Circulating Fibroblast Growth Factor-23 Is Associated With Fat Mass and Dyslipidemia in Two Independent Cohorts of Elderly Individuals

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Objective—Disturbances in mineral metabolism define an increased cardiovascular risk in patients with chronic kidney disease. Fibroblast growth factor-23 (FGF23) is a circulating regulator of phosphate and vitamin D metabolism and has recently been implicated as a putative pathogenic factor in cardiovascular disease. Because other members of the FGF family play a role in lipid and glucose metabolism, we hypothesized that FGF23 would associate with metabolic factors that predispose to an increased cardiovascular risk. The goal of this study was to investigate the relationship between FGF23 and metabolic cardiovascular risk factors in the community.

Methods and Results—Relationships between serum FGF23 and body mass index (BMI), waist circumference, waist-to-hip ratio, serum lipids, and fat mass were examined in 2 community-based, cross-sectional cohorts of elderly whites (Osteoporotic Fractures in Men Study: 964 men aged 75±3.2; Prospective Investigation of the Vasculature in Uppsala Seniors study: 946 men and women aged 70). In both cohorts, FGF23 associated negatively with high-density lipoprotein and apolipoprotein A1 (7% to 21% decrease per 1-SD increase in log FGF23; $P<0.01$) and positively with triglycerides (11% to 14% per 1-SD increase in log FGF23; $P<0.01$). A 1-SD increase in log FGF23 was associated with a 7% to 20% increase in BMI, waist circumference, and waist-to-hip ratio and a 7% to 18% increase in trunk and total body fat mass ($P<0.01$) as determined by whole-body dual x-ray absorptiometry. FGF23 levels were higher in subjects with the metabolic syndrome compared with those without (46.4 versus 41.2 pg/mL; $P<0.05$) and associated with an increased risk of having the metabolic syndrome (OR per 1-SD increase in log FGF23, 1.21; 95% CI, 1.04 to 1.40; $P<0.05$).

Conclusion—We report for the first time on associations between circulating FGF23, fat mass, and adverse lipid metabolism resembling the metabolic syndrome, potentially representing a novel pathway(s) linking high FGF23 to an increased cardiovascular risk. **(*Arterioscler Thromb Vasc Biol*. 2011;31:219-227.)**

Key Words: cardiovascular disease prevention ■ diabetes mellitus ■ elderly ■ epidemiology ■ growth factors ■ lipids ■ metabolism ■ FGF-23 ■ FGF23 ■ Fibroblast growth factor-23

Alterations in mineral metabolism, including hyperphosphatemia, vitamin D insufficiency, and elevated parathyroid hormone (PTH) levels, are frequently present in chronic kidney disease (CKD) and substantially contribute to the high prevalence of cardiovascular morbidity and mortality observed in these patients.[1] Fibroblast growth factor-23 (FGF23) is a bone-derived circulating hormone that directly controls serum levels of phosphate, 1,25-dihydroxy vitamin D_3, and PTH and may therefore affect cardiovascular risk.[2–6]

High serum FGF23 levels in CKD are linked to adverse outcomes such as increased mortality in patients receiving hemodialysis[7,8] and mortality and cardiovascular events in patients with coronary artery disease.[9] Similarly, we and others recently reported that higher FGF23 levels, both in

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CKD and in subjects with normal renal function, are associated with cardiovascular risk factors such as vascular dysfunction, atherosclerosis, and left ventricular hypertrophy.[10–14] Putative pathogenic pathways of FGF23 in cardiovascular disease development remain unknown.

Phylogenic and sequence analyses have shown that FGF23 shares common structural and biological features with FGF19 and FGF21, which are members of the FGF19 subfamily.[15] The FGF19 subfamily has emerged as a set of novel factors that regulate diverse metabolic processes.[16] These FGFs are secreted factors that act systemically, in contrast to other FGFs that mainly have autocrine or paracrine functions, and require α-Klotho or β-Klotho in addition to canonical FGF receptors for their action.[17–21] Importantly, both FGF19 and FGF21 have been implied in the regulation of lipid and glucose metabolism.[22–26]

Because of the central role of FGF23 in mineral metabolism and its common structural features with FGF19 and FGF21, we aimed to test whether FGF23 associates with an adverse lipid and glucose metabolism that predisposes to an increased cardiovascular risk in 2 independent cohorts of elderly individuals.

Methods

The Osteoporotic Fractures in Men Study Cohort

The Osteoporotic Fractures in Men Study (MrOS) is an international multicenter prospective epidemiological investigation of elderly men. The Swedish part consists of 3014 men aged 69 to 80 years.[27] The participants were randomly selected from population registries and invited by mail in the cities of Uppsala (n=999), Malmö (n=1005), and Göteborg (n=1010). To be eligible for the study, the subject had to be able to walk without aid, and subjects with bilateral hip replacements were excluded. There were no other exclusion criteria. At the clinic visit, participants completed questionnaires about their medical history, current medication used, and lifestyle characteristics. Informed consent was obtained for all subjects, and the study was approved by the local ethics committees at Uppsala (ethical approval number Ups 01-057), Malmö (LU-693-00), and Göteborg (Gbg M 014-01) universities and conducted in accordance with the guidelines in the Declaration of Helsinki.

Serum parameters reflecting fat mass and glucose homeostasis were analyzed only in the Göteborg part of the Swedish MrOS. Thus, participants from other Swedish study sites were not included in the present study. Baseline characteristics were, as previously shown, similar among the 3 study sites.[28] Thus, for the present study, only subjects from the Göteborg part (75.3±3.2 years of age, n=1010) were included. Of the 1010 subjects in the MrOS cohort, 28 were missing FGF23 measurements (n=28), 7 were missing biochemistry measurements (n=7), and 11 were missing lipid measurements (n=11). In all, 46 subjects were excluded, and 964 subjects were included in subsequent analyses. Of those, 582 (60.4%) used at least 1 medication, as follows: diabetes treatment, 79 (8.2%); high thyroid treatment, 3 (0.3%); low thyroid treatment, 17 (1.8%); osteoporosis treatment, 9 (0.9%); stroke treatment, 43 (4.5%); Parkinson disease treatment, 6 (0.6%); high blood pressure treatment, 328 (34.0%); myocardial infarction treatment (including statins), 122 (12.7%); angina treatment, 101 (10.5%); congestive heart failure treatment, 84 (8.7%); chronic obstructive pulmonary disease, asthma, or emphysema treatment, 58 (6.0%); prostatis treatment, 62 (6.4%); glaucoma treatment, 37 (3.8%); medication for arthritis or joint pain, 87 (9.0%); and currently treated for kidney stones, 3 (0.3%).

Basic Investigation

Height was measured using a wall-mounted stadiometer, and weight was measured to the nearest 0.1 kg. All measurements were performed by the same staff. Body mass index (BMI) was calculated as weight (kg)/height (m^2).

Serum Biochemistries

Plasma and serum samples were collected at 8 AM after at least 10 hours of fasting and nonsmoking and were immediately stored at −80°C. Cystatin C was analyzed with polyclonal antibodies against human cystatin C and measured by immunoturbidimetry (Cystatin C Immunoparticles, Dako Denmark A/S, Glostrup, Denmark). Estimated glomerular filtration rate (eGFR) was calculated using the following estimate: glomerular filtration rate=$79.901\times(\text{cystatin C})^{-1.4389}$. This proxy for glomerular filtration rate has good precision, good linearity, and strong correlation with iohexol clearance (R^2=0.956).[29] Serum PTH was analyzed using the Immulite 2000 Intact PTH Assay (Diagnostic Products Corp, Los Angeles, Calif), and 25-hydroxy vitamin D_3 ($25(OH)D_3$) was measured on the Nichols Advantage automated assay system (Nichols Institute Diagnostics, San Juan Capistrano, Calif). Leptin was analyzed using a commercially available kit (Diagnostic Systems Laboratories Inc, Webster, Texas; interassay coefficient of variation [CV], 5.3%). All other routine serum biochemistries were measured at the Department of Clinical Chemistry, Sahlgrenska University Hospital.

Serum Lipids

Serum lipid analyses were performed on a Konelab 20 autoanalyzer (Thermo Electron Corporation, Vantaa, Finland). Total cholesterol and triglyceride levels were determined by fully enzymatic techniques. High-density lipoprotein (HDL) was determined after precipitation of apolipoprotein B (apoB)–containing lipoproteins with magnesium sulfate and dextran sulfate. Low-density lipoprotein (LDL) was calculated using the Friedewald formula. apoB and apolipoprotein A1 (apoA1) were determined by immunoprecipitation enhanced by polyethylene glycol at 340 nm. Interassay CVs were below 5% for all Konelab analyses.

Serum Levels of Insulin and Glucose

Fasting plasma glucose was quantitated by an enzymatic method on a Modular instrument (Roche, Stockholm, Sweden) with an interassay CV of less than 4%. Homeostasis model assessment (HOMA) index was calculated as the product of fasting serum insulin level (microunits per milliliter) and fasting plasma glucose level (millimoles per liter) divided by 22.5.[30]

Body Composition and Fat Mass

Total and trunk fat mass and lean mass were assessed by dual x-ray absorptiometry (DXA) analysis, using a Hologic QDR 4500/A-Delphi (Hologic, Waltham, Mass). The CVs for the measurements ranged from 0.5% to 3%, depending on the application.

The Prospective Investigation of the Vasculature in Uppsala Seniors Cohort

Participants from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study were aged 70 and living in the community of Uppsala, Sweden. They were invited by mail within 1 month of their 70th birthday in a randomized order from April 2001 to June 2004. Of the 2025 subjects invited, 1016 subjects were investigated.[31] An analysis of nonparticipants showed the present sample to be representative of the total cohort regarding cardiovascular disorders and drug intake.[31] Of 1016 subjects, 13 were missing FGF23 measurements, 42 were missing biochemistry, 4 were missing lipid measurements, and 11 were missing waist circumference or waist-to-hip ratio (WHR). In total, 70 subjects were excluded, and 946 subjects were included in subsequent analyses. All participants answered a questionnaire about their medical history, smoking habits, and regular medication and were investigated under standardized conditions in the morning after an overnight fast. No medication or smoking was allowed after midnight. In total, 662 (70.0%) subjects used any type of medication (in monotherapy or in combination), including hypertensive medication, 291 (30.8%); bronchdilators, 57 (6.0%); nitroglycerin, 29 (3.1%); β-blockers, 199 (21.0%); calcium antagonists, 110 (11.6%); diuretics, 115 (12.2%); angiotensin-

converting enzyme (ACE) inhibitors, 81 (8.6%); angiotensin receptor blockers, 77 (8.1%); statins, 140 (14.8%); other lipid lowering drugs, 12 (1.3%); warfarin, 28 (3.0%); acetylsalicylic acid (ASA), 171 (18.1%); antiarrhythmics, 1 (0.1%); insulin, 18 (1.9%); and oral antidiabetics, 55 (5.8%). Hypertension was defined as noninvasively measured supine systolic blood pressure≥140 mm Hg, diastolic blood pressure≥90 mm Hg, or current use of antihypertensive medication. The ethics committee of Uppsala University approved the study, and the participants gave informed consent.

Basic Investigation

Height, weight, and BMI were recorded as for the MrOS cohort. The circumference of the abdomen (waist circumference) was measured at the umbilical level and the hip circumference at the trochanter level. WHR was then calculated.

Serum Biochemistries

Plasma cystatin C (reagent 1014, Gentian, Moss, Norway) and phosphate (reagent 7D71-30) analyses were performed on an Architect Ci8200 analyzer (Abbott Laboratories, Abbott Park, Ill). eGFR was determined as described for the MrOS. PTH levels were analyzed using the Immulite 2000 Intact PTH Assay (Diagnostic Products Corp, Los Angeles, Calif). The LIAISON 25(OH)D_3 assay (DiaSorin Inc, Saluggia, Italy) was performed on a Liaison analyzer according to the manufacturer's instructions. Leptin and adiponectin were analyzed with double-antibody radioimmunoassays (Linco Research, St. Louis, Mo). Total CV for leptin was 4.7% at both low (2 to 4 ng/mL) and high (10 to 15 ng/mL) levels, and for adiponectin the total CV was 15.2% at low (2 to 4 μg/mL) and 8.8% at high (26 to 54 μg/mL) levels. High-sensitivity C-reactive protein was measured by an ultrasensitive particle enhanced immunoturbidimetric assay (Orion Diagnostica, Espoo, Finland) on a Konelab 20 autoanalyzer (Thermo Clinical Labsystems, Espoo, Finland). The interassay CV was 3.2%.

All other routine serum biochemistries were measured at the Department of Clinical Chemistry at Uppsala University Hospital.

Serum Lipids

Serum total cholesterol, triglycerides, and HDL were assayed by enzymatic techniques. LDL was calculated by the Friedewald formula. apoA1 and apoB were determined by a 2-site immunoradiometric assay, using commercial kits from Pharmacia (Uppsala, Sweden).

Insulin Resistance, Diabetes, and the Metabolic Syndrome

Serum insulin was measured by an enzymatic-immunologic assay (Boehringer Mannheim). HOMA insulin resistance index was defined as in MrOS[30] and was not evaluated in subjects on insulin treatment. Diabetes mellitus was defined as a self-reported history of diabetes or fasting blood glucose of 6.2 mmol/L or above.

Metabolic syndrome was defined by the National Cholesterol Education Program/Adult Treatment Panel III criteria.[32] Three of the following 5 criteria should be fulfilled: blood pressure >130/85 mm Hg or antihypertensive treatment, fasting blood glucose >5.6 mmol/L, serum triglycerides >1.7 mmol/L, waist circumference >102 cm in men and >88 cm in women, HDL-cholesterol <1.0 mmol/L in men and <1.3 in women.

Body Composition and Fat Mass

Total fat mass, trunk fat mass, and lean mass were assessed by DXA analysis (DPX Prodigy, Lunar Corp, Madison, Wis) on average 2 years after baseline investigation in 898 of 1016 cohort members. By triple measurements in 15 subjects, the precision error of the DXA measurements in our laboratory has been calculated to be 1.5% for total fat mass and 1.0% for total lean mass.

Serum FGF23

FGF23 was measured in both cohorts using an ELISA (Kainos Laboratories International; Tokyo, Japan).[33] This second-generation, 2-site, monoclonal antibody ELISA recognizes only the biologically active, intact FGF23.[33] The assay has a lower limit of detection of 3 pg/mL and intra- and interassay CVs of less than 5%, and it was the most sensitive among 3 different ELISAs for FGF23 measurements.[34]

Statistical Analyses

Initially, the distributional properties of all baseline variables were examined, and nonnormally distributed variables (triglycerides, HDL, leptin, calcium, PTH, and FGF23) were log-transformed before being used in subsequent analyses. Linear relationships were investigated using linear regression models, and standardized β-values are given for all analyses. All models were defined a priori, and the variables included were chosen based on known associations with the outcome variable or FGF23.

Relationships between FGF23 and clinical markers of general obesity (weight and BMI), central obesity (waist circumference and WHR), and body composition were evaluated in 2 models: adjusted for age and gender and adjusted for age, gender, and known FGF23-regulatory variables and factors of mineral metabolism (serum phosphate, albumin, calcium, 25(OH)D_3, PTH, and eGFR).

Relationships between FGF23 and serum lipids (total cholesterol, triglycerides, HDL, LDL, apoA1, apoB, and leptin) were examined in 3 sets of models: a crude model; a model adjusted for age, gender, and BMI; and a model adjusted for age, gender, BMI, serum phosphate, albumin, calcium, 25(OH)D_3, PTH, and eGFR.

Finally, we investigated whether FGF23 levels could predict the fulfillment of the National Cholesterol Education Program metabolic syndrome criteria[32] using logistic regression.

Probability values <0.05 from 2-sided tests were considered statistically significant. SAS 9.2 (SAS Institute Inc) was used for all calculations.

Results

Clinical characteristics and serum biochemistries for MrOS and PIVUS cohorts are presented in Table 1. The same variables are presented over FGF23 tertiles in Supplemental Tables I and II, available online at http://atvb.ahajournals.org. After exclusion of participants with missing data for any variable, 964 and 946 subjects were included in subsequent analysis in MrOS and PIVUS, respectively. Median serum FGF23 was 42.1 pg/mL (interquartile range, 33.1 to 53.7 pg/mL) in MrOS and 42.3 pg/mL (interquartile range, 33.5 to 54.0 pg/mL) in PIVUS. FGF23 levels were not influenced by gender ($P>0.05$).

FGF23, Anthropomorphic Measurements of Obesity and Fat Mass

In both cohorts, a 1-SD increase in log FGF23 was associated with 7% to 20% higher body weight and 7% to 17% higher BMI (Table 2), translating into a 3% increase in body weight and BMI for a 10% increase in FGF23. In PIVUS, a 1-SD increase in log FGF23 was associated with 9% to 10% higher waist circumference and 6% to 7% higher WHR (central obesity determined only in PIVUS; Table 2), equivalent to a 2% increase in waist circumference and WHR for a 10% increase in FGF23.

The relationship between FGF23 and overweight (BMI≥26 as defined by the WHO criteria) was analyzed. In total, 471 (48.9%) and 542 (57.3%) were classified as overweight in MrOS and PIVUS, respectively. In MrOS, a 1-SD increase in log FGF23 was associated with an increased risk for overweight both in crude and multivariate adjusted models (OR 1.30, CI 1.14 to 1.49; OR 1.38, CI 1.19 to 1.59, respectively). We found no significant evidence for this association in PIVUS when modeling FGF23 as a continuous variable. However, in both cohorts, individuals in the highest

Table 1. Clinical Characteristics, Medical History, Serum Biochemistries, and Body Composition Measured by DXA in MrOS and PIVUS

	MrOS cohort (n=964)	PIVUS cohort (n=946)
Females (n)	0 (0)	470 (49.7)
Age (years)	75.3 (3.2)	70
Clinical measurements		
Height (cm)	175.6 (6.4)	168.9 (9.1)
Weight (kg)	80.8 (12.2)	77.4 (14.5)
BMI (kg/m^2)	26.2 (3.5)	27.1 (4.3)
Waist circumference (cm)	…	91.2 (11.7)
WHR	…	0.9 (0.1)
Cardiovascular risk factors		
Systolic blood pressure (mm Hg)	…	149.4 (22.4)
Diastolic blood pressure (mm Hg)	…	78.7 (10.1)
Previous cardiovascular disease (n)	304 (31.5)	155 (16.4)
Hypertension	356 (36.9)	582 (60.4)
Smoker (n)	76 (7.9)	103 (10.9)
Diabetes (n)	220 (22.8)	111 (11.7)
Medication	582 (60.4)	662 (70.0)
Serum lipids		
Cholesterol (mmol/L)	5.4 (1.0)	5.4 (1.0)
LDL (mmol/L)	3.5 (1.0)	3.4 (0.9)
HDL (mmol/L)	1.2 (0.9 to 1.8)	1.4 (1.0 to 2.0)
Triglycerides (mmol/L)	1.3 (0.8 to 2.2)	1.1 (0.7 to 2.0)
Leptin (ng/mL)	15.9 (5.7 to 45.6)	10.4 (3.4 to 27.9)
apoA1 (mmol/L)	1.6 (0.3)	1.6 (0.3)
apoB (mmol/L)	1.1 (0.8 to 1.4)	1.0 (0.2)
Fasting glucose, insulin, and HOMA index		
Fasting glucose (mmol/L)	5.8 (1.4)	5.3 (1.6)
Insulin (mU/L)	7.9 (3.9 to 19.0)	7.6 (4.2 to 16.1)
HOMA index (mmol/L mU/L)	2.0 (0.9 to 5.2)	1.7 (0.9 to 4.0)
FGF23 and related serum biochemistries		
Intact FGF23 (pg/mL)	42.1 (25.8 to 69.4)	42.3 (26.2 to 70.4)
eGFR (mL/min per 1.73m^2)	71.4 (19.3)	76.6 (19.5)
Calcium (mmol/L)	2.3 (2.1 to 2.4)	2.3 (2.2 to 2.5)
Phosphate (mmol/L)	1.1 (0.2)	1.1 (0.2)
25-OH-vitamin D_3 (nmol/L)	66.8 (19.0)	57.6 (19.8)
Intact PTH (pg/mL)	51.3 (31.4 to 83.6)	43.7 (25.1 to 72.6)
Body composition		
Total fat mass (kg)	18.5 (5.8)	25.6 (9.1)
Total lean mass (kg)	59.3 (6.8)	47.7 (10.2)
Trunk fat (kg)	9.7 (3.6)	14.0 (5.1)
Body fat (%)	22.7 (4.8)	33.4 (9.1)

Values are mean (standard deviation) for normally distributed continuous variables, median (10th to 90th percentiles) for nonnormally distributed variables, and n (%) for categorical variables.

FGF23 tertile were at a significantly higher risk for overweight than those in the lowest tertile (Figure 1).

As supported by anthropomorphic measurements, FGF23 was associated with total body fat mass, trunk fat mass (Table 2), and fat mass in arms and legs (data not shown), as determined by DXA. In both cohorts, a 1-SD increase in log FGF23 was associated with 7% to 16% higher total body fat mass and 9% to 18% higher trunk fat mass (Table 2).

In MrOS, a 10% increase in FGF23 translated into a 4% increase in total body fat mass and trunk fat mass and 3% higher body fat. FGF23 explained 2% and 3% of the variations in total fat mass and trunk fat mass, respectively.

FGF23, Serum Lipids, Leptin, and Adiponectin

A 1-SD increase in log FGF23 was associated with 7% to 22% and 7% to 19% lower HDL and apoA1, respectively. In contrast, a corresponding 11% to 14% increase in triglycerides was observed (Table 3). These associations were significant and essentially unaltered in crude and multivariate-adjusted models in both cohorts (Table 3). In MrOS, a 10% increase in log FGF23 corresponded to 4% to 5% lower HDL and apoA1 and a 3% increase in triglycerides. FGF23 explained 5%, 3%, and 2% of the variations in HDL, apoA1, and triglycerides, respectively. There was no significant association between FGF23 and LDL or apoB1 (data not shown). Finally, a 1-SD increase in log FGF23 was associated with 9% to 12% higher leptin; however, this relationship was nonsignificant in multivariate-adjusted models (Table 3). Importantly, the association between FGF23 and fat mass remained significant but was attenuated when leptin was added to multivariate model 3 (Table 2).

Furthermore, in multivariate models, FGF23 was inversely related to serum adiponectin (analyzed in PIVUS only) (Table 3). FGF23, in addition to BMI, calcium, and eGFR, was also retained as a variable significantly associated with adiponectin in a post hoc stepwise regression model (data not shown).

FGF23, Fasting Glucose, Insulin, and HOMA Index

A 1-SD increase in log FGF23 was associated with 8% to 12% higher insulin and HOMA index in both cohorts; however, it was statistically nonsignificant in multivariable adjusted models. We found no association between FGF23 and fasting glucose (data not shown).

FGF23 and the Metabolic Syndrome

We evaluated a potential role of FGF23 in the metabolic syndrome because of its link to dyslipidemia and increased fat mass. On the basis of the updated National Cholesterol Education Program criteria,[32] the metabolic syndrome was diagnosed in 23.3% (n=220) of all PIVUS subjects (all components of the metabolic syndrome were recorded in PIVUS only). These participants had significantly higher FGF23 levels compared with subjects without the metabolic syndrome (median FGF23, 46.4 versus 41.2 pg/mL; $P<0.05$). Similarly, FGF23 levels were higher in subjects fulfilling the National Cholesterol Education Program triglyceride criteria (>1.7 mmol/L) compared with those who did not (median

Table 2. Correlation Between FGF23 and Anthropomorphic Measurements of General and Central Obesity and Fat Mass Determined by DXA Analysis

	Adjusted for Age and Gender	Adjusted for Age, Gender, Phosphate, Albumin, Calcium, 25(OH)D, PTH, and eGFR	Adjusted for Age, Gender, Phosphate, Albumin, Calcium, 25(OH)D, PTH, eGFR, and Leptin
MrOS cohort (n=964)			
Body weight	**0.18§ (0.12 to 0.24)**	**0.20§ (0.13 to 0.27)**	**0.12§ (0.07 to 0.18)**
BMI	**0.13§ (0.07 to 0.20)**	**0.17§ (0.10 to 0.24)**	**0.08† (0.03 to 0.14)**
Total body fat mass	**0.15§ (0.09 to 0.22)**	**0.16§ (0.09 to 0.22)**	**0.07† (0.03 to 0.12)**
Total body lean mass	**0.11‡ (0.05 to 0.17)**	**0.14§ (0.07 to 0.20)**	**0.12‡ (0.06 to 0.19)**
Trunk fat mass	**0.17§ (0.10 to 0.23)**	**0.18§ (0.11 to 0.24)**	**0.09‡ (0.04 to 0.13)**
Percentage body fat mass	**0.13§ (0.07 to 0.20)**	**0.13‡ (0.06 to 0.19)**	0.04 (−0.01 to 0.08)
PIVUS cohort (n=946)			
Body weight	**0.07* (0.01 to 0.12)**	**0.07* (0.02 to 0.13)**	0.03 (−0.01 to 0.08)
BMI	0.06 (−0.00 to 0.13)	**0.07* (0.01 to 0.14)**	0.03 (−0.02 to 0.07)
Waist circumference	**0.09† (0.03 to 0.15)**	**0.10† (0.04 to 0.16)**	**0.06* (0.01 to 0.10)**
WHR	**0.06* (0.00 to 0.11)**	**0.07* (0.01 to 0.12)**	0.04 (−0.01 to 0.09)
Total body fat mass	**0.07* (0.01 to 0.14)**	**0.09† (0.02 to 0.15)**	**0.05* (0.00 to 0.09)**
Total body lean mass	0.07 (−0.00 to 0.13)	0.02 (−0.02 to 0.06)	−0.00 (−0.04 to 0.04)
Trunk fat mass	**0.09* (0.02 to 0.16)**	**0.09* (0.02 to 0.15)**	**0.05* (0.00 to 0.09)**
Percentage body fat mass	0.03 (−0.04 to 0.10)	**0.06* (0.01 to 0.12)**	**0.04* (0.01 to 0.07)**

Note that the strongest associations were found between FGF23 and central obesity. Values are β-values for 1-SD increase in log FGF23 (95% confidence intervals).

*$P<0.05$, †$P<0.01$, ‡$P<0.001$, §$P<0.0001$. Statistically significant results are in boldface.

FGF23, 46.9 versus 41.5 pg/mL; $P<0.01$). In MrOS, both subjects fulfilling the triglyceride and HDL criteria (<1.0 mmol/L in men and <1.3 mmol/L in women) had significantly higher FGF23 level compared with those who did not (median FGF23, 44.2 versus 41.4, $P<0.001$, and 43.1 versus 41.4 pg/mL, $P<0.01$, respectively). We did not find any significant evidence for the relationship between FGF23 levels and the number of metabolic syndrome criteria met (data not shown). Hence, FGF23 may represent the burden of the metabolic syndrome risk factors rather than the clustering of these factors.

A 1-SD increase in log FGF23 was associated with a 21% (95% CI, 4% to 41%) increased risk of having the metabolic syndrome, although it was borderline significant in multivariate adjusted models (Table 4). FGF23 was also associated with an increased risk of fulfilling the triglyceride and HDL criteria of the metabolic syndrome (Table 4). Subjects within the highest FGF23 tertile were at a nearly 2-fold increased risk of having the metabolic syndrome (Figure 2).

Subgroup Analyses

All associations remained significant and unaltered after removal of subjects with diabetes (MrOS: n=220; PIVUS: n=111), and FGF23 did not predict the presence of diabetes (data not shown). A multiplicative interaction term between FGF23 and diabetes was significant for FGF23 associations with HDL and apoA1 in PIVUS but not in MrOS. In stratified analyses, the crude association between FGF23 and lipid variables were essentially consistent in subjects with diabetes compared with subjects without; however, it was nonsignificant in some multivariate models, likely because of loss of power (data not shown).

The associations also remained significant after removal of subjects with previous cardiovascular disease (MrOS, n=304; PIVUS, n=155), and there was no evidence for an interaction between FGF23 and previous cardiovascular disease (data not shown).

Finally, we did not find any significant evidence for an interaction between FGF23 and diminished renal function (glomerular filtration rate, <60 mL/min per 1.73 m^2).

Discussion

In the current study, we evaluated the relationship between FGF23 and metabolic cardiovascular risk factors in 2 independent community-based, cross-sectional cohorts of elderly individuals. We report on novel associations between serum FGF23 levels and higher BMI, larger waist circumference, elevated triglycerides, lower HDL cholesterol and apoA1, and increased total and trunk fat mass.

This study is important from the point of view that FGF23 may be a biomarker of cardiovascular disease in CKD. The current understanding of FGF23 and cardiovascular risk is incomplete and mainly relates to the role of FGF23 in mineral metabolism. As such, changes in serum phosphate, PTH, or vitamin D levels, factors that are regulated by FGF23, are associated with increased cardiovascular morbidity and mortality.[35] Similarly, FGF23 was suggested as a biomarker of phosphate-induced cardiovascular toxicity in CKD, possibly explaining its link to increased mortality risk in CKD patients receiving hemodialysis.[7,8] We provide evidence that the relationship between FGF23 and cardiovascular risk may, in part, be mediated through classical risk factors beyond mineral metabolism, that is, obesity and dyslipidemia.

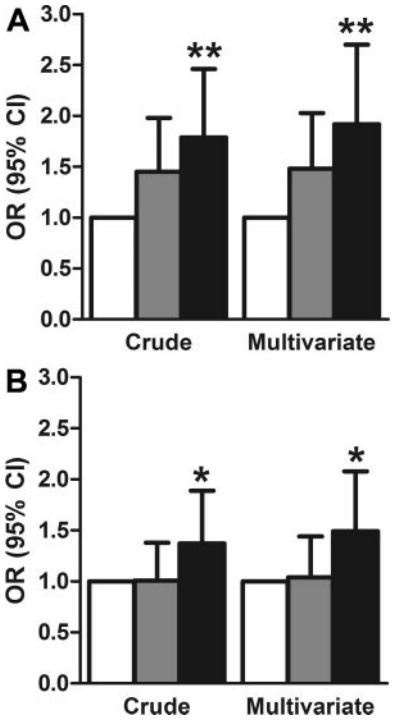

Figure 1. FGF23 and the risk for overweight (BMI≥26 as defined by the WHO criteria) in the MrOS (A) and PIVUS study (B), respectively. The association between FGF23 tertiles (white column: tertile 1 as referent) and the risk for overweight was analyzed using logistic regression models. Left, Crude models (age-adjusted in MrOS and gender-adjusted in PIVUS). Right, Additional adjustments for phosphate, albumin, calcium, 25(OH)D, PTH, and eGFR. FGF23 tertile 1≤33.1 pg/mL; FGF23 tertile 2=33.1 to 53.7 pg/mL; FGF23 tertile 3>53.7 pg/mL. *$P<0.05$, **$P<0.01$.

There may be several explanatory factors for the observed association among FGF23, fat mass, and dyslipidemia. FGF23 could exert indirect metabolic effects because of variations in mineral metabolism or, alternatively, indicate as-yet-unidentified end-organ effects of FGF23 beyond kidneys and parathyroid glands. In support for the first hypothesis, elevated PTH levels and vitamin D insufficiency are associated with increased fat mass and the metabolic syndrome.[36,37] Additionally, *Fgf23*-null mice experience both cardiovascular calcifications and disturbed glucose and lipid homeostasis[38]; however, these metabolic alterations and calcification phenotype are largely normalized after genetic and dietary alterations in phosphate and vitamin D pathways.[39–41] It should also be noted that phosphate-lowering therapy with sevelamer in CKD patients leads to an improved blood lipid profile, including higher HDL and lower LDL,[42] which

Table 4. Relationship Between FGF23 and Risk of the Metabolic Syndrome

	Odds Ratio for 1-SD Increase in Log FGF23	
	Crude Model	Adjusted for Age, Gender, BMI, Phosphate, Albumin, Calcium, 25(OH)D, PTH, and eGFR
PIVUS cohort (n=946)		
Metabolic syndrome present (fulfillment of at least 3 criteria)	**1.21* (1.04 to 1.41)**	1.17 (0.98 to 1.40)
Triglycerides criteria	**1.29† (1.09 to 1.54)**	**1.31† (1.08 to 1.57)**
HDL criteria	1.18 (0.99 to 1.40)	1.11 (0.92 to 1.35)
Waist circumference criteria	1.06 (0.93 to 1.22)	1.11 (0.88 to 1.41)
MrOS cohort (n=964)		
Triglycerides criteria	**1.29‡ (1.11 to 1.49)**	**1.30† (1.10 to 1.54)**
HDL criteria	**1.25† (1.08 to 1.44)**	1.14 (0.96 to 1.34)

Values are odds ratios (95% CI).
*$P<0.05$, †$P<0.01$, ‡$P<0.001$. National Cholesterol Education Program/Adult Treatment Panel III criteria[32] for triglycerides: >1.7 mmol/L; for HDL: <1.0 mmol/L in men and <1.3 mmol/L in women. Statistically significant results are in boldface.

Table 3. Correlation Between FGF23 and Serum Lipids

	Crude Model	Adjusted for Age, Gender, and BMI	Adjusted for Age, Gender, BMI, Phosphate, Albumin, Calcium, 25(OH)D, PTH, and eGFR
MrOS cohort (n=964)			
Total cholesterol	**−0.08* (−0.15 to −0.02)**	**−0.07* (−0.14 to −0.00)**	−0.05 (−0.13 to 0.02)
HDL	**−0.22§ (−0.28 to −0.15)**	**−0.18§ (−0.24 to −0.12)**	**−0.17§ (−0.24 to −0.10)**
Triglycerides	**0.14§ (0.07 to 0.20)**	**0.11† (0.04 to 0.17)**	**0.11† (0.04 to 0.18)**
apoA1	**−0.19§ (−0.25 to −0.13)**	**−0.16§ (−0.22 to −0.09)**	**−0.16§ (−0.23 to −0.09)**
Leptin	**0.12§ (0.07 to 0.18)**	**0.05* (0.01 to 0.10)**	0.03 (−0.02 to 0.08)
PIVUS cohort (n=946)			
Total cholesterol	−0.04 (−0.11 to 0.02)	−0.02 (−0.08 to 0.04)	−0.03 (−0.09 to 0.04)
HDL	**−0.11‡ (−0.18 to −0.05)**	**−0.07* (−0.13 to −0.02)**	**−0.08† (−0.14 to −0.02)**
Triglycerides	**0.13§ (0.07 to 0.19)**	**0.11‡ (0.05 to 0.17)**	**0.12‡ (0.06 to 0.18)**
apoA1	**−0.11‡ (−0.17 to −0.04)**	**−0.07* (−0.13 to −0.01)**	**−0.10‡ (−0.15 to −0.04)**
Adiponectin	−0.05 (−0.11 to 0.02)	−0.04 (−0.10 to 0.03)	**−0.07* (−0.13 to −0.01)**
Leptin	**0.09† (0.03 to 0.15)**	0.04 (−0.00 to 0.09)	0.01 (−0.03 to 0.06)

Values are β-values for 1-SD increase in log FGF23 (95% confidence intervals).
*$P<0.05$, †$P<0.01$, ‡$P<0.001$, §$P<0.0001$. Statistically significant results are in boldface.

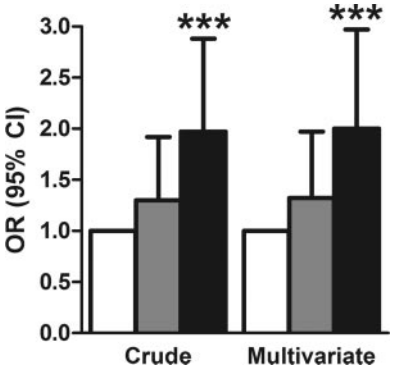

Figure 2. The relationship between FGF23 and the metabolic syndrome in the PIVUS study. Subjects in the highest FGF23 tertile (white column: tertile 1 as referent) had an increased risk for the presence of the metabolic syndrome. Left, Crude model. Right, Multivariate model adjusted for age, gender, BMI, phosphate, albumin, calcium, 25(OH)D, PTH, and eGFR. FGF23 tertile 1≤33.1 pg/mL; FGF23 tertile 2=33.1 to 53.7 pg/mL; FGF23 tertile 3>53.7 pg/mL. ***$P<0.001$.

speculatively could be attributed to a reduction in net serum phosphate balance in addition to intestinal binding of bile salts. Collectively, given the critical role of phosphate in generation of ATP and protein production, the link between FGF23 and fat mass may represent a regulatory mechanism to coordinate systemic phosphate metabolism with the presence of long-term energy stores.

On the other hand, it is tempting to infer parallels to other bone-derived factors, such as osteocalcin, which exerts profound effect on glucose homeostasis, insulin sensitivity, and fat metabolism.[43,44] In this regard, the FGF23-related growth factors FGF19 and FGF21 directly control fat mass and glucose metabolism in rodents[23,26] and our findings should prompt mechanistic studies addressing potential similar functions of FGF23. It should, however, be pointed out that the clinical significance of FGF19 and FGF21 in humans remains unclear, although a higher FGF21 level is associated with obesity, type 2 diabetes mellitus, and the metabolic syndrome.[45–47]

A recent report showed that leptin directly stimulates FGF23 expression in bone,[48] further accentuating the biological relevance of this report. In support for leptin regulation of FGF23, the association between FGF23 and fat mass was attenuated when adjusting for serum leptin and FGF23 correlated to serum leptin. FGF23 was also inversely related to adiponectin, which is highly expressed in adipose tissue[49] and protects against endothelial damage and subsequent cardiovascular disease.[50,51] This may be yet another explanatory factor for FGF23 association to vascular dysfunction.

Because there is a strong interplay between fat mass and diabetes, we examined whether diabetes modified the relationship of FGF23 to fat mass. FGF23 did not predict the presence of diabetes or related to HOMA index or blood glucose in multivariate models. Although the relationship FGF23 to HDL and apoA1 was somewhat strengthened in subjects with diabetes in PIVUS, diabetes is not a major modifier of the relationship of FGF23 to fat mass and dyslipidemia.

Although we found no gender difference in FGF23 level, its association to fat mass and lipid metabolism was consistently stronger in male than in female subjects. At this point, it remains elusive whether this represents a true gender difference or is due to other confounding factors, but it is possible that the effects of FGF23 may be influenced by sex hormones.

This study has several strengths. Our data are validated in 2 large, independent cohorts with inclusion of both genders, and we have as far as possible adjusted for relevant confounders. Fat mass and serum biochemistries were determined by accurate quantification methods. Potential limitations are lack of adjustments for dietary intake, especially of phosphate and vitamin D. The study cohorts represent only elderly whites with normal renal function. Because FGF23 levels increase dramatically with declining renal function,[52–54] we cannot conclude whether these elevated levels of FGF23 will still be associated with an adverse lipid profile in CKD or in patients of other ethnic backgrounds. Interestingly, black and Hispanic patients on hemodialysis have a survival advantage compared with whites[55–58] and also have lower FGF23 levels,[7] yet caution must be taken when generalizing our results to these populations. Finally, causal relationships between FGF23, fat mass, and serum lipids cannot be evaluated in a cross-sectional study design. Regardless, the importance of this study is evidenced by the established roles of other FGFs in regulation of lipid metabolism and fat mass, as well as the emerging concept of FGF23 as a biomarker for cardiovascular risk.

In conclusion, circulating FGF23 is associated with increased fat mass and dyslipidemia in humans, supporting a novel link between FGF23 and cardiovascular risk.

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Disclosures

None.

References

1. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol*. 2004;15:2208–2218.
2. Shimada T, Mizutani S, Muto T, Yoneya T, Hino R, Takeda S, Takeuchi Y, Fujita T, Fukumoto S, Yamashita T. Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. *Proc Natl Acad Sci U S A*. 2001;98:6500–6505.
3. Larsson T, Marsell R, Schipani E, Ohlsson C, Ljunggren O, Tenenhouse HS, Juppner H, Jonsson KB. Transgenic mice expressing fibroblast growth factor 23 under the control of the α1(i) collagen promoter exhibit growth retardation, osteomalacia, and disturbed phosphate homeostasis. *Endocrinology*. 2004;145:3087–3094.
4. Liu S, Tang W, Zhou J, Stubbs JR, Luo Q, Pi M, Quarles LD. Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D. *J Am Soc Nephrol*. 2006;17:1305–1315.
5. Krajisnik T, Bjorklund P, Marsell R, Ljunggren O, Akerstrom G, Jonsson KB, Westin G, Larsson TE. Fibroblast growth factor-23 regulates parathyroid hormone and 1α-hydroxylase expression in cultured bovine parathyroid cells. *J Endocrinol*. 2007;195:125–131.
6. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V, Goetz R, Kuro-o M, Mohammadi M, Sirkis R, Naveh-Many T, Silver J. The parathyroid is a target organ for FGF23 in rats. *J Clin Invest*. 2007;117:4003–4008.

7. Gutierrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, Smith K, Lee H, Thadhani R, Juppner H, Wolf M. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med*. 2008;359:584–592.
8. Jean G, Terrat JC, Vanel T, Hurot JM, Lorriaux C, Mayor B, Chazot C. High levels of serum fibroblast growth factor (FGF)-23 are associated with increased mortality in long haemodialysis patients. *Nephrol Dial Transplant*. 2009;24:2792–2796.
9. Parker BD, Schurgers LJ, Brandenburg VM, Christenson RH, Vermeer C, Ketteler M, Shlipak MG, Whooley MA, Ix JH. The associations of fibroblast growth factor 23 and uncarboxylated matrix Gla protein with mortality in coronary artery disease: the Heart and Soul study. *Ann Intern Med.* 152:640–648.
10. Mirza MA, Larsson A, Lind L, Larsson TE. Circulating fibroblast growth factor-23 is associated with vascular dysfunction in the community. *Atherosclerosis*. 2009;205:385–390.
11. Mirza MA, Hansen T, Johansson L, Ahlstrom H, Larsson A, Lind L, Larsson TE. Relationship between circulating FGF23 and total body atherosclerosis in the community. *Nephrol Dial Transplant*. 2009;24:3125–3131.
12. Mirza MA, Larsson A, Melhus H, Lind L, Larsson TE. Serum intact FGF23 associate with left ventricular mass, hypertrophy and geometry in an elderly population. *Atherosclerosis*. 2009;207:546–551.
13. Gutierrez OM, Januzzi JL, Isakova T, Laliberte K, Smith K, Collerone G, Sarwar A, Hoffmann U, Coglianese E, Christenson R, Wang TJ, deFilippi C, Wolf M. Fibroblast growth factor 23 and left ventricular hypertrophy in chronic kidney disease. *Circulation*. 2009;119:2545–2552.
14. Hsu HJ, Wu MS. Fibroblast growth factor 23: a possible cause of left ventricular hypertrophy in hemodialysis patients. *Am J Med Sci*. 2009;337:116–122.
15. Itoh N, Ornitz DM. Functional evolutionary history of the mouse FGF gene family. *Dev Dyn*. 2008;237:18–27.
16. Itoh N, Ornitz DM. Evolution of the FGF and FGFR gene families. *Trends Genet*. 2004;20:563–569.
17. Lin BC, Wang M, Blackmore C, Desnoyers LR. Liver-specific activities of FGF19 require klotho β. *J Biol Chem*. 2007;282:27277–27284.
18. Kurosu H, Choi M, Ogawa Y, Dickson AS, Goetz R, Eliseenkova AV, Mohammadi M, Rosenblatt KP, Kliewer SA, Kuro-o M. Tissue-specific expression of betaklotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. *J Biol Chem.* 2007;282:26687–26695.
19. Ogawa Y, Kurosu H, Yamamoto M, Nandi A, Rosenblatt KP, Goetz R, Eliseenkova AV, Mohammadi M, Kuro-o M. BetaKlotho is required for metabolic activity of fibroblast growth factor 21. *Proc Natl Acad Sci U S A*. 2007;104:7432–7437.
20. Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T. Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature*. 2006;444:770–774.
21. Kurosu H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, Rosenblatt KP, Baum MG, Schiavi S, Hu MC, Moe OW, Kuro-o M. Regulation of fibroblast growth factor-23 signaling by klotho. *J Biol Chem.* 2006;281:6120–6123.
22. Tomlinson E, Fu L, John L, Hultgren B, Huang X, Renz M, Stephan JP, Tsai SP, Powell-Braxton L, French D, Stewart TA. Transgenic mice expressing human fibroblast growth factor-19 display increased metabolic rate and decreased adiposity. *Endocrinology*. 2002;143:1741–1747.
23. Fu L, John LM, Adams SH, Yu XX, Tomlinson E, Renz M, Williams PM, Soriano R, Corpuz R, Moffat B, Vandlen R, Simmons L, Foster J, Stephan JP, Tsai SP, Stewart TA. Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes. *Endocrinology*. 2004;145:2594–2603.
24. Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski VJ, Li DS, Mehrbod F, Jaskunas SR, Shanafelt AB. FGF-21 as a novel metabolic regulator. *J Clin Invest*. 2005;115:1627–1635.
25. Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y, Moller DE, Kharitonenkov A. Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology*. 2008;149:6018–6027.
26. Xu J, Lloyd DJ, Hale C, Stanislaus S, Chen M, Sivits G, Vonderfecht S, Hecht R, Li YS, Lindberg RA, Chen JL, Jung DY, Zhang Z, Ko HJ, Kim JK, Veniant MM. Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. *Diabetes*. 2009;58:250–259.
27. Mellstrom D, Johnell O, Ljunggren O, Eriksson AL, Lorentzon M, Mallmin H, Holmberg A, Redlund-Johnell I, Orwoll E, Ohlsson C. Free testosterone is an independent predictor of BMD and prevalent fractures in elderly men: MRoS Sweden. *J Bone Miner Res*. 2006;21:529–535.
28. Marsell R, Mirza MA, Mallmin H, Karlsson M, Mellstrom D, Orwoll E, Ohlsson C, Jonsson KB, Ljunggren O, Larsson TE. Relation between fibroblast growth factor-23, body weight and bone mineral density in elderly men. *Osteoporos Int*. 2009;20:1167–1173.
29. Flodin M, Jonsson AS, Hansson LO, Danielsson LA, Larsson A. Evaluation of Gentian cystatin C reagent on Abbott Ci8200 and calculation of glomerular filtration rate expressed in ml/min/1.73 m(2) from the cystatin c values in mg/l. *Scand J Clin Lab Invest*. 2007;67:560–567.
30. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–419.
31. Lind L, Fors N, Hall J, Marttala K, Stenborg A. A comparison of three different methods to evaluate endothelium-dependent vasodilation in the elderly: the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. *Arterioscler Thromb Vasc Biol*. 2005;25:2368–2375.
32. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *J Am Med Assoc.* 2001;285:2486–2497.
33. Yamazaki Y, Okazaki R, Shibata M, Hasegawa Y, Satoh K, Tajima T, Takeuchi Y, Fujita T, Nakahara K, Yamashita T, Fukumoto S. Increased circulatory level of biologically active full-length FGF-23 in patients with hypophosphatemic rickets/osteomalacia. *J Clin Endocrinol Metab*. 2002;87:4957–4960.
34. Imel EA, Peacock M, Pitukcheewanont P, Heller HJ, Ward LM, Shulman D, Kassem M, Rackoff P, Zimering M, Dalkin A, Drobny E, Colussi G, Shaker JL, Hoogendoorn EH, Hui SL, Econs MJ. Sensitivity of fibroblast growth factor 23 measurements in tumor-induced osteomalacia. *J Clin Endocrinol Metab*. 2006;91:2055–2061.
35. Covic A, Kothawala P, Bernal M, Robbins S, Chalian A, Goldsmith D. Systematic review of the evidence underlying the association between mineral metabolism disturbances and risk of all-cause mortality, cardiovascular mortality and cardiovascular events in chronic kidney disease. *Nephrol Dial Transplant*. 2009;24:1506–1523.
36. Ahlstrom T, Hagstrom E, Larsson A, Rudberg C, Lind L, Hellman P. Correlation between plasma calcium, parathyroid hormone (PTH) and the metabolic syndrome (METS) in a community-based cohort of men and women. *Clin Endocrinol (Oxf)*. 2009;71:673–678.
37. Foss YJ. Vitamin D deficiency is the cause of common obesity. *Med Hypotheses*. 2009;72:314–321.
38. Shimada T, Kakitani M, Yamazaki Y, Hasegawa H, Takeuchi Y, Fujita T, Fukumoto S, Tomizuka K, Yamashita T. Targeted ablation of FGF23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. *J Clin Invest*. 2004;113:561–568.
39. Stubbs JR, Liu S, Tang W, Zhou J, Wang Y, Yao X, Quarles LD. Role of hyperphosphatemia and 1,25-dihydroxyvitamin D in vascular calcification and mortality in fibroblastic growth factor 23 null mice. *J Am Soc Nephrol*. 2007;18:2116–2124.
40. Hesse M, Frohlich LF, Zeitz U, Lanske B, Erben RG. Ablation of vitamin D signaling rescues bone, mineral, and glucose homeostasis in FGF-23 deficient mice. *Matrix Biol*. 2007;26:75–84.
41. Sitara D, Razzaque MS, St-Arnaud R, Huang W, Taguchi T, Erben RG, Lanske B. Genetic ablation of vitamin D activation pathway reverses biochemical and skeletal anomalies in FGF-23-null animals. *Am J Pathol*. 2006;169:2161–2170.
42. Burke SK, Dillon MA, Hemken DE, Rezabek MS, Balwit JM. Meta-analysis of the effect of sevelamer on phosphorus, calcium, PTH, and serum lipids in dialysis patients. *Adv Ren Replace Ther*. 2003;10:133–145.
43. Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G. Endocrine regulation of energy metabolism by the skeleton. *Cell*. 2007;130:456–469.
44. Lee NK, Karsenty G. Reciprocal regulation of bone and energy metabolism. *J Musculoskelet Neuronal Interact*. 2008;8:351.
45. Mraz M, Bartlova M, Lacinova Z, Michalsky D, Kasalicky M, Haluzikova D, Matoulek M, Dostalova I, Humenanska V, Haluzik M. Serum concentrations and tissue expression of a novel endocrine regulator fibroblast growth factor-21 in patients with type 2 diabetes and obesity. *Clin Endocrinol (Oxf)*. 2009;71:369–375.

46. Zhang X, Yeung DC, Karpisek M, Stejskal D, Zhou ZG, Liu F, Wong RL, Chow WS, Tso AW, Lam KS, Xu A. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes*. 2008;57:1246–1253.
47. Chen WW, Li L, Yang GY, Li K, Qi XY, Zhu W, Tang Y, Liu H, Boden G. Circulating FGF-21 levels in normal subjects and in newly diagnose patients with type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes*. 2008;116:65–68.
48. Tsuji K, Maeda T, Kawane T, Matsunuma A, Horiuchi N. Leptin stimulates fibroblast growth factor 23 expression in bone and suppresses renal 1α,25-dihydroxyvitamin D(3) synthesis in leptin-deficient ob/ob mice. *J Bone Miner Res.*
49. Kadowaki T, Yamauchi T, Kubota N. The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. *FEBS Lett*. 2008;582:74–80.
50. Ohashi K, Kihara S, Ouchi N, Kumada M, Fujita K, Hiuge A, Hibuse T, Ryo M, Nishizawa H, Maeda N, Maeda K, Shibata R, Walsh K, Funahashi T, Shimomura I. Adiponectin replenishment ameliorates obesity-related hypertension. *Hypertension*. 2006;47:1108–1116.
51. Wu X, Mahadev K, Fuchsel L, Ouedraogo R, Xu SQ, Goldstein BJ. Adiponectin suppresses ikappab kinase activation induced by tