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Original Article

Fibroblast growth factor-23 in patients with homozygous familial hypercholesterolemia

Jarrod M. Zamparini, MBChB*, Andrew R. Immelman, BSc, Frederick J. Raal, FRCP, FRCPC, FCP(SA), Cert Endo, MMed, PhD

Department of Medicine, Faculty of Health Sciences, University of the Witwatersrand, Parktown, South Africa (Drs Zamparini, Immelman, and Raal); and Carbohydrate & Lipid Metabolism Research Unit, Department of Medicine, Faculty of Health Sciences, University of the Witwatersrand, Parktown, South Africa (Drs Immelman and Raal)

KEYWORDS:
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BACKGROUND: Patients with homozygous familial hypercholesterolemia (HoFH) develop significant vascular calcification early in life, the cause of which is not yet fully understood. Patients with chronic kidney disease have similar vascular calcification, with fibroblast growth factor-23 (FGF23) implicated in these patients.

OBJECTIVE: To determine whether there was a difference in FGF23 between patients with HoFH and age- and gender-matched controls and whether there is a correlation between FGF23 and serum low-density lipoprotein, total cholesterol, and carotid intima-media thickness in patients with HoFH.

METHODS: The study was a cross-sectional review involving 30 patients with HoFH attending the Charlotte Maxeke Johannesburg Academic Hospital Lipid Clinic in Parktown, South Africa, as well as 30 age- and gender-matched healthy controls. FGF23, fasting lipid profiles, calcium, and phosphate were measured. B-mode ultrasonography of the carotid arteries was done to assess the extent and severity of arterial calcification.

RESULTS: There was no difference in mean FGF23 between the patient and control groups (62.07 ± 26.42 pg/mL vs 63.69 ± 19.84 pg/mL; $P = .4621$) nor was there any correlation between FGF23 and low-density lipoprotein cholesterol ($P = .9483$ and .8474) or total cholesterol ($P = .9261$ and .859). In the HoFH patients, FGF23 did not correlate significantly with any cardiovascular disease.

CONCLUSIONS: Serum FGF23 is not elevated in patients with HoFH when compared to non–familial hypercholesterolemia age- and gender-matched controls, and there is no correlation between serum FGF23 and cardiovascular disease in patients with HoFH. FGF23 does not appear to be a major factor for arterial calcification in HoFH.

Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disorder resulting from mutations that affect the function of the low-density lipoprotein (LDL) receptor. It remains an important condition in South Africa, given the greater prevalence, due to a founder effect, when compared to that reported globally.[1–3] FH can be divided into 2 broad categories: the more common (1 in 200–500) heterozygous

* Corresponding author. Department of Medicine, Faculty of Health Sciences, University of the Witwatersrand, 29 Princess of Wales Terrace, PO Box 688, Parktown, Johannesburg, 2193 South Africa.
E-mail address: jarmarzam@gmail.com

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FH, where patients tend to have an elevation of their low-density lipoprotein cholesterol (LDL-C) to approximately double the normal level and the much less common (1 in 300,000–1,000,000), but more severe, homozygous FH (HoFH).[4,5] HoFH patients effectively express few, if any, LDL receptors on their cell surfaces and thus have extremely elevated LDL-C levels (often 4 to 6 times normal). These patients develop premature accelerated atherosclerotic cardiovascular disease that, if untreated, can lead to fatal myocardial infarction, often in the second or third decade of life.[5]

The quadrupled level of plasma LDL-C at birth in patients with HoFH leads to atherosclerosis in all arterial beds from early on in life.[6] This "accelerated atherosclerosis"[7] is not limited to the coronary vasculature—carotid arteries, renal arteries, aortic valve cusps, aortic root, and descending aorta are all frequently affected.[6,7] Inflammation within the atherosclerotic plaque produces inflammatory mediators that are thought to interact with vascular cells capable of osteogenic differentiation, in turn leading to the vascular calcification seen in these patients.[8] Importantly, the distribution of vascular calcification tends to be localized to the intima of blood vessels as opposed to the medial calcification (Monckeberg's sclerosis) seen in subjects with end-stage renal disease, the elderly, and in those with diabetes mellitus.[9] Nevertheless, both types of calcification are associated with increased cardiovascular morbidity and mortality.[8]

