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FINAL REPORT

Project / Program Title		Phosphate, FGF23 and Klotho in chronic kidney disease
Name		Associate Professor Nigel D Toussaint
Award Received		2015 Jacquot Research Establishment Fellowship
Report Date		7 August 2017
Chief Investigator / Supervisor		Professor Stephen G Holt
Administering Institution		The Royal Melbourne Hospital
Funding Period	Start Date:	1 January 2015
	Finish Date:	31 December 2016

PROJECT SUMMARY

Patients with chronic kidney disease (CKD) suffer from the formation of bone-like deposits in blood vessels ('vascular calcification'). Imbalance in regulation of the mineral phosphate, which occurs in kidney disease, may be central to this process, leading to a so-called 'mineral bone disorder'. Elevated blood phosphate levels are associated with increased mortality. The discovery of fibroblast growth factor- 23 (FGF23) and its co-receptor klotho, have highlighted a possible mechanism by which the body can regulate mineral metabolism and phosphate handling. As with elevated phosphate, high FGF23 levels are also associated with both mortality and increased cardiovascular disease. However, it is still not known if one causes the other.

A key role of FGF23 is to induce phosphate excretion and klotho is an essential co-receptor in this process. This research project set out to examine the relationships between FGF23, klotho and phosphate excretion by studying these parameters in several groups of individuals, including patients undergoing kidney surgery, where a small section of kidney tissue was available for study together with blood and urine samples. We also studied other individuals such as those with normal kidney function, including living kidney donors, and patients with CKD and kidney transplant recipients, and assessed different parameters of phosphate metabolism. This study has improved our understanding of urinary phosphate excretion and the relationships with FGF23 and klotho involved in this process, especially at early stages of kidney dysfunction. This may, in turn, lead to further insight into possible mechanisms involved in improving phosphate balance to reduce the cardiovascular disease burden associated with ongoing kidney disease.

PROJECT AIMS / OBJECTIVES

Aims of this project included:

1. To assess the associations between urinary phosphate excretion, intact FGF23 and c-terminal FGF23 levels, soluble klotho and membrane-bound klotho/FGF-Receptor in both patients with normal kidney function and CKD.

Specific Experimental and Clinical Objectives included:

a. To (i) characterise expression of membrane-bound klotho in human kidney tissue and (ii) determine if it is co-localised with expression of the FGF receptor.

b: To (i) quantitatively determine klotho mRNA expression in human kidney tissue (RT-PCR) and (ii) determine if it is associated with plasma and urine soluble klotho

c: To (i) test for an association between intact and c-terminal FGF23 levels, and urinary phosphate excretion and (ii) determine if membrane-bound klotho impacts on this association.

d: To establish klotho as an obligate co-receptor in FGF23 signalling in cultured human kidney cells.

e: To study the change in serum FGF23 and klotho levels in living kidney donors and renal transplant recipients.

2. To test the variability of soluble klotho levels and the impact of exercise on this parameter.

(See next section for summary of results)

SIGNIFICANCE AND OUTCOMES

Outcomes of clinical and experimental studies were summarised together with studies/methodologies above.

Significance:

Taken together, the outcomes from the above studies suggest soluble klotho is proportional to kidney mass/function, and is also affected directly in response to change in kidney mass either from

kidney transplantation or due to kidney donation (via a donor nephrectomy). Although the benefits of higher soluble Klotho levels were not directly examined in this project, others have reported on survival advantages associated with higher soluble Klotho levels.

In the final pilot study of healthy volunteers, we were able to establish that soluble Klotho levels can increase, albeit transiently, in response to physical activity. This requires further investigation with longer duration of physical activity and in disease populations (eg CKD) in order to determine the overall benefit of such programs in those with kidney disease. We would hope to improve overall survival outcomes in those with kidney disease by increasing soluble Klotho levels with such lifestyle modifications.

PUBLICATIONS / PRESENTATIONS

Tan SJ, Chu M, Toussaint ND, Cai MMX, Hewitson TD, Holt SG. High-intensity Physical Exercise Increases Serum a-Klotho Levels In Healthy Volunteers. 2017 (submitted, undergoing peer review)

Krishnasamy R, Tan SJ, Hawley CM, Johnson DW, Stanton T, Lee K, Mudge OW, Campbell S, Toussaint ND, Isbel NM. Progression of aortic stiffness in advanced CKD correlates with increasing fibroblast growth factor 23. BMC Nephrology 2017, in press

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Tan SJ, Holt SG, Hewitson TD, Hughes PD, Toussaint ND. Changes in markers of mineral adaptations following living kidney donation. Transplantation Direct 2017; 3(4): e150

Tan SJ, Smith ER, Holt SG, Hewitson TD, Toussaint ND. Soluble klotho may be a marker of phosphate reabsorption. Clinical Kidney Journal 2017; 10: 397-404

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Tan SJ, Satake, S, Smith ER, Toussaint ND, Hewitson TD, Holt SG. Parenteral iron polymaltose changes i:c-terminal FGF23 ratios in iron deficiency, but not in dialysis patients. European Journal of Clinical Nutrition 2017; 71: 180-184

Tan SJ, Cai MMX, Holt SG, Smith ER, Hewitson TD, Toussaint ND. Relationship between timed and spot urine collections for measuring phosphate excretion. International Urology and Nephrology 2016; 48: 115-124

Tan SJ, Smith ER, Hewitson TD, Holt SG, Toussaint ND. Diurnal variation and short-term pre-analytical stability of serum soluble aklotho in healthy volunteers: a pilot study. Annals of Clinical Biochemistry 2015; 52: 506-509

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