic process. Structural changes of the vessel wall could be assessed non-invasively by using ultrasound technique, and the composite measure of the intima media thickness (IMT) has been shown to correlate with traditional risk factors such as arterial blood pressure, plasma cholesterol and smoking habit. [47-50] Furthermore, IMT conveys important information regarding the risk of future CHD. [51, 52] Increased IMT of the carotid artery has been demonstrated in individuals with slightly elevated blood pressures. [53, 54]

## 2.4 Myocardial repolarization

The QRS complex of the electrocardiogram (ECG) is generated when the ventricles depolarize before contraction. The T-wave of the ECG is caused by potentials generated during the repolarization process. During depolarization the normal negative potential inside the myocyte is lost and the membrane potential becomes positive inside and negative outside the cell. During the repolarization the electric potential returns to the normal state. A normal repolarization process is dependent on intracellular ion channel function and reflects cycling of potassium, sodium and calcium ions. The QT interval and the T-wave of the ECG reflect the myocardial repolarization. There are various methods to assess the cardiac repolarization, such as measuring the prolongation of the QT interval, assessing the temporal and spatial variation of QT interval length. Another well-known method to assess the temporal lability of myocardial repolarization is T-wave alternans, where heart rate changes are induced by pacing or exercise. [55] Prolongation and lability of the QT interval have been coupled to ventricular arrhythmias and sudden cardiac death. [56-60] The mechanisms are unknown, but prolongation of the QT interval induces early afterdepolarisations, increased spatial gradients in transmembrane potentials and beat-to-beat variation in action potential duration may occur. [61, 62] Data indicate that beat to beat repolarization lability arises at the level of the single cell. Possible explanations are impairment in intracellular ion cycling at the cellular and sub-cellular levels. [63] The main depolarizing current are the inward sodium current, giving rise to the fast phase of the action potential and the inward L-type calcium current during the plateau phase. [64] The main repolarizing currents are the rapid and the slow components of the delayed rectifier potassium current. The balance between those depolarizing and repolarizing currents determine the duration and shape of the action potential. The ventricular action potential in the heart myocyte is very long, compared to action potentials of skeletal muscle and neural tissue. [64] This is important for the intracellular cycling of calcium currents. During each heart beat, activation of the L-type calcium current causes free calcium to cross the membrane into the cell, and this cytosolic calcium binds to ryanoidine receptors of the sarcoplasmatic reticulum (Figure 2). These processes lead to release of abundant calcium from the sarcoplasmatic reticulum into the cytosol. Release of calcium from the sarcoplasmatic reticulum can also be stimulated by inward calcium current from the sodium-calcium exchanger operating in reverse mode (Figure 2). Before next beat, the majority of calcium is transfered back into the sarcoplasmatic reticulum by the SR-Ca-ATPase and also, to a lesser extent, via the sodium-calcium exchanger. The cycling of calcium is completed when the calcium of the sarcoplasmatic reticulum is translocated to the junctional sarcoplasmatic reticulum prior to its

release by the ryanodine-sensitive release channel (Figure 2). [63] An impairment in the process described above, at any level, could cause a situation where cytoplasmic calcium cannot be fully reclaimed during each beat and therefore resulting in lability in the duration of action potentials and increased lability of temporal repolarization.

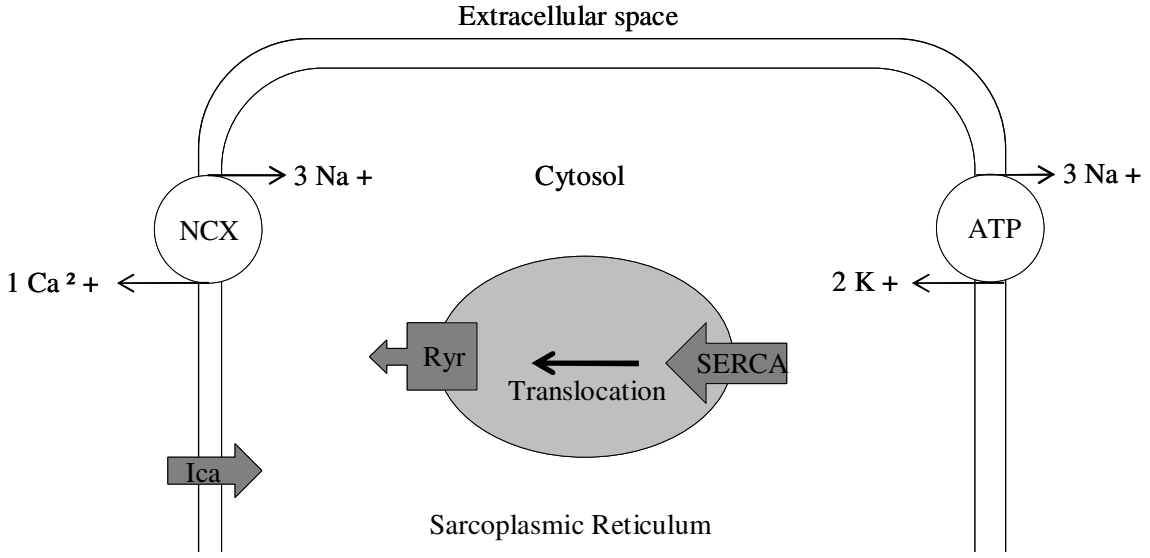


**Figure 2** *Schematic illustration of intracellular calcium ion cycling. Calcium (Ca) flows into the cell via L-type calcium channels (Ica) which trigger the release of Ca from the sarcoplasmic reticulum (SR) via ryanodine-sensitive release channels (Ryr). This promotes inactivation of Ica. Before next beat, the cell eliminates the extra cytosolic calcium via the sodium-calcium exchanger (NCX) in the cellmembrane or by reuptake into the SR via the SR-calcium-ATPase channel (SERCA). Ca is translocated through the SR to be available for release in the next cardiac cycle. Adopted from Walker and Rosenbaum 2003.*

Stimulation of the sympathetic nervous system affects both the L-type calcium channel (increase current, probably due to increased opening time) and the potassium currents which could affect the repolarizing process. [65, 66] Furthermore sympathetic stimulation increases the dispersion of the action potential duration and QT interval (probably due to inhomogenous distribution of receptors), decrease the refractory periods and can cause early afterdepolarisations. [64] All of these effects could increase the susceptibility of ventricular arrhythmias.

## 2.5 Ventricular arrhythmias and elevated blood pressure

In the general population, more than half of the coronary mortality is attributed to sudden cardiac death (SCD). [67] The risk for SCD increases with increasing blood pressure, beginning at SBP of 125 mmHg and patients with SBP >155 mmHg have three times higher risk of SCD compared to normotensives. [68] An incidence of SCD of 0,4 /1000 individuals and year has been reported in subjects with blood pressure in the prehypertensive range. [68] LVH is known to further increase the risk for SCD and LVH quadruples the risk of ventricular tachycardia. [69, 70] The mechanisms linking elevated blood pressure to ventricular arrhythmias are complex and incompletely understood.