Coronary artery calcification, which can be detected and scored by computed tomography scanning, is an early marker of coronary artery atherosclerosis and together with carotid intima-media thickness (CIMT) can be used as surrogate markers for atherosclerosis in other vessels. Wiegman et al. report that 25% of 11- to 23-year-old patients with heterozygous FH have coronary calcification, whereas it is barely, if ever, detected in the same age group in the general population.[10]

The extensive vascular calcification seen particularly in HoFH leads to surgical challenges at the time of aortic valve and coronary surgery. In a cohort of 25 HoFH patients, followed up for a mean of 18 years, 45% required coronary artery bypass graft surgery and over 50% had evidence of either aortic stenosis or aortic root calcification of which half required aortic valve replacement. All but the 2 youngest patients in this cohort had calcification of the aorta.[5] Similarly, aortic root calcification was found in all patients of a smaller cohort of 7 HoFH patients from the United Kingdom.[11] This is significant considering the incidence of aortic calcification in the general population in the Western world is only 3% in adults older than 75 years.[8]

Fibroblast growth factor-23 (FGF23) is a hormone that acts with its cofactor (α-klotho) via FGF receptors to decrease circulating levels of 1,25-dihydroxycholecalciferol (vitamin D_3) and increase renal excretion of phosphate by inhibiting renal tubular reabsorption of phosphate. Raised FGF23 is an early finding in patients with chronic kidney disease, and elevated FGF23 is an independent marker for cardiovascular events and mortality in these patients. A positive correlation has been noted between elevated levels of FGF23 and coronary artery stenosis as well as between FGF23 and "total body atherosclerosis" (defined as the sum of vascular calcification at vasculature in the neck, aorta, kidney, upper leg, and lower leg).[12]

In their 2015 study, Turan et al. demonstrated a 17% increase in risk for severe coronary artery calcification, as determined by computed tomography scan, for every 50 pg/mL increase in serum FGF23. Of significance is that this independent correlation continued to exist in patients with a glomerular filtration rate > 60 mL/min/1.73 m^2.[13] A further independent correlation between FGF23 and aortic calcification was noted by Nasrallah et al.,[14] Desjardins et al.,[15] and Schoppet et al.[16]

A statistically significant positive association between FGF23 and coronary artery calcification was revealed in a study of 545 African-American patients with type 2 diabetes mellitus implying a race-independent correlation between FGF23 and vascular calcification. However, there was no correlation between carotid and aortic calcification and FGF23.[17]

In patients with FH, vascular calcification continues to progress despite statin therapy and reduction in LDL-C, suggesting that "the calcification process may proceed independently of cholesterol levels, once subendothelial damage has occurred".[5,8] A two-hit hypothesis in the development of vascular calcification in patients with FH has therefore been proposed.[8,9]

Given that FGF23 is an independent risk factor for vascular calcification, we hypothesized that FGF23 could be one of the additional factors responsible for the vascular calcification seen in patients with FH.

Methods

This was a cross-sectional study undertaken at the Carbohydrate and Lipid Metabolism Research Unit at the Charlotte Maxeke Johannesburg Academic Hospital in Parktown, South Africa. Permission to conduct the study was obtained from the University of the Witwatersrand Human Research Ethics Committee (medical), reference number M160537.

The main objective of the study was to compare serum FGF23 levels between patients with HoFH and age- and gender-matched healthy controls without hypercholesterolemia to determine whether FGF23 could be implicated in the pathogenesis of the severe vascular calcification seen in patients with HoFH. The secondary objective was to assess whether any correlation exists between FGF23 levels and total and LDL-C as well as CIMT in patients with HoFH.

