Title

High plasma FGF21 levels predicts major cardiovascular events in patients treated with atorvastatin (from the Treating to New Targets [TNT] Study)

Authors

Ong, Kwok Leung
Hui, Nicholas
Januszewski, Andrzej S
et al.

Publication Date

2019-04-01

DOI

10.1016/j.metabol.2018.11.006

Peer reviewed

Metabolism Clinical and Experimental 93 (2019) 93–99

Contents lists available at ScienceDirect

Metabolism Clinical and Experimental

journal homepage: www.metabolismjournal.com

High plasma FGF21 levels predicts major cardiovascular events in patients treated with atorvastatin (from the Treating to New Targets [TNT] Study)☆,☆☆

Kwok Leung Ong [a,*], Nicholas Hui [a], Andrzej S. Januszewski [b], Nadeem O. Kaakoush [a], Aimin Xu [c,d], Rana Fayyad [e], David A. DeMicco [e], Alicia J. Jenkins [b], Anthony C. Keech [b], David D. Waters [f], Philip J. Barter [a], Kerry-Anne Rye [a]

[a] Lipid Research Group, School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia
[b] National Health and Medical Research Council Clinical Trials Centre, University of Sydney, Sydney, NSW, Australia
[c] Department of Medicine, University of Hong Kong, Hong Kong, China
[d] State Key Laboratory of Pharmaceutical Biotechnology, University of Hong Kong, Hong Kong, China
[e] Pfizer, Inc., New York, NY, United States
[f] Division of Cardiology, San Francisco General Hospital, the University of California at San Francisco, San Francisco, CA, United States

ARTICLE INFO

Article history:
Received 5 October 2018
Accepted 13 November 2018

ABSTRACT

Background: Higher plasma fibroblast growth factor 21 (FGF21) levels predict incident cardiovascular events in type 2 diabetes patients. However, whether FGF21 levels predict cardiovascular events in statin-treated patients in the general population is unknown. We investigated whether FGF21 levels predict major cardiovascular event (MCVE) in the Treating to New Targets (TNT) trial participants.

Methods: After 8-week run-in on atorvastatin 10 mg/day, 10,001 patients with stable coronary disease in the TNT trial were randomized to 10 mg or 80 mg/day of atorvastatin for a median of 4.9 years. We analyzed data from 1996 patients with plasma FGF21 levels measured at randomization. Among them, 1835 patients had FGF21 measured one-year post-randomization.

Results: Higher ln-transformed FGF21 levels at randomization were associated with higher risk of incident MCVE (adjusted hazards ratio per SD increase = 1.18, $P = 0.019$). At 1-year post-randomization, FGF21 levels were lower in patients randomized to receive 80 mg versus 10 mg atorvastatin (186.9 versus 207.5 pg/mL respectively, $P = 0.006$). Higher ln-transformed FGF21 levels at 1-year post-randomization were also associated with higher subsequent risk of MCVEs (adjusted hazards ratio per SD increase = 1.24, $P = 0.009$). However, changes in FGF21 levels over 1-year were not related to subsequent MCVE risk. FGF21 levels had significant incremental value in net reclassification improvement in MCVE risk prediction.

Conclusions: Higher plasma FGF21 levels are associated with higher CVD risk in statin-treated high-risk patients. Higher dose atorvastatin is associated with a reduction in FGF21 levels. FGF21 provides incremental value in CVD risk prediction in statin-treated patients.

1. Introduction

Fibroblast growth factor 21 (FGF21), which is mainly produced and secreted by the liver [1,2], plays an important role in glucose and lipid metabolism [3–5]. It has anti-inflammatory, anti-diabetic and hypolipidaemic effects in animal studies [1,2]. However, in clinical studies, circulating FGF21 levels are often elevated in obesity, dyslipidemia, insulin resistance, metabolic syndrome, type 2 diabetes (T2D), non-alcoholic fatty liver disease and coronary artery disease, and has been identified as a potential biomarker for the early detection of cardiometabolic dysfunction [1,2]. Elevated FGF21 levels in this context may be due to FGF21 resistance resulting from impaired FGF21 signaling or compensatory responses to the underlying metabolic stress [1,2].

Abbreviations: ALT, alanine aminotransferase; AST, Aspartate aminotransferase; BMI, body mass index; CAD, coronary artery disease; CHD, coronary heart disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FFA, free fatty acids; FGF21, fibroblast growth factor 21; HDL, high-density lipoprotein; HR, hazards ratio; LDL, low-density lipoprotein; MCVE, major cardiovascular event; NAFLD, non-alcoholic fatty liver disease; NRI, net reclassification improvement; T2D, type 2 diabetes; TNT, Treating to New Targets.

☆ To be presented in part at the American Heart Association Annual Scientific Sessions, Chicago, Illinois, November 2018.

☆☆ Clinical Trial Registration: NCT00327691.

* Corresponding author at: School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia.
E-mail address: oklws@yahoo.com.hk (K.L. Ong).

https://doi.org/10.1016/j.metabol.2018.11.006
0026-0495/

Elevated circulating FGF21 levels have been reported to be associated with coronary heart disease (CHD) [6], carotid atherosclerosis [7], and acute myocardial infarction [8]. In T2D patients, elevated FGF21 levels can predict the development of incident total cardiovascular disease (CVD) events [9]. However, it is unknown whether circulating FGF21 levels can predict CVD events in statin-treated patients. This is particularly important given that statins are widely used for CVD prevention, and hepatic FGF21 expression is repressed in statin-treated mice [10].

The Treating to New Targets (TNT) trial was a double-blind randomized controlled trial [11]. Briefly, 10,001 patients with stable coronary artery disease (CAD) and an low-density lipoprotein (LDL) cholesterol level off-therapy of 3.4–6.5 mmol/L (130–250 mg/dL), decreasing to <3.4 mmol/L (130 mg/dL) after an 8-week run-in period on atorvastatin 10 mg/day, were randomized to 10 mg or 80 mg/day of atorvastatin with a median follow-up period of 4.9 years [11]. The mean LDL cholesterol during follow-up was 2.6 mmol/L (101 mg/dL) in the 10 mg/day group and 2.0 mmol/L (77 mg/dL) in the 80 mg/day group. The primary endpoint of total major cardiovascular events (MCVEs) occurred in 10.9% of patients in the 10 mg group and 8.7% of patients in the 80 mg group (hazards ratio [HR] 0.78, 95% CI 0.69–0.89, $P < 0.001$) [11].