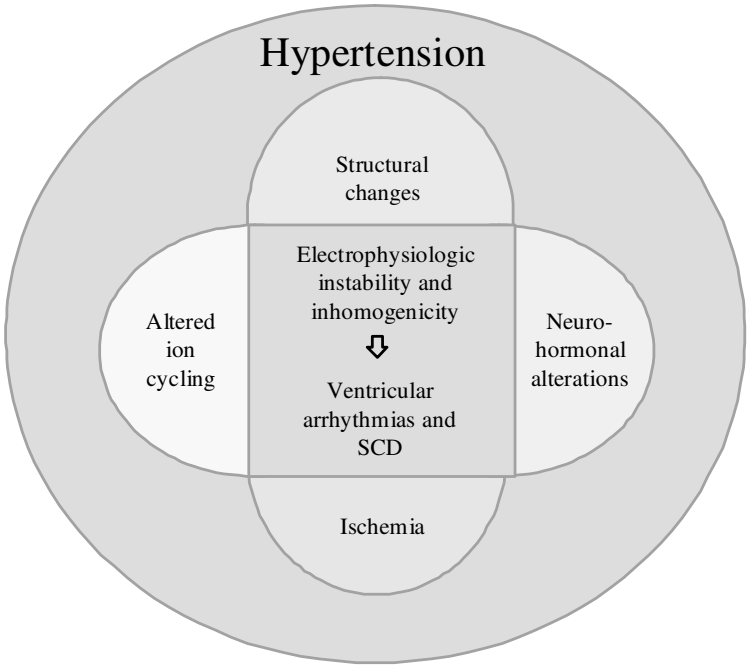


**Figure 3** *Possible cardiac alterations linking hypertension to an increased risk for ventricular arrhythmias and sudden cardiac death.*

Structural changes of hte heart, such as LVH, myocardial fibrosis, and silent myocardial infarctions may increase the susceptibility for ventricular arrhythmias (Figure 3). In prehypertension a higher incidence of LVH and left ventricular diastolic dysfunction compared to normotensives have been reported. [71-75] Myocyte ion channel function is fundamental for normal cardiac repolarization. Hence, dysfunction of the myocyte ion channels may contribute to the development of ventricular arrhythmias. [64] Furthermore, intracellular ion cycling is influenced by the sympathetic nervous activity which enhances the susceptibility to ventricular

arrhythmias. [76, 77] Activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system has been demonstrated in primary hypertension and also in the developement of LVH. [78-80] Several studies link increased sympathetic nervous activity and decreased parasympathetic nervous activity, independently to the risk of ventricular arrhythmias and SCD. [81, 82] Hence, dysfunction of the ANS may provide a link between hypertension and ventricular arrhrythmias (Figure 3).

## 2.6 Ventricular arrhythmias and coronary artery by-pass grafting (CABG)

Sustained ventricular tachycardia during the recovery period after CABG is uncommon (1-8%) but the incidence is high in certain subsets of patients, for example in those with a history of previous myocardial infarction, congestive heart failure and low left ventricular ejection fractions (an incidence of 30% has been reported). [83-85] Furthermore, when ventricular arrhythmia occurs, the 30 day mortality after CABG is 30% compared to 1-2% among subjects without ventricular arrhythmias. [83] In particular the early recovery phase, lasting up to three months after CABG, appears hazardous regarding the risk of ventricular arrhythmia. [86] Female sex, reduced left ventricular ejection fraction, the presence of pulmonary hypertension, the presence of arterial hypertension and pump time have been associated with an increased risk of ventricular arrhythmias after CABG but the underlying mechanisms remain unclear. [83, 84, 86]

# 3 Aims

## 3.1 General aim

Recent guidelines point out the increased risk for cardiovascular disease associated with slightly elevated arterial blood pressure and there is an ongoing discussion about threshold values for diagnosis of hypertension. Characterization of patophysiological mechanisms in early stages of hypertension is important. The general aim of the current thesis was to explore cardiovascular function and structure in othervise healthy individuals with blood pressure in the prehypertensive range in comparison to healthy subjects, to patients with established primary and secondary hypertension and also investigate patients with manifest CHD.

## 3.2 Hypothesis

- Arterial baroreflex dysfunction, altered cardiac repolarization and structural changes in the radial artery, probably in the media layer, prevail in individuals with prehypertension.
- Otherwise healthy individuals with a reduced blood pressure dip at night have increased temporal QT variability and reduced arterial baroreflex function measured as BRS and BEI.
- The temporal QT variability is increased among patients with severe renovascular hypertension and among patients with CHD after CABG surgery.

# 4 Methodological considerations

## 4.1 Study groups

All studies were conducted at Varberg Hospital, Sweden, with the exception for the patients with renovascular hypertension who were examined at Sahlgrenska University Hospital, Gothenburg. The local ethics committees of Lund and Gothenburg, respectively, approved the studies and all subjects gave their informed consent to participate.

***Study I*** was conducted during 2002 until 2004. Subjects (n=132) were recruited from a health control and via advertisement in the local press. Thirty-one subjects were excluded due to established hypertension (SBP >140 mmHg or DBP >90 mmHg). The subjects were otherwise healthy and had never received antihypertensive treatment. All were non-smokers and had normal body mass index (range 20-25kg/m²). Twenty-one subjects had prehypertension with blood pressures between 130/80 mmHg and 140/90 mmHg (average values of the cuff blood pressure measurements on three different occasions at least one week apart). Nineteen subjects in the prehypertensive group had a normal ECG and two had LVH according to Cornells ECG (electrocardiogram) criteria. [87] Eighty subjects were normotensive having blood pressures <130/80 mmHg (average values of the cuff blood pressure measurements on three different occasions at least one week apart). Normotensive subjects were younger compared to the prehypertensive group and female gender was more common. After matching for age and gender, 45 normotensive subjects remained for comparison with the prehypertensive subjects. All of the HS had normal ECGs. In the HS group, it was not possible to perform the QTVI analysis due inadequate ECG signal for T-wave detection or frequent extra-systoles (>5%) in 5 subjects.

Twenty hypertensive patients with renal artery stenosis >50% were recruited at the Department of Nephrology, Sahlgrenska University Hospital. The duration of hypertension was on average 7±9 years. Patients with diabetes mellitus or congestive heart failure were excluded. Renal angioplasty was performed in 13 patients and nephrectomy in one patient. After renal angioplasty or nephrectomy, 9 were improved regarding hypertension ( i.e normotensive without or with a 50% reduction of the antihypertensive medication) and thus, had proven renovascular hypertension. None of the patients with renovascular hypertension had renal artery stenosis due to fibromuscular dysplasia. A history of smoking was reported in 10 of the patients with renovascular hypertension, 2 had claudicatio, 2 suffered from chronic obstructive pulmonary disease, 3 had coronary artery disease and 3 had a history of stroke. Among the patients with

renovascular hypertension 40% were on treatment with more than three antihypertensive drugs, 65% were on treatment with betareceptor blockers, 15% on angiotensin converting enzyme (ACE) inhibitors, 5% on angiotensin II (A II) receptor blockers and 60% on diuretics. In one patient with renovascular hypertension the BRS analysis was omitted due to frequent extra-systoles. The QTVI analysis was not performed in 4 patients due to inadequate sampling rate (below 1000 Hz), whereas the analysis was omitted due to inadequate ECG signal for T-wave detection or frequent extra-systoles (>5%) in 4 patients.