Thirty HoFH patients who follow up at the Charlotte Maxeke Johannesburg Academic Hospital Lipid Clinic and 30 age- and gender-matched control patients were studied. Patients with a creatinine clearance of less than 60 mL/min/1.73 m^2 were excluded from the study.

Table 1 Demographic data

Variable	Patient (n = 30)	Control (n = 30)
Age	29.8 ± 13.6	28.8 ± 13.01
Gender		
Male	57% (n = 17)	60% (n = 18)
Female	43% (n = 13)	40% (n = 12)
Race		
White	83.3% (n = 25)	90% (n = 27)
Black	13.3% (n = 4)	10% (n = 3)
Indian	3.3% (n = 1)	0% (n = 0)

Total cholesterol, triglyceride, high-density lipoprotein cholesterol, serum calcium, and serum phosphate were measured using a Siemens ADVIA 1800 assay (Siemens Healthcare Pty Ltd, South Africa). LDL-C was calculated using the Friedewald formula.[18]

FGF23 was measured using an Elisa assay (Kainos Laboratories, Japan). This assay has an intraassay and interassay coefficient of variation of 10%.

B-mode ultrasound measurement, using a Toshiba Nemio Ultrasound SSA-550A (Toshiba Medical Systems Corporation, Japan), of the carotid arteries was also done to measure CIMT and to determine if plaque or calcification was present. The transducer frequency was set at 11 MHz for all measurements.

Measurements of the CIMT were carried out at the optimum angle of interrogation, which allows visualization of the flow tip divider, the common carotid artery, external carotid artery, and the internal carotid artery from a single selected angle of the carotid arteries at the bifurcation. Doppler was used to verify the identification of the external carotid artery and internal carotid artery. The intima-medial thickness was measured when the two echogenic lines, representing the lumen-intima interface and the media-adventitia interface, are visualized over a length of ≥1 cm.

We used the Kolmogorov-Smirnov and Shapiro-Wilk tests to assess normality of distribution of FGF23, serum calcium, and serum phosphate in patient and control groups. The data were not normally distributed for FGF23; thus, the two groups were compared using the Mann-Whitney U test. Serum calcium and phosphate were evenly distributed, and so the two groups were compared using Student's t test. Pearson's correlation coefficient was used to assess for correlation between FGF23, serum calcium, and serum phosphate, and other variables.

Results

Demographic data for the patient and control groups are presented in Table 1. All 30 HoFH patients included in the study were on lipid-lowering therapy at the time of blood sampling with 13.3% (n = 4) of the patients on high-intensity statin monotherapy and the remaining patients (86.7%, n = 26) on a combination of a statin and ezetimibe. Mean duration of therapy was 10.9 ± 5.2 years for statins and 3.4 ± 2.3 years for combination therapy. None of the 30 control patients had hypercholesterolemia, and none were on lipid-lowering therapy.

Premature coronary artery disease (CAD) was present in 37% (n = 11) of the patient group, 57% (n = 17) had evidence of carotid artery calcification, and 16.7% (n = 5) had undergone previous aortic valve replacement.

There was a statistically significant difference in mean LDL-C and mean total cholesterol between the patient and control groups (both $P < .0001$); however, no statistically significant difference existed in mean FGF23 between the two groups ($P = .4621$) nor was there any correlation, as demonstrated in Figures 1 and 2, between FGF23 and total cholesterol, LDL-C, serum calcium, or serum phosphate in either group. In the patient group, CIMT did not correlate with FGF23 ($r = -0.2656$). Values for the various means are shown in Table 2.

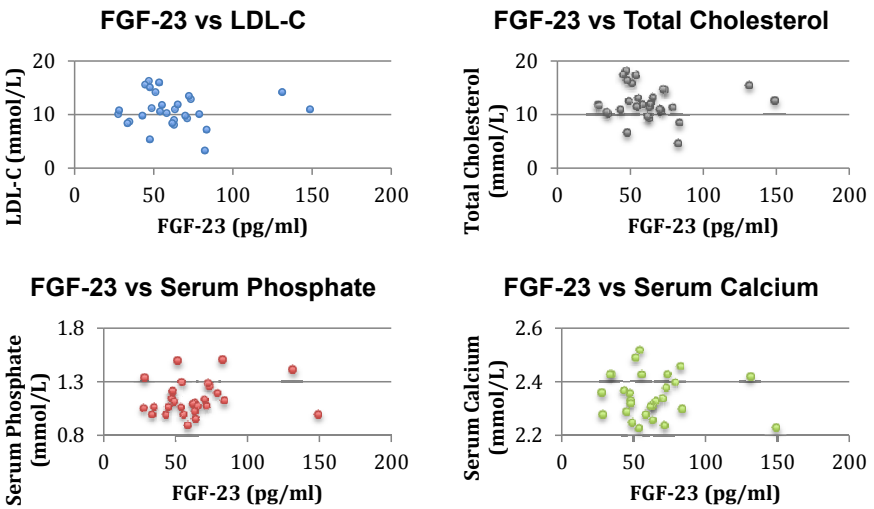

Figure 1 Correlation between FGF23 and other variables in patient group. FGF23, fibroblast growth factor-23; LDL-C, low-density lipoprotein.

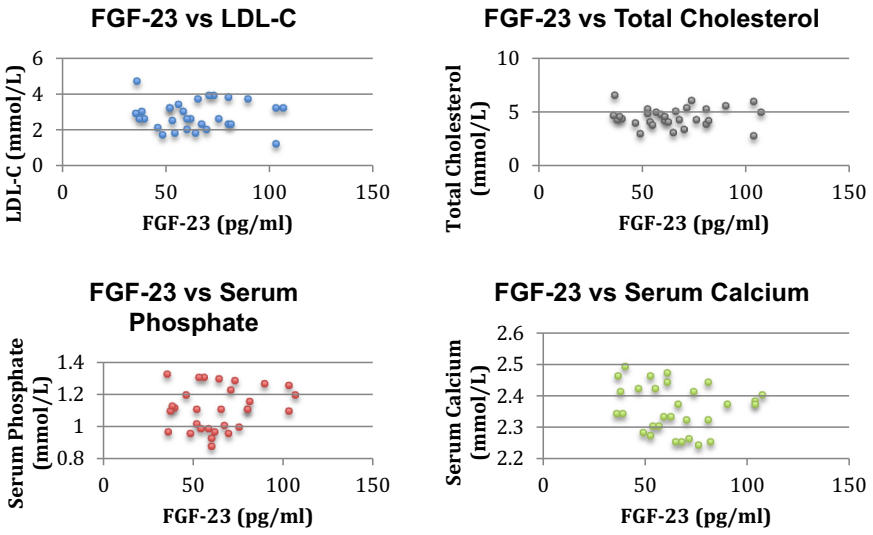

Figure 2 Correlation between FGF23 and other variables in control group. FGF23, fibroblast growth factor-23; LDL-C, low-density lipoprotein.

The difference in mean FGF23 in the patient group for those with and without CAD (67.82 pg/mL vs 59.10 pg/mL), those with and without history of aortic valve replacement (74.47 pg/mL vs 59.49 pg/mL), and those with and without carotid artery calcification (59.44 pg/mL vs 70.71 pg/mL) was not significant ($P = .4516$, $P = .4791$, and $P = .3061$, respectively). This is represented in Figure 3.

