Primary Hyperparathyroidism

A Study of Cardiovascular Dysfunction and its Reversibility After Parathyroidectomy

BY

INGA-LENA NILSSON
ABSTRACT


Cardiovascular risk in primary hyperparathyroidism (HPT) is controversial, and studies mainly from Europe associate HPT with increased cardiovascular morbidity and mortality. Cardiovascular morphology and function were evaluated prospectively in 31 consecutive HPT patients (mean serum calcium 2.97±0.04) and randomly enrolled controls matched for age and sex. Patients were re-examined at normocalcemia about one year after parathyroidectomy.

HPT patients showed an operatively reversible disturbance in endothelial vasodilatory function that seemed unrelated to an early sign of atherosclerosis, i.e. thickness of carotid artery intima-media complex. Acute hypercalcemia in healthy subjects induced a similar impairment in endothelial function, which suggests a dependence on biochemical rather than structural vascular changes in HPT. Echocardiography showed left ventricular diastolic dysfunction and supernormal systolic performance being reversed after operation. Left ventricular mass tended to be irreversibly increased. During exercise HPT patients exhibited greater rise in systolic blood pressure compared to controls and an increased number of premature ventricular beats. This indicated increased work load and a propensity for fatal cardiac events. Following surgery, an improvement with less pronounced ST-segment depression was seen. 24-hour ambulatory blood pressure monitoring showed irreversibly increased levels despite maintained diurnal rhythm, while 24-hour heart rate variability analysis displayed blunted nocturnal increase of low and very low frequency bands that was corrected postoperatively.

Parathyroidectomy seems to alleviate most of the cardiovascular disturbances in HPT, except for hypertension. This is consistent with the normalised longevity in HPT treated with parathyroidectomy and supports active treatment of HPT.

Key words: primary hyperparathyroidism, vascular endothelium, heart, heart rate variability, blood pressure, parathyroidectomy, calcium, parathyroid hormone

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This thesis is based on the following original studies, which will be referred to in the text by their Roman numerals:


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<tr>
<td>A</td>
<td>peak transmitral flow velocity during atrial contraction</td>
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<td>ABP</td>
<td>ambulatory blood pressure</td>
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<td>AVPD</td>
<td>left systolic atrio-ventricular plane displacement</td>
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<td>BMI</td>
<td>bone mass index</td>
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<td>BSA</td>
<td>body surface area</td>
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<td>E</td>
<td>peak transmitral flow velocity during early diastole</td>
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<td>ECG</td>
<td>electrocardiography</td>
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<td>EDV</td>
<td>endothelium-dependent vasodilation</td>
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<td>EIDV</td>
<td>endothelium-independent vasodilation</td>
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<td>FBF</td>
<td>forearm blood flow</td>
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<tr>
<td>HF</td>
<td>high frequency power (defined in the band between 0.15 and 0.4 Hz)</td>
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<td>HPT</td>
<td>sporadic primary hyperparathyroidism</td>
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<td>HRV</td>
<td>heart rate variability</td>
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<tr>
<td>iCa</td>
<td>ionised calcium</td>
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<td>IVRT</td>
<td>isovolemic relaxation time</td>
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<td>IVS</td>
<td>interventricular septal thickness</td>
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<tr>
<td>LF</td>
<td>low frequency power (defined in the band between 0.04 and 0.15 Hz)</td>
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<tr>
<td>LVEDD</td>
<td>left ventricular end-diastolic diameter</td>
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<tr>
<td>LVESD</td>
<td>left ventricular end-systolic diameter</td>
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<td>LVM</td>
<td>left ventricular mass</td>
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<tr>
<td>MCh</td>
<td>methacholine</td>
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<tr>
<td>MDT</td>
<td>mitral deceleration time</td>
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<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>nitric oxide synthase</td>
</tr>
<tr>
<td>pNN50</td>
<td>percent of differences &gt;50ms between adjacent RR-intervals</td>
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<td>PTH</td>
<td>parathyroid hormone</td>
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<td>rMSSD</td>
<td>root mean square differences between adjacent RR-intervals</td>
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<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SDANN-i</td>
<td>standard deviation of the means of all 5-minute segments</td>
</tr>
<tr>
<td>SDNN</td>
<td>standard deviation of all RR-intervals</td>
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SDNN-i - mean of standard deviation of all RR-intervals for all 5-min segments
SEM - standard error of the mean
SNP - sodium nitroprusside
ST/HR - ST- and heart rate changes from a four-minute period before end exercise
VLF - very low frequency power (between 0.003-0.04 Hz)
INTRODUCTION

Background

Sporadic primary hyperparathyroidism (HPT) has become a rather frequently diagnosed disorder since the introduction in the early 1970s of automated methods for serum calcium determination (Bilezikian et al. 1994). Nevertheless, data on the prevalence of primary HPT are sparse from most countries. About 1% of the adult population has been suggested to be affected, and there is an undisputed female predominance and increase in prevalence with increasing age of both genders. Screening programs for HPT have mostly used the serum calcium concentration and indeed also different degrees of hypercalcemia for patient identification. Most prevalence figures consequently comprise underestimates, and it remains to be determined if the apparently increased prevalence is due to a mere ascertainment bias (Äkerström et al. 1997). Concomitantly with the increased incidence, the clinical presentation of HPT has changed. Once it was a rare disease accompanied by pronounced renal, skeletal and mental symptoms, while it nowadays- at least in the Western world- is characterised by subtle, if any such obvious clinical manifestations (Söreide et al. 1996; Hasse et al. 2000; Talpos et al. 2000). Today, HPT instead appears to be associated with an over-representation of hypertension, diabetes mellitus, dyslipidemia and hyperuricemia (Lind et al. 1994a; Fardella et al. 1995; Lundgren et al 1998 a). There is, however, no simple relationship between the extent of hypercalcemia and the severity of such cardiovascular risk factors. Indeed these risk factors have been described also in patients with normocalcemia and in those with an otherwise asymptomatic clinical presentation (Ljunghall et al. 1989; Lundgren et al. 1998b).

The idea of an interaction between HPT and cardiovascular functions was substantiated rather dramatically by the demonstration of premature death from cardiovascular diseases in untreated hypercalcemia and HPT (Sivula et al. 1996; Palmér et al. 1987b). Moreover there was a suggestion of a causal relationship as normalisation of the longevity was noted with time after parathyroidectomy (Hedbäck
et al. 1998b; Lundgren et al. 2001). Indeed increased morbidity has also been found in a population-based screening study, which shows that sick-leave for cardiovascular disorders is elevated before diagnosis of biochemically mild HPT even in the absence of an apparent symptomatology (Lundgren et al. 1998b). It should be emphasised, however, that the incidence and clinical significance of cardiovascular disturbances and the risk of premature cardiovascular death in HPT are controversial especially in North America (Söreide et al. 1997; Wermers et al. 1998). Also mechanistically the basis for cardiovascular derangements in HPT is unsatisfactorily clarified.

Today parathyroidectomy is the only active treatment available for HPT outside prospective trials. However, the indication for surgery in asymptomatic cases is controversial, and controlled trials exploring surgery versus conservative follow-up have not been conducted with cardiovascular morbidity and mortality as end-points. Moreover, prevalence data from Sweden and USA indicate that only about one tenth of patients with HPT undergo parathyroidectomy, and it could be assumed that a majority of these would be undiagnosed cases lacking the well-recognised and non-controversial symptoms of the disorder (Melton 1991; Palmér et al. 1987b; Lundgren et al. 1997). It is also a fair assumption that detailed studies on the association of HPT with cardiovascular diseases and their reversibility by treatment could influence the urge to diagnose and actively treat many patients with HPT.

**Diagnosis of hyperparathyroidism**

The medical history and physical manifestations of HPT are often uncharacteristic and diagnosis has mostly to rely on laboratory tests. Since the introduction of two-site immunometric assays for intact parathyroid hormone (PTH), the diagnostic accuracy has improved substantially and the disease no longer has to be recognised on the basis of excluding other possible causes for hypercalcemia (Ljunghall et al. 1991a). It is often rapidly established by documenting an increase in total serum calcium and intact PTH above the normal range. An accurate laboratory test is to measure the ionised or diffusible calcium fraction iCa. About 40% of the calcium in circulation is
bound to albumin and the remaining 10% is in soluble complexes with anions (Moore
1970). In most clinical situations the total serum calcium value suffices for diagnostic
purposes provided that adjustments for the degree of protein- binding (e.g., serum
albumin) are performed (Ladenson 1991; Ljunghall et al. 1991a). The basis for this
reality is that diagnosis always requires investigation of both the serum calcium
(Schluter et al. 1998) and PTH value, whereby minor imprecision in the calcium
determination has little impact. In addition the urinary calcium concentration will be
normal or increased (Clark 1997), and secondary causes for stimulated PTH release
have to be excluded by the demonstration of an apparently retained kidney function.