***Study II*** was conducted during 2005-2009. Ninety-five healthy subjects without any clinical symptoms who were participitating in a health control program were recruited from a primary care unit and from occupational health service. They did not suffer from any chronic disease and were all non-smokers at present. None of the participants were on anti-hypertensive medication. All subjects underwent 24 hour ambulatory blood pressure recordings using Spacelabs ultralight ambulatory blood pressure monitor 90217 and very high resolution ultrasound measurements of the radial artery. Daytime hours were defined from 06.00 am to 10.00 pm. A dipping blood pressure pattern were defined as >10% reduction of SBP and DBP during night. The subjects were divided into three study groups; HS ( n=29) with average 24 hours SBP <120 mmHg and DBP < 75 mmHg, prehypertensives (n=32) with average SBP between 120 and 130 mmHg or average DBP between 75 and 85 mmHg, hypertensives (n=34) with average SBP > 130 mmHg or average DBP > 85 mmHg. In one subject with hypertension it was not possible to measure IMT.

**Study III** was conducted during 2006-2009. Seventy-nine subjects participating in study II were included in study III. In addition, twenty-three subjects were recruited via advertising in the local press. All subjects were healthy and had never received antihypertensive treatment. All were non-smokers and they underwent measurement of office blood pressure and 24 hour ambulatory blood pressure recordings. Individuals with non-dipping blood pressure pattern (n=36) had <10% reduction in SBP and DBP during night. Sixty-five individuals had dipping blood pressure pattern showing at least 10% reduction of the SBP or DBP during night. The individuals with dipping blood pressure pattern were younger and female gender were more common. Hence, in order to obtain study groups matched for age and gender the 7 youngest women were omitted, and hence 59 individuals with dipping pattern entered the study. In one subjects with NDP and in one subject with dipping blood-pressure pattern, the BRS analysis was omitted due to frequent extra-systoles. The QTVI analysis was not performed in 3 subjects with NDP due to inadequate ECG signal for T-wave detection or frequent extra-systoles (>5%) in 4 patients.

***Study IV*** was conducted during 2002-2005. All patients (n=153) who were referred to Varberg Hospital for rehabilitation after CABG between January 2002 to November 2003 were invited to participate in the study. Exclusion criteria were atrial fibrillation, atrial flutter, bundle branch blocks, frequent (>5%) extra systoles or inability to detect the end of the T-wave on ECG which interfered with the QT analyses. Fifteen patients refused to participate, 15 were excluded due to inability to speak Swedish, 36 were excluded because they had atrial fibrillation and 13 were excluded due to bundle branch blocks. The investigations were performed at the scheduled visits at the hospital 5 weeks and 5 months after CABG surgery. Thirteen of the patients had frequent extra systoles and and therefore QTVI were not possible to analyze. Hence, sixty-one patients (average age 63±7 years, range 41-75 years, 16 females) were included in the study and underwent investigations 5 weeks and 5 months after CABG. Twelve patients had pathological Q waves on the ECG and 11 patients had signs of left ventricular hypertrophy. Four patients refused to attend the 5 months visit and the ECG signals were inadequate for the QTVI analysis in 3 patients at the last visit. Hence, fifty-four patients underwent both investigations. Thirty-two HS were recruited by advertisement in a local newspaper. HS had similar age (69 ±9 years, range 36-77 years) and gender distribution (8 females) as the patients. All were non-smokers, had no significant past medical history and were not taking any regular medication. They were normotensive (<140/90 mm Hg) and had a normal ECG.

## 4.2 Arterial baroreflex sensitivity and baroreflex effectiveness index

Arterial baroreflex sensitivity reflects the modulation of heart rate in response to blood pressure changes and there are several methods in use to assess the baroreflex sensitivity. The Oxford technique, were blood pressure changes are induced by injection of vasoactive drugs, is a reference method. [88, 89] However, there are some limitations of the Oxford method such as the invasiveness and the use of vaso-active drugs which could affect the vessels. In the current studies, a non-invasive method to evaluate the arterial baroreflex sensibility was used.[90] The current method evaluates the sensitivity of the entire baroreflex loop in a physiologic way and provides the opportunity to assess not only the correlation between the blood pressure and heart rate (HR), but also how often the baroreflex is active. All individuals refrained from caffeine-containing beverages for the 12 hours preceding the investigations and patients on medication did

not take their medication for the preceding 24 hours. After 10 minutes of supine rest in a quiet room, ECG and beat-to-beat blood pressure were registered over 20 minutes by a Portapres equipment. [91] The time series of SBP and RR interval from the entire period of recording (20 minutes) were scanned to identify baroreflex sequences, which were defined as three or more consecutive beats in which successive SBP and RR intervals concordantly increased or decreased, with the threshold set at 1.0 mmHg and 5.0 ms, respectively, and a shift of +1 between the blood pressure pulse and the RR interval, resembling the classical criteria suggested by Bertineri et al. (threshold values of 1.0 mmHg and 4.0 ms, respectively and shift 1). [92] Linear regression was applied to each sequence and only those for which the square of the correlation coefficient ($r^2$) was greater than 0.85 were accepted for further analysis. The arterial baroreflex function was estimated by calculating the following; i) The spontaneous baroreflex sensitivity (BRS), reflecting the average regression slope for all the linear regressions plotted for accepted baroreflex sequences within the whole time frame. For each blood pressure ramp the overall blood pressure change was calculated and the slope of the ramp was estimated by the maximum of the first derivative of the blood pressure signal within the time interval of the ramp (max dP/dt). ii) The baroreflex effectiveness index (BEI), defined as the ratio between the number of SBP ramps followed by the respective reflex RR interval ramps that fulfilled the BRS criteria and the total number of SBP ramps were calculated during the recording period. Previous studies have shown good correlation between spontaneous BRS and BRS obtained by Oxford technique [90]. Whereas BRS reflects the gain of the arterial baroreflex for a given blood pressure change, BEI reflects the number of times the arterial baroreflex is being active. Previous studies have demonstrated that both BRS and BEI are affected by modulation of the parasympathetic nervous system. Denervation of arterial baroreceptors in cats resulted in markedly decreased BRS and reduced BEI. [92] Furthermore, parasympathetic blockade by atropine showed a major reduction of BRS indicating that these measurements are greatly influenced by the parasympathetic nervous system. [90] There are also data indicating that the sympathetic nervous system could affect the BRS such that when giving beta blockade and clonidine an increased BRS have been reported. [90] BRS and BEI have been shown to convey prognostic information after myocardial infarction, ischemic stroke and in end-stage renal disease. [93-95] Although the mechanisms behind the prognostic value of BRS and BEI are unclear, data suggest that a normal modulation of cardiac parasympathetic nervous activity could protect against ventricular arrhythmia. [96] The baroreflex response is impaired in hypertension and this impairment is observed in early phases of hypertension. [23, 97] Previous studies have shown that the inter- and intra examiner variability of

the BRS measurements is low, between 1 and 5%, whereas the within-subject reproducibility over time is moderate, with a coefficient of variation of 27% in healthy subjects. [98]

## 4.3 Temporal QT variability and QT variability index

The temporal QT variability could be assessed with several methods. In the current studies we used the QT variability index (QTVI), a method first described by Berger in 1997. [99] QTVI is a method to detect subtle beat-to-beat changes in the QT interval, using a template-matching scheme. ECG is recorded and the operator defines a template QT interval by defining the beginning of the QRS and the end of the T-wave. The algorithm then finds the QT interval for each beat such that the T-wave shape best matches the template T wave under the time-stretch model. Identifying the T-wave peak or the T-wave end could sometimes be difficult, and in contrast to several other methods, this algorithm comprises the entire T-wave shape to match the template. The QT variance and QT mean are calculated from the recordings. Since the QT interval is highly dependent of the heart rate, the RR variance and RR mean is also calculated, and the QTVI is calculated with the formula below:

$$QTVI = \log 10\left[\frac{QTv / QTm^2}{RRv / RRm^2}\right]$$

We also calculated variance of QT (QTVN) normalized for mean QT as the numerator in the formula:

$$QTVN = \left[\frac{QTv}{(QTm)^2}\right]$$

In the current studies, we excluded individuals with atrial flutter, atrial fibrillation, left bundle branch block, right bundle branch block and recordings with >5% ectopic beats, and if other arrhythmias and noices lead to >5 % omitted RR intervals. An interval of 300 sec was analyzed. From the QT and RR recordings the squared coherence function, which is a measure of the degree of linear interaction between RR- and QT fluctuations, was calculated from the power spectra of the RR and QT interval time series and the cross spectrum between the two series derived by fast Fourier transform. The mean squared coherence was obtained by averaging the coherence function over the frequency band from 0 to 0,45 Hz. All subjects in the studies refrained from caffeine-containing beverages 12 hours preceding the investigation and patients on medication did not take their medication for the 24 preceding hours. After 10 minutes of rest,

ECG was registered during 20 minutes of rest in supine position in a quiet room. The mechanisms behind increased temporal QT variability is unknown, but alternans of intracellular ion changes is an important mechanism for cellular alternans [63].

We have previously reported an inter and intra examiner variability of the QTVI measurements between 5 and 10%, indicating that the effect of variation in QT template definition by the same or different examiners on QTVI value derived from the same time section was small. [98] The within-subject reproducibility over time was moderate, with a coefficient of variation being 18% in healthy subjects. [98]

## 4.4 Very high resolution ultrasound measurements

Although clinically relevant atherosclerosis of the upper limb arteries is rarely seen, there are evidence suggesting that intimal hyperplasia of the arteries in the upper limbs reflect global atherosclerosis. Firstly, histological studies indicate that intimal hyperplasia prevails in the majority of the radial artery grafts used in patients undergoing coronary artery by-pass grafting (CABG). [100, 101] Secondly, established risk factors for atherosclerotic vascular disease such as age, smoking, peripheral vascular disease, diabetes and hypertension are independent predictors of intima hyperplasia in the radial artery. [100, 101] In the current studies, the radial arteries of healthy subjects with various blood pressures were examined using a 55 MHz probe (Visualsonics, Toronto, Can). The radial arteries of the right hands were investigated with the subjects in a supine position. The radial artery was measured 1-2 cm proximal to the skinfold separating the palma manus from regio antebrachii anterior. At the position of the thickest part of the far wall (visually judged) 4 consecutive beats were recorded in real time and saved. Analyses were then made offline. Three measurements of the intima thickness and IMT were performed in systole when the artery showed its largest diameter. Intima thickness was defined as the distance from the leading edge of the lumen-intima interface to the interface between intima and media of the far wall. IMT was defined as the distance from the lumen-intima interface of the far wall to the leading edge of the media-adventitia interface (Figure 4). The media thickness was then calculated by subtracting intima thickness from IMT. Lumen diameter was defined by the distance between the leading edges of the intima-lumen interface of the near wall and the lumen-intima interface of the far wall (Figure 4). [102]

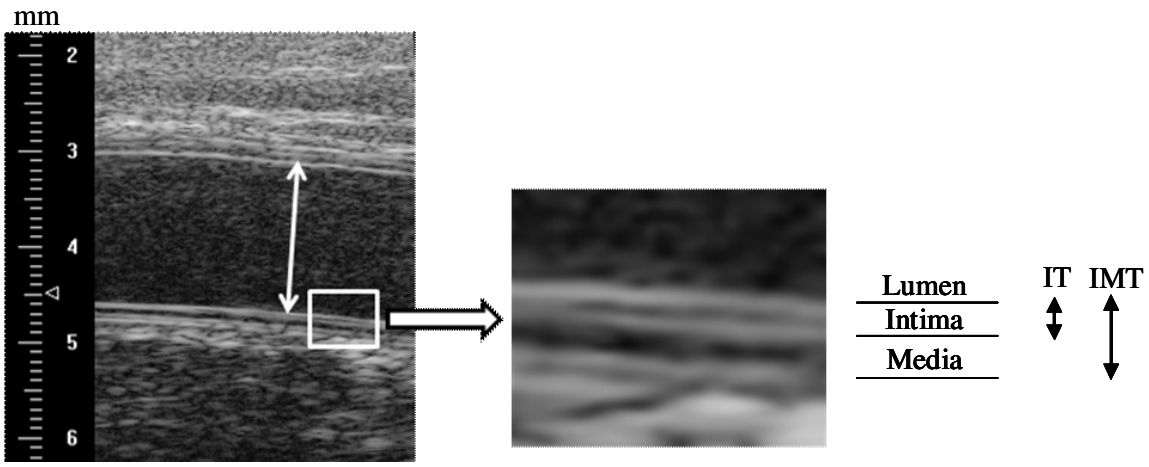


**Figure 4** *Very high resolution ultrasound investigation of the radial artery (left panel) and a close-up picture (right panel) in a prehypertensive subject. IT: intima thickness, IMT: intima-media thickness, arrow depicts lumen diameter.*

The very high resolution ultrasound is a novel method and the reports in humans are so far limited. [103-105] There are, however, several lines of evidence to support the very high resolution ultrasound as being an accurate method for non-invasive in-vivo measurement of intima thickness in superficial arteries. Validation of these technique against silicone layers of different thicknesses and sections of human superior mesenteric arteries have shown correlation coefficients of 0,98 and 0,92, respectively measuring structures from 1µm to 200 µm. [103] Recently, Razuvaev and co-workers reported a significant correlation (r=0,74) between histological and very high resolution ultrasound in-vivo measurements of the rat carotid artery intima thickness, which in magnitude is comparable to the human radial artery (the median IMT in the carotid artery of the rat was about 0,12 mm and the mean IMT in human radial artery 0,23 mm). [106] In that study, a series of experiments were carried out where arteries with partially removed intimal layers were visualized ex vivo, verifying that the intimal layers of the vessel walls were correctly assessed by very high resolution ultrasound.[106] Similar validation experiments have been performed in our laboratory in human superior mesenteric artery (Figure 5).