In the present study, we asked whether plasma FGF21 levels at the time of randomization (after the 8-week atorvastatin 10 mg run-in period) predict MCVEs in participants from the TNT trial. We also assessed the effect of higher dose of atorvastatin on plasma FGF21 levels at one year after randomization, and whether plasma FGF21 levels at one year predict subsequent risk of MCVEs. The underlying rationale of the study is to provide an insight into whether FGF21 is a CVD risk biomarker in statin-treated patients.

2. Material and Methods

2.1. Study Population

The study design and results of the TNT trial have been published [11]. Patients with stable CAD were randomized to two doses of atorvastatin (either 10 or 80 mg daily). Participants were recruited in 14 countries and randomization occurred between July 1998 and December 1999 [11]. The primary endpoint was MCVE, a composite of (i) CHD death, (ii) nonfatal, non–procedure-related myocardial infarction, (iii) resuscitated cardiac arrest, and (iv) fatal or nonfatal stroke. All patients gave written informed consent. The study was approved by local research ethics committees or institutional review boards at each center and was performed in accordance with the Helsinki Declaration.

Among 10,001 patients, 9960 had blood samples available at the time of randomization. Plasma FGF21 levels were measured in a random sub-sample of 2032 participants, in which informed consent was obtained for measuring non-lipid biomarkers (in addition to that originally collected for the primary study).

2.2. FGF21 Level Measurement

Blood samples were collected in 10-mL EDTA Vacutainer (BD, Franklin Lakes, New Jersey) venous blood collection tubes using standard phlebotomy practices. Immediately after collection, tubes were gently inverted six times, and centrifuged at 2000 ×*g* (10 min). Plasma samples were transferred into 8-mL freezer vials, frozen (−70 °C), shipped on dry ice, and thawed for biomarker analysis. FGF21 levels were measured from stored plasma samples obtained at randomization (after the 8-week atorvastatin 10 mg run-in period) and again one year after randomization. FGF21 levels were measured using ELISA kits from the Antibody and Immunoassay Services, University of Hong Kong, Hong Kong (www.antibody.hku.hk) as described previously [9,12]. Briefly 60 μL of plasma sample was diluted 1:1 (v:v) with assay diluent and analyzed together with quality controls as per the manufacturer's instruction. The intra-assay and inter-assay CVs were <6%. All samples were analyzed masked for participant identity, treatment allocation and time-point.

2.3. Statistical Analysis

Statistical analysis was performed using SPSS 24 (IBM, Armonk, NY) or STATA 14.0 (StataCorp, College Station, TX). Data are presented as mean ± SD or n (percentage). Comparisons of participant characteristics at the time of randomization between treatment groups or outcomes were performed using a chi-square test for categorical variables, and an independent *t*-test for continuous variables. For non-normally-distributed variables, data were presented as median (interquartile range) and compared using Wilcoxon rank sum test. Treatment allocation, age, sex, and other variables that showed a trend of difference ($P < 0.1$) in either treatment group were used as covariates in subsequent multiple Cox regression analyses.

Associations of baseline FGF21 levels with incident MCVE over the follow-up period was assessed using Cox proportional hazards regression analysis. In this analysis, for each participant that had an event, the time to event (in days) was taken as that between the randomization date and the date of the visit at which the earliest event was ascertained. For participants who remained event-free, the follow-up time was censored at last visit or last day known to be alive, whichever was later. For subjects who died, follow-up time was censored at their death date. As plasma FGF21 levels were highly skewed, data were ln-transformed in the Cox regression analyses to prevent unstable estimates of effects since extreme values may have undue influence on the estimate of the regression coefficient. Associations of FGF21 levels and change in FGF21 levels at 1-year post-randomization with subsequently incident MCVE were analyzed similarly while excluding patients with any prior event (or censored) during the first year post-randomization. Survivals were estimated by the Kaplan-Meier method and compared by the log-rank test. The proportional hazards assumption was checked using Schoenfeld residuals and no significant violation was found. In all analyses, we also investigated whether there was an interaction by treatment allocation. *P* for interaction was estimated by including the interaction term in the regression models in the full sample after adjustment for the main effects of the covariates. The incremental value of the addition of ln-transformed FGF21 levels in the Cox regression model was assessed by the change in Harrell's C-statistic using a method adapted for survival models [13]. The goodness of fit of the models was assessed using the Gronnesby and Borgan test [14]. Net reclassification improvement (NRI) were also assessed as described previously [15]. As the NRI method is highly sensitive to the chosen cut-points of risk and there are no pre-specified cut-points that can be applied to the outcome appropriately, the category-free NRI (NRI > 0) approach was utilized with both "event NRI" and "nonevent NRI" calculated [16]. NRI was calculated using a macro in SAS 9.4 (SAS Institute, Cary, NC) [17]. A two-tailed $P < 0.05$ was considered statistically significant. In all the analyses, participants with missing data were excluded.

3. Results

3.1. Clinical Characteristics

As shown in Supplementary Table 1, there were no significant difference in age, sex, body mass index (BMI) and percentage of participants receiving high-dose statin treatment between these 2032 participants and the remaining 7928 participants (all $P > 0.10$). Among these 2032 participants, results from 36 participants (1.8%) were excluded from further analysis as the FGF21 levels were below the assay limit of detection (<8.69 pg/mL). Therefore, a total of 1996 participants were included in the analysis of plasma FGF21 levels at the time of randomization. As shown in Supplementary Table 2, there were no significant difference in age, sex, BMI, percentage of participants receiving high-dose statin treatment and other clinical characteristics between these

1996 participants and the excluded 36 participants, except that excluded participants were more likely to be current smokers and had higher total cholesterol than those not excluded ($P = 0.039$ and 0.031, respectively).

Table 1 shows the clinical characteristics of the 1996 participants at the time of randomization. Clinical characteristics were similar in the groups randomized to remain on 10 mg atorvastatin ($n = 1012$, 50.7%) and to receive atorvastatin 80 mg ($n = 984$, 49.3%). Within the same treatment group, participants who developed an MCVE were more likely to be older, and have hypertension, diabetes and higher white blood cell count at time of randomization, compared to participants who did not developed an MCVE. In the 10 mg atorvastatin group, participants who developed an MCVE were also less likely to be Caucasian, and more likely to be a current smoker, have lower high-density lipoprotein (HDL) cholesterol and have higher triglyceride levels than those who did not. In the 80 mg atorvastatin group, participants who developed an MCVE were also more likely to have a higher BMI, and blood urea nitrogen levels.

