Evaluation, Prevention, and Treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl*. 2017;7:1–59.
5. Tran L, Pannier B, Lacolley P, et al. A case-control study indicates that coagulation imbalance is associated with arteriosclerosis and markers of endothelial dysfunction in kidney failure. *Kidney Int*. 2021;99:1162–1172.
6. Murshed M, Schinke T, McKee MD, et al. Extracellular matrix mineralization is regulated locally; different roles of two gla-containing proteins. *J Cell Biol*. 2004;165:625–630.
7. Holden RM, Morton AR, Garland JS, et al. Vitamins K and D status in stages 3-5 chronic kidney disease. *Clin J Am Soc Nephrol*. 2010;5:590–597.
8. De Vriese AS, Caluwe R, Pyfferoen L, et al. Multicenter randomized controlled trial of vitamin K antagonist replacement by rivaroxaban with or without vitamin K2 in hemodialysis patients with atrial fibrillation: the Valkyrie Study. *J Am Soc Nephrol*. 2020;31:186–196.
9. Verbeke FH, Agharazii M, Boutouyrie P, et al. Local shear stress and brachial artery functions in end-stage renal disease. *J Am Soc Nephrol*. 2007;18:621–628.

Parathyroid hormone oxidation in chronic kidney disease: clinical relevance?

Tilman B. Drüeke[1] and Jürgen Floege[2]

In chronic kidney disease, parathyroid hormone (PTH), like all proteins, can undergo post-translational modifications, including oxidation. This can lead to structural and functional changes of the hormone. It has been hypothesized that currently used PTH measurement methods do not adequately reflect PTH-related bone and cardiovascular abnormalities in chronic kidney disease owing to the presence of oxidized, biologically inactive PTH in the circulation. Ursem *et al.* now report a strong correlation between serum non-oxidized and total PTH, and comparable associations with histomorphometric and circulating bone turnover markers, pleading against this hypothesis.

Kidney International (2021) **99,** 1070–1072; https://doi.org/10.1016/j.kint.2021.01.019

see clinical investigation on page 1173

Chronic kidney disease (CKD) is associated with post-translational modifications of proteins, peptides, lipids, and DNA through carbamylation, glycation, and oxidation.[1] There is ample evidence that many of these modifications can substantially alter the biological role of the molecules. Among a host of uremic toxins, post-translationally modified substances have long been known to play an important role in the pathogenesis of cardiovascular disease and mortality. They favor inflammation, atherosclerosis, arterial stiffening, hypertension, heart failure, and stroke. They may also contribute to the progression of CKD.

Parathyroid hormone (PTH) is but one among the numerous potential targets of reactive oxygen and nitrogen species in CKD, which create peptide-bound cleavage or oxidation of amino acid side chains and lead to structural and functional changes of oxidized proteins. Aromatic amino acids, as well as methionine and cysteine residues, are preferred target sites for hydroxyl radicals, and such modified proteins have been named advanced oxidation protein products.[2]

PTH is an important player in the pathogenesis of the CKD-associated bone and mineral disorder. Its circulating concentrations increase with the progression of CKD, although to a highly variable degree from one patient to the other, because of differences in synthesis and secretion, effects on target tissues, and catabolism. Furthermore, partial resistance to the skeletal action of PTH develops in many patients with CKD. Such resistance can start early in the course of CKD and is eventually overcome by more intensive PTH secretion. Several factors have been incriminated in PTH resistance, including disturbances of vitamin D metabolism, hyperphosphatemia, acidosis, uremic toxins, PTH/PTH-related peptide receptor down-regulation, and a reduction of the ability of PTH to bind to and activate the PTH/PTH-related peptide receptor.

The hormone is released from the parathyroid glands both in its active, intact form (PTH1–84) as well as PTH fragments. In severe secondary hyperparathyroidism, secretion of the active form prevails. PTH1–84 is catabolized by the liver into the N-terminal PTH1–34 fragment, which retains full activity, and C-terminal fragments with no PTH activity. The latter, in turn, are degraded mainly by the kidneys. With the progression of CKD, the renal removal of C-terminal fragments is progressively impaired, resulting in their accumulation in the circulation. It is noteworthy that different C-terminal fragments may exert actions opposite to those of PTH1–84. At present, second-generation PTH assays measuring the so-called "intact" PTH are mainly used in clinical practice. Third-generation PTH assays measuring the so-called "whole" PTH have a theoretical

[1]*Inserm U-1018, Centre de recherche en épidémiologie et santé des populations (CESP), Paris-Ile-de-France-Ouest University, Paris-Sud University, and Paris Saclay University, Villejuif, France; and* [2]*Department of Nephrology and Clinical Immunology, University Hospital, Rheinisch Westfälische Technische Hochschule Aachen, Aachen, Germany*

Correspondence: *Tilman B. Drüeke, Inserm Unit 1018, Team 5, CESP, Hôpital Paul Brousse, 16 Ave Paul Vaillant Couturier, 94807 Villejuif Cedex, France. E-mail: tilman.drueke@inserm.fr*

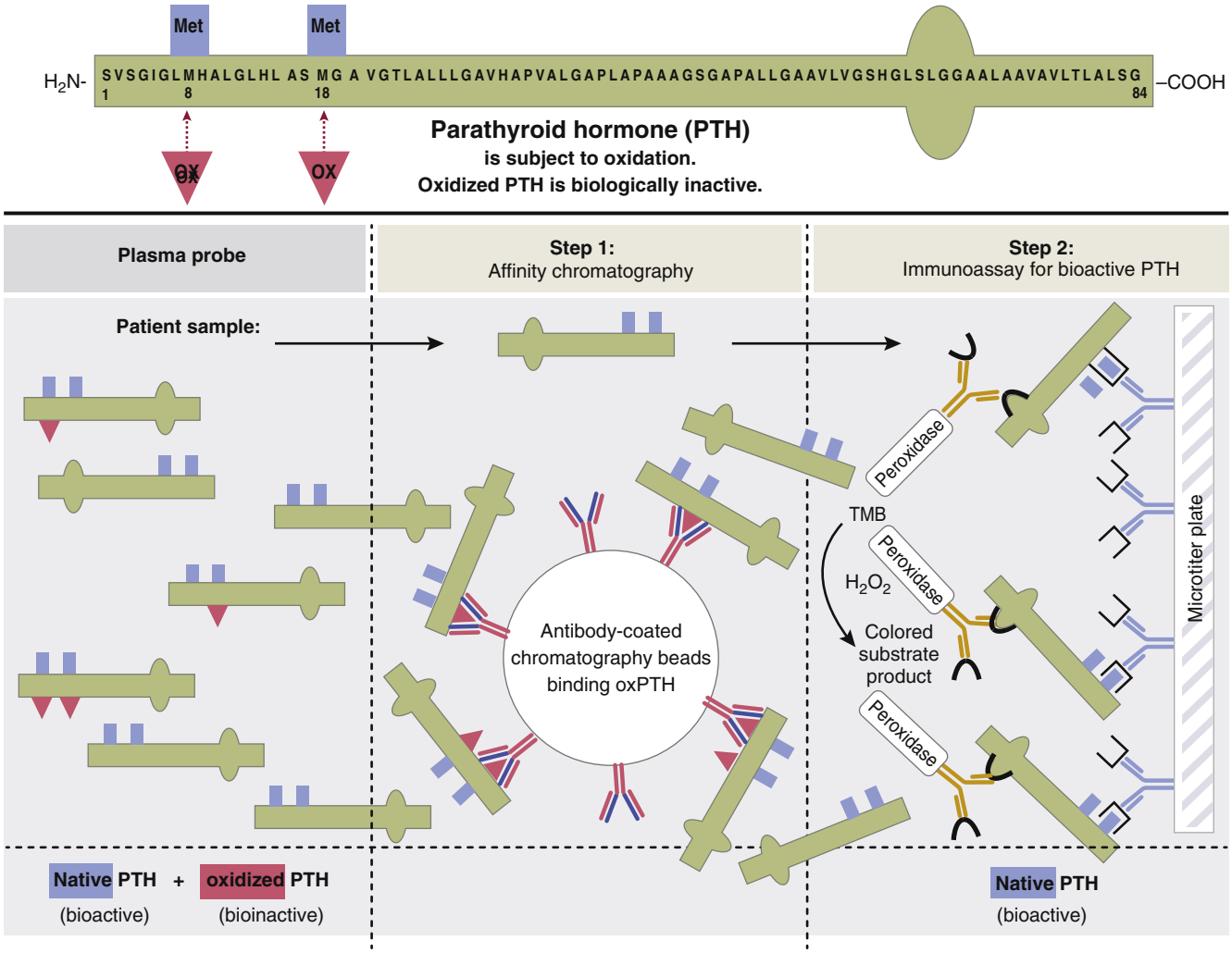

Figure 1 | Basic principles of the new assay system for detection of intact and "real" intact parathyroid hormone (PTH) in human samples. The new detection process for "real" intact bioactive PTH consists of 2 steps: First, any forms of PTH oxidized at position Met8 and/or position Met18 are removed from the plasma sample by a specific affinity chromatography column. The column contains monoclonal rat/mouse antibody raised against oxidized human PTH(1–34) (oxPTH) fragments. These antibodies are able to remove any oxidized forms of PTH. Second, the remaining non-oxidized PTH is analyzed in conventional 2-site "sandwich" immunoassay systems. The antibody on the left (capture antibody) is bound to solid phase. The antibody on the right (label antibody) relays the signal. These antibodies must bind different sites on the PTH analyte to produce a positive result in the assay. OX, oxidation; TMB, 3,3′,5,5′-tetramethylbenzidine (peroxidase substrate). Figure adapted with permission from Hocher B, Armbruster FP, Stoeva S, et al. Measuring parathyroid hormone (PTH) in patients with oxidative stress–do we need a fourth generation parathyroid hormone assay? *PLoS One*. 2012;7:e40242.[5] © 2012 Hocher *et al.* under the terms of the Creative Commons Attribution License.

advantage over "intact" PTH assays because they measure PTH1–84 alone, whereas the "intact" PTH assays measure in addition some PTH fragments. However, this is only of academic, not of clinical, interest.

Whether in addition to their well-established catabolic fate the PTH peptides may undergo post-translational modifications in CKD, and whether structural PTH changes induced by oxidative stress may play a role in the skeletal resistance to its action, remains a matter of debate. PTH has 2 methionine residues at positions 8 and 18 whose oxidation has long been shown to impair or abolish its biological activity.[3,4] Based on these observations, Hocher *et al.* developed an assay able to distinguish between oxidized PTH and biologically active PTH, using a specific adsorption column with monoclonal antibodies raised against oxidized human PTH.[5] Figure 1 shows the basic principles of the measurement method. Applying this assay to patients with CKD, including those with end-stage kidney disease (ESKD), and to kidney transplant recipients, they observed substantial interindividual variation of measured non-oxidized PTH concentrations after removal of oxidized PTH.[5,6] They claimed that because of the presence of oxidized, biologically inactive PTH, currently used PTH measurement methods might not adequately reflect PTH-related bone and cardiovascular abnormalities in CKD. However, another group failed to

confirm this claim as far as cardiovascular disease is concerned. They observed an association of second-generation "intact" PTH with all-cause mortality in patients with CKD after adjustment for cardiovascular and kidney risk factors, whereas selective measurement of non-oxidized PTH did not allow them to identify any relationship.[7]

In the present issue of *Kidney International*, Ursem *et al.* now report a new study devoted to the above issue.[8] They asked whether non-oxidized PTH outperformed total PTH as a biomarker of bone turnover in patients with ESKD. To this end, they selected 10 ESKD patients with low bone turnover, 10 ESKD patients with normal bone turnover, and 11 ESKD patients with high bone turnover, all categorized on the basis of histomorphometric criteria. First, they observed a strong correlation between serum non-oxidized PTH and total PTH. Second, both histomorphometric and circulating bone turnover markers showed similar correlations with non-oxidized PTH and total PTH. They therefore concluded that non-oxidized PTH is not superior to total PTH as a biomarker of bone turnover in patients with ESKD.

Is this the final verdict? Probably not. Because the sample size of the study by Ursem *et al.* was relatively small, their study had limited statistical power. However, as rightly pointed out by the authors, the strong correlation between non-oxidized PTH and total PTH rendered a type 2 statistical error highly unlikely. All study participants had ESKD and were of White ethnicity; therefore, extrapolation of the findings to non–dialysis-dependent CKD and patients with other ethnic backgrounds may not be warranted.

In another vein, one would have liked to know whether there is no correlation at all of cardiovascular or skeletal complications with circulating oxidized PTH. However, Ursem *et al.* did not provide data on directly measured oxidized PTH. The problem is that the current non-oxidized PTH assay eliminates 3 different forms of oxidized PTH (namely, PTH oxidized at either methionine 8 or methionine 18, or both). These 3 forms are not all biologically inactive. Therefore, it is possible that only fully oxidized PTH participates at the phenomenon of skeletal PTH resistance. Ideally, one would like to have an assay that measures bioactive PTH alone. Such assays have been proposed in the past but were subsequently abandoned because of the advent of more reliable, clinically useful PTH immunoassays. Because the correlation of total PTH with bone histomorphometry parameters and various clinical end points in CKD is generally either weak or null, it would be interesting to measure different oxidized forms separately or to develop a fully reliable PTH bioassay, not so much for clinical routine but for the purpose of clinical investigation. Whether a fully reliable PTH bioassay will ever be set up that reflects the net effect of circulating agonistic and antagonistic PTH forms on bone and other tissues in CKD remains to be seen.

Another interesting question is that of "falsely" elevated oxidized PTH levels due to preanalytical errors (i.e., PTH oxidation of serum or plasma post-sampling), depending on sample handling and storage under various conditions before effective PTH measurements. This issue has been solved by the same group of authors. They recently reported that *ex vivo* oxidation of PTH was negligible.[9]

In conclusion, *in vivo* PTH oxidation clearly occurs in patients with CKD. However, presently available methods do not allow a precise distinction of biologically active from inactive PTH forms, be it through oxidative or other post-translational modifications of the hormone. Unless better performing assays are developed, we should stay with second- or third-generation PTH assays, as currently used in clinical practice.

DISCLOSURE

TBD reports personal fees from Akebia, Amgen, Astellas, Chugai, FMC, Kyowa Hakko Kirin, Sanofi, and Vifor. JF reports personal fees from Amgen, Astellas, Bayer, Fresenius, and Vifor.

REFERENCES

1. Gajjala PR, Fliser D, Speer T, et al. Emerging role of post-translational modifications in chronic kidney disease and cardiovascular disease. *Nephrol Dial Transplant*. 2015;30:1814–1824.
2. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int*. 1996;49:1304–1313.
3. Galceran T, Lewis-Finch J, Martin KJ, Slatopolsky E. Absence of biological effects of oxidized parathyroid hormone-(1-34) in dogs and rats. *Endocrinology*. 1984;115:2375–2378.
4. Horiuchi N. Effects of oxidation of human parathyroid hormone on its biological activity in continuously infused, thyroparathyroidectomized rats. *J Bone Miner Res*. 1988;3:353–358.
5. Hocher B, Armbruster FP, Stoeva S, et al. Measuring parathyroid hormone (PTH) in patients with oxidative stress–do we need a fourth generation parathyroid hormone assay? *PLoS One*. 2012;7:e40242.
6. Hocher B, Oberthür D, Slowinski T, et al. Modeling of oxidized PTH (oxPTH) and non-oxidized PTH (n-oxPTH) receptor binding and relationship of oxidized to non-oxidized PTH in children with chronic renal failure, adult patients on hemodialysis and kidney transplant recipients. *Kidney Blood Press Res*. 2013;37:240–251.
7. Seiler-Mussler S, Limbach AS, Emrich IE, et al. Association of non-oxidized parathyroid hormone with cardiovascular and kidney disease outcomes in chronic kidney disease. *Clin J Am Soc Nephrol*. 2018;13:569–576.
8. Ursem SR, Heijboer AC, D'Haese PC, et al. Non-oxidized parathyroid hormone (PTH) measured by current method is not superior to total PTH in assessing bone turnover in chronic kidney disease. *Kidney Int*. 2021;99:1173–1178.
9. Ursem SR, Vervloet MG, Hillebrand JJG, et al. Oxidation of PTH: in vivo feature or effect of preanalytical conditions? *Clin Chem Lab Med*. 2018;56:249–255.