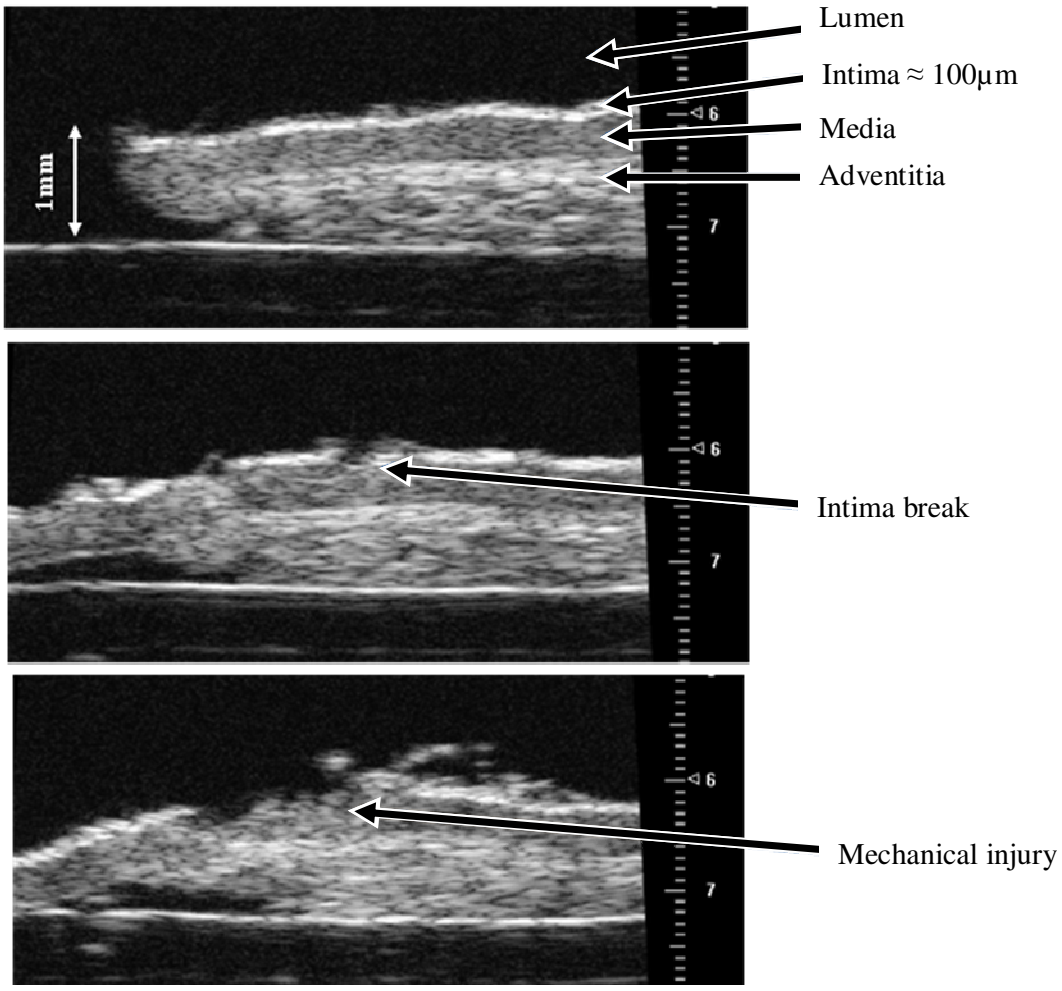


**Figure 5** *Experiment showing imaging of vascular wall ex vivo, a non-fixed section of the human superior mesenteric artery. The vessel was cut obliquely and scanned along this cut. Upper panel intact intima, the middle and bottom panel showing that the white layer adjacent to the lumen disappears when the intima layer is removed.*

We have previously reported intra-observer coefficients of variation for radial arterial intima thickness and IMT of 7% and 5%, respectively. [104] The intra individual variation (the coefficient of variation of repeated measurements by the same operator) was 8,1 % for the intima thickness and 4,0 % for IMT of the radial artery and 1,5% for the diameter of the radial artery. [104]

## 4.5 Physical activity

The participants answered a validated questionnaire regarding physical activity comprehending four categorical questions about leisure-time physical activity during the last 12 months [107] comprising 4 categories: (1) sedentary leisure-time (2 hours of activity such as walking and biking per week); (2) sporadic leisure-time moderate activity (at least 2 hours of moderate-intensity activity such as bicycling, walking and gardening per week); (3) sporadic regular exercise ( regular exercise once or twice per week for at least 30 min at each occasion, such as jogging, aerobics, weight training, soccer, etc.); and (4) regular exercise (regular exercise at least three times per week for at least 30 minutes at each occasion, such as jogging, aerobics, weight training, soccer etc.). Since very few participants rated themselves in the first category, the first and second categories were lumped together and categorized as sedentary and the results are presented for three groups.

## 4.6 Statistical analyses

Data are presented as mean ± standard deviation (SD). Student's t-test for unpaired comparisons was used for data with a normal distribution and paired t-test was used for paired comparisons when the variable showed a normal distribution. The Mann–Whitney test was used for unpaired data with a non-normal distribution and Wilcoxon signed rank test was used for paired analysis of data with a non-normal distribution. Comparisons of proportions were carried out using cross-tabulation and Fischer's exact test. For comparison of paired proportions, McNemar's test was used. The differences between three groups with respect to normally distributed variables were assessed by analysis of variance; Scheffe 's test was used as a post-hoc test and for non-normal distributed data the Kruskal–Wallis test was used. The relationship between two variables was assessed from bivariate scatter plots and by calculation of the rank correlation coefficient according to Spearman. Statistical significance was defined as $P < 0.05$ in paper I, II and III whereas statistical significance was defined as $p<0,01$ in paper IV. In paper II the results were adjusted for other covariates with impact on the result, using a multiple linear regression model.

# 5 Review of results and discussion

## 5.1 Cardiovascular regulation and vascular structure in individuals with prehypertension (I, II)

In study I, we assessed the QTVI, RR variances and BRS among individuals with prehypertension and compared them to HS and patients with renovascular hypertension. In subjects with prehypertension, the QTVI was increased by 19% compared to HS ($p<0,05$, Figure 6). The renovascular hypertensive patients demonstrated a 15% increase of QTVI compared to subjects with prehypertension and 47% versus HS ($p<0,05$, Figure 6). The QT variances were elevated among both subjects with prehypertension and patients with renovascular hypertension compared to HS ($p<0.05$ for both), whereas the RR variances did not differ. The BRS was reduced by 24% in subjects with prehypertension compared to HS and with 44% in patients with renovascular hypertension compared to HS, ($p<0,05$ for all, Figure 6) whereas patients with renovascular hypertension and subjects with prehypertension did not differ.

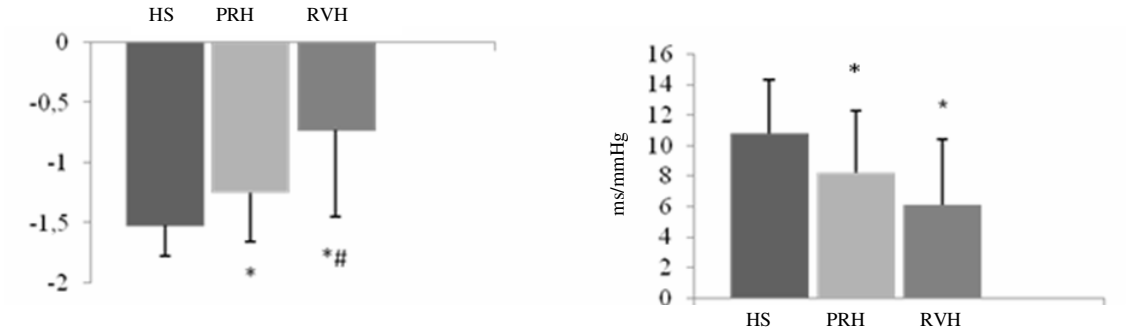


**Figure 6** *Bar graphs showing QT variability index (left panel) and spontaneous baroreflex sensitivity (right panel) in healthy subjects (HS), subjects with prehypertension (PRH) and patients with renovascular hypertension (RVH). Data are presented as mean value and standard deviation, *denotes p<0,05 compared to HS and # denotes p<0,05 compared to PRH.*

Several factors are involved in the regulation of myocardial repolarization and previous data suggest that patients with established primary hypertension and especially those with LVH have an elevated QTVI reflecting increased myocardial repolarization lability. [108] Hence, LVH among the prehypertensive subjects is a possible explanation to the elevated QTVI observed.