3.2. FGF21 Levels at Randomization and Risk of MCVE

At randomization plasma FGF21 levels were significantly higher in participants with versus without incident MCVE (median [interquartile range]: 253.5 [164.3–398.5] vs 204.6 [127.0–316.9] respectively, $P < 0.001$). Similar significant differences was present within the 10 mg and 80 mg atorvastatin group (Table 1). Fig. 1A shows the Kaplan-Meier cumulative curves for incident MCVE over time across FGF21 level tertiles at randomization. Higher FGF21 tertiles at randomization had a higher risk of incident MCVE (log-rank test $P < 0.001$). As shown in Table 2, the association of ln-transformed FGF21 levels with incident MCVE was significant after adjusting for confounding variables, including treatment allocation, age, sex, Caucasian race, BMI, smoking status, hypertension, diabetes, HDL-cholesterol, triglycerides, blood urea nitrogen, and white blood cell count at baseline ($P = 0.019$). Similar significant results were obtained when continuous FGF21 levels were assessed as a categorical tertile variable. No significant treatment interaction was found. In a sensitivity analysis with exclusion of participants with FGF21 levels in the top and bottom 5% (Supplementary Table 3), higher FGF21 levels were still associated with higher risk of incident MCVE after adjusting for confounding variables ($P = 0.004$ for ln-transformed FGF21 levels and $P = 0.020$ for FGF21 tertiles).

3.3. Effect of High and Low Dose Atorvastatin on Plasma FGF21 Levels over One Year

Among these 1996 participants, FGF21 levels were also measured at one year after randomization in 1849 participants, of whom 14 participants (0.8%) were excluded from analysis due to FGF21 levels below the assay limit. Therefore, a total of 1835 participants were included in the analysis of FGF21 levels at one year. Table 3 shows the FGF21 levels at randomization and 1-year post-randomization, as well as the absolute and relative changes in FGF21 levels over one year by treatment groups and status of incident MCVE. At the time of randomization, there was no significant difference in FGF21 levels between treatment groups ($P = 0.83$). However, at 1-year post-randomization, FGF21 levels in the 80 mg atorvastatin group, were significantly lower than in the 10 mg atorvastatin group ($P = 0.006$). FGF21 levels decreased more significantly over time in the 80 mg atorvastatin group ($P = 0.001$), compared to the 10 mg atorvastatin group ($P = 0.034$), with both absolute and relative decreases in FGF21 levels being approximately twice that with 80 mg atorvastatin than with 10 mg atorvastatin (both $P = 0.011$). Although, in both treatment groups, the FGF21 levels at randomization and at 1-year post-randomization were significantly higher in patients with incident MCVE, compared to those without MCVE, the change in FGF21 levels did not differ significantly between participants with and without incident MCVE in each treatment group.

3.4. FGF21 Levels at One Year after Randomization and Subsequent Risk of MCVE

Fig. 1B shows the Kaplan-Meier cumulative curves for incident MCVE over time across FGF21 tertiles at 1-year post-randomization. Participants in the higher FGF21 tertiles at 1-year post-randomization had a higher subsequent risk of incident MCVE (log-rank test $P < 0.001$). As shown in Table 4, the association of ln-transformed FGF21 levels at one year with subsequent incident MCVE was significant after adjusting for confounding variables ($P = 0.009$). Similar significant results were obtained when continuous FGF21 levels were assessed as a categorical tertile variable. No significant treatment interaction was found. However, when assessing the changes in FGF21 levels over 1-year post-randomization as continuous variables, a larger relative change in FGF21 levels was associated with higher subsequent risk of MCVE ($P = 0.002$, Supplementary Table 4). However, such significant association was not found when assessing absolute changes in FGF21 level, or when assessing the changes as categorical variables (Supplementary Fig. 1 and Supplementary Tables 4 & 5).

3.5. Incremental Value of FGF21 Levels for Risk Prediction

Addition of the FGF21 levels to a model adjusted for treatment allocation, age, sex, Caucasian race, BMI, smoking status, hypertension, diabetes, HDL-cholesterol, triglycerides, blood urea nitrogen, and white blood cell count only modestly increased the C-statistic, with a borderline non-significance $P = 0.082$ (Supplementary Table 6). When assessing reclassification using the category-free NRI(>0), addition of FGF21 levels at randomization to a model with traditional cardiovascular risk factors significantly increased the NRI(>0) of 20.9% for incident MCVE (Table 5). This was the result of both correct upward reclassification of those with events (net gain in reclassification of 16.4%), and from correct downward reclassification of nonevents (net gain in reclassification of 4.5%). Similar results were obtained when assessing the incremental value of FGF21 levels at 1-year post-randomization.

4. Discussion

The present findings support FGF21 as a CVD biomarker in high risk patients, even on statins. This is the first study to investigate the association of plasma FGF21 levels and CVD events in a clinical trial of statin-treated subjects. Higher plasma FGF21 levels were associated with higher CVD risk in statin-treated patients. Plasma FGF21 levels were also decreased in the 80 mg atorvastatin group compared to the 10 mg atorvastatin group, but the changes in FGF21 levels over 1-year post-randomization were not robustly related to subsequent CVD risk over a median of 3.9 years, it follows that statin-associated FGF21 reduction do not play a major role in statin-mediated CVD risk reduction.

The role of FGF21 in CVD has been reported in earlier pre-clinical studies. Cardiomyocytes have been found to express and release FGF21 in an autocrine-paracrine manner [18,19]. FGF21's anti-inflammatory, anti-oxidative and anti-apoptotic properties of FGF21 also protect against CVD events, including cardiac hypertrophy and myocardial infarction, in mice [18–21]. Global FGF21-knockout mice are also more likely to develop cardiac hypertrophy than wild-type mice [18].

Despite exhibiting cardioprotective effects in cell culture and pre-clinical animal studies, elevated circulating FGF21 levels have been reported in patients with CHD [22], carotid atherosclerosis [7,23], subclinical atherosclerosis [24], and acute myocardial infarction [8]. These observations could be due to the presence of FGF21 resistance in these conditions. In rodent models, cardiomyocytes can secrete FGF21 in response to global cardiac ischemia [19]. However, some human studies have shown no associations between FGF21 levels and CVD [25,26]. Nevertheless, these studies are limited by having a cross-sectional design and relatively small sample size ($n < 420$).

Table 1
Clinical characteristics at time of randomization ($n = 1996$).

Clinical characteristics	n	Atorvastatin 10 mg			Atorvastatin 80 mg			$P^{\dagger}$
		With MCVE	Without MCVE	P value*	With MCVE	Without MCVE	P value*	
n		137	875	–	112	872	–	–
Age, years	1996	63.3 (9.1)	60.8 (8.8)	0.003	63.4 (7.8)	61.2 (8.9)	0.006	0.53
Female, n (%)	1996	28 (20.4)	185 (21.1)	0.85	22 (19.6)	163 (18.7)	0.81	0.21
Caucasian race, n (%)	1996	117 (85.4)	814 (93.0)	0.002	106 (94.6)	795 (91.2)	0.21	0.73
Body mass index, kg/m^2	1993	29.2 (5.1)	28.5 (4.7)	0.124	29.9 (5.7)	28.2 (4.3)	0.003	0.39
Smoking status, n (%)	1996	–	–	0.035	–	–	0.98	0.23
Current		26 (19.0)	98 (11.2)	–	12 (10.7)	93 (10.7)	–	–
Former		84 (61.3)	581 (66.4)	–	73 (65.2)	561 (64.3)	–	–
Hypertension, n (%)	1996	90 (65.7)	492 (56.2)	0.037	77 (68.8)	473 (54.2)	0.004	0.47
Systolic blood pressure, mmHg	1995	133.7 (16.7)	130.3 (15.9)	0.020	133.7 (17.8)	129.6 (17.1)	0.019	0.39
Diastolic blood pressure, mmHg	1995	76.5 (8.6)	77.2 (9.6)	0.40	77.3 (10.6)	76.8 (9.7)	0.59	0.55
Diabetes mellitus, n (%)	1996	34 (24.8)	112 (12.8)	<0.001	28 (25.0)	125 (14.3)	0.003	0.48
Fasting glucose, mg/dL	1995	105 (95–127)	98 (90–110)	<0.001	102 (93–116)	98 (91–111)	0.035	0.69
Total cholesterol, mg/dL	1995	175.6 (23.7)	174.0 (24.0)	0.48	176.8 (24.6)	173.7 (22.9)	0.17	0.85
LDL cholesterol, mg/dL	1994	98.7 (15.6)	96.9 (17.4)	0.25	98.4 (17.3)	96.5 (16.6)	0.27	0.62
HDL cholesterol, mg/dL	1995	44.4 (9.9)	47.5 (10.9)	0.002	47.2 (11.4)	47.9 (11.2)	0.57	0.14
Triglycerides, mg/dL	1995	150 (107–201)	133 (102–179)	0.028	137 (101–191)	133 (100–180)	0.25	0.45
Blood urea nitrogen, mg/dL	1996	16 (14–19)	16 (14–19)	0.49	17 (14–21)	16 (14–19)	0.022	0.45
White blood cell, 10^3/mm^3	1996	6.5 (5.5–7.6)	6.1 (5.2–7.2)	0.007	6.5 (5.8–7.6)	6.1 (5.1–7.0)	<0.001	0.17
ALT, U/L	1996	15 (12−21)	16 (12−20)	0.88	15 (12–20)	16 (12–20)	0.36	0.76
AST, U/L	1996	16 (13–19)	16 (13–19)	0.59	16 (13–19)	16 (13–19)	0.72	0.57
eGFR, mL/min/1.73 m^2‡	1996	62.2 (13.2)	64.1 (11.4)	0.11	62.7 (14.1)	64.3 (11.8)	0.25	0.65
FGF21, pg/mL	1996	258.9 (173.1–386.9)	205.4 (126.6–313.7)	<0.001	241.2 (162.6–427.2)	203.1 (127.6–320.8)	0.005	0.59

Data are expressed as mean (SD), n (%), or median (interquartile range). Baseline characteristics at randomization were using a chi-square test for categorical variables, and a Wilcoxon rank sum test or t-test for continuous variables, where appropriate.
Abbreviations: AST, Aspartate aminotransferase; ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate; FGF21, fibroblast growth factor 21; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MCVE, major cardiovascular event.
* P value for patients who experienced MCVE versus those who did not within each treatment group.
† P value for patients randomized to remain on 10 mg atorvastatin versus patients up-titrated to 80 mg atorvastatin.
‡ Calculated using the creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

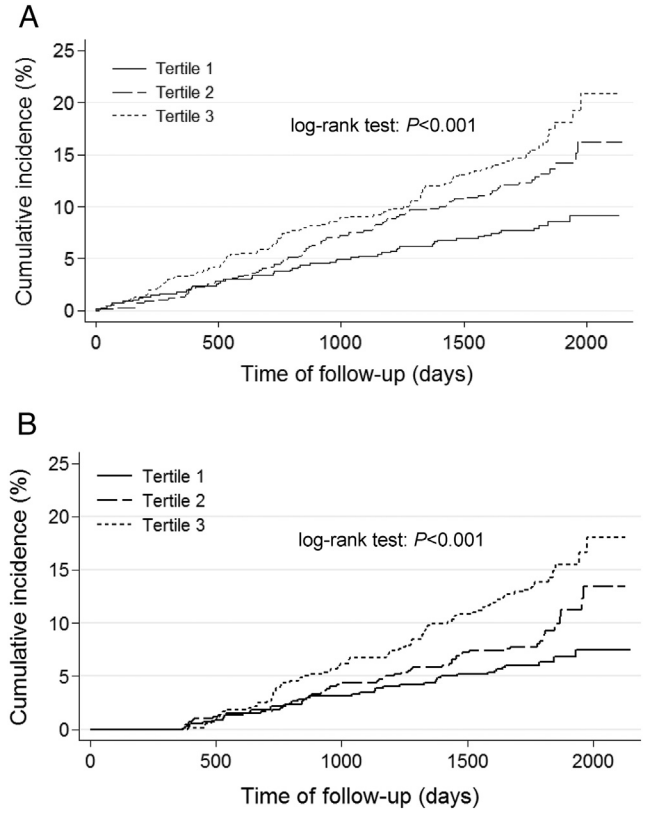

Fig. 1. Kaplan-Meier cumulative incidence curves for MCVEs across tertiles of FGF21 levels at (A) randomization and (B) 1-year post-randomization. Abbreviations: FGF21, fibroblast growth factor 21; MCVE, major cardiovascular event.

