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Cardiovascular Consequences of Parathyroid Dysfunction: Implications for Management of PTH Excess, PTH Insufficiency, and PTH Resistance

Dwight A. Towler MD, PhD
J.D. and Maggie E. Wilson Distinguished Chair in
Biomedical Research
Department of Internal Medicine
Division of Endocrinology

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Name: Dwight A Towler MD, PhD
Rank: Professor
Division: Endocrinology
Research / academic interests profile: Tremendous unmet needs exist in musculoskeletal medicine. Osteoporosis and osteoarthritis are recognized as common and clinically important, but other serious skeletal disorders also afflict our society. In the setting of type 2 diabetes mellitus (T2DM), lower-extremity musculoskeletal disease is prevalent, costly, and exceedingly difficult to manage, with fracture, arthropathy, ischemia, ulcer, infection, and amputation commonly confronting patients and clinicians. Aortofemoral medial artery calcification is a strong predictor of risk for lower extremity amputation in patients with T2DM. While not occluding the lumen, mural elastinolysis and medial calcification compromise arterial elasticity -- a material property necessary for Windkessel physiology that ensures normal tissue perfusion throughout the cardiac cycle. During aortic calcification, the Msx2-Wnt signaling cascade that controls orthotopic craniofacial bone formation is activated ectopically in the aortic valve and vessel wall. Diabetes and dyslipidemia induce expression of Msx2 in arterial myofibroblasts, upregulate aortic Wnt3a and Wnt7a gene expression, and activate pro-calcific canonical Wnt signaling in the valve and tunica media. By studying Msx2 actions, we have identified that paracrine Wnt/Dkk signals control arterial calcification and fibrosis in T2DM by regulating osteogenic lineage allocation of vascular mesenchymal progenitors. Prosclerotic inflammatory Wnt signals initiated by TNF-alpha and osteopontin -- but inhibited by vascular LRP6 and PTH1R -- modulate the sustained activation of this arterial injury response. Intracellular protein arginine methylation / demethylation has recently emerged as a novel feature of the Wnt/LRP6 regulatory relay. We now study how strategies that differentially modulate skeletal vs. arterial Wnt signaling can preserve bone homeostasis and cardiovascular health in diabetes and uremia.

Purpose and Overview: The purpose of the presentation is to highlight known and novel interactions between the prototypic osteotropic hormone, parathyroid hormone, and cardiovascular disease. Preclinical genetic models, patient-oriented investigation, and epidemiology converge to indicate that there is a normal “set point” for parathyroid hormone receptor signaling with respect to cardiovascular physiology -- and that PTH excess, insufficiency, or acquired resistance arising in the setting of metabolic disorders negatively impacts cardiovascular health. While some of the negative consequences can be attributed to globally altered calcium and phosphate homeostasis, powerful data emerging from preclinical models demonstrate that the cardiovascular system is a direct target for regulation by PTH and the PTH-related polypeptide, PTHrP.

Educational Objectives: At the conclusion of this lecture, the listener should be able to:

1. Describe the actions of parathyroid hormone (PTH) on cardiovascular physiology as directly conveyed by vascular signaling vs. indirect actions via the global regulation of calcium and phosphate homeostasis.

2 Explain how chronic elevations in endocrine PTH production perturb paracrine signals dependent upon PTH related polypeptide (PTHrP) - regulation of their shared PTH/PTHrP receptor.

3. Anticipate the untoward cardiovascular consequences of excessive or insufficient PTH levels in patients as influenced by the presence or absence of intact renal function.

1. Introduction

All osteotropic hormones have vasculotropic actions(1). This pithy statement of fact is most certainly true for PTH and PTHrP. The cardiovascular actions of PTH have been known for 9 decades, first identified when James Collip injected anesthetized Dog 164 with intravenous parathyroid extract and documented hypotensive actions(2); PTH might have very well been named a "hypotensin" due to this vascular response (**Figure 1**). Moreover, PTH tone permits the co-registration of capillary blood flow and nutrient supply with the demands of skeletogenesis as an manifestation of bone-vascular interactions. PTH1R signaling also controls arteriosclerotic calcification as a perfect example of the broader osteotropic-vasculotropic relationship and its emerging relevance to cardiovascular health(3). Discovery of the critical roles played by the **PTH/PTHrP receptor (PTH1R)** in vertebrate heart and valve development and disease have begun to engage the cardiovascular research community(4). Thus, a fundamental understanding of PTH/PTHrP biology must encompass a better understanding of actions within and upon the cardiovascular system.

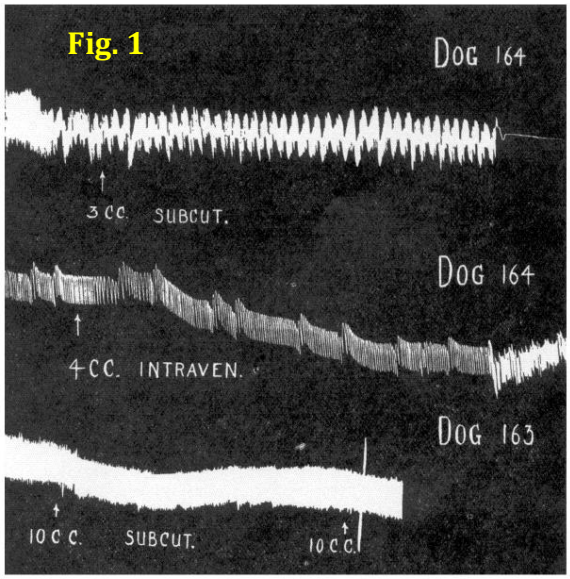
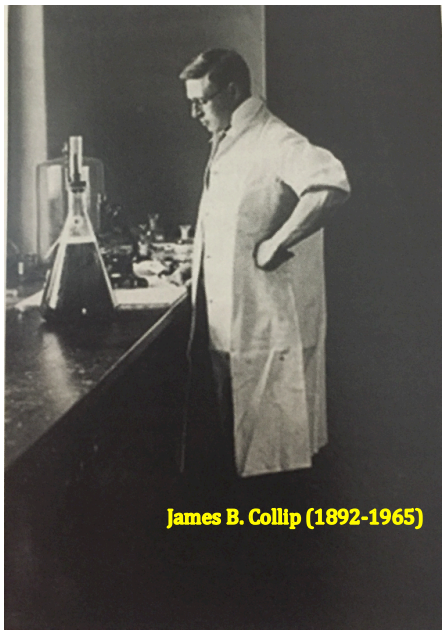


CHART 19. Showing effect of injection of extract upon blood pressure in anesthetized animals. Middle tracing fast drum.

In this presentation, I give a very brief overview of PTH/PTHrP biology and vasculature, emphasizing relationships with respect to cardiovascular biology and pathophysiology. I point to how these important relationships become clearly manifest in chronic kidney disease – mineral and bone disorder (CKD-MBD) -- a perfect storm of cardiometabolic risk arising in significant part from perturbed PTH1R signaling – and point to the need to develop new strategies that define and refine an integrated "set point" for PTH1R signaling tone in an emerging bone-vascular endocrine axis (3).



2. PTH / PTHrP biology in cardiovascular development

Some of the first evidence that PTH/PTHrP signaling might contribute to cardiovascular development arose from studies of Strewler et al., wherein they identified the expression of PTHrP in the chicken embryonic heart as well as in other mesodermally derived tissues. Additional studies in the rat identified that mesenchymal PTH1R expression lay adjacent to epithelial or endothelial sources of PTHrP. Inductive (i.e. local paracrine) epithelial-mesenchymal signals were inferred to be key in the spatial relationships revealed from these in situ hybridization studies. In dogs, PTH1R was also identified early on as being expressed in heart and aorta albeit at levels much lower than those observed in kidney and bone. However, the important role of PTH/PTHrP in cardiovascular development and disease was not truly appreciated until Kronenberg, Clemens and colleagues began their systematic analysis of why PTH1R knockout mice exhibit prenatal lethality(5). They confirmed that PTH1R message was abundantly expressed in developing mouse cardiomyocytes - and then went on to show that mice lacking PTH1R abruptly die between

mouse embryonic days E11.5 and E12.5 due to massive cardiomyocyte apoptosis(5). Primary abnormalities in cardiomyocyte mitochondrial morphology and pump function arising with myocardial PTH1R deficiency were followed by secondary hepatic injury and tissue necrosis. The mechanisms whereby PTH1R signaling regulates mitochondrial metabolism to prevent apoptotic cell death in the cardiomyocyte have yet to be fully explored. Interestingly, however, classical PTH1R activation has been demonstrated to inhibit apoptosis in other settings, including in osteoblasts challenged with glucocorticoids and in TNF–treated chondrocytes. Thus, PTH1R expression and signaling represents a fundamental component of cardiomyocyte cell physiology necessary for cardiovascular morphogenesis. A better understanding of the pro-survival mechanisms afforded by PTH1R signaling in the heart may prove useful in therapeutic approaches to ischemic heart disease (vide infra).

Recent studies using other vertebrate models have robustly confirmed the important role for PTH/PTHrP signaling in cardiovascular development. In zebrafish, 3 PTH receptors (*pth1r, pth2r, pth3r*) are expressed, with all 3 responsive to PTH. Knockdown of *pth1r* or the *pth1r / pth2r* ligand *pthrp* results in zebrafish morphants (developmental mutants) with aortic coarctation defects. Because of the important role for *notch* gene integrity in mammalian aortic valve development(6), Chico and colleagues examined whether *notch* signaling might be perturbed – and indeed demonstrated that restoration of *notch* signaling in *pth1r* targeted morphants prevented the aortic developmental defect. It is tempting to speculate that variable penetrance for preductal aortic coarctation in the lethal Blomstrand chondrodysplasia – a rare human disorder due to loss of function mutations in the human – may also relate to partially compensatory changes in notch co-regulatory pathways.

3. PTHR signaling in arterial biology: Vascular smooth muscle cell and endothelial responses to PTH and PTHrP.

The acute vasodilatory actions of PTH administration were recognized in some of the very earliest studies of the hormone's physiological response (2). Following Collip and Clark's lead, Pang et al confirmed that bovine PTH(1-34) reduced blood pressure in anesthetized dogs and rats. Imai demonstrated coronary vasodilation in response to PTH that same year(7). Ex vivo, human and bovine middle cerebral arteries were also shown to undergo vasodilatation as well, followed by a rapid progression of studies demonstrating similar responses in multiple vertebrate tissue beds, but with varying potency and efficacy. In 1986, using a preclinical model of myocardial ischemia, the salutary actions of PTH was reported; enhanced collateral blood flow was thought to represent the primary mechanism of benefit (see below). However, as noted above, PTH1R signaling exerts direct anti-apoptotic actions within cardiomyocytes during fetal development – and this may also contribute to post-natal PTH1R actions in the setting of ischemia.

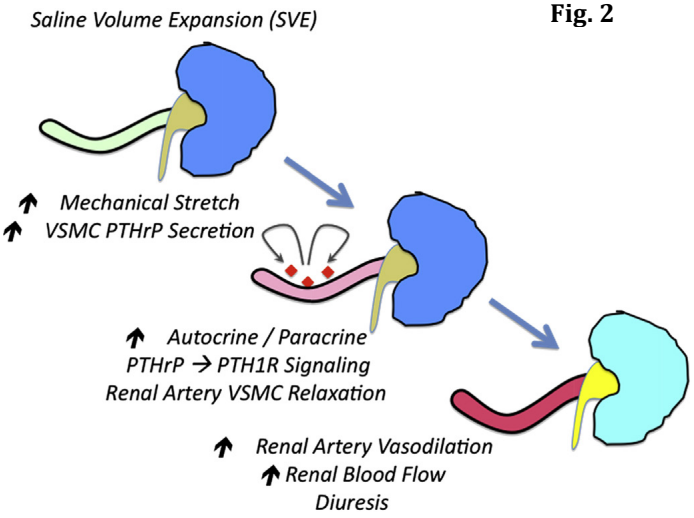
Nickols, Barthelmebs, Helwig and colleagues went on to confirm widespread vasodilatory responses to PTHrP. While endothelial nitric oxide production certainly contributes to PTH/PTHrP actions, direct PTH1R signaling in VSMCs appears sufficient to mediate many salutary actions. However, this again may vary with vascular bed; Prisby et al very recently concluded that the endothelium was vital to PTH actions enhancing blood flow to the skeleton in studies of the femoral principal nutrient artery. Whether age-dependent increases in vascular sensitivity to pressors combines with altered nutrient artery endothelial PTH1R signaling to compromise bone health remains to be fully explored.

Bilezikian et al first established that PTH and PTHrP signals exert acute inotropic actions in the isolated perfused heart that are also largely dependent upon coronary vasodilatation and independent of direct chronotropy. In the heart, the endothelium itself may function as an important source of paracrine PTHrP tone that maintains myocardial functions. Schlüter and colleague identified that coronary endothelial produce PTHrP under ischemic conditions and enhance myocardial inotropy (contraction velocity) and lusitropy (relaxation velocity). Others proposed a role for PTHrP as a stretch-inducible paracrine vasodilator -- important for homeostatic roles of PTH1R signaling that control tissue perfusion via vascular

tone. Clemens et al demonstrated that augmenting local PTHrP/PTH1R signaling could impact blood pressure and vascular contractility; implementing novel vascular smooth muscle cell – specific transgenic mice expressing either PTHrP or the PTH1R, these investigators elicited sustained reductions in blood pressure and increases in volume-dependent renal tissue perfusion. Moreover, pressor responses to angiotensin II were significantly reduced by VSMC PTH1R activation. Given that wild-type PTH1R activation is ligand-dependent, these data strongly suggest that endogenous paracrine PTHrP production -- modulated by mechanical, inflammatory and endocrine cues -- helps to locally regulate vasodilatation and sustain tissue perfusion.

In 2013, this working model of paracrine PTHrP signaling was confirmed in studies of renovascular perfusion (8). Using mice possessing floxed PTHrP alleles and a smooth muscle cell transgene driving tamoxifen-inducible Cre recombinase, conditional deletion of VSMC PTHrP was achieved that permitted assessment of effects on renal blood flow. As compared to control mice (untreated ERT2-Cre; PTHrP(flox/flox), Cre-negative PTHrP(flox/flox), VSMC PTHrP knockout mice (smooth muscle ERT2-Cre;PTHrP(flox/flox) treated with tamoxifen) exhibited increased renal vascular resistance with concomitant reductions in perfusion and glomerular filtration (8). Moreover, renovascular perfusion in response to mechanical stimulation with **saline volume expansion (SVE)** was also impaired. While normal mice increase renal plasma flow and diuresis – an index of vasodilation – in response to SVE as depicted in **Figure 2**, mice lacking VSMC PTHrP were unable to do so. Interestingly, no change in systemic blood pressure was noted – even though pharmacological dosing with PTHrP simultaneously reduces blood pressure while increasing renal plasma flow(8). Thus, endogenous VSMC PTHrP regulates renal perfusion in response to mechanical challenge.

Fig. 2



The physiological responses described above seem somewhat paradoxical against the backdrop of the vascular disease burden observed in settings of primary **hyperparathyroidism (HPT)** or secondary HPT with chronic kidney disease (CKD) – viz., hypertension, arteriosclerosis, valve and vascular calcification, increased cardiovascular mortality. However, the vasculature rapidly becomes refractory to PTH1R signaling in response to sustained PTH or PTHrP exposure(9). By studying vital rat femoral artery segments contracted with norepinephrine (NE) ex vivo, Brickman and colleagues demonstrated that vasodilatory responses to PTHrP or PTH were markedly diminished (by > 50%) following 40 minutes of prior exposure to either of these two PTH1R ligands (**Figure 3**). Similarly, isolated rat VSMCs were shown to exhibit similar

Fig. 3

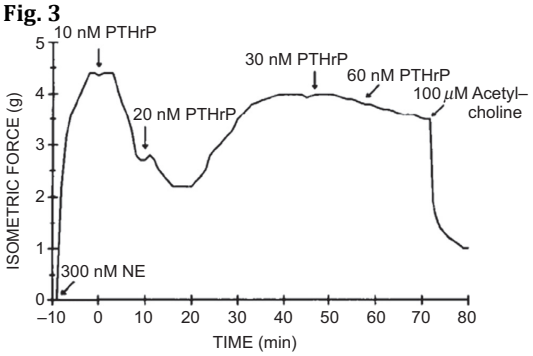
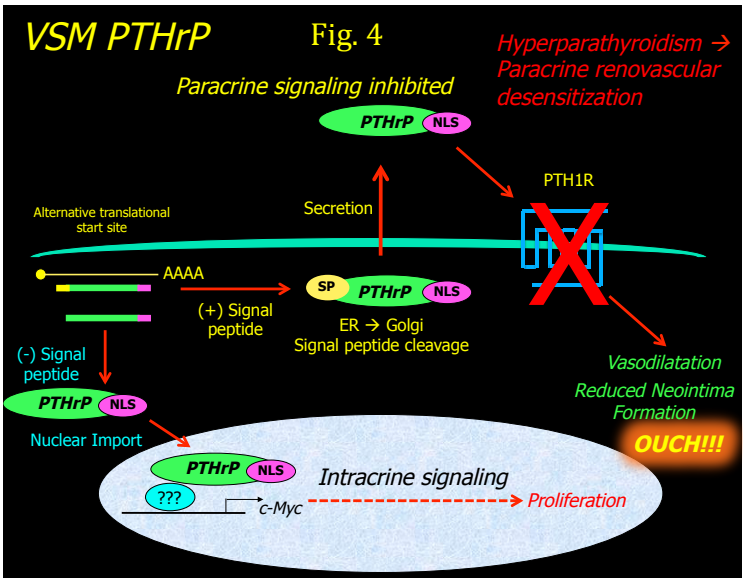


FIGURE 12.2 **Desensitization to the vasodilatory actions of PTHrP in isolated rat femoral artery segments.** Brickman and colleagues established that following maximal contraction with the pressor norepinephrine (NE), 10 nM PTHrP(1–34) treatment elicited vasorelaxation that became rapidly unresponsive to subsequent PTHrP challenges. Response to other vasodilators remained intact, as exhibited by preserved vasorelaxation to acetylcholine. Similar responses occur with PTH(1–34). *Reprinted with permission from The Endocrine Society.*[48]

tachyphylaxis within 30 minutes of exposure by using cAMP production as an assay of PTH1R signaling(9). Of note, renovascular tachyphylaxis to PTH has been independently observed as well in the isolated perfused rabbit kidney model. Thus, the vasculopathy of primary and secondary HPT may relate in part to arterial de-sensitization to the important paracrine, PTHrP-dependent regulation of vascular tone and cell function(9)(**Figure 4**). In this view, sustained exposure to PTH and antagonistic PTH degradation fragments can down-regulate vascular PTH1R signals that serve a protective role in vascular health.



In 2010, our group began to explore the potential benefits of PTH1R activation in diabetic arteriosclerosis; we did this by generating a transgenic mouse expressing a constitutively active caPTH1R transgene, PTH1R(H223R), in vascular smooth muscle of LDLR-/- mice using the SM22 promoter as a delivery module (10). The PTH1R(H223R) Jansen metaphyseal chondrodysplasia variant is ligand independent and thus does not undergo homologous desensitization. In the LDLR-deficient mouse model of diet-induced diabetic arteriosclerosis, we demonstrated that the VSMC PTH1R(H223R) transgene significantly reduced aortic calcification and fibrosis while maintaining arterial compliance (10). This occurred in part via PTH1R-dependent **inhibition of arterial pro-sclerotic Wnt signaling** and arterial oxidative stress in VSMCs (**Figure 5**). Additionally, intermittent dosing with PTH(1-34) was also shown to reduce vascular osteogenic programs, mineralization, and oxidative stress(10; 11). In a related line of study, Friedman subsequently showed that intermittent PTH(1-34) administration reduces vascular calcification in uremic rats. Of note, similar effects were reported by Morii et al using cultured VSMC treated with PTHrP.

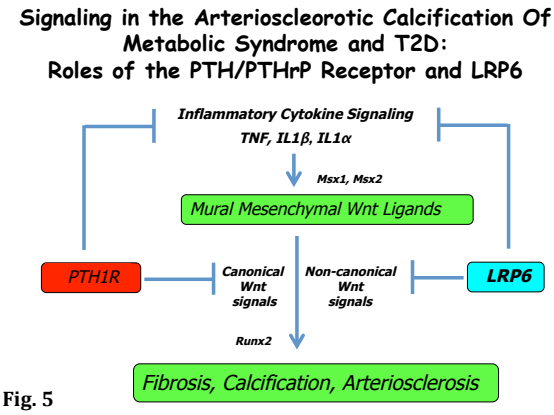


Fig. 5

Thus, strategies that selectively preserve normal paracrine PTHrP/PTH1R pathway are predicted to exert cardiovascular benefits with respect to enhanced tissue perfusion*, reduced arteriosclerotic calcification and vascular stiffness, and restricted neointimal proliferation**. This, in sum, preserves arterial structure, cardiac pump functions, and tissue perfusion. Therefore, in addition to alterations in circulating calcium phosphate levels, the physiology of direct cardiovascular PTH1R signaling and the associated "pharmacokinetic – pharmacodynamic" (PK-PD) relationships deserve full consideration. This concept is very important to embrace as we seek to better define medical and surgical strategies to combat cardiovascular disease in the settings of primary HPT and CKD(12). ***Unfortunately, no facile or clinically validated method has been identified as a metric for establishing or monitoring the healthy cardiovascular PTH1R signaling "set point." This is all the more difficult since beneficial actions of paracrine PTHrP signaling will be influenced

by endocrine PTH tone and circulating inhibitory fragments that accumulate with declining renal function(13). Moreover, because variable post-translational oxidation occurs at residues Met-8 and Met-18 of intact PTH that alter its bioactivity, establishing these relationships in certain disease states (CKD, diabetes) may require implementation of a newer generation PTH assay.

4. Impact of hyperparathyroidism on cardiovascular mortality, coronary flow reserve, and vascular stiffness: An emerging concern in cardiovascular endocrinology.

PEARS, the Parathyroid Epidemiology and Audits Research Study, was retrospective, population based outcomes study that investigated patients diagnosed with mild primary hyperparathyroidism (**HPT**) in Tayside, Scotland, between 1997 and 2006(14). Many other observational studies have supported the relationship between primary HPT and cardiovascular disease(15); however, this large study of 2.99 million person years of followup – 5735 in patients with mild primary HPT – presents a clinically compelling epidemiological data set for this pathophysiological relationship. These HPT patients were included in the study after a positive diagnosis only if (a) the first two calcium concentrations above the upper limits of normal (2.6 mmol/L or 10.4 mg/dL) never exceeded 3 mmol/L (12 mg/dL); (b) they had never been surgically treated for hyperparathyroidism; and (c) had no known renal complications. These mild primary HPT patients were compared to the whole Tayside population, adjusted for age group, sex and calendar year. In the 1683 subjects with mild primary HPT identified (2/3rd female), age- and gender- adjusted **cardiovascular mortality was increased 2.7-fold** (14). After adjusting for pre-existing conditions, cardiovascular morbidity was still increased 2.5-fold, and renal failure increased over 10-fold (**Figure 6**). The HEALTH ABC cohort, focused upon individuals in Memphis and Pittsburgh, confirmed increases in cardiovascular mortality with elevated PTH; elevation conveyed a 1.8-fold increased risk, independent of serum calcium and 25-hydroxyvitamin D levels(16). Importantly, recent follow up analyses revealed that baseline PTH levels, NOT calcium levels, best predicts long-term outcomes in this untreated mild pHPT cohort (**Figure 7**)(17). Thus, even though hypercalcemia itself – signaling through the calcium sensing receptor – can engender hypertension as well, preclinical, clinical, and epidemiological data point to the importance of PTH "tone" independent of prevailing calcium levels as but one index of PTH actions.

Fig. 6 **Increased mortality and morbidity in mild primary hyperparathyroid patients**
The Parathyroid Epidemiology and Audit Research Study (PEARS)

Table 6. Standardised incidence ratios (SIRs) showing the observed and expected number of events adjusted for pre-existing conditions*

Observed morbidity endpoints	Obs	Exp	SIR	95% CI
Cardiovascular disease	218	86·1	2·53	2·21–2·89
Cerebrovascular disease	121	39·1	3·10	2·57–3·70
Renal failure	272	19·2	14·14	12·51–15·92
Renal stones	20	4·1	4·85	2·96–7·49
Psychiatric disease	31	5·6	5·56	3·78–7·89
Hypertension	159	42·1	3·77	3·21–4·41
All fractures	101	51·5	1·96	1·60–2·38

Clinical Endocrinology Vol73:30, 2010

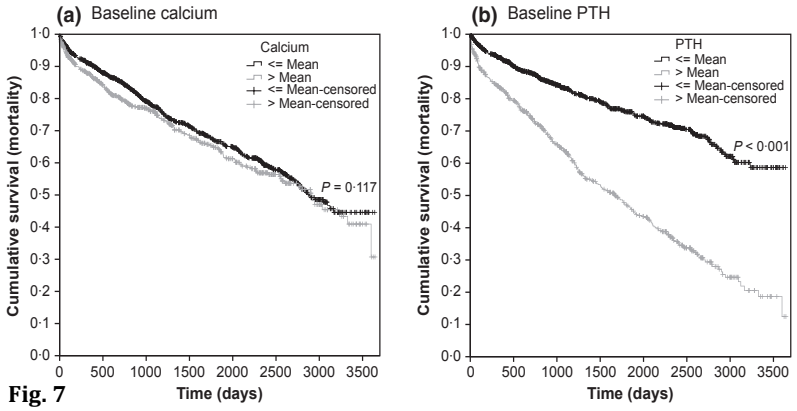


Fig. 7

As presented above, preclinical models evaluating PTH/PTHrP actions have long indicated the role of the cardiovascular system as a physiologically relevant target;

mechanisms relevant to disease biology are rapidly emerging from genetically manipulated animals(8; 10). However, in the past several years, it is the physiological studies of human subjects that have confirmed and extended these observations in important ways that are likely to alter our recommendations for treatment of HPT in otherwise “asymptomatic” patients(18). Macrovascular compliance and conduit functions, known as Windkessel physiology, are altered by HPT (19-21). With every heartbeat, part of the kinetic energy of each systolic pulse is stored as potential energy within the rubbery elasticity of conduit vessels. During diastole, this energy is released to provide smooth perfusion of the coronary arteries, myocardium, and distal tissues(1). In addition, with arterial stiffening, reflected retrograde pulse waves generated at vessel branchpoints travel faster, and begin to sum with the latter p art of orthograde pulse waves in late systole to increase systolic blood pressure. Thus, with arterial stiffening, perfusion during diastole not only becomes more erratic but the workload placed upon the heart increases due to systolic hypertension (**Figure 8**). Silverberg, Bilezikian and colleagues established that arterial stiffening is in fact increased in patients with mild HPT. Using the augmentation index (AIx), a measure of the retrograde wave reflection that characterizes vascular stiffening and increases systolic pressure, they uncovered a positive linear relationship between PTH and AIx after adjusting for heart rate, height, gender, blood pressure, age, diabetes mellitus, smoking, and hyperlipidemia(19). Importantly, Smith and colleagues reported similar responses in their smaller patient cohort. Importantly, in patients with pre-existing cardiovascular abnormalities, parathyroidectomy improves indices of cardiovascular stiffness(22).

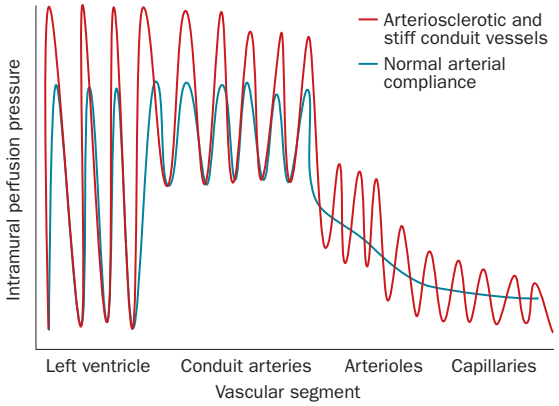


Fig. 8 | Consequences of arterial stiffening and impaired Windkessel physiology. During systole, some kinetic energy is stored as potential energy in the elastic conduit arteries. This stored energy permits not only coronary perfusion but also smooth distal capillary perfusion during diastole (blue tracing). With arteriosclerotic stiffening (red tracing), less potential energy is stored during systole, giving rise to impaired, pulsatile and erratic flow during diastole (two-thirds of the cardiac cycle).[210] Systolic blood pressure is also increased. The topic has been reviewed elsewhere.[81]

Problems also arise with the regulation of myocardial blood flow in response to metabolic demand in the setting of hyperparathyroidism. Coronary flow reserve (CFR) is a physiological index of epicardial conduit function; CFR assesses both the significance of any coronary stenosis present and downstream microvascular dysfunction. When changes in myocardial oxygen demand downstream of a conduit artery segment cannot be met by vasodilation, CFR is said to be compromised. Importantly, reduced CFR portends adverse cardiovascular outcomes and health in men and women. CFR was initially evaluated at the time of angiography following intracoronary administration of vasodilators with Doppler imaging; however, transthoracic Doppler echocardiography has now been implemented with peripheral adenosine administration as a mechanism for non-invasively assessing CFR without angiography. **In 2012, Osto and colleagues performed a highly important, longitudinal analysis of CFR in 100 primary HPT patients with solitary adenomas before and after adenoma resection, comparing responses elicited in 50 gender- and age-matched controls**(23). In this landmark study, they established that PTH, age, and heart rate were the only major variables altering CFR -- and that CFR independent of serum calcium. Moreover, in the 27 primary HPT patients with clearly abnormal CFR (<= 2.5 following adenosine infusion), all of these individuals exhibited normal CFR (> 2.5) following adenoma resection (**Figure 9**)(23). Using a completely independent method of assessment, nuclear perfusion imaging, Marini and colleagues also demonstrated improvement of CFR in primary HPT undergoing surgical treatment(24). In a clinically important way, these two studies help to confirm and

extend the previous work of Kosch et al. published a decade prior. These investigators identified that brachial artery flow-mediated vasodilation, an index of endothelial function and NOS activation, is impaired with primary HPT but reversed following surgical treatment(25). Thus, primary HPT is consistently associated with reversible alterations in coronary and peripheral artery endothelial function that improve following surgical intervention. The improvements in CFR following surgery for HPT has significant implications as the risks vs. benefits of parathyroidectomy in otherwise asymptomatic hyperparathyroidism are considered.

Fig. 9

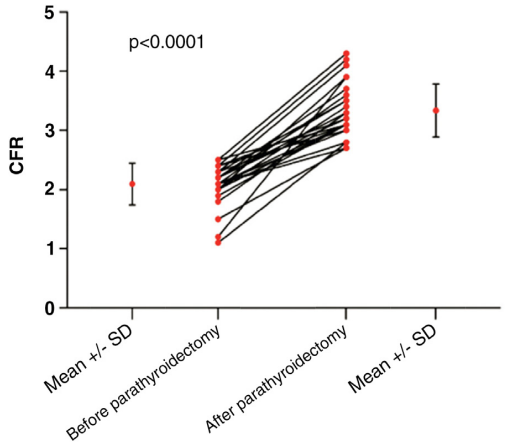


FIGURE 12.3 **Improvement in coronary flow reserve (CFR) following adenoma resection for primary hyperparathyroidism.** In their landmark study, Osto and colleagues established that coronary microvascular dysfunction occurs in the left anterior descending artery of patients with asymptomatic hyperparathyroidism due to a solitary adenoma. Curative resection of the adenoma restores normal CFR. *Reprinted with permission from Lippincott Williams & Wilkins.*[91]

6. Secondary hyperparathyroidism of chronic kidney disease (CKD); the metabolic "perfect storm" of cardiovascular risk.

CKD is a highly significant cardiovascular risk factor, synergizing with diabetes to increase morbidity and mortality at least 5- to 10-fold. While cholesterol-lowering therapies have impact, reducing non-fatal cardiovascular events by ca. 20% in pre-dialysis CKD, no significant reduction in cardiovascular mortality has been achieved with this strategy in CKD patients on dialysis. This appears to occur in large part due to the contributions of perturbed calcium phosphate metabolism to cardiovascular risk(26). Indeed, the chronic kidney disease – mineral and bone disorder (CKD-MBD) designation was created as an imperfect mechanism meant to capture the implications of this clinical setting(27; 28).

Phosphate-Induce Vascular Smooth Muscle Dysfunction

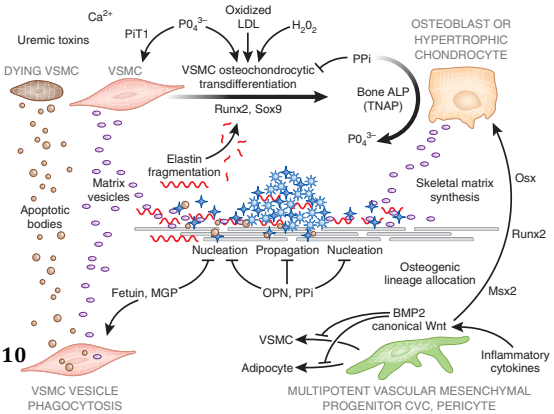


Fig. 10

In the setting of CKD, serum phosphate exerts a stepwise increase in cardiovascular mortality. Vascular toxicity is mediated in part via arterial calcification, and cardiovascular mortality tracks the presence and extent of vascular mineralization. Phosphate-dependent activation of sodium phosphate co-transporter (PiT) signaling in VSMCs drives osteogenic mineralization programs(29) and pro-apoptotic responses that perturb mineralizing matrix vesicle clearance – thus augmenting vascular calcium load(30) (**Figure 10**). At every level of renal function, serum phosphate is a cardiovascular risk factor(31; 32) and PTH is a key defense against hyperphosphatemia(33). PTH (a) directly induces renal phosphate excretion when renal function is intact; (b) recruits osteoblast – derived FGF23 to assist in these phosphaturic actions; (c) and maintains bone formation even in the setting of CKD -- a state of variable skeletal resistance to PTH(34). **Thus, actions of PTH play an important role along with FGF23 to prevent phosphate – dependent vascular toxicity when renal function is intact and normal bone turnover is maintained.** Extremes of bone turnover – too high or too low –negatively impact both serum