It must be remembered, however, that even under routine circumstances at least 20%
of clinically recognised patients with HPT have intact serum PTH levels within the
reference range. As serum calcium and PTH fluctuate and the hypercalcemia and
increased PTH may be intermittent, the diagnosis of HPT must rely on the presence
of an inappropriately elevated serum concentration of PTH relative to the serum
calcium value (Ljunghall et al. 1991a; Siperstein et al. 1992). Rather little, however,
has been done to systematically define what an inappropriately elevated serum PTH
level actually is (Lundgren et al. 1997).

HPT and malignancy account for more than 90% of all causes of hypercalcemia. By
using a two-site assay, PTH will not cross-react with the parathyroid hormone-related
peptide associated with some malignancies of nonparathyroid origin (Budayr et al.
1989; Clark 1997). Other causes of hypercalcemia include e.g. immobilisation,
treatment with thiazide diuretics and granulomatous disease, when the the PTH level
will be low to normal. Benign familial hypocalciuric hypercalcemia could mimic
HPT with an increase in serum calcium and an inappropriately normal serum PTH
level, whilst the urinary calcium clearance will be low in virtually all cases (Clark
1997).
**Parathyroid hormone**

The PTH secretion is tightly regulated by the serum calcium concentration, although several other endogenous substances have been found to influence the release. Extracellular calcium-sensing receptors on the parathyroid cell surface mediate an inhibitory effect on PTH secretion and probably also affect the parathyroid cell proliferation (Brown 1999; Hellman et al. 2000). Binding of external calcium to the receptor is associated with calcium influx and mobilisation of intracellular calcium. Acute changes in the extracellular calcium concentration induce rapid changes in the PTH secretion. The dose-relationship is inversely sigmoidal with a considerable secretory response even to minor alterations in the external calcium level (Rastad J 1997 & Ridefelt P). HPT is associated with a right-shifted dose-response relationship between calcium and PTH secretion and a decreased steepness of the slope of the dose-response curve. There is reduced expression of the calcium-sensing receptors, which probably contributes to the relative hypersecretion and calcium-insensitivity of PTH (Ljunghall et al. 1991b; Farnebo et al. 1997; Malberti et al. 1999).

The PTH level fluctuates with a frequency of about 6-7 bursts per hour. About two thirds of PTH in the circulation is attributable to tonic secretion, while the pulsations account for about one third. In HPT there are proportional increases in both secretory phases (Harms et al. 1989; Ljunghall et al. 1991b; Schmitt et al. 1996). Circadian variation in serum PTH values with differences between men and women seems to occur and this variation is probably blunted in HPT (Logue et al. 1990; Kitamura et al. 1990; Calvo et al 1991; Samuels et al 1993).

**Calcium and the cardiovascular system**

The concentration of iCa is a strongly guarded parameter and the ion serves as a key regulator of a host of processes, like in muscle contraction-relaxation, central and peripheral neural transmission, endocrine and exocrine secretion, and in intracellular
signalling pathways. Calcium receptors are widely distributed, sensitive to small 
changes of iCa and regulate the hormone release from the parathyroid gland (Brown 
et al. 1998; Brown 2000). They are also expressed in e.g. the perivascular nerve 
network. An increase in the concentration of intracellular iCa in vascular smooth 
muscle cells is essential for the tension (Fardella & Rodriguez-Portales 1995). Acute 
hypercacemia is associated with an increase of the mean arterial pressure by an 
increased peripheral vascular resistance (Scheidegger et al. 1980; Marone et al. 1981). 
Indeed a positive correlation between blood pressure and intracellular iCa in platelets 
has been described in essential hypertension and raised iCa also seems to occur with 
aging (Fardella & Rodriguez-Portales 1995; Barbagallo et al. 1999). Paradoxically, 
essential hypertension has been associated with both low serum calcium and high 
PTH levels (McCarron et al. 1987; Reichel et al. 1992).

Calcium infusion at hypocalcemia exerts a positive inotropic effect, while effects are 
marginal at normocalcemia (Mori 1978; Drop et al. 1981). In uremic 
hyperparathyroidism exogenous calcium has been reported to impair left ventricular 
diastolic function without any positive inotropic effect (Virtanen et al. 1998). Indeed 
hypercacemia associates with electrocardiographic changes with shortening of the 
QT- interval to an extent that correlates with the serum calcium level (Saikawa et al. 
1988; Lind et al. 1994c). Besides, signs of decreased atrial activity like bradycardia , 
sinus arrest and ectopic ventricular beats have been induced by calcium infusion in 
animals (Littledike et al. 1976). Thus it is apparent that there are multiple levels for 
interaction between calcium and the cardiovascular system. This is consistent with 
findings in population-based analyses where high serum calcium is a predictor of 
cardiovascular morbidity and mortality (Hedbäck & Oden 1998b; Palmér et al. 
1987b; Jorde et al. 1999). Even the risk of dying in cardiovascular diseases for young 
and middle-aged men has been correlated with serum calcium in the normal range 
(Leifsson et al. 1996).
Parathormone and the cardiovascular system

PTH has been claimed to play a permissive role in the hypertensive action of hypercalcemia, and elevated serum PTH has been reported in essential hypertension (Reichel et al. 1992) (Oshima et al. 1995; Young et al. 1995). PTH receptors have been demonstrated in the vascular endothelium and smooth muscle cells (Jiang et al. 1998; Isales et al. 2000). Although acute PTH infusion causes vasodilation in experimental animals, chronic infusions seem to increase the blood pressure (Pang et al. 1981; Hulter et al. 1986). PTH also affects the heart with induced chronotropism, inotropism and even hypertrophy (Bogin et al. 1981; Ogino et al. 1995; Hui et al. 1996; Schluter & Piper 1998). At the cellular level PTH has been suggested to act as an ionophore that stimulates calcium entry (Fardella & Rodriguez-Portales 1995). Endothelin is a potent smooth muscle cell constrictor that is produced in the vascular endothelium. It is also synthesised by parathyroid cells and endothelin receptors are expressed by the parathyroid parenchyma (Fujii et al. 1991; Eguchi et al. 1992). Endothelin seems to inhibit PTH release by interfering with parathyroid iCa and consequently may be an autocrine regulator of PTH release (Takahashi et al. 1995; Rastad J & Ridefelt P 1997).

Cardiovascular disease and risk factors in hyperparathyroidism

HPT has been associated with premature death in mainly cardiovascular diseases in a handful of European studies containing over 6,500 patients (Ronni-Sivula 1985; Palmér et al. 1987a; Hedbäck et al. 1990; Hedbäck & Oden 1998b; Walgenbach et al. 2000). It should however be pointed out that a significantly increased risk of dying could not be found in two HPT materials from North America (Söreide et al. 1997; Wermers et al. 1998). Nevertheless serum PTH was found to be an independent risk factor for death and a significantly raised mortality was found for HPT patients without a history of urolithiasis. The increased risk of dying from HPT in Europe has been found to diminish with time after surgery, although this important circumstance
has been explored in only a minority of the cases referred to above (Palmér et al. 1987a; Hedbäck et al. 1991). The risk of dying notably has been related to the degree of hypercalcemia and the weight of parathyroid adenomas, whereby it has been hypothesised that the duration of HPT may be important for its fatality (Hedbäck et al. 1995b; Hedbäck et al. 1995a). Indeed a short duration of disease and well-preserved renal function seem to have protective roles (Hedbäck et al. 1998a). Little, however, is known of the type of morbidity that can cause such mortality. Insight into this morbidity was provided by the finding of increased sick-leave utilisation for disorders of the cardiovascular system before diagnosis of even overtly asymptomatic and biochemically mild degrees of HPT (Lundgren et al. 1998b). This finding suggests that cardiovascular complications cannot be considered to occur in advanced HPT alone.

Hypertension is over-represented in HPT and left ventricular hypertrophy, impaired left ventricular diastolic function and metastatic myocardial and valvular calcifications have been reported (Niederle et al. 1990; Ohara et al. 1995; Piovesan et al. 1999; Näppi et al. 2000). Increased cardiac output, augmented central aortic pressures and autonomic nervous system dysfunction have also been found even in mild HPT not associated with hypertension or other overt cardiocascular diseases (Smith et al. 2000; Georgiannos et al. 1996; Barletta et al. 2000). Additionally, mild HPT in postmenopausal women has been associated with type IV dyslipidemia that normalises after surgery (Lundgren et al. 1998a). Peripheral insulin resistance seems to occur and an increased prevalence of diabetes mellitus type II has been reported with prospects of improvement, albeit rarely cure after parathyroidectomy (Valdemarsson et al. 1998; Richards et al. 1999; Chiu et al. 2000). The correlation between the degree of hypercalcemia and these complications is weak and even overtly asymptomatic HPT can be associated with cardiovascular risk factors (Rastad 2001).
Hypertension

Despite rather convincing findings of over-representation of arterial hypertension in HPT, the blood pressure usually remains unaltered after parathyroidectomy (Ljunghall et al. 1989; Lind et al. 1991; Sancho et al. 1992). By no means, however, does this argue strongly against a causal relationship as is the case with the parallel situation in operatively treated primary hyperaldosteronism. Contrary to the situation in some endocrine diseases, hypertensive HPT patients exhibit a normal circadian blood pressure (Middeke et al. 1991). Ambulatory blood pressure measurement is a better predictor or of cardiovascular risk than office recordings of blood pressure by elimination of the ‘white coat effect’ (Staessen et al. 1999; Ohkubo et al. 1998). The parathyroid glands are necessary for maintenance of hypertension in spontaneously hypertensive rats and a vasopressor factor has been partially purified from the human parathyroid (Pang et al. 1991; Schluter et al. 1992). Normotensive patients with HPT also have an abnormal response to pressor agents similar to that found in patients with idiopathic hypertension (Rodriguez-Portales & Fardella 1994).