Echocardiographic examination was not performed in the current study and this is a study limitation. Sympathetic and parasympathetic nervous activity could both affect the RR and QT intervals. Hence, increased sympathetic activity and reduced parasympathetic activity could contribute to the elevated QTVI observed. [109, 110] Reduced BRS and a trend towards reduced RR variance were observed in subjects with prehypertension in the present study, which supports the notion that reduced modulation of cardiac parasympathetic nervous activity prevails already at slightly elevated blood pressures. There are a variety of possible mechanisms to reduced BRS in subjects with prehypertension. Apart from reduced modulation of parasympathetic nervous activity, reduced arterial elasticity has been reported in patients with borderline hypertension and could have contributed to the reduced BRS in subjects with slightly elevated blood pressure. [111]

In study II we assessed the intima, media and intimamedia thickness (IMT) of the radial arteries in otherwise healthy subjects with normotension, prehypertension and established hypertension. Subjects with prehypertension showed a 14% increase of intima thickness compared to healthy subjects (Figure 7, $p<0,05$) , whereas the media thickness and IMT did not differ. Individuals with established hypertension showed a 12% increase of intima thickness, whereas no differences were observed regarding IMT and media thickness (Figure 7, $p<0,05$). Individuals with prehypertension and those with established hypertension did not differ in intima thickness and media thickness.

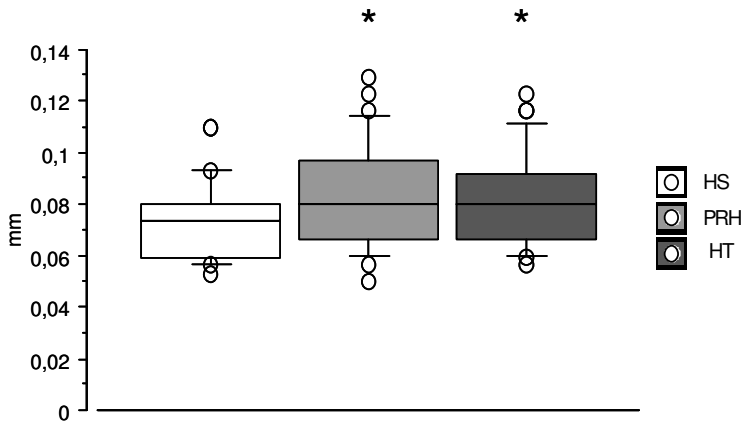


**Figure 7** *Box plots showing individual values at the extremes in conjunction to 10th to 90th and 25th to 75th percentiles and median values of intima thickness in healthy subjects (HS), subjects with prehypertension (PRH) and subjects with hypertension (HT). *denotes p<0,05 compared to HS.*

There are several possible mechanisms to the increased intima thickness observed in subjects with prehypertension or hypertension. Firstly, elevated blood pressure per se exerts hemodynamic stress on the vessel wall and hyperkinetic circulation, which could damage the endothelium. [112-114] A previous study comprehending young men with borderline hypertension (130-140/85-89 mmHg) reported increased IMT of the carotid and the brachial arteries compared to normotensives and an association between IMT and 24 hour ambulatory SBP was found. [53] Moreover, a study using high-frequency ultrasound (25 MHz probe) recently reported increased intima thickness of the carotid artery in individuals with hypertension. [115] Secondly, elevated blood pressure is a component of the metabolic syndrome with increased lipids, visceral obesity, decreased glucose tolerance and inflammation which could damage the endothelium and cause intimal hyperplasia. In the current study individuals with prehypertension or hypertension had elevated BMI and increased waist circumference. After adjusting the results for BMI, waist circumference, age and physical activity as covariates, the differences in intima thickness between HS and the group of individuals with prehypertension and established hypertension taken together remained statistically significant. We did not observe any difference between subjects with prehypertension and subjects with hypertension regarding measurements of intima thickness. The current study was not designed to detect differences between different levels of blood pressure elevations and there is a possibility that small differences in intima thickness were not detected due to the small sample sizes.

Previous experimental research in hypertension has focused on the small resistance arteries and arterioles with a lumen size between 50 µm and 400 µm. These vessels are of major importance for the vascular resistance, and there are reports suggesting that hypertrophy of the resistance arteries plays an important role in the development of hypertension. [8, 116-120] Resistance to flow varies inversely with the fourth power of the blood vessel radius according to Poiseuille´s law. Thus, small decreases in lumen size will greatly increase the resistance to blood flow. Previous studies of larger arteries have provided conflicting results. In renovascular hypertension, several studies have shown increased intima thickness of the thoracic aorta, whereas other studies suggest increased media thickness. [114, 122 123] Extensive studies in the spontaneously hypertensive rat model, have established media hypertrophy and an increased vascular resistance at maximal dilatation. [8, 124-126] However, intimal thickening of elastic and muscular arteries (mesenteric arteries) as well as media hypertrophy has been reported. [127, 128] Using salt sensitive rats, Lee et al reported increased media thickness in mesenteric arteries of various magnitude (elastic, muscular, small arteries) and intimal lesions in the superior mesenteric artery (an elastic artery). [129] Furthermore, intimal lesions were linked to hypertension and the amount of salt in the diet. In

a rat model, with hypertension induced by inhibition of nitric oxide synthesis, intimal thickening of the thoracic aorta was shown. [113] Interestingly, intima thickening prevailed also when compared to rats with inhibition of nitric oxide synthesis and controlled blood pressure with captopril, suggesting that hypertension per se induces intimal thickening. [113] A previous study in humans using 25MHz ultrasound, showed that individuals with hypertension had increased intimal thickness in the carotid artery. [115] These previous human data support the current findings of increased intimal thickness of the radial arteries. The current study did not show any differences in the media thickness of the radial arteries. One could speculate that increased media thickness occur later in the development of hypertension. Data in patients with end-stage renal disease show increased intima as well as media thickness. (Johansson et al, in manuscript) Secondly, arterial medial hypertrophy may not occur in the radial artery in the same magnitude as in some other arteries. There are data indicating that different arteries respond differently to elevated blood pressure, with the elastic arteries (aorta and carotid artery) increasing the diastolic diameters, whereas muscular arteries (brachial, radial and femoral) remain unchanged. [130, 131] One may conclude that both the type of artery (muscular, elastic or resistance vessels) and the experimental model influence the effects exerted by the elevated blood pressure on the arterial wall.

Although the association between IMT of the carotid artery and CHD is well established the prognostic value of radial arterial IMT, intima thickness and media thickness is uncertain. [132, 133] Frick et al reported that the wall thickness of the brachial artery predicted long-term cardiovascular events in a population with chest pain, with or without CHD. [134] Furthermore, flow-mediated dilatation in the brachial artery predicts cardiovascular events in the population.[135] Intimal hyperplasia of the radial artery may reflect global atherosclerotic vascular disease. Unpublished data obtained in our laboratory in patients undergoing radionuclide myocardial perfusion studies for evaluation of chest pain, suggest that radial arterial intima thickness convey information on the risk of future cardiovascular events. (Gan et al, accepted for oral presentation at American Heart Association Congress 2009)

In study II we assessed the effect of physical activity on the vasculature. Subjects were divided into three groups according to their physical activity. There was a difference in radial arterial intima thickness between the three groups; the sedentary category subjects showed increased intima thickness compared to the regular exercise category (Figure 8, $p<0,05$). Furthermore, there was a trend towards increased intima thickness among the sedentary category subjects compared to the sporadic exercise subjects (Figure 8, $p=0,06$), whereas no difference were seen

between the sporadic exercise subjects and the regular exercise subjects regarding intima thickness measurements. Media thickness did not differ between the three groups.