phosphate and calcium homeostasis in the setting of CKD(34). However, the specific relationship between bone formation (potentially functioning as a "buffer" mitigating vascular phosphate toxicity), changes in PTH and FGF23 tone with declining renal function, and maintenance of healthy serum phosphate homeostasis with respect to vascular physiology have not yet been established. Indeed, the initial short-term adaptive responses of PTH and FGF23 with respect to acute dietary phosphate loads and phosphate vascular toxicity are emerging as maladaptive with respect to cardiometabolic health over the long-term. FGF23 levels are elevated in patients with primary HPT, and decrease following parathyroidectomy.

Gerard London and colleagues have provided the most important insights into the relationship between PTH, arterial calcification, and bone formation in the setting of CKD (34). Implementing an ultrasound based method for scoring calcification in the common carotid arteries, the abdominal aorta, the iliofemoral axis, and the lower extremities, his group related the extent of arterial calcification with dynamic histomorphometric assessment of cellular bone functions with circulating PTH. In this important study, they established that patients with the lowest levels of PTH (with or without prior parathyroid surgery) exhibited low turnover bone disease and the most extensive vascular calcification (34). The relationship between low PTH values and increased coronary artery calcification was subsequently confirmed by others (35). Interestingly, similar observations were made by Chertow and colleagues upon comparison of the relationships between bone mineral density, vascular calcium load, and PTH levels in patients treated with calcium-based phosphate binders vs. sevelamer (does not contain calcium);(36). Suppression of PTH levels in those subjects given calcium-based binders was associated with lower bone mass and increased vascular calcium load.

Basal PTH tone exerts beneficial actions in part via maintenance of bone formation(34). As discussed(1), it has become increasingly clear that skeletal maintenance of hematopoiesis, regulation of calcium phosphate exchange, and production of phosphaturic hormones such as FGF23 play important roles in vascular health. Surgery is the standard of care for severe primary and severe secondary / tertiary hyperparathyroidism. However, surgery is not the first line approach to the vast majority of patients with significant secondary hyperparathyroidism in CKD because of the clinical need to "titrate" PTH levels to maintain bone formation(34). In the setting of uremia, circulating fragments of PTH and uremic toxins give rise to a skeletal resistance to PTH(13). Based upon dynamic histomorphometry performed in patients with CKD on dialysis, PTH levels between 150 – 300 pg/ml were associated with maintenance of normal bone turnover in this setting. However, similar analyses have not been performed in CKD patients prior to the initiation of renal replacement therapy. Moreover, the specific PTH assay used to establish this treatment goal is no longer available, calling into question how one co-registers these prior guidelines with current generation PTH assays. Hence, current KDIGO guidelines recommend levels of PTH between 2 and 9 times the upper limit of normal in any given assay – although not strictly co-registered with skeletal or cardiovascular indices of health (37). Nevertheless, striving to achieve this skeletally defined "set point" also appears to improve cardiovascular disease risk – and the cardiovascular benefits of pharmacological management of HPT were first established in the setting of CKD. Treatment with injectable calcitriol or paricalcitol to maintain PTH levels to approximately 2-4 times the upper limit of normal reduces cardiovascular mortality (38; 39). Recent epidemiological data point to a biphasic relationship between

Biphasic Mortality Relationship With PTH CKD5 on Hemodialysis

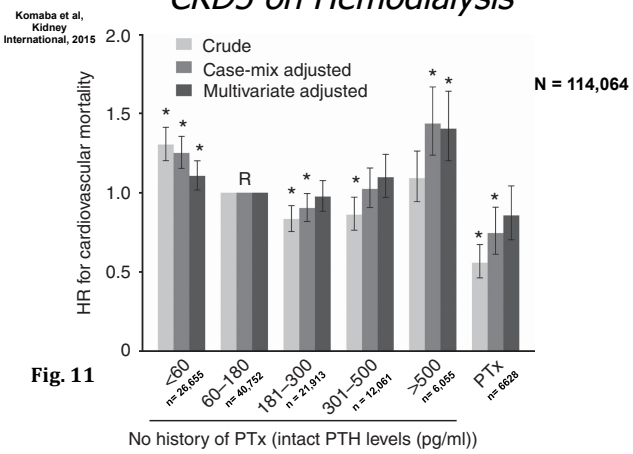
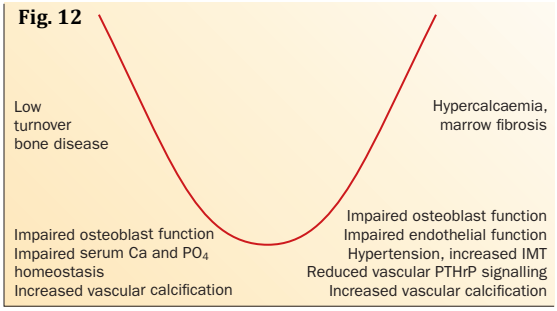


Fig. 11

PTH levels and mortality in CKD5 – and points once again to the importance of mineral metabolism as a key contributor to cardiovascular disease in this setting (**Figure 11**)(40). The emerging potential reasons for this relationship are summarized in **Figure 12**(1).