Vascular endothelium and vasodilatory function

The vascular endothelium is important for the vascular tone and endothelial dysfunction is an early event in the atherosclerotic process (Panza et al. 1990). Acetylcholine causes relaxation of arterial smooth muscles by acting on muscarinic receptors on the endothelial cells (Furchgott et al. 1980). Nitric oxide (NO) is a potent vasoactive substance synthesised in the endothelium from L-arginine by nitric oxide synthase (Fig. 1). NO causes smooth muscle relaxation by a decreased intracellular iCa concentration (Vane et al. 1990). Inhibition of NO-synthesis in man causes vasoconstriction indicating the presence of an active, tonic vasodilation that is maintained by NO. Inhibited NO-synthesis also attenuates the vasodilatory response of muscarinic stimulation, which links the contribution of endothelium-derived NO to the stimulation of regional blood flow in man (Vallance et al. 1989).
Figure 1. A model for NO-mediated smooth muscle relaxation (Martina et al. 1998). NO is synthesised in the endothelial cell, and diffuses to the smooth muscle cell where it causes relaxation. The synthesis of NO from L-arginine is initiated by muscarinic receptor for acetylcholine (mACh) stimulation and catalysed by the calcium-dependent NO synthase (NOS). NO stimulates guanylate cyclase (GC) that converts guanosine-triphosphate (GTP) to cyclic guanosine-monophosphate (cGMP) and causes muscle relaxation chiefly via decreased intracellular ionised calcium (Ca^{2+}).

Forearm endothelial dysfunction has been associated with left ventricular hypertrophy and increased frequency of cardiovascular events in non-treated hypertension (Perticone et al. 1999; Perticone et al. 2001). NO is a short-lived radical that can be measured only by sophisticated methods (Vallance et al. 1995), and mostly analyses have utilised evaluation of break-down products and physiologic all actions (Lind et al. 2000). Most animal models and human forms of hypertension are characterised by impaired NO release, contributing to an attenuated endothelial-dependent vasodilation (EDV) and probably also to the cardiovascular complications of hypertension (Vane et al. 1990). An increased NO production in platelets with normalisation after parathyroidectomy has been demonstrated in HPT suggesting a role of calcium and/or PTH in regulating the activity of constitutive NO synthase (Martina et al. 1998). Hypothetically this increase could down-regulate the vasoconstriction that could be caused by high iCa in smooth muscle cells.
**Myocardial function**

The calcium ion is important for the contractile force of the heart and changes in cytoplasmic levels of iCa regulate the myocardial contraction-relaxation process. The influx of iCa is modulated by adrenergic and cholinergic neurotransmitters through their action on the membrane potential (Reuter 1987). Calcium is stored in the cardiac sarcoplasmic reticulum and released by sarcolemmal depolarisation, whereby contraction is induced (Inui et al. 1990). The iCa release seems to be triggered by the calcium influx through the sarcolemma, and the calcium level in the sarcoplasmic reticulum may play a crucial role in regulating the release process (Shannon et al. 2000). During cardiac relaxation, calcium is reaccumulated by the reticulum’s calcium pump and extruded by the sarcolemmal Na/Ca exchange transporter.

Diastolic dysfunction with prolongation of the early diastolic phase and a relative decrease of the passive volume filling of the left ventricle has been reported in most, but not all studies of HPT (Barletta et al. 2000; Dalberg et al. 1996; Näppi et al. 2000; Ohara et al. 1995). The diastolic dysfunction has been related to decreased compliance from calcium deposition in the myocardium, decreased mitochondrial energy production and chronic calcium excess in the myocytes (Baczynski et al. 1985). In addition, left ventricular hypertrophy is reported in HPT and to some extent this could be explained by concomitant hypertension. Also a continuos excess of intracellular iCa may induce cardiac hypertrophy, and through this or other mechanisms PTH has been assumed to promote cardiac fibrosis (Pearce et al. 1985; Amann et al. 1994). iCa is important for generation of pacemaker potentials, and a shortening of the repolarisation phase (QT-interval) has been found in HPT (Lind et al. 1994b). Less attention, however, has been paid to systolic function in HPT despite the fact that normotensive and asymptomatic HPT patients can have an operatively reversible increase in cardiac output (Bogin et al. 1981). Another issue awaiting clarification is the link between HPT and of myocardial ischemia, since this might be a cause for the increased cardiovascular morbidity and mortality in HPT. Indeed,
also an increased risk of arrhythmia in HPT has been suggested by case reports (Koch 1992; Carpenter et al. 1994; Chang et al. 2000).

The autonomic nervous system is important for the regulation of cardiovascular function. Simplified, the sympathetic system mediates the chronotropism and inotropism of the heart and promotes vessel constriction in some tissues, but dilation of skeletal muscle vessels. Vagus, on the other hand, mediates cardiovascular ‘protection’ by decreased heart rate and increased heart rate variability (HRV). Abnormal circadian HRV measured over a 24-h period has been found to be a predictor of cardiovascular death in subjects with and without heart disease (Rich et al. 1988; Otsuka et al. 1997; Burger et al. 1999). However, mechanisms responsible for this association are incompletely clarified (Rich et al. 1988; Burger et al. 1999; Huikuri et al. 1999).

**Treatment of hyperparathyroidism**

Parathyroidectomy is the only active treatment of HPT that has been evaluated during long-term follow-up. The procedure can be performed with exceptionally low morbidity and expectations of a success rate of 95% when serum calcium is accepted as the most appropriate outcome variable (Clark & Duhbb 1989; Hasse et al. 2000; Walgenbach et al. 2001). However, there are less encouraging results with respect to the persistence and recurrence of an inappropriately elevated serum PTH value (Lundgren et al. 1992; Proye et al. 2000; Heuser et al. 2000; Mimura et al. 1998). The indications for surgery in asymptomatic cases and in those with no, marginal or rather moderate degree of hypercalcemia are debated. Alternate active treatments are medicinal, and include CaR-interactive compounds (calcimimetics) and hormone replacement therapy (estrogen and/or gestagen) (Duarte et al. 1988; Brown 1999). In general, neither of these can be recommended outside clinical trials.

In 1990, NIH held a consensus conference on the treatment of asymptomatic HPT (Proceedings of the NIH Consensus Development Conference on diagnosis and
management of asymptomatic primary hyperparathyroidism. Bethesda, Maryland, October 29-31, 1990). Noteworthy is that asymptomatic HPT essentially was defined by the absence of overt symptoms of bone loss, current or recent history of nephrolithiasis and unequivocal psychiatric and muscular derangements. It was suggested that individuals fulfilling these criteria were eligible for conservative follow-up provided serum calcium is less than 2.80 mM, age exceeds 50 years, renal function is satisfactory maintained (creatinine clearance reduced by less than 30%), the 24-h urine calcium excretion is below 400 mg, and the bone mass is greater than minus two standard deviations of age- and gender-matched persons. It was also concluded that parathyroidectomy could be motivated whenever the rather laborious program of surveillance was considered unsuitable. Adherence to these recommendations was analysed in 1998, and the criteria for parathyroidectomy were found to vary widely even among highly experienced surgeons (Sosa et al. 1998).

**Natural history and conservative surveillance in hyperparathyroidism**

Natural history should reflect many of the expectations on the outcome of conservative surveillance in HPT. Lessons from the past suggest that a small, albeit significant proportion of untreated patients will develop a more or less marked hypercalcemia, recurrent renal stones, renal impairment, and skeletal complications. However, the disease may have changed over time. A hypothetical explanation for such a shift may relate to an improved vitamin D and calcium balance in the population, since HPT of the developing countries mimics the experience from our past (Mishra et al. 2001). Development of progressive hypercalcemia, a deteriorated kidney function or symptom development cannot be predicted in the individual case and can occur unexpectedly also under careful monitoring (Corlew et al. 1985; Corsello et al. 1991).