Discussion

Patients with HoFH are known to undergo severe premature vascular calcification with fatal complications early on in life. Multiple studies have been done to assess this calcification and potential causative factors; however, as far as we are aware, no studies have been reported to date, to assess FGF23 in patients with HoFH.[5,8–11,19–21]

The lack of a statistically significant difference in FGF23 between patient and control groups suggests that FGF23 does not play a major role in the vascular calcification seen in patients with FH. This is an important finding as it shows that the pathophysiology of the vascular calcification seen in patients with FH differs from that seen in patients with end-stage renal disease.[6–8,10] Vascular calcification seen in patients with renal failure occurs in the media of blood vessels, and it is this vascular calcification that is associated with elevated levels of FGF23 as opposed to the intimal calcification seen in patients with dyslipidemia.[8–10]

Similarly, FGF23 did not correlate with any lipid variables (in either patient or control groups) nor was there a correlation with CIMT or carotid artery calcification in the patient group. Interestingly there was no correlation between FGF23 and phosphate in either group despite evidence of a strong correlation in previous studies.[22,23]

Statins remain the cornerstone of therapy in the management of FH as they have been shown to delay the onset of cardiovascular disease in these patients.[7,21,24,25] However, despite proven benefits in causing atherosclerotic plaque regression, multiple studies have shown no effect on aortic and coronary calcification that is already present.[5,8,26]

Given the lack of a statistically significant difference in FGF23 between study groups, it is not surprising that neither total cholesterol nor LDL-C correlated with FGF23. This lack of correlation seems to suggest that FGF23 is not

Table 2 Serum mean values

Variable	Mean		Statistical significance (P)
	Patients	Controls	
Total cholesterol mmol/L (mg/dL)	12.02 ± 3.35 (464.09 ± 129.34)	4.68 ± 0.96 (180.69 ± 37.07)	<.0001
LDL-cholesterol mmol/L (mg/dL)	10.51 ± 3.31 (405.79 ± 127.80)	2.90 ± 0.82 (111.97 ± 31.66)	<.0001
FGF23 pg/mL	62.07 ± 26.42	63.69 ± 19.84	.4621
Calcium mmol/L (mg/dL)	2.32 ± 0.07 (9.33 ± 0.28)	2.36 ± 0.07 (9.44 ± 0.28)	.3331
Phosphate mmol/L (mg/dL)	1.14 ± 0.14 (3.53 ± 0.43)	1.13 ± 0.14 (3.50 ± 0.43)	.4886

FGF23, fibroblast growth factor-23; LDL, low-density lipoprotein.

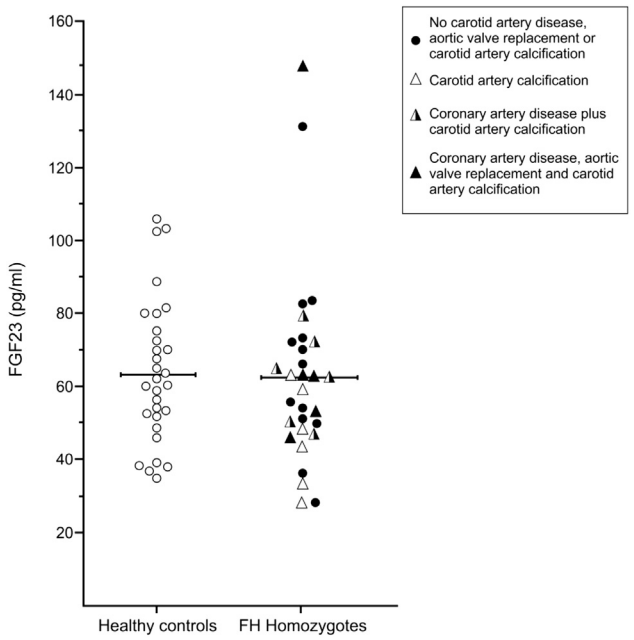

Figure 3 Scatter plot showing individual and mean FGF23 levels in healthy controls and in subjects with HoFH. FGF23, fibroblast growth factor-23; FH, familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia.

implicated in the two-hit hypothesis, proposed by both Fantus[8] and Awan,[9] described previously.