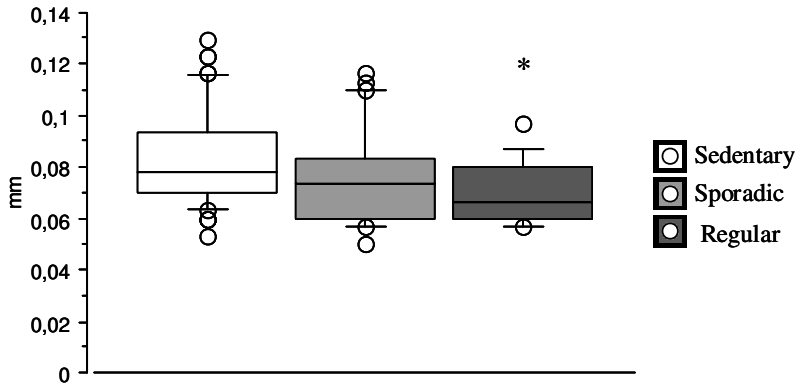


**Figure 8** *Box plots showing individual values at the extremes in conjunction to 10th to 90th and 25th to 75th percentiles and median values of intima thickness in subjects with sedentary lifestyle, sporadic exercise and regular exercise. *denotes p<0,05 compared to subjects with sedentary lifestyle.*

Previous studies have reported conflicting results regarding the association between physical activity and IMT of the carotid artery. [136-139] The current results with increased intima thickness in individuals with a sedentary lifestyle compared to individuals who exercised regularly suggest that physical activity may reduce primarily the intima thickness of the radial artery. One may speculate that physical activity may improve the endothelial function or exert an anti-inflammatory effect on the vessel walls [140-142]

## 5.2 Cardiovascular regulation in healthy subjects with non-dipping blood pressure during night (III)

In study III, a healthy population was investigated regarding QTVI, BRS and BEI. Subjects with a non-dipping blood pressure pattern (NDP) were investigated and compared to healthy subjects with normal nocturnal dipping blood pressure. There were no differences regarding 24 hour mean blood pressure, sphygmomanometer blood pressure, age and BMI between the study groups. Individuals with NDP showed elevated temporal QT variability and arterial baroreflex dysfunction compared to subject with a dipping blood pressure pattern. The QTVI was increased

by 15% in subjects with a NDP compared to those with a dipping BP pattern ($p<0.05$, Figure 9). The QT variances, RR variances, QT means and RR means did not differ between the study groups. Moreover, subjects with a NDP pattern showed a 21 % reduction of BEI compared to individuals with a dipping blood pressure pattern, whereas BRS did not differ ($p<0,05$, Figure 9). Subjects with a dipping blood pressure pattern had a larger reduction in heart rate during night compared to non-dippers (12±5 beats per minute for dippers versus 8±6 beats per minute for non-dippers, $p<0,05$), whereas no difference was observed regarding average HR.

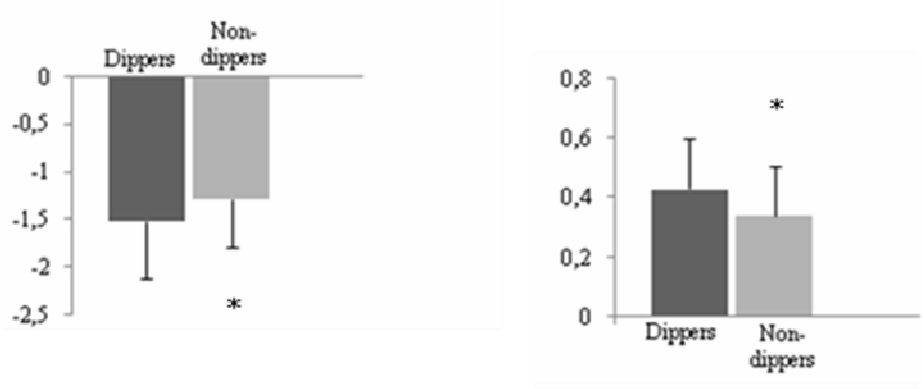


**Figure 9** *Bar graphs showing the QT variability index (left panel) and Baroreflex effectiveness index (right panel) in healthy subjects with dipping and non-dipping blood pressure pattern during night. Data presented as mean and standard deviation, *denotes p<0,05 compared to subjects with dipping blood pressure pattern*

Several previous studies have pointed out the importance of blood pressure reduction during night, even among normotensive subjects. [143] [144] [145] Both the cause of NDP and the mechanisms behind the increased risk are incompletely understood, but there are several lines of evidence to suggest that dysfunction of the autonomic nervous system may play a role. [36-41] Moreover, autonomic nervous dysfunction has been proposed as a pathophysiological mechanism to NDP. [39-41, 109, 110] Previous studies report that a reduced BEI may reflect neuropathy of the autonomic peripheral nerves and hence, one may speculate that a hampered autonomic nervous control of the circulation may add to current results with reduced BEI among subjects with NDP. [95] This notion is supported by previous reports of dysfunction of the autonomic nervous system in hypertensive subjects with NDP.[36, 39-41] There were no differences in BRS between subjects showing NDP and those with a dipping blood pressure pattern corroborating earlier studies. [37, 151, 152] Collectively, BRS and BEI provide complementary information on the arterial baroreflex function and previous data have shown that BEI and BRS react differently during sleep and

during different cognitive and attentional tasks. [153-155] Mechanism behind the reduced BEI in NDP subject cannot be deduced from the present data. One may surmise that BEI is affected by central mechanisms during sleep and also while being awake.

Grassi et al reported a close inverse relationship between sympathetic nervous activity and the day-night blood pressure reduction in hypertensive subjects. [37] In diabetes mellitus, neuropathy may affect the cardiac autonomic nervous system and there are several reports linking diabetic neuropathy to NDP. [145, 146] In a recently published study of type 2 diabetic patients, NDP was associated to mortality after adjustment for traditional risk factors such as LVH, mean AMBP, glomerular filtration rate and long term glucose control. [43] Furthermore, reduced heart rate variation in expiration/inspiration, reflecting cardiac autonomic neuropathy, was a predictor of mortality. [43] Sympathetic and parasympathetic nervous activity could both affect the RR and QT intervals and previous studies have demonstrated increased QTc and QT dispersion among primary hypertensives with NDP. [39-41, 109, 110 36, 38, 147] Sympathetic nervous activation may increase the temporal lability of cardiac repolarization and hence, elevated sympathetic nervous activity could be a mechanism linking NDP to an elevated QTVI. [37, 148, 149] NDP has been associated with LVH in patients with primary hypertension and previous data indicate that hypertensive patients with LVH have an elevated QTVI compared to hypertensive subjects without LVH. [150 108] Hence, LVH among subjects with non-dipping blood pressure could be one explaination to the elevated QTVI observed in the current study. According to ECG, one subject in the NDP group had LVH (Cornells criteria).

## 5.3 Repolarization lability after CABG (IV)

In study IV we assessed the QTVI, RR variances, QT variances and QTVN after CABG. Five weeks after surgery, QTVI, QT variances and QTVN were elevated, whereas the RR variances were reduced, compared to HS ($p<0.01$ for all, Figure 10). The QTVI values decreased between 5 weeks and 5 months after surgery but remained elevated compared to HS ($p<0.01$, Figure). Likewise, the QT variances decreased ($p<0.01$), whereas the RR variances increased during the recovery after CABG ($p<0.01$).