Although hypercalcemia seems to decrease over time, premature cardiovascular death seems to persist in patients younger than 70 years during even 25 years of follow-up (Palmér et al. 1987a; Lundgren et al. 2001). Indeed even normal aging has been
associated with elevation of intracellular iCa and reciprocal reduction in extracellular iCa (Barbagallo et al. 1999). An increased serum PTH level may become noticeable despite the fact that serum calcium is stable (Rudnicki et al. 1992). Even a significantly reduced bone mass may remain unaltered, why it has been hypothesised that the loss of bone preferentially occurs early in HPT (Parfitt et al. 1991). Elevation of the diastolic and systolic blood pressures in comparison with controls, and other risk factors for cardiovascular complications should be expected to persist (Lind et al. 1991).

**POTENTIAL OUTCOMES OF THE STUDY**

Clarification of the cardiovascular derangements in HPT and their prospects for normalisation after surgery may have several potential consequences:

- Increase knowledge of the possible role of calcium and PTH in cardiac and vascular functions and diseases
- Enable better prediction of the type and extent of risks in untreated HPT
- Improve motivation for routine serum calcium determination for diagnosis at even minimal suspicion of HPT
- Determine the expedience by which different degrees of HPT should be treated actively
- Provide guidelines for the selection of active (today operative) treatment vs. conservative surveillance in HPT
- Improve the knowledge of necessary requirements for follow-up in treated HPT patients
Being an endocrine surgeon it was quite natural for me to start by exploring any potential effect of treatment with parathyroidectomy on the registered pathophysiology of the cardiovascular system. It should be emphasised, however, that I am fully aware of the necessity to make structured comparisons also with other treatment modalities and preferably also the time course of these events to fully appreciate the clinical importance of cardiovascular derangements in actively treated and untreated HPT.

**AIMS OF THE STUDY**

Specifically the studies were designed to explore:

- Endothelial vasodilatory function in relation to blood pressure and the carotid artery intima-media thickness in patients with HPT, and any influence of parathyroidectomy on these characteristics

- Effects of induced hypercalcemia on endothelial vasodilatory function and blood pressure in young healthy subjects

- Left ventricular morphology and function in HPT and the potential reversibility of abnormalities after parathyroidectomy

- Myocardial ischemia and arrhythmias in patients with HPT and any effect of parathyroidectomy

- Autonomous nervous function in terms of ambulatory heart rate variability and blood pressures as contributors to the increased cardiovascular morbidity in HPT
MATERIAL AND METHODS

Patients and control subjects

At the initiation of the program 31 consecutive HPT patients, 24 women and 7 men, were recruited from the routine referrals for parathyroidectomy at the County Hospital of Sundsvall, Sweden (Table 1). The overall age range was 33 to 80 and their total serum calcium concentration averaged 2.97±0.24 mM (normal range 2.20-2.60 mM). A majority of them (70%) could be considered to have ‘classical’ symptoms of HPT. By random sampling 31 normocalcemic control subjects were enrolled, with matching for age and gender, from the same underlying population. The intent was to subject patients and controls to several cardiovascular function tests to allow future comparison between them. However, patients and controls had a variety of recognised cardiovascular diseases at recruitment or developed requirement for medication during the course of studies. Overall 10 patients and 8 controls were on regular antihypertensive treatment, while 3 patients received such treatment (n=1) or had increased dosages of preexisting β-blocking agents (n=2) after surgery (Table 1). Also the initial investigations revealed e.g. undiagnosed arrhythmia that prevented participation in some of the examinations. Finally some patients refused postoperative re-examination, and there were also technical failures that prevented analysis of individual examinations.

The actual number of patients and controls having undergone the examinations in studies I, III, and IV are shown in Table 2. Despite the loss of subjects particularly in study IV, patients and controls remained satisfactorily matched with respect to age and sex. Also serum calcium and serum PTH values of the investigated patients mirrored those in the initial recruitment group. All patients and controls were asked to discontinue any antihypertensive therapy during the week before the study day, except for the patients with angina pectoris.
**Table 1.** Clinical characteristics and relevant medications of all recruited patients and their matched controls with variables both before and after parathyroidectomy in the patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=31)</th>
<th>Cases (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>after</td>
</tr>
<tr>
<td>Age (yr.)</td>
<td>64±2</td>
<td>63±2</td>
</tr>
<tr>
<td>Sex (females:males)</td>
<td>24:7</td>
<td>24:7</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Coronary artery disease (n)</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Medically treated hypertension (n)</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Nephrolithiasis (n)</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Diabetes mellitus (n)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Vasoactive medications (n)</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>ß-blocking agents</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Angiotensin enzyme inhibitors</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>slow releasing nitrocompounds</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Diuretics (n)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Digitalis (n)</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 2.** Number and gender (female:males) of patients and controls that were investigated in studies I, II and IV. Patients were analysed before and after parathyroidectomy and the time (months±SEM) between operation and postoperative re-examination is given.

<table>
<thead>
<tr>
<th>Study number and technique</th>
<th>Controls</th>
<th>Cases</th>
<th>time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I Venous occlusion plethysmography</td>
<td>25 (18:7)</td>
<td>25 (18:7)</td>
<td>17 (13:4)</td>
</tr>
<tr>
<td>Carotis duplex 'Office' blood pressure</td>
<td>30 (23:7)</td>
<td>30 (23:7)</td>
<td>30 (23:7)</td>
</tr>
<tr>
<td>Study III Echocardiography</td>
<td>28 (21:7)</td>
<td>27 (21:6)</td>
<td>29 (22:7)</td>
</tr>
<tr>
<td>Exercise test</td>
<td>20 (15:5)</td>
<td>18 (14:4)</td>
<td>15 (12:3)</td>
</tr>
<tr>
<td>Study IV Heart rate variability</td>
<td>20 (13:7)</td>
<td>16 (11:5)</td>
<td>13 (8:5)</td>
</tr>
<tr>
<td>Ambulatory blood pressure</td>
<td>20 (13:7)</td>
<td>16 (11:5)</td>
<td>13 (8:5)</td>
</tr>
</tbody>
</table>

§ Ambulatory blood pressure was analysed in 21 cases and 24 controls.
Venous occlusion plethysmography - the forearm model (study I, II)

Venous occlusion plethysmography is a method to estimate forearm blood flow (FBF) that is well established (Vane et al. 1990; Panza et al. 1990; Lind et al. 1998). Vasodilatory infusions were given through a cannula in the brachial artery with the contra-lateral arm as control. With the subjects supine and the forearms elevated slightly above the level of the heart, venous occlusion was achieved by blood pressure cuffs applied proximal to the elbow. The cuffs were inflated to 40 mm Hg approximately four times per minute by a rapid inflator, and each inflation lasted seven seconds. The increase in forearm circumference due to the preserved arterial inflow during venous occlusion was monitored by a strain gauge, which was placed on the upper third of the forearm and connected to a calibrated plethysmograph. EDV and endothelium-independent vasodilation (EIDV) were evaluated during local infusion of methacholine (Mch, 2 and 4 µg/min) and sodium nitroprusside (SNP, 5 and 10 µg/min), respectively.

Metacholine in this model has been shown to increase the release of the NO waste products nitrite and nitrate in healthy volunteers (Lind et al. 1998). Nitroprusside, on the other hand acts as a NO donor that affects the vascular smooth muscle cells directly and evaluates the NO-reactivity. Each drug dose was given during 5 minutes with a 20-minute wash-out period in between. FBF of both arms was measured before and at the end of the drug infusions, and evaluated as the mean of 5 consecutive recordings. The endothelial function index was calculated as the ratio of FBF during the highest doses of Mch and SNP (EDV/ EIDV). This index can be considered to express the contribution of endothelial NO to the vasodilatory process.

Carotid artery intima-media thickness (study I)

Thickening of the intima-media part of the carotid artery wall is considered as a sign of early atherosclerosis (Ghiadoni et al. 1998). This thickness was measured with ultrasound in the far wall of both common carotid arteries one centimeter proximal to
the bifurcation. A 10-5/12-5 MHz broadband transducer (HDI 3000/5000 system, ATL Inc, US) was used, and the mean value of both carotids was calculated for each person.

Systemic and local calcium infusion (study II)

Calcium-Sandoz™ (5ml calciumglubionate, 9mg/ml) in 45 ml saline was infused at a rate of 30-60ml/h for 1 hour in the brachial artery. The aim was to achieve a steady-state iCa of 1.40- 1.60 mM in an ipsilateral antecubital vein. After a 30-minute wash out, the effects of systemic hypercalcemia were studied by adding 60 ml Calcium-Sandoz™ to 440 ml saline and infusing the mixture for 1 hour at 230- 250 ml/h dependent on the body weight. A similar extent of hypercalcemia was the goal, and the infusion rate was adjusted to measurements of blood iCa values. EDV and EIDV were evaluated by the forearm model before and at the end of local and systemic calcium-infusion.