The predictive value of FGF21 levels for CV-related adverse events has been explored in earlier prospective studies. In a study of 87 T2D patients, above median FGF21 levels at baseline predicted higher CV morbidity and mortality, but not total mortality, over 2 years follow-up [27]. In another study of 1668 CAD patients, both lower and higher FGF21 levels predicted all-cause and CVD mortality over a median of 4.9 years follow-up, in which the smallest HR was associated with second quartile of FGF21 levels [28]. In 253 Chinese subjects that underwent a coronary angiography, serum FGF21 levels were independently associated with prevalent CAD [6] and left ventricular systolic dysfunction at baseline, and higher risk of cardiac death over 5 years [29]. In the same cohort of subjects, higher serum FGF21 levels predicted development of major adverse cardiovascular events in 169 patients with CAD at baseline [30]. In another study of 3528 T2D patients, the optimal cut-off value of serum FGF21 levels at baseline was 206 pg/mL, levels above which predicted incident CHD over a median follow-up of 3.8 years [31]. In our previous study of 9697 T2D patients not on statin at baseline, higher plasma FGF21 levels predicted CVD events over 5 years follow-up [9]. When considered together, these results support FGF21 levels as a potential CVD biomarker, though few participants were on statin therapy.

In the present study, higher plasma FGF21 levels at randomization were associated with a higher risk of incident MCVE after adjusting for confounding variables including demographics and traditional CVD risk factors. Similar results were obtained with FGF21 levels assessed as either a continuous or categorical (tertile) variable. Similar results were obtained when subjects with FGF21 levels in the top or bottom 5% were excluded in the sensitivity analysis, or based on FGF21 levels at 1-year post-randomization. This supports the robustness of our findings. The present larger study extends previous findings by showing that plasma FGF21 levels can predict CVD events in high-CVD risk statin-treated patients with stable CHD. Moreover, as no significant treatment interaction was found in all the analyses, it follows that the association of FGF21 levels with MCVEs did not differ significantly between low- and high-dose atorvastatin. This also suggests that FGF21-

Table 2
Association of FGF21 levels at randomization with risk of MCVEs (n = 1996).

Model	n	Outcome, n (%)	All patients		Atorvastatin 10 mg		Atorvastatin 80 mg		*P* for treatment interaction
			HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	
ln-transformed FGF21*									
Unadjusted model	1996	249 (12.5)	1.32 (1.17–1.49)	<0.001	1.33 (1.14–1.57)	<0.001	1.31 (1.10–1.57)	0.003	0.92
Adjusted model†	1992	247 (12.4)	1.18 (1.03–1.36)	0.019	1.19 (0.99–1.44)	0.062	1.13 (0.92–1.39)	0.25	0.93
FGF21 tertile									
Unadjusted model									0.39
Tertile 1 (≤155.0 pg/mL)	665	55 (8.3)	1.00 (referent)	–	1.00 (referent)	–	1.00 (referent)	–	
Tertile 2 (155.1–280.9 pg/mL)	666	88 (13.2)	1.67 (1.19–2.34)	0.003	1.46 (0.92–2.32)	0.10	1.93 (1.17–3.17)	0.010	–
Tertile 3 (≥281.0 pg/mL)	665	106 (15.9)	2.10 (1.51–2.90)	<0.001	2.22 (1.44–3.41)	<0.001	1.95 (1.19–3.21)	0.008	–
Overall *P* value			–	<0.001	–	0.001	–	0.016	–
Adjusted model†									0.35
Tertile 1 (≤155.0 pg/mL)	664	54 (8.3)	1.00 (referent)	–	1.00 (referent)	–	1.00 (referent)	–	
Tertile 2 (155.1–280.9 pg/mL)	665	88 (13.2)	1.45 (1.03–2.05)	0.034	1.28 (0.80–2.06)	0.30	1.74 (1.05–2.89)	0.033	–
Tertile 3 (≥281.0 pg/mL)	663	105 (15.9)	1.56 (1.10–2.22)	0.013	1.71 (1.07–2.73)	0.025	1.37 (0.80–2.34)	0.25	–
Overall *P* value			–	0.037	–	0.071	–	0.099	–

Abbreviations: FGF21, fibroblast growth factor 21; HR, hazards ratio; MCVE, major cardiovascular event.
* Data are expressed as HR (95% CI) in terms of per SD (0.8016) increase in ln-transformed levels.
† Data were adjusted for treatment allocation, age, sex, Caucasian race, body mass index, smoking status, hypertension, diabetes, high-density lipoprotein cholesterol, triglycerides, blood urea nitrogen, and white blood cell count at baseline. Four patients (two with MCVE events) were excluded from adjusted model due to missing baseline body mass index for 3 subjects and missing baseline high-density lipoprotein cholesterol and triglycerides for 1 subject.

based therapies may confer cardioprotection in statin-treated patients, which merits study. Statins are commonly used for CVD prevention. In the present study, FGF21 levels decreased significantly after 1-year of atorvastatin, and the decreases in FGF21 levels were twice that with high-dose atorvastatin than with low-dose atorvastatin. We speculate this reduction could be due to reduced FGF21 resistance, but underlying mechanisms of statin-associated reduction in FGF21 levels are not clear. FGF21 is mainly produced and secreted by the liver. Notably, liver enzymes can be elevated by statins [32], in keeping with altered liver homeostasis, which may affect FGF21 production. A recent meta-analysis of controlled clinical trials has shown reduction of plasma free fatty acids (FFAs) by atorvastatin [33], and FFAs can stimulate FGF21 expression in vitro [34]. Short-term simvastatin suppresses hepatic FGF21 expression and reduces circulating FGF21 levels in mice as well as FGF21 expression in mouse primary hepatocytes [10]. All these findings are consistent with our clinical study results.

In this study, we also assessed the relationship of changes in FGF21 levels over 1-year post-randomization with subsequent risk of MCVEs and found no robustly significant associations. This suggests that FGF21 may not play a major causal role in the development of MCVEs over the short-term, even though its circulating levels can predict in future MCVEs. However, it should be noted that the change in FGF21 levels found was small compared to the supraphysiological doses that were used in two proof-of-concept clinical trials of the FGF21 variants, LY2405319 and PF-05231023 [35,36]. In those trials, treatment with FGF21 variants improved body weight, lipid and adiponectin levels in overweight/obese T2D patients [35,36]. Further studies are warranted to investigate the effects of these FGF21 variants on CVD risk reduction.

Our study suggests FGF21 may be a CVD biomarker with potential prognostic value as assessed by its ability to improve net reclassification. Although FGF21 levels did not result in a significant change in the C-statistic, the C-statistic is a less sensitive measure than the NRI(>0) approach [15]. Further studies are needed to compare the prognostic value of FGF21 with other biomarkers, especially NT-proBNP, as well as their prognostic ability with considered in combination.

The present study has several strengths. It takes advantage of the large sample size, well-defined inclusion and exclusion criteria, the well-characterized double-blinded parallel study design and the uniform follow-up of the TNT trial, in which all patients took low-dose atorvastatin for 8 weeks pre-randomization. There are also some limitations. Participants were not further stratified into subgroups based on the number of coronary arteries involved or degree of stenosis, however another cross-sectional study found no significant difference in plasma FGF21 levels among CHD patients by numbers of stenosed coronary arteries involved [26]. The present study was a post-hoc analysis of the TNT trial, though all analyses were performed after pre-stating

Table 3
FGF21 levels at randomization and 1-year post-randomization (*n* = 1835).

Parameter	Atorvastatin 10 mg				Atorvastatin 80 mg				*P* value‡
	All	With MCVE	Without MCVE	*P* value†	All	With MCVE	Without MCVE	*P* value†	
n	946	120	826	–	889	93	796	–	–
FGF21, pg/ml									
Time of randomization	213.8 (132.7–328.0)	257.8 (156.5–387.8)	208.4 (129.8–318.2)	0.002	209.5 (134.1–325.7)	245.9 (164.6–398.5)	207.7 (132.3–323.8)	0.015	0.83
1-year post-randomization	207.5 (127.2–330.1)	277.3 (154.9–383.4)	200.2 (122.7–313.5)	<0.001	186.9 (114.9–301.9)	227.3 (138.1–365.9)	180.1 (114.6–295.9)	0.008	0.006
P for change*	0.034	0.34	0.054	–	<0.001	0.34	<0.001	–	–
Change in FGF21 levels									
Absolute change, pg/mL	−10.8 (−78.8 to 60.1)	−13.7 (−100.8 to 82.0)	−9.4 (−75.8 to 57.7)	0.68	−19.6 (−96.0 to 40.2)	−13.2 (−97.7 to 61.7)	−20.8 (−95.4 to 40.0)	0.51	0.011
Relative change, %	−4.7 (−34.3 to 37.3)	−3.5 (−38.1 to 38.8)	−4.8 (−34.0 to 37.3)	0.87	−10.5 (−39.0 to 27.9)	−8.2 (−31.8 to 36.9)	−11.1 (−39.4 to 27.3)	0.27	0.011

Data are expressed as median (interquartile range).
Abbreviations: FGF21, fibroblast growth factor 21; MCVE, major cardiovascular event.
* *P* value for the difference in FGF21 levels between time of randomization and 1-year post-randomization within each group using Wilcoxon signed rank test.
† *P* value for patients who experienced MCVEs versus those who did not in both treatment groups using Wilcoxon rank sum test.
‡ *P* value for patients randomized to remain on 10 mg atorvastatin versus patients up-titrated to 80 mg atorvastatin using Wilcoxon rank sum test.

Table 4
Association of FGF21 levels at 1-year post-randomization with subsequent risk of MCVE ($n = 1801$).

Model	n	Outcome, n (%)	All patients		Atorvastatin 10 mg		Atorvastatin 80 mg		*P* for treatment interaction
			HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	
ln-transformed FGF21 (per SD increase)*									
Unadjusted model	1801	180 (10.0)	1.40 (1.22–1.61)	<0.001	1.41 (1.17–1.71)	<0.001	1.38 (1.12–1.69)	0.002	0.89
Adjusted model†	1797	178 (9.9)	1.24 (1.05–1.46)	0.009	1.27 (1.00–1.60)	0.046	1.17 (0.93–1.47)	0.19	0.99
FGF21 tertile									
Unadjusted model									0.66
Tertile 1 (≤143.5 pg/mL)	600	39 (6.5)	1.00 (referent)	–	1.00 (referent)	–	1.00 (referent)	–	
Tertile 2 (143.6–265.5 pg/mL)	601	56 (9.3)	1.47 (0.98–2.22)	0.063	1.65 (0.91–3.00)	0.098	1.33 (0.75–2.34)	0.33	–
Tertile 3 (≥265.6 pg/mL)	600	85 (14.2)	2.33 (1.59–3.40)	<0.001	2.78 (1.61–4.83)	<0.001	1.92 (1.13–3.27)	0.017	–
Overall *P* value			–	<0.001	–	<0.001	–	0.050	–
Adjusted model†									0.77
Tertile 1 (≤143.5 pg/mL)	599	38 (6.3)	1.00 (referent)	–	1.00 (referent)	–	1.00 (referent)	–	
Tertile 2 (143.6–265.5 pg/mL)	600	56 (9.3)	1.34 (0.88–2.04)	0.18	1.61 (0.87–2.99)	0.13	1.11 (0.62–1.99)	0.72	–
Tertile 3 (≥265.6 pg/mL)	598	84 (14.1)	1.70 (1.12–2.60)	0.013	2.07 (1.12–3.83)	0.021	1.31 (0.72–2.37)	0.38	–
Overall *P* value			–	0.045	–	0.068	–	0.66	–

1835 patients had valid plasma FGF21 levels at 1-year post-randomization, but 34 patients with any prior event over year one were excluded from the analysis, resulting in a total sample size of 1801 patients.
Abbreviations: FGF21, fibroblast growth factor 21; HR, hazards ratio; MCVE, major cardiovascular event.
* Data are expressed as HR (95% CI) in terms of per SD (0.8045) increase in ln-transformed levels.
† Data were adjusted for treatment allocation, age, sex, Caucasian race, body mass index, smoking status, hypertension, diabetes, high-density lipoprotein cholesterol, triglycerides, blood urea nitrogen, and white blood cell count at baseline. Four patients (two with MCVE events) were excluded from adjusted model due to missing baseline BMI for 3 subjects and missing baseline high-density lipoprotein cholesterol and triglycerides for 1 subject.

hypotheses and as per a pre-specified statistical analysis plan. Plasma samples used for FGF21 measurement were stored at −80 °C for >15 years, and we cannot exclude the possibility of confounding effects due to long-term sample storage. However, in our previous pilot study, serum FGF21 levels were stable after 1–6 freeze-thaw cycles a CV of 8.1% [12]. In an exploratory analysis, we did not find any correlation between FGF21 levels and length of sample storage (Spearman correlation coefficient = 0.008, $P = 0.72$). During the FGF21 ELISA assay, not all samples from the same participant were analyzed in the same analytical run. However, the intra-assay and inter-assay CVs were small, and the potential bias should be random in direction and weaken the observed association.