The difference between serum calcium and phosphate was not statistically significant between the 2 groups in this study. Interestingly, there was a statistically significant negative correlation between serum calcium and CIMT but not between serum phosphate and CIMT. The low-powered nature of this study makes it difficult to draw firm conclusions from this finding. Previous studies have shown that high serum phosphate is associated with an increased risk of cardiovascular disease, even in patients without CKD.[27] The evidence for the association between serum calcium and cardiovascular disease is conflicting with some studies finding no association between the 2 and others[28] finding that patients with previous CAD had elevated levels of serum calcium compared to those without.

Considering the role of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] in calcium and phosphate metabolism, it is unfortunate that there was insufficient serum to assess levels in the study cohort. Furthermore, deficiency of vitamin D has been associated with an increased risk for cardiovascular disease and cardiovascular mortality.[29] An inverse correlation between 1,25$(OH)_2$D and vascular (both coronary and aortic) calcification has been noted in both FH and non-FH patients in the literature with the negative correlation being stronger in FH patients.[30]

This study had a number of limitations that must be taken into account when interpreting the data. The study had a small sample size; however, HoFH is a rare condition and, to the best of our knowledge, this is one of the largest cohorts of FH in the world, so expanding the study would have required a multicentre study design. The nature of the study meant that we were unable to assess for coronary artery calcification in all patients as has been done in other studies. Similarly parathyroid hormone and 1,25$(OH)_2$D were not measured, and thus, their correlation with FGF23, serum calcium, and serum phosphate could not be assessed.

However, despite these limitations, our results show that further research is needed into the pathophysiology of vascular calcification in both FH patients and all patients with lipid-related atherosclerosis.

Given the known links between vitamin D and vascular calcification,[29,30] it may be important to evaluate vitamin D status in this patient cohort. Furthermore, there may be a role for investigating newer biomarkers such as proprotein convertase subtilisin/kexin type and lipoprotein(a) and their role in the vascular calcification seen in FH.

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Authors' contributions: JMZ. and FJR. were responsible for data collection and processing. ARI. was responsible for sample processing and measurement of FGF23. All authors contributed equally to the preparation of the manuscript and all authors have approved the final article.

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References

1. Jenkins T. Medical genetics in South Africa. *J Med Genet*. 1990;27:760–779.
2. Ramachandran S, Deshpande O, Roseman CC, Rosenberg NA, Feldman MW, Cavalli-Sforza LL. Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. *Proc Natl Acad Sci U S A*. 2005;102:15942–15947.
3. Rubinsztein DC, Van der Westhuyzen DR, Coetzee GA. Monogenic primary hypercholesterolaemia in South Africa. *S Afr Med J*. 1994;84:339.
4. Hobbs HH, Russell DW, Brown MS, Goldstein JL. The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. *Annu Rev Genet*. 1990;24:133–170.
5. Awan Z, Alrasadi K, Francis GA, et al. Vascular calcifications in homozygote familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol*. 2008;28:777–785.
6. Gidding SS, Champagne MA, de Ferranti SD, et al. The agenda for familial hypercholesterolemia: a scientific statement from the American Heart Association. *Circulation*. 2015;132:2167–2192.