Blood pressure (study I-IV)

Conventional systolic and diastolic blood pressure (‘office pressure’) were recorded after a 10-minute supine rest with a regular auscultatory technique. In study II, an automatic device (OMRON HEM 705C, Tokyo, Japan) was used. In study III the blood pressure was followed every second minute during exercise with an automatic device (Tango, SunTech Medical Instruments US, Raleigh N.C, USA).

Ambulatory 24-hour blood pressure (ABP) was measured in studies I and IV. Recordings were obtained automatically with a portable device (Diasys Integra, Novacor SAA, France). Systolic and diastolic pressure and heart rate were registered every 30 min during daytime and every 45 min at night. In study I daytime was defined as the interval from 07.00 to 23.00 and night-time from 23.00 to 07.00. Since better discrimination between periods of sleep and activity may be obtained with
another definition (Fagard et al. 1997), daytime in study IV was defined as the interval from 10.00 to 20.00 and night-time from midnight to 06.00.

**Echocardiography** (study III)

Echocardiography was performed with an HDI 3000 or HDI 5000 and ATL 3-2 MHz or 4-2 MHz transducers (ATL Inc., Seattle, USA). The recordings were made with the patient recumbent in the left semilateral position. Myocardial thickness and left ventricular diameter in diastole (LVEDD) and systole (LVESD) were measured by M-mode (Park et al. 1996). Left ventricular mass (LVM) was related to the body surface area (BSA) and determined from measurements of the interventricular septal and posterior wall thickness and end-diastolic diameter using the M-mode formula of Troy. The relative left ventricular wall thickness was calculated as the sum of the interventricular and posterior wall thicknesses divided by the end-diastolic diameter. Left ventricle volumes were calculated according to the Teichholz M-mode formula (Teichholz et al. 1976) and used to calculate the ejection fraction. Fractional shortening was determined as $\frac{\text{LVEDD} - \text{LVESD}}{\text{LVEDD}}$. The left systolic atrio-ventricular plane displacement (AVPD) was recorded from the apical four- and two-chamber views, by M-mode echo, at four left ventricular sites, corresponding to the septal, lateral, posterior and anterior walls.

The maximal velocities of early diastolic (E) and late atrial (A) mitral flow were recorded with an apical transducer position and pulsed Doppler from the level between the tips of the mitral leaflets. The E to A ratio was calculated. The mitral deceleration time was measured from the peak of the early diastolic mitral flow velocity to its extrapolation at baseline. The interval from aortic valve closure to mitral valve opening, the isovolemic relaxation time (IVRT), was recorded with pulsed or continuos Doppler after angulating the transducer toward the left ventricular outflow tract until aortic valve closure and mitral opening appeared.
**Exercise test** (study III)

Upright bicycle ergometry was performed with the electronically braked Ergomed 940 (Ergofit, GMBH Pirmasens, Germany). The initial workload was 30 W for the women and 50 W for the men and it was increased by 5 W every 30 seconds. It was limited by the occurrence of clinical symptoms (exhaustion, dyspnea or chest pain) or significant electrocardiography (ECG) findings. A 12-lead ECG was continuously recorded (Megacart, Siemens-Elema, Solna, Sweden), which permitted graphical and numerical presentation of the time course of the ST-slope, the heart rate and the ST-segment 60 ms after the junction point. Maximal ST-segment depression was evaluated among complexes with horizontal or down-sloping segments, which were defined as a slope not exceeding 0.1mV/second at rest or 0.3 mV/second during exercise. The ST/HR slope was calculated by measuring ST and heart rate changes in lead V5 from at least a 4-minute period before the end of the exercise (Suurküla M 1999). The number of ventricular ectopic beats during the exercise test was counted at the ECG recordings.

**Heart rate variability** (study IV)

24-hour ECG-recordings were made using a cassette-based two-channel recorder (Oxford Medilog 4500) with recordings stored and analysed with the Oxford Medilog Excel 2 system (Oxford Instruments Ltd, England). Beat templates were manually edited. Recordings with predominantly sinus rhythm and normal atrioventricular conduction were analysed, while those with frequent supraventricular or ventricular ectopic rhythms or atrial fibrillation were excluded.

Time domain measures of heart rate variation (HRV) were calculated from the interpolated tachogram and frequency domain variables obtained with an autoregressive algorithm after resampling the tachogram at 292 milliseconds (ms). The time domain variables were expressed in ms. SDNN was defined as the standard deviation of all RR-intervals, SDNN-i as the mean of the standard deviations of all
RR-intervals for all 5-minute segments of the analysis, SDANN-i as the standard deviation of the means of all 5-minute segments, rMSSD as the root mean square differences between adjacent RR intervals and pNN50 as the percent of differences >50ms between adjacent intervals.

Frequency domain variables were expressed in absolute units (ms²) with the high frequency power (HF) defined in the band between 0.15 and 0.40 Hz, the low frequency power (LF) in the band between 0.04 and 0.15 Hz and very low frequency power (VLF) between 0.003-0.04 Hz. The ratio between LF and HF was calculated.

**Biochemistry in blood** (study I- IV)

Blood was sampled anaerobically after an overnight fast. Clinical routine methods were used to estimate total calcium (adjusted for albumin), creatinine, total alkaline phosphates, fasting glucose, triglycerides and cholesterol. Intact serum PTH and iCa values were measured with commercial kits (Ciba-Corning Magic Lite, and Ciba-Corning 288; Ciba-Corning, UK). After 1999, PTH measurements used the DPC Immulite kit (Diagnostics Products Corporation, US). The correlation coefficient between the two PTH assays was 0.92448 (p=0.00001). Recalculation of data with the Immulite assay utilised the formula 0.40799 x (measured value - 3.1174). These recalculated values were utilised in the statistical analysis of the data. In study II, iCa was measured by an ion sensitive electrode (AVL OMNI, Graz, Austria, normal range is 1.09-1.33 mM) and serum PTH with the Allegro immunoradiometric sandwich assay (Nichols Institute, San Juan, CA, normal range 12-55 ng/l. BSA was evaluated according to the formula of Du Bois and the body mass index (BMI) as weight (in kg) divided by the square of the height (in cm).

**Statistics**

Values are expressed as the mean and standard deviation (SD), the mean and standard error of the mean (SEM) or the median and interquartile range. Student’s unpaired
and paired two-tailed tests were used for inter- and intraindividual analyses of normally distributed data; the Mann-Whitney U-test and the Wilcoxon signed rank sum test were used for skewed data. Pearson’s correlation coefficient was used to analyse relationships between pairs of normally distributed variables and Spearman’s correlation coefficient for the others. P less than 0.05 was regarded as significant.

RESULTS AND DISCUSSION

Biochemistry in blood and histopathology (study I- IV)

There were the expected differences between patients and controls in studies I, III and IV with respect to serum calcium, PTH and phosphate levels (Table 3). Total and iCa values normalised postoperatively in all the cases, and the postoperative change was significant of total and ionised calcium, PTH, alkaline phosphatases (p<0.001) and phosphate (p<0.05).

Single parathyroid adenoma was excised in 28 cases and double adenomas in the other three. All excised parathyroid specimen were examined histologically with the use of conventional histologic criteria. The total pathological parathyroid weight was 0.10-5.74 g (1.15±1.14g). It correlated to preoperative serum PTH (r=0.447, p<0.05), and the total and iCa values in blood (r=0.432, 0.406, p<0.05). In general it may be concluded that the investigated patient sample should be considered to represent a moderately severe extent of HPT. This assumption is consistent with the average serum calcium and intact PTH concentration, the proportion of symptomatic individuals, and the total weight of the abnormal parathyroid tissue (Rastad et al. 1995; Kleerekoper et al. 1994). Indeed these characteristics probably reflect the prevailing tradition among physicians in the region of what currently is considered the necessary degree of symptomatology and biochemical disturbance at which diagnosis and active treatment for HPT are indicated. Moreover the majority of recruited patients should be eligible for parathyroidectomy even according to the recommendations of the NIH consensus document (NIH 1991).
Table 3. Biochemical findings in the entire group of patients and matched controls (mean±SEM). Values in parentheses represent normal ranges, *p<0.05, **p<0.01 and ***p<0.001 in patients before surgery vs controls (Mann-Whitney) and in patients before vs. after surgery (Wilcoxon).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>Cases (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=31</td>
<td>Before</td>
</tr>
<tr>
<td>Body mass index</td>
<td></td>
<td>26.2±0.6</td>
</tr>
<tr>
<td>Total s-calcium (2.20-2.60 mM)</td>
<td>2.37±0.02</td>
<td>2.97±0.04***</td>
</tr>
<tr>
<td>Ionised b-calcium (1.00-1.30 mM)</td>
<td>nd</td>
<td>1.55±0.03</td>
</tr>
<tr>
<td>s-PTH (1.2-5.7 pM)</td>
<td>4.5±0.6</td>
<td>15.7±1.6***</td>
</tr>
<tr>
<td>s-Phosphorous (0.9-1.3 mM)</td>
<td>1.0±0.05§</td>
<td>0.9±0.1**</td>
</tr>
<tr>
<td>s-Creatinine (45-115 µM)</td>
<td>84±2</td>
<td>90±4</td>
</tr>
<tr>
<td>b-Glucose (3.9–5.8 mM)</td>
<td>5.4±0.2</td>
<td>5.5±0.1</td>
</tr>
<tr>
<td>s-Alk phosphatases (&lt;4.6 μkat/l)</td>
<td>nd</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>Total s-cholesterol (4.0-8.0 mM)</td>
<td>5.8±0.2</td>
<td>5.5±0.2</td>
</tr>
<tr>
<td>s-HDL cholesterol (0.8-1.9 mM)</td>
<td>nd</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>s-LDL cholesterol (3.5-6.1 mM)</td>
<td>nd</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td>Total s-triglycerides (0.4-1.8 mM)</td>
<td>1.5±0.2</td>
<td>1.6±0.1</td>
</tr>
</tbody>
</table>

nd:not done; § n= 17

Naturally it cannot be concluded with certainty whether the investigated patient group can be considered to be representative of contemporary HPT in general. This potential limitation, however, essentially occurs in any primary study of new concepts, in which limited sample size is more or less obligatory. Nevertheless patients had a wide age range and there were cases with both mild degrees of hypercalcemia and modest serum PTH elevations. Despite this variation it is difficult to envision that extrapolation can be made with any greater degree of certainty into the probably rather large group of HPT patients with no, intermittent or very mild degrees of hypercalcemia (Clark et al. 1991; Lundgren et al. 1998a).
It should also be noted that a significant proportion of the examined patients had recognised cardiovascular diseases and appropriate medication for them. In general, this is consistent with notions of the coupling of HPT to both morbidity and mortality in cardiovascular diseases (Lundgren et al. 1998b; Palmér et al. 1987b; Hedbäck & Oden 1998a). Assuming skewed recruitment in this respect, inclusion of all patients into the examination may bias findings towards an over-representation of abnormal cardiovascular functions. However, there was no such trend in study I as inclusion and exclusion of patients with vasoactive medications yielded similar outcomes. On the other hand, exclusion of the cardiovascularly compromised individuals may bias results in the other direction. This may be a limitation particularly in study IV, where abnormalities displayed by the eligible patients consequently could be regarded to represent a ‘minimal’ level.

**Endothelial vasodilatory function in hyperparathyroidism** (study I)

The endothelial function index was lower (p<0.05) in the HPT patients before surgery (1.01± 0.26) in comparison with the controls (Fig. 2). This discrepancy mainly related to an elevated EIDV (p<0.05), while EDV was similar in the cases and controls. The function index normalised at postoperative follow-up, when there were trends to decreased EIDV and increased EDV (p=0.08 and 0.14, respectively). The total serum calcium value was inversely related to the endothelial function index when cases and controls were analysed together (r = -0.30, p<0.05).

The impaired endothelial vasodilatory function is consistent with the over-representation of cardiovascular complications and hypertension in HPT (Lundgren et al. 1998b; Ljunghall et al. 1989; Sancho et al. 1992; Stefenelli et al. 1997). It also substantiates causal coupling with the hyperparathyroid state per se, since the decreased endothelial function index was reversible after parathyroidectomy and not limited to the HPT patients with a history of hypertension. Despite consistency with regard to the occurrence of a vasodilatory disturbance in HPT, our findings differ from previous findings of a decreased EIDV (Neunteufl et al. 1998) and decreased
EDV that was reversed after surgery (Kosch et al. 2000). Both these studies, however, used the change of brachial artery diameter during reactive hyperemia as a model for EDV and sublingual nitroglycerine to estimate EIDV. Moreover the materials differ with respect to the patient’s age and the presence of cardiovascular risk factors. Although the impaired endothelial function index in HPT was of a similar magnitude as in essential hypertension (Panza et al. 1990), it was EIDV rather than EDV that was abnormal and seemed to become reversed after parathyroidectomy. The increased EIDV in HPT may be explained by an up-regulation of the NO-receptor, the soluble guanylate cyclase, and thereby an increased sensitivity to vasodilators like SNP that act on this receptor (Moncada et al. 1991). Martina and colleagues found an increase in platelet iCa together with an increased NO production in HPT that normalised after surgery (Martina et al. 1998). They proposed this as a mechanism to down-regulate the vasoconstriction that may be caused by an elevated iCa level in the smooth muscle cells. Hypothetically this could also relate to the observation that calcium, at higher concentrations, can act as a calcium channel blocker, decrease calcium entry into the vascular smooth muscle cells, and elevate EIDV in a manner similar to what has been found with verapamil (Millgard et al. 1998).

**Endothelial vasodilatory function during induced hypercalcemia** (study II)

This analysis was designed to test the hypothesis that the endothelial vasodilatory disturbance in HPT is a consequence of hypercalcemia rather than a sign of early atherosclerosis. Baseline iCa before calcium infusion was 1.26 ± 0.05 mM. The local iCa concentration in response to the local infusion was 1.50 ± 0.08 mM (p<0.05), while the systemic level remained unchanged. The systemic (venous) calcium infusion raised the systemic iCa to 1.58 ± 0.15 mM (p<0.05) and resulted in a significant drop (p<0.05) of the PTH level (n=6).

The endothelial function index declined after the systemic calcium infusion (1.28±0.40 to 1.00±0.14, p<0.05). This reduction was mainly related to an increased EIDV (15.0±5.2 to 18.8±5.9 ml/min/100ml, p<0.05), while EDV was not
significantly altered (fig.3). A similar reduction, although not significant, in endothelial function index was seen during the local calcium infusion (1.04±0.21, p=0.3). Also in this case, it was mainly EIDV that tended to increase. The endothelial function index after local calcium infusion correlated to the local iCa level (r=-0.58, p<0.05).

**Figure 2.** Forearm blood flow (basal, EDV, EIDV) before and at the end of local (intraarterial) and systemic (venous) calcium infusion. One measurement during systemic calcium was excluded due to technical failure.

The findings substantiate that serum calcium and acute hypercalcemia either directly or indirectly may induce a disturbance in endothelial vasodilatory function that is very similar to that seen in HPT. If this is a secondary phenomenon it surely cannot rely on an increased PTH in HPT, since the systemic calcium infusion elicited the HPT-like endothelial dysfunction despite a dramatic reduction in the serum PTH level.
**Carotid artery intima-media-thickness** (study I)

The intima-media thickness of the carotid arteries did not differ significantly between the HPT patients and the controls and was unchanged after surgery. There was a significant correlation between the mean arterial pressure at 24-hour determination and the thickness of the intima-media complex in the cases and controls \(r=0.43, p<0.05\), but not between this thickness and the indices of endothelial function.

Both an impaired endothelial vasodilatory function and an increased intima-media thickness in the carotid arteries have been suggested to be early signs of atherosclerosis (Panza et al. 1990; Vane et al. 1990; Ghiadoni et al. 1998). However, the carotid intima-media complex was of a similar thickness in the cases and controls, and did not correlate with the endothelial vasodilatory capacity. This corroborates the assumption that the endothelial vasodilatory derangement is an effect of the biochemical alterations rather than a sign of atherosclerosis in HPT. Furthermore the findings strengthen the view that cardiovascular derangements could be an important indication, rather than a mere contraindication, for parathyroidectomy, as the defect vasodilatory capacity is normalised postoperatively - as are the biochemical disturbances.

**Blood pressure** (study I-IV)

The ‘office blood pressure’ and heart rate at rest were similar in patients and controls, but for a significant increase in diastolic pressure in study IV. Moreover blood pressure did not change after parathyroidectomy. This may be taken as an argument against over-representation of hypertension in HPT. However it should be recognised that studies I, III and IV essentially relate to one, rather limited sample of patients with HPT. Moreover, observation numbers became quite small and risks for chance effects rather substantial when auscultatory blood pressures were analysed in the persons without vasoactive medication. Also ABP, however, was similar in cases and controls in study I. Such 24-hour determination should be more reliable than the
‘office pressure’, since it involves repetitive measurement in a non-healthcare environment. However, evaluation of ABP in subjects without vasoactive medication in study IV, where we used a previously validated division of periods of activity and rest (Fagard et al. 1997), showed that HPT can be coupled with higher systolic and diastolic pressures at both day-time and night-time (p<0.05). Also under these circumstances parathyroidectomy had no discernible influence (Fig. 4). Diastolic ABP at daytime and systolic ABP correlated with the PTH level (r=0.47, 0.43; p<0.05). Such correlation was also found between systolic ABP and total serum calcium (r= 0.38; p<0.05).

**Figure 3.** Ambulatory 24-h systolic (SBP) and diastolic (DBP) blood pressure in control subjects (ctrl) and patients with HPT before (HPT) and after surgery (PTX) in subjects without vasoactive medication.
An increase in blood pressure could depend on an increased cardiac output and/or an elevated peripheral resistance. Furthermore, an increased pressure wave reflection could augment blood pressure recordings in the arms. Mechanistically it was interesting to note the influence of acute hypercalcemia (study II). The systemic calcium infusion increased the systolic blood pressure (from 114±13 mm Hg to 121±10 mm Hg, p< 0.05), while no effects were noted on heart rate or diastolic pressure. Such response to the local calcium infusion was not noted. Increased wave reflection is consistent with the augmented central aortic pressure that was recently documented in mild HPT (Smith et al. 2000).

In addition to serum calcium, however, there was a correlation between the systolic and diastolic ABP and the serum PTH level. An action of PTH fits with experimental findings of raised blood pressure in humans exposed to PTH infusion during 2 weeks (Hulter et al. 1986). The intracellular iCa level is probably a major determinant of vascular tone and has been found to be elevated in hypertension (Resnick 1992; Gonzalez et al. 1995). PTH might act as a calcium ionophore increasing the tone of vascular smooth muscle cells through such a mechanism (Barbagallo et al. 1999). A circulating parathyroid hypertensive factor has been found in a proportion of humans with essential hypertension and in spontaneously hypertensive rats (Lewanczuk et al. 1993). Some data also suggest that noradrenaline and the renin-angiotensin-aldosterone systems may be involved in blood pressure dysregulation in HPT (Vlachakis et al. 1982; Jespersen et al. 1994; Gennari et al. 1995; Schiffl et al. 1997).

In accordance with what has been found in most other studies, the blood pressure remained unchanged after parathyroidectomy. This suggests an occurrence of structural changes in consistency with the correlation between carotid artery wall thickness and mean blood pressure (study I). Perhaps such structural changes elicited by the biochemical alterations progress with the duration of HPT and become irreversible at some point. Since even mild HPT is associated with atherosclerotic risk factors amenable to improvement after parathyroidectomy, surgical therapy at early stages of the disease may be advantageous. Not unexpectedly there have been no
controlled studies on the longitudinal development of blood pressure in parathyroidectomised and conservatively followed HPT patients.

**Left ventricular morphology and function** (study III)

Left ventricle hypertrophy was defined as $>120$ g/m$^2$ in women and $>150$ g/m$^2$ in men. It was present in 11 patients (9 women) and 7 controls (all women). There was a trend to a higher mass in the patients, especially the men ($144\pm23$ vs. $114\pm28$ g/m$^2$, p=0.06) that persisted postoperatively. Measurements of left ventricular systolic function, but displacement of the atrioventricular plane, tended to be elevated in the patients (ejection fraction, p=0.07; shortening fraction, p=0.06). They decreased after parathyroidectomy (p=0.04, p=0.07), while the end-systolic diameter of the left ventricle increased (p=0.04). The isovolemic relaxation time was prolonged (p=0.009 vs. controls), and decreased postoperatively. The deceleration time of the early mitral flow tended to be prolonged in the patients (p=0.08) and normalised after surgery (p=0.05). The differences between cases and controls persisted when those on vasoactive medication were excluded from the analysis.

In consistency with previous studies, HPT at rest was coupled with an impaired diastolic function of the left ventricle (Ohara et al. 1995; Dalberg et al. 1996). The initial relaxation phase, the isovolemic relaxation time, was significantly increased. Also the later phases of early diastole, evaluated by the deceleration time, were attenuated in the HPT subjects and normalised postoperatively. Relaxation during diastole is believed to depend on the efficacy of the calcium adenosine triphosphatase that extrudes calcium to extracellular or other compartments of the myocardial cells. However, mild asymptomatic HPT has previously been associated with a shortened, albeit numerically normal isovolemic relaxation time (Barletta et al. 2000). An inverse IVRT and an impaired early transmitral filling of the left ventricle is also usually seen in hypertension. However, in the total sample used in this study no significant difference in blood pressure was seen nor could the use of
antihypertensive medication explain the differences in IVRT and MDT between HPT cases and controls. Furthermore, the difference persisted when cases and control subjects with hypertension were excluded from analysis (fig. 4). Thus, these impairments in left ventricular diastolic function are possibly due to the HPT state as they normalised postoperatively.

Systolic function, as the ejection fraction and fractional shortening, was elevated before and decreased after parathyroidectomy. This performance could be explained by positive inotropic effect of excess calcium and PTH. The systolic variables, however, were only marginally altered and within the normal interval. Sparse data have previously been presented on systolic functions in HPT. Asymptomatic patients with normal heart rate have shown increased cardiac output that decreased after parathyroidectomy (Georgiannos et al. 1996). Such increased workload might be harmful and over time induce cardiac hypertrophy in concert with an increased blood pressure. The persistent elevation of blood pressure might be the cause why no reduction in LVM was seen postoperatively.

**Figure 4.** Isovolemic relaxation time (IVRT) during echocardiography in control subjects (ctrl) and cases with HPT before and after parathyroidectomy (PTX). Subjects without hypertension or antihypertensive treatment (normotensive; 22 controls, 20 cases before and 19 after PTX) are shown separately.
**Exercise test** (study III)

The symptom-limited exercise test involved a similar maximal workload in patients and controls. The patients had a significantly higher systolic blood pressure (p<0.05) at the maximal load than the controls (221±27 vs. 206±31 mmHg, table 4). In addition there was an increased (p<0.05) number of ectopic ventricular complexes that decreased to some extent after parathyroidectomy. At the maximal load there was also a significant ST-segment depression in 9 patients (8 controls), which decreased postoperatively in 3 of them. When the ST-depression was normalised for heart rate, the postoperative improvement was significant (p<0.05). Serum PTH correlated with the maximal systolic blood pressure during exercise (r= 0.32, p<0.05) and negatively with the normalised ST-segment depression (r=-0.32, p<0.05). These correlations were not found for the blood calcium levels.

**Table 4.** Systolic blood pressure (SBP), ventricular extrasystolies and ST-segment depression (mean±SEM) during the symptom-limited exercise test in the controls and the HPT patients before and after parathyroidectomy; *p<0.05 in patients before surgery vs controls (Mann-Whitney) and **p<0.05 in patients before vs. after surgery (Wilcoxon).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=28)</th>
<th>Cases (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP at max load (mm Hg)</td>
<td>206±6</td>
<td>221±5*</td>
</tr>
<tr>
<td>Ventricular extrasystolic beats (n)</td>
<td>0.9±0.5</td>
<td>17±12*</td>
</tr>
<tr>
<td>ST-segment depression at max load (mm)</td>
<td>-0.76±0.15</td>
<td>-0.90±0.19</td>
</tr>
<tr>
<td>ΔST/Δ Heart rate (mm per beats/min)</td>
<td>-0.93±0.5</td>
<td>-1.65±0.4</td>
</tr>
</tbody>
</table>

Examination of cardiovascular functions during exercise has, to my knowledge, not been presented in HPT previously. In principle there were three key findings in study III. The first of these is the abnormal increase in systolic blood pressure during the maximal work load. Such elevation has been shown to be a more accurate predictor
of cardiovascular disorders than blood pressure at rest (Kjeldsen 1999). It could represent increased cardiac output or increased peripheral resistance due to arterial stiffness. Since the exercise-related rise in systolic blood pressure failed to decline after parathyroidectomy, dependence on structural alterations seems most probable. The second key finding was the increased number of ventricular extrasystolies, which could contribute to the observed cardiovascular morbidity and mortality in HPT (Hedbäck & Oden 1998b; Palmér et al. 1987b; Rastad et al. 1995).

The third key finding was the coupling of HPT with enhanced ST-segment depression during the maximal workload. ST-segment depression exceeding one millimetre in amplitude is generally regarded as a sign of myocardial ischemia (Mc Henry PL et al 1984). Standardisation of the depression for the heart rate improves diagnostic accuracy of the test regarding coronary artery disease (Suurküla M 1999). This ratio was numerically higher in the HPT patients before surgery. However, the difference to controls did not reach statistical significance, possibly due to the limited sample size. Nevertheless the ST/HR-ratio improved significantly after parathyroidectomy, when intraindividual analysis of the data could be performed. Also the correlation of serum PTH with the standardised ST-depression suggests causal coupling with the parathyroid disease. Since chest pain was equally rare in the patients and controls during the exercise, other causes of the ST-segment depression could characterise HPT, as the disease in itself reduces the ST-segment period (Lind et al. 1995). In order to ascertain the benefit from parathyroidectomy in this respect, more sensitive tests of myocardial ischemia have to be performed, such as scintigraphy or Dobutamine-stress echocardiography or magnetic resonance imaging (MRI).

**Heart rate variability** (study IV)

Patients tended to have a lower maximal heart rate on ambulatory HRV analysis. Time domain variables obtained by analysis of the RR-intervals did not differ between patients and controls. Neither did the common frequency domain variables HF, LF or VLF. There was a tendency (p=0.06) to a lower LF/HF-ratio in the patients
(2.6±1.6 vs. 3.3±1.5) that normalised postoperatively (p=0.04). The ratio correlated inversely with the PTH (r=-0.46, p=0.004) and total calcium level (r=-0.32; p=0.05).

Further analysis of the circadian rhythm was done with daytime defined as 10.00 to 20.00 and night-time from midnight to 06.00 in an attempt to strictly separate periods of activity and rest. A blunted nocturnal increase was found in HPT patients for LF (103±128 ms² vs. 430±171 ms² in controls, p=0.03) and a corresponding trend for VLF (212±165 ms² vs. 675±262 ms², p=0.10). VLF at night-time correlated inversely with the PTH level (r=-0.33; p <0.05) and increased postoperatively (p<0.05).

Multiple biologic functions such as blood pressure and vascular tone show diurnal variation and blunting appears to contribute to adverse cardiac events (Giles 2000). Spectral analysis of 24-hour recordings has shown that HRV normally has a circadian rhythm (Malliani et al. 1991). This variation was blunted in HPT and normalised after surgery. We also found a tendency to lower LF/HF ratio that increased significantly after parathyroidectomy. Modulation of circadian HRV reflects an imbalance in the autonomic regulation. The findings in HPT may indicate modification of the autonomic input to the heart or diminished responsiveness to this input by reduced nocturnal vagal activity or increase in sympathetic activity (Furlan et al. 1990; Lombardi et al. 1992). The tendency of a lower maximal heart rate in HPT could be an indication of less physical activity. Analyses of the variability during night-time could perhaps diminish any effect on HRV of this possible confounder. The significant postoperative increase in the power of the VLF band at night-time should be clinically important, since this variable has shown more powerful association with mortality after myocardial infarction than the HF and LF bands. Only VLF of these variables is also more strongly associated with arrhythmic death than with non-arrhythmic death of cardiac or any cause (Bigger et al. 1992). Power of the VLF band correlated inversely with the PTH level.
Figure 5. Heart rate variability, spectral components in control subjects (ctrl) and cases (HPT) before and after parathyroidectomy (PTX).

VLF (ms²)

LF (ms²)

HF (ms²)

[Graphs showing heart rate variability spectral components for control subjects (ctrl) and cases (HPT) before and after parathyroidectomy (PTX)]
Interestingly also PTH has been found to have a bimodal diurnal rhythm with probable blunting in HPT (Lobaugh et al. 1989). Because VLF has been considered to reflect rhythmic neuroendocrine alterations, it is tempting to speculate that the impairment in PTH secretion could be involved in the alterations in circadian HRV in HPT.

Although the blood pressure was increased, the nocturnal decline was intact in HPT. Thus, it is likely that the cardiac autonomic imbalance demonstrated by the HRV is not responsible for the elevated blood pressure.

**Potential impact on treatment options**

Hitherto, parathyroidectomy is the only treatment with demonstrated effects on the signs, risks and adverse outcome of cardiovascular derangements in HPT. There is a rather contradictory interest for a surgeon in these matters, since cardiovascular complications – disregarding age – probably are the most common contraindications to parathyroidectomy today. It is also fair to assume that the virtually negligible perioperative mortality for parathyroidectomy during at least the most recent decade should involve a consensus, albeit not public, on how to define cardiopulmonary contraindications to parathyroidectomy under general anaesthesia. Naturally this probable consensus should not be altered by the result of the present and other studies on the cardiovascular derangements of HPT. Instead I strongly favour that the outcome of such studies should be utilised to consider whether re-evaluation of the indication for active treatment is pertinent, and if any such re-evaluation should consider including cardiovascular risk to the complications of untreated HPT. Today one would be inclined to limit such risks essentially to nephrolithiasis (in the younger patient group) and renal damage, fragility fracture and arthralgia (in preferentially the older patient group), progression to crisis (truly rare in the Western hemisphere) and perhaps also neuromuscular complications.
It is also important to emphasise that controlled studies of larger materials will have to be performed to substantiate the cardiovascular derangements of HPT and the prospects for normalisation after not only parathyroidectomy but also other types of treatment. The really critical issue then boils down to whether or not the results available today should bear any practical clinical consequences before conclusive studies have been evaluated. For obvious reason surgeons may be more inclined to choose active surgical treatment. On the other hand, it is not unfair to argue that proponents for other treatment strategies, including surveillance, should feel a similar motivation for critical appraisal. Today surveillance requires a committed patient and the recommendations by the NIH Consensus Conference are expensive and time-consuming.
GENERAL SUMMARY

The findings of study I, III and IV are summarised in table 5.

- Primary hyperparathyroidism is associated with a vasodilatory dysfunction, and the diminished endothelial contribution to the vasodilatory process is normalised after parathyroidectomy. Thickness of the intima-media complex of the carotid arteries is similar to that of controls suggesting that the vasodilatory disturbance is explained by biochemical rather than structural changes.

- Acute hypercalcemia in healthy subjects induced a vasodilatory dysfunction, which substantiates that serum calcium and acute hypercalcemia may be involved, either directly or indirectly in the endothelial vasodilatory disturbance of HPT.

- The systolic blood pressure of healthy subjects increased during systemic calcium infusion, while no effects were noted on heart rate or diastolic pressure.

- Echocardiography demonstrated left ventricular diastolic dysfunction and supernormal systolic function in HPT with partial alleviation after parathyroidectomy. The supersystolic performance could increase the workload and promote cardiac hypertrophy.

- Primary hyperparathyroidism was associated with exercise-related increase of systolic blood pressure, number of ventricular extrasystolic beats and depression of the ST- segment standardised for heart rate. This suggests susceptibility to cardiac ischemia and arrhythmia that could contribute to premature death in HPT.

- Ambulatory blood pressure with strict definitions of periods of activity and rest demonstrated increased blood pressures during daytime and night-time that persisted after parathyroidectomy in HPT subjects without vasoactive medication.
Modulation of the circadian heart rate variability was found in HPT. This possibly reflects autonomic imbalance with prospects of normalisation after surgery.

Since parathyroid surgery can be performed with high success and a low risk of complications, the findings argue for operative intervention in all cases with a reasonable surgical risk and life expectancy, in order to improve a number of aspects of cardiovascular performance in HPT patients.

**Table 5. Schematic summary of cardiovascular alterations in HPT and changes noted after parathyroidectomy.** ↓↑ represent significant decrease and increase respectively with trends (p = 0.06-0.08) in parenthesis; n.s: not significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HPT vs. controls</th>
<th>Effects of parathyroidectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial function index</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Intima media thickness of the carotides</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ambulatory blood pressure</td>
<td>↑</td>
<td>n.s.</td>
</tr>
<tr>
<td>Left ventricular mass</td>
<td>(↑)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>(↑)</td>
<td>↓</td>
</tr>
<tr>
<td>Fractional shortening</td>
<td>(↑)</td>
<td>(↓)</td>
</tr>
<tr>
<td>Isovolemic relaxation time</td>
<td>↑</td>
<td>n.s.</td>
</tr>
<tr>
<td>Mitral deceleration time</td>
<td>(↑)</td>
<td>↓</td>
</tr>
<tr>
<td>E-wave/A-wave</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ventricular extrasystolic beats</td>
<td>↑</td>
<td>n.s.</td>
</tr>
<tr>
<td>ST-depression related to heart rate</td>
<td>n.s.</td>
<td>↓</td>
</tr>
<tr>
<td>Workload</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Maximal systolic blood pressure during exercise</td>
<td>↑</td>
<td>n.s.</td>
</tr>
<tr>
<td>Standard deviation of all RR-intervals (SDNN)</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Low to high frequency power</td>
<td>(↓)</td>
<td>↑</td>
</tr>
<tr>
<td>Night-time low frequency power</td>
<td>n.s.</td>
<td>(↑)</td>
</tr>
<tr>
<td>Night-time very low frequency power</td>
<td>n.s.</td>
<td>↑</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

This study was performed as a collaboration between the Departments of Surgical Sciences and Medical Sciences at Uppsala University and the Departments of Surgery and Clinical Physiology at the Sundsvall County Hospital.

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ERRATA

Page 7
Insert $r$ equals correlation coefficient.

Page 19, § 3, line 4
The reference Clark & Duhbb should be Clark & Duh.

Page 24
Table 1: angiotensin enzyme inhibitors were used by 2 controls. Table 2 includes the studies I, III and IV.

Page 40
Table 4: the unit for $\Delta ST/\Delta HR$ should be $\mu V/\text{beats/min}$