As a type II calcium sensing receptor mimetic with a short half-life, cinacalcet successfully reduces circulating PTH levels and enables facile titration to goal. Cinacalcet is very effective in treating primary and secondary hyperparathyroidism, but its actions on cardiovascular and fracture risks are still emerging(41). A meta-analysis of 4 studies suggested reductions in both hip fracture and cardiovascular hospitalization rates with cinacalcet(42). In EVOLVE (Evaluation of Cinacalcet Hydrochloride Therapy to Lower Cardiovascular Events), a prospective study to treat secondary HPT in dialysis patients with CKD5, cinacalcet failed to achieve significant reductions in cardiovascular mortality ($p = 0.11$) in the unadjusted intention to treat analysis(43). However, when stratified by age, significant differences in cardiovascular mortality were in older patients on RRT(44) – and cinacalcet-induced reductions in FGF23 were associated with reductions in cardiovascular events as a secondary endpoint. After adjustment for other baseline clinical characteristics, the relative hazard ratio for the primary composite end point (risk of death or first myocardial infarction, hospitalization for unstable angina, heart failure, or a peripheral vascular event) was 0.88 in EVOLVE (95% CI, 0.79 to 0.97; $p = 0.008$)(43). However, the milieu of CKD5 and dialysis represents a metabolic "perfect storm" for cardiovascular disease; in this setting, secondary hyperparathyroidism is but one important contributor that must be addressed. Moving forward, the multifactorial high-risk metabolic and genetic milieu of CKD must be prospectively embraced as strategies targeting PTH biology are evaluated for mitigating cardiovascular risk.

7. Chronic PTH1R activation and the renin-angiotensin-aldosterone (RAA) axis in cardiovascular disease: A feed-forward vicious cycle.

As mentioned above, the relationships between hyperparathyroidism and hypertension are well-appreciated(45). In otherwise healthy adults, while singular administration lowers blood pressure, sustained PTH administration over a period of 12 days actually increases blood pressure in humans(46). In addition to PTH1R desensitization mechanisms described above(9) that impair paracrine PTHrP-mediated vasorelaxation, PTH may have direct effects on aldosterone biosynthesis(45). However, PTH1R-dependent signals in the juxtaglomerular apparatus also support renin production. Following surgery for HPT, renin and aldosterone levels fall(47) albeit with variable improvements in blood pressure and cardiac hypertrophy. Conversely, it has recently become clear that angiotensin II augments circulating PTH levels in part via aldosterone / mineralocorticoid receptor signaling pathways in humans. Thus, a "feed-forward" vicious cycle may exist between dysregulated PTH and RAA axis that promotes cardiovascular disease arising from the metabolic derangements of aging, declining renal function, and HPT (4). This potential relationship is currently being examined in the EPATH trial, testing the impact of the mineralocorticoid antagonist eplerenone in patients with primary HPT.

8. Summary, conclusions, and some future directions.

The cardiovascular actions of PTH have been known for almost a century. However, the clinical and pharmacological implications of altered PTH/PTHrP signaling with respect to cardiovascular endocrinology have been underappreciated except in the settings of extreme excess with sever hypercalcemia or calciphylaxis. In addition to mild primary hyperparathyroidism, secondary hyperparathyroidism associated with advancing age and/or

declining renal function is increasingly prevalent. In addition to the uremic milieu of CKD(13), dyslipidemia also induces a state of PTH/PTHrP resistance at least in the skeleton (48; 49), and potentially within the vasculature. As such, perturbations in PTH/PTHrP physiology are amongst the most prevalent endocrinopathies impacting human health and healthcare today. Heart disease remains the #1 cause of mortality worldwide and is a growing burden(50). Given the contributions of PTH/PTHrP signaling in cardiovascular health and disease – and the prevalence of perturbed PTH/PTHrP signaling in our aging populace – it is stunning that so very little is known about the fundamentals of PTH/PTHrP biology within the vasculature. The specific protein-protein interactions and signaling cascades conveying PTH1R actions in VSMCs, endothelial cells, and interstitial/adventitial cell populations need to be defined for each relevant vascular bed; coronary, renal, aortic valve, and peripheral vascular venues may represent the most clinically relevant with respect to cardiovascular and metabolic bone diseases. The mechanisms controlling paracrine vs. intracrine PTHrP bioactivities have yet to be identified and integrated with the mechanical and neuroendocrine cues that together coordinate tissue perfusion. Perhaps most importantly, biomarkers and molecular / functional imaging methods are required to quantify tissue-specific PTH1R actions as a first step towards establishing the healthy "set point" for signaling tone and dynamics within the vasculature. Because post-translational modifications and/or dysmetabolic states induce PTH/PTHrP resistance in the very settings where this biology becomes most clinically significant with respect to cardiovascular health, titration to a single circulating PTH value or ionized calcium level will likely prove to be inadequate. In addition, extent to which skeletally –derived endocrine hormones such as FGF23, osteocalcin, and osteopontin contribute to the cardiometabolic risks of HPT have yet to be established. Moreover, whereas surgery is a standard of care in primary hyperparathyroidism that yields reductions in hip fracture risk, renal disease, and hypercalcemic crises, the impact of parathyroid surgery on cardiovascular morbidity and mortality has yet to be unambiguously established for mild or asymptomatic disease. However, beneficial changes with curative surgery in important cardiovascular parameters such as vascular stiffness and coronary flow reserve are truly encouraging. Adjunctive medical strategies will likely be required(4), and it remains unclear whether surgical intervention and pharmacological intervention for treatment of HPT are functionally equivalent with respect to both skeletal and cardiovascular outcomes. As occurred during the history of the cholesterol controversy, discovery and implementation of clearly effective pharmacotherapy will be important(51). Clearly, detailed examination of the cardiovascular physiology regulated by the PTH superfamily will continue to yield important new insights – and will be necessary to devise novel therapeutic strategies that better treat our patients afflicted with HPT, CKD, and cardiometabolic diseases.

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