Table 5
Assessing the incremental value of ln-transformed FGF21 levels using the category-free NRI (NRI >0) approach.

Outcome	FGF21 at randomization	FGF21 at 1-year post-randomization
n	1992‡	1797‡
NRI (>0)	0.2086	0.2071
SE	0.0621	0.0704
P value	<0.001	0.003
Event NRI (>0)*	0.1635	0.1319
SE	0.0286	0.0329
P value	<0.001	<0.001
Non-event NRI (>0)†	0.0451	0.0752
SE	0.0121	0.0126
P value	<0.001	<0.001

Comparison are to be made for the addition of FGF21 (ln-transformed) to a model containing treatment allocation, age, sex, Caucasian race, body mass index, smoking status, hypertension, diabetes, high-density lipoprotein cholesterol, triglycerides, blood urea nitrogen, and white blood cell count at baseline. The category-less NRI(>0) was calculated to quantify the improvement gained due to correct upward or downward change in predicted risks and is calculated as the proportion of event patients with correct upward or downward change minus incorrect upward or downward change plus the corresponding proportion among non-event patients. Values above zero for the NRI indicate improved risk prediction and discrimination with the addition of FGF21 to the model.
Abbreviations: FGF21, fibroblast growth factor 21; NRI, net reclassification improvement.
* Percentage correctly reclassified among subjects who had events.
† Percentage correctly reclassified among subjects who did not have events.
‡ Four patients (two with MCVE events) were excluded from adjusted model due to missing baseline BMI for 3 subjects and missing baseline high-density lipoprotein cholesterol and triglycerides for 1 subject.

In summary, our TNT trial based study has demonstrated that plasma FGF21 levels can predict incident MCVE over 5 years in statin-treated patients with stable CHD. Our findings support the use of FGF21 as a potential CVD risk biomarker in high-risk, statin-treated patients.

Author Contributions

K.L.O., D.A.D., D.D.W., P.J.B. and K.A.R. conceived and designed the study. K.L.O., A.S.J., and A.X. contributed to FGF21 level measurement. K.L.O., N.H., N.O.K. and R.F. contributed to data analysis. K.L.O., N.O.K., A.J.J. and A.C.K. contributed to data interpretation. K.L.O. and N.H. drafted the manuscript. All authors contributed to discussion and reviewed/edited the manuscript. K.L.O. is the guarantor of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data. All Authors approved the final version of the manuscript.

Disclosure Statement

KLO has consulted for Pfizer. AJJ has served as a diabetes advisory panel member for Abbott, Medtronic and Sanofi, has received remuneration for lectures from Novo, and has received research support from Abbott and Medtronic. ACK has served as an Advisory Board member for Amgen, Bayer and Sanofi, and has received speaker and/or advisor honoraria from Abbott, Astra-Zeneca and Pfizer, research support from Mylan, Novartis and Sanofi, and honoraria from Abbott and Amgen. DDW has consulted for Pfizer, and has received remuneration for participating in clinical trial committees from CSL Ltd., the Medicines Company, Pfizer, Regeneron, Resverlogix and Sanofi, and remuneration for lectures from Pfizer. PJB has been a member of advisory boards for Amgen, Pfizer, and Sanofi-Regeneron; received honoraria from Amgen, Merck, Pfizer and Sanofi-Regeneron. RF and DAD are Pfizer employees. Other authors have no conflict with regards to the content of this manuscript.

Funding

The FGF21 level measurement was supported by the NSW CVRN Research Development Project Grant (100715) from the National Heart Foundation of Australia to Dr. Kwok Leung Ong. Dr. Ong was supported

by the Vice-Chancellor's Postdoctoral Fellowship from the University of New South Wales (RG134592) and the Australian National Health and Medical Research Council (NHMRC) Career Development Fellowship (1122854). Alicia J Jenkins was supported by the NHMRC Practitioner Fellowship (1121272) and is a Sydney Medical Foundation Fellow. Anthony C. Keech was supported by the NHMRC Program grant (1037786) and Research Fellowship grant (1137071). The authors thank the investigators, the staff, and the participants of the TNT trial for their valuable contributions. The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, and final approval of manuscript.

Role of Sponsor

The TNT trial was sponsored by Pfizer. The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, and final approval of manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2018.11.006.