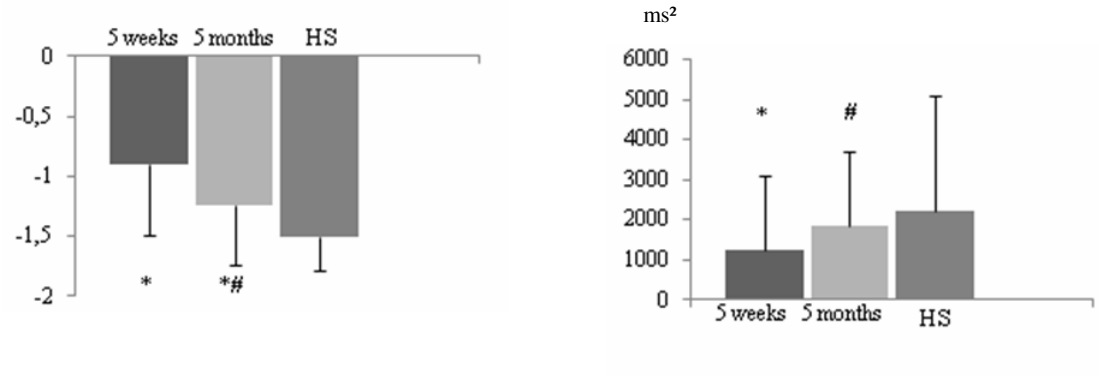


**Figure 10** *Bar graphs showing QT variability index (QTVI) (left panel) and RR variance (right panel) in patients 5 weeks (5 weeks) and 5 months (5 months) after coronary artery by-pass surgery (CABG) and in healthy subjects (HS). Data presented as mean value and standard deviation, *denotes p<0,01 compared to HS and # denotes p<0,01 compared to patients 5 weeks after CABG.*

The early recovery period after CABG up to 3 months after surgery has been associated with an elevated risk of ventricular arrhythmias. [86] The current data establish elevated temporal lability of myocardial repolarization during the recovery phase after CABG. Previous studies have reported an increased QTVI 4 days after CABG surgery, which further decreased and was comparable to measurements before surgery 4 weeks later. [156] The present study which assessed QTVI during a longer follow-up period, suggests that QTVI decreases further between 5 weeks and 5 months after surgery. Left ventricular ejection fraction was on average 57% (range 30 to 70%). During a 3 year follow-up, symptoms of angina pectoris occurred in 4 patients, myocardial infarction occurred in 2 patients and one underwent percutaneous coronary intervention. One patient died during follow-up due to a cerebral aneurysm.

QTVI in patients undergoing CABG could be elevated due to structural changes in the heart (hypertrophy, fibrosis and ischemia), abnormal intracellular ionic cycling, changes of intercellular coupling and altered ion channel function. [63, 157] Moreover, sympathetic and parasympathetic nervous activity could both affect the RR and QT intervals and alterations in the autonomic nervous system after CABG may have affected QTVI. [109, 110] A previous study reported attenuation of the heart rate response to deep breathing and valsalva manoeuvre after CABG. [158] The authors speculated that perioperative local damage on the effector organ or autonomic nerves were possible causes. [158]

In postinfarction patients with severe left ventricular dysfunction enrolled in the Multicenter Automatic Defibrillator Implantation II (MADIT II), a top-quartile QTVI was independently associated with the occurrence of ventricular arrhythmia. [58] Furthermore, Piccirillo et al has reported that QTVI predicts the risk of sudden cardiac death among asymptomatic patients with moderately depressed left ventricular ejection fractions. [59] Given this prognostic information on QTVI, one could speculate that the increased QTVI in the early rehabilitation phase after CABG may increase the risk for ventricular arrhythmias.

In conclusion, elevated temporal lability of myocardial repolarization prevails in particular during the early recovery phase after CABG and improves during the rehabilitation process. During the rehabilitation process, patients participated in a training program lead by a nurse. One may speculate that physical activity contribute to the reduction of temporal repolarization lability, perhaps through an effect on the autonomic nervous system with increased cardiac vagal activity and decreased cardiac sympathetic nervous activity. [159] A previous study showed that QTc (QT interval corrected for heart rate using Bazetts formula QT$\sqrt{RR}$) in women decrease with physical activity and the QT / RR slope in both sexes were improved (reduced) by physical acitivity. [160]

# 6 Summary and conclusions

*What was known before the study?*

- Subjects with established primary hypertension have increased lability of myocardial repolarization and reduced baroreflex sensitivity
- Individuals with prehypertension and established hypertension have increased carotid artery IMT.
- In hypertensive subjects, decreased nocturnal blood pressure dipping during night is associated with increased cardiovascular mortality, increased sympathetic nervous activity and reduced parasympathetic nervous activity.
- Patients with coronary heart disease have increased lability of myocardial repolarization early after CABG, which seems to decrease during rehabilitation.

*What this study adds*

- Reduced baroreflex function and increased myocardial repolarization lability were observed in prehypertensive subjects, suggesting that these phenomena occur early in the development of primary hypertension.
- Reduced nocturnal blood pressure dipping in otherwise healthy individuals is associated with increased lability of myocardial repolarization and impaired baroreflex function.
- Individuals with prehypertension and primary hypertension have increased intima thickness of the radial artery, suggesting an effect of slightly elevated blood pressure on the vascular wall, which may constitute an early sign of atherogenesis.
- Individuals who exercise regularly have reduced intima thickness of the radial artery compared to sedentary subjects, suggesting the beneficial of physical activity on the vascular structure.

- Patients with renovascular hypertension and CHD have markedly elevated temporal QT variability, which may increase the risk for malignant arrhythmias. After CABG, QTVI is elevated in particular during the early recovery phase and then decreases. Elevated lability of myocardial repolarization may contribute to the increased incidence of ventricular arrhythmias observed previously.

# 7 Clinical relevance and perspectives

This thesis demonstrates reduced baroreflex sensitivity, increased myocardial repolarization lability and increased intima thickness among individuals with prehypertension. Reduced baroreflex function and increased myocardial repolarization is already seen in healthy subjects without nocturnal blood pressure dip. These findings support the contention that elevated blood pressure exerts adverse effects on the vasculature and cardiac regulation already in the prehypertensive state. Tools to identify subsets of pre-hypertensive individuals at high risk for end-organ damage who will benefit the most from treatment are needed. Taken that 60% of a western adult population will be classified as pre- or primary hypertensives, it is unreasonable to treat everyone. In the future, we need to focus on the whole risk factor profile. Sir George Pickering´s statement about hypertension (and prehypertension as well) as an arbitrary division in the continous blood pressure distribution is more actual than ever.

# Acknowledgements

I wish to express my sincere and deep gratitude to:

**Mats Johansson**, MD, PhD, my supervisor

**Peter Friberg**, Professor, my co-supervisor

**Ruth Jonsson**, research nurse Varberg Hospital

**Li-Ming Gan**, Professor, co-author

**Sinsia Gao**, MD, PhD, co-author

**Walter Osika**, MD, PhD, co-author

**Ann-Kristin Karlsson**, PhD, co-author

My other co-authors

**Gun Bodehed-Berg** and **Marika Bring Friman** at Department of Clinical Physiology

The staff at Varbergshälsan, Varberg

The staff at Håsten Primary Care Unit, Varberg

Colleagues and co-workers at Department of Internal Medicine and Research Department, Varberg Hospital

And in particular, my family

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