# Format for ANSWERING REVIEWERS

March 24, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 2429-review.doc).

**Title:** Potential pathophysiological role for the vitamin D deficiency in essential hypertension
**Author:** Federico Carbone, François Mach, Nicolas Vuilleumier, Fabrizio Montecucco

**Name of Journal:** *World Journal of Cardiology*

**ESPS Manuscript NO:** 7225

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers:

**Reviewer 1:**

Comment 1: "Vitamin D mainly activates two signaling pathways: the renin-angiotensin-aldosterone pathway and the fibroblast growth factor 23/klotho signaling cascade. Please describe in details these two signaling systems."

Reply: we attempted to better address this critical point on page 7, as follows: "…Overall, 1,25(OH)2 vitamin D and FGF23 are involved in a classical hormonal loop also including PTH. High levels of 1,25(OH)2 vitamin D raise the serum concentrations of both calcium and phosphate,. Concomitantly, the feedback by PTH reduces only calcium levels by enhancing its urinary excretion. Increased levels of FGF suppress the expression of sodium-phosphate cotransporter NaPi-2a on renal proximal tubules, thus resulting in increased phosphaturia [Ref]. Therefore, phosphorus homeostasis might be maintained by 1,25(OH)2 vitamin D via a direct regulation on FGF23 levels.…"

and

"…For instance, the age-associated suppression of Klotho expression [Ref] may promote a vitamin D toxicosis during therapeutic supplementation characterized by over-hyperphosphatemia and thus increased cardiovascular risk[Ref]. Although it is likely a failure of the normal feedback mechanism regulating vitamin D and FGF23, the molecular bases of these clinical features have not been identified yet. Furthermore, Camalier and co-workers recently provided evidence of both rapid and late effects induced by FGF23 on mesenchymal stromal cells, involving cell proliferation and extracellular matrix (ECM) regulation[Ref]. In addition, Jimbo and colleagues showed that FGF23 promoted osteoblastic differentiation of aortic vascular smooth muscle cells (VSMCs) from uremic rats by inducing ERK1/2 phosphorylation pathway[Ref]. However, it should be noted that these features were shown only in primary rat VSMCs and other studies failed to recognize the relevance of FGF23-Klotho signalling in mouse arteries[Refs].…".

In addition, as required, we have improved the section about the interaction between vitamin D and renin-angiotensin-aldosterone pathway on page 9, as follows: "…From a molecular point of view, the research group directed by Li discovered a direct effect of 1,25(OH)2 vitamin D on renin gene transcription. They identified that vitamin D is capable of suppressing renin gene transcription by a cAMP response element, identified on the promoter region of Ren-1c gene[Ref]. In addition, the same authors confirmed a central role of active vitamin D by excluding the

control of PTH or serum calcium levels on renin expression[Ref]. …".

Comment 2: "Please describe, in brief, the effects of the above signalling pathways on vascular cell phenotypes including endothelial cells, smooth muscle cells, and matrix.".
Reply: we agree with the reviewer's comment and we included a paragraph about the detrimental role of fibroblast growth factor 23/klotho signalling within the vessel wall on page 7, as follows: "…Although it is likely a failure of the normal feedback mechanism regulating vitamin D and FGF23, the molecular bases of these clinical features have not been identified yet. Furthermore, Camalier and co-workers recently provided evidence of both rapid and late effects induced by FGF23 on mesenchymal stromal cells, involving cell proliferation and extracellular matrix (ECM) regulation[Ref]. In addition, Jimbo and colleagues showed that FGF23 promoted osteoblastic differentiation of aortic vascular smooth muscle cells (VSMCs) from uremic rats by inducing ERK1/2 phosphorylation pathway[Ref]. However, it should be noted that these features were shown only in primary rat VSMCs and other studies failed to recognize the relevance of FGF23-Klotho signalling in mouse arteries[Refs]..."

Comment 3: "3. Please describe the effects of vitamin D deficiency on arterial remodelling in hypertension (molecular histopathology).".
Reply: as recommended, we have described the role of vitamin D in arterial remodelling on page 9, as follows "…Angiotensin II is a main mediator responsible for adverse vascular remodelling in hypertension[Ref]. By promoting endothelial dysfunction and vascular permeability, RAAS induces recruitment and activation of inflammatory cells within the vessel wall. This inflammatory behaviour stimulates hyperplasia and hypertrophy of VSMCs, but also their release of pro-inflammatory molecules (VCAM-1, monocyte chemoattractant protein-1, interleukin 6 and 8)[Ref]. Furthermore, angiotensin II was shown to mediate the shift of VSMCs toward a fibroblast phenotype that alters the ECM composition by suppressing the activity of matrix metalloproteinases and enhancing the production of their inhibitors[Ref]. Among the intracellular signalling pathways involved in angiotensin II signalling a key role is played by oxidants and their downstream signalling cascades including mitogen-activated protein kinase, protein kinase C, phospholipase A2 and the transcription factors NFκB and AP-1[Ref]…".

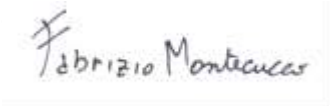
**Reviewer 2:**

Comment 1: "Pg. 5, 2nd paragraph line 1 - Although the effects of vitamin D on blood pressure are known since decades,…- should be revised to read something like - Although the effects of vitamin D on blood pressure have been known for several decades,…-"".
Reply: as recommended, this change was done (page 8).

Comment 2: "Pg. 10, Top paragraph, 3 lines from the bottom: there is a circle degree symbol on the number 25 that needs to be corrected or clarified."
Reply: as required we have deleted the symbol 25° and replaced it with as follows: "…first quartile…" (page 12).

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Cardiology*.



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