References

[1] Woo YC, Xu A, Wang Y, Lam KS. Fibroblast growth factor 21 as an emerging metabolic regulator: clinical perspectives. Clin Endocrinol (Oxf) 2013;78:489–96.
[2] Kokkinos J, Tang S, Rye KA, Ong KL. The role of fibroblast growth factor 21 in atherosclerosis. Atherosclerosis 2017;257:259–65.
[3] Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, et al. FGF-21 as a novel metabolic regulator. J Clin Invest 2005;115:1627–35.
[4] Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E. Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metab 2007;5:426–37.
[5] Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, et al. Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. Cell Metab 2007;5:415–25.
[6] Shen Y, Ma X, Zhou J, Pan X, Hao Y, Zhou M, et al. Additive relationship between serum fibroblast growth factor 21 level and coronary artery disease. Cardiovasc Diabetol 2013;12:124.
[7] Chow WS, Xu A, Woo YC, Tso AW, Cheung SC, Fong CH, et al. Serum fibroblast growth factor-21 levels are associated with carotid atherosclerosis independent of established cardiovascular risk factors. Arterioscler Thromb Vasc Biol 2013;33:2454–9.
[8] Zhang W, Chu S, Ding W, Wang F. Serum level of fibroblast growth factor 21 is independently associated with acute myocardial infarction. PLoS One 2015;10:e0129791.
[9] Ong KL, Januszewski AS, O'Connell R, Jenkins AJ, Xu A, Sullivan DR, et al. The relationship of fibroblast growth factor 21 with cardiovascular outcome events in the Fenofibrate Intervention and Event Lowering in Diabetes study. Diabetologia 2015;58:464–73.
[10] Ziros P, Zagoriti Z, Lagoumintzis G, Kyriazopoulou V, Iskrenova RP, Habeos EI, et al. Hepatic FGF21 expression is repressed after simvastatin treatment in mice. PLoS One 2016;11:e0162024.
[11] Larosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, et al. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med 2005;352:1425–35.
[12] Ong KL, Rye KA, O'Connell R, Jenkins AJ, Brown C, Xu A, et al. Long-term fenofibrate therapy increases fibroblast growth factor 21 and retinol-binding protein 4 in subjects with type 2 diabetes. J Clin Endocrinol Metab 2012;97:4701–8.
[13] Newson RB. Comparing the predictive powers of survival models using Harrell's C or Somers' D. Stata J 2011;10:339–58.
[14] May S, Hosmer DW. A simplified method of calculating an overall goodness-of-fit test for the Cox proportional hazards model. Lifetime Data Anal 1998;4:109–20.
[15] Pencina MJ, D'Agostino Sr RB, D'Agostino Jr RB, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157–72.
[16] Pencina MJ, D'Agostino Sr RB, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. Stat Med 2011;30:11–21.
[17] Cook N. Risk Prediction Modeling. http://ncook.bwh.harvard.edu/sas-macros.html, Accessed date: July 2018.
[18] Planavila A, Redondo I, Hondares E, Vinciguerra M, Munts C, Iglesias R, et al. Fibroblast growth factor 21 protects against cardiac hypertrophy in mice. Nat Commun 2013;4:2019.
[19] Patel V, Adya R, Chen J, Ramanjaneya M, Bari MF, Bhudia SK, et al. Novel insights into the cardio-protective effects of FGF21 in lean and obese rat hearts. PLoS One 2014;9:e87102.
[20] Liu SQ, Roberts D, Kharitonenkov A, Zhang B, Hanson SM, Li YC, et al. Endocrine protection of ischemic myocardium by FGF21 from the liver and adipose tissue. Sci Rep 2013;3:2767.
[21] Planavila A, Redondo-Angulo I, Ribas F, Garrabou G, Casademont J, Giralt M, et al. Fibroblast growth factor 21 protects the heart from oxidative stress. Cardiovasc Res 2014;106:19–31.
[22] Lin Z, Wu Z, Yin X, Liu Y, Yan X, Lin S, et al. Serum levels of FGF-21 are increased in coronary heart disease patients and are independently associated with adverse lipid profile. PLoS One 2010;5:e15534.
[23] An SY, Lee MS, Yi SA, Ha ES, Han SJ, Kim HJ, et al. Serum fibroblast growth factor 21 was elevated in subjects with type 2 diabetes mellitus and was associated with the presence of carotid artery plaques. Diabetes Res Clin Pract 2012;96:196–203.
[24] Xiao Y, Liu L, Xu A, Zhou P, Long Z, Tu Y, et al. Serum fibroblast growth factor 21 levels are related to subclinical atherosclerosis in patients with type 2 diabetes. Cardiovasc Diabetol 2015;14:72.
[25] Kim WJ, Kim SS, Lee HC, Song SH, Bae MJ, Yi YS, et al. Association between serum fibroblast growth factor 21 and coronary artery disease in patients with type 2 diabetes. J Korean Med Sci 2015;30:586–90.
[26] Miyazaki Y, Saita E, Kishimoto Y, Ibe S, Seki T, Miura K, et al. Low plasma levels of fibroblast growth factor-21 in patients with peripheral artery disease. J Atheroscler Thromb 2018;25:821–8.
[27] Lenart-Lipińska M, Matyjaszek-Matuszek B, Gernand W, Nowakowski A, Solski J. Serum fibroblast growth factor 21 is predictive of combined cardiovascular morbidity and mortality in patients with type 2 diabetes at a relatively short-term follow-up. Diabetes Res Clin Pract 2013;101:194–200.
[28] Li Q, Zhang Y, Ding D, Yang Y, Chen Q, Su D, et al. Association between serum fibroblast growth factor 21 and mortality among patients with coronary artery disease. J Clin Endocrinol Metab 2016;101:4886–94.
[29] Shen Y, Zhang X, Pan X, Xu Y, Xiong Q, Lu Z, et al. Contribution of serum FGF21 level to the identification of left ventricular systolic dysfunction and cardiac death. Cardiovasc Diabetol 2017;16:106.
[30] Shen Y, Zhang X, Xu Y, Xiong Q, Lu Z, Ma X, et al. Serum FGF21 is associated with future cardiovascular events in patients with coronary artery disease. Cardiology 2018;139:212–8.
[31] Lee CH, Woo YC, Chow WS, Cheung CY, Fong CH, Yuen MM, et al. Role of circulating fibroblast growth factor 21 measurement in primary prevention of coronary heart disease among Chinese patients with type 2 diabetes mellitus. J Am Heart Assoc 2017;6:e005344.
[32] Cohen DE, Anania FA, Chalasani N. An assessment of statin safety by hepatologists. Am J Cardiol 2006;97:S77–81.
[33] Sahebkar A, Simental-Mendía LE, Pedone C, Ferretti G, Nachtigal P, Bo S, et al. Statin therapy and plasma free fatty acids: a systematic review and meta-analysis of controlled clinical trials. Br J Clin Pharmacol 2016;81:807–18.
[34] Mai K, Andres J, Biedasek K, Weicht J, Bobbert T, Sabath M, et al. Free fatty acids link metabolism and regulation of the insulin-sensitizing fibroblast growth factor-21. Diabetes 2009;58:1532–8.
[35] Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, et al. The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab 2013;18:333–40.
[36] Talukdar S, Zhou Y, Li D, Rossulek M, Dong J, Somayaji V, et al. A long-acting FGF21 molecule, PF-05231023, decreases body weight and improves lipid profile in non-human primates and type 2 diabetic subjects. Cell Metab 2016;23:427–40.