7. Cuchel M, Bruckert E, Ginsberg HN, et al. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. *Eur Heart J*. 2014;35:2146–2157.
8. Fantus D, Awan Z, Seidah NG, Genest J. Aortic calcification: novel insights from familial hypercholesterolemia and potential role for the low-density lipoprotein receptor. *Atherosclerosis*. 2013;226:9–15.
9. Awan Z, Alwaili K, AlShahrani A, et al. Calcium homeostasis and skeletal integrity in individuals with familial hypercholesterolemia and aortic calcification. *Clin Chem*. 2010;56:1599–1607.
10. Wiegman A, Gidding SS, Watts GF, et al. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. *Eur Heart J*. 2015;36:2425–2437.
11. Allen JM, Thompson GR, Myant NB, Steiner R, Oakley CM. Cardiovascular complications of homozygous familial hypercholesterolaemia. *Br Heart J*. 1980;44:361–368.
12. Xiao Y, Peng C, Huang W, et al. Circulating fibroblast growth factor 23 is associated with angiographic severity and extent of coronary artery disease. *PLoS One*. 2013;8:e72545.
13. Turan MN, Kircelli F, Yaprak M, et al. FGF23 levels are associated with vascular calcification, but not with atherosclerosis, in hemodialysis patients. *Int Urol Nephrol*. 2016;48:609–617.
14. Nasrallah MM, El-Shehaby AR, Salem MM, Osman NA, El Sheikh E, Sharaf El Din UA. Fibroblast growth factor-23 (FGF23) is independently correlated to aortic calcification in haemodialysis patients. *Nephrol Dial Transplant*. 2010;25:2679–2685.
15. Desjardins L, Liabeuf S, Renard C, et al. FGF23 is independently associated with vascular calcification but not bone mineral density in patients at various CKD stages. *Osteoporos Int*. 2012;23:2017–2025.
16. Schoppet M, Hofbauer LC, Brinskelle-Schmal N, et al. Serum level of the phosphaturic factor FGF23 is associated with abdominal aortic calcification in men: the STRAMBO study. *J Clin Endocrinol Metab*. 2012;97:E575–E583.
17. Freedman BI, Divers J, Russell GB, et al. Plasma FGF23 and calcified atherosclerotic plaque in African Americans with type 2 diabetes mellitus. *Am J Nephrol*. 2015;42:391–401.
18. Knopfholz J, Disserol CC, Pierin AJ, et al. Validation of the Friedewald formula in patients with metabolic syndrome. *Cholesterol*. 2014;2014:261878.
19. Morrisett JD, Vickers KC. Vascular calcification in homozygote familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol*. 2008;28:606–607.
20. Tang FT, Chen SR, Wu XQ, et al. Hypercholesterolemia accelerates vascular calcification induced by excessive vitamin d via oxidative stress. *Calcif Tissue Int*. 2006;79:326–339.
21. Najam O, Ray KK. Familial hypercholesterolemia: a review of the natural history, diagnosis, and management. *Cardiol Ther*. 2015;4:25–38.
22. Weber TJ, Liu S, Indridason OS, Quarles LD. Serum FGF23 levels in normal and disordered phosphorus homeostasis. *J Bone Miner Res*. 2003;18:1227–1234.
23. Quarles LD. Role of FGF23 in vitamin D and phosphate metabolism: implications in chronic kidney disease. *Exp Cell Res*. 2012;318:1040–1048.
24. Raal FJ, Santos RD. Homozygous familial hypercholesterolemia: current perspectives on diagnosis and treatment. *Atherosclerosis*. 2012;223:262–268.
25. Jensen MD, Ryan DH, Apovian CM, et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults. *Circulation*. 2014;129:S102–S138.
26. Alrasadi K, Alwaili K, Awan Z, Valenti D, Couture P, Genest J. Aortic calcifications in familial hypercholesterolemia: Potential role of the low-density lipoprotein receptor gene. *Am Heart J*. 2009;157:170–176.
27. Dhingra R, Sullivan LM, Fox CS, et al. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. *Arch Intern Med*. 2007;167:879–885.
28. Jorde R, Sundsfjord J, Fitzgerald P, Bonaa KH. Serum calcium and cardiovascular risk factors and diseases: the Tromso study. *Hypertension*. 1999;34:484–490.
29. de Boer IH, Kestenbaum B, Shoben AB, Michos ED, Sarnak MJ, Siscovick DS. 25-hydroxyvitamin D levels inversely associate with risk for developing coronary artery calcification. *J Am Soc Nephrol*. 2009;20:1805–1812.
30. Watson KE, Abrolat ML, Malone LL, et al. Active serum vitamin D levels are inversely correlated with coronary calcification. *Circulation*. 1997;96:1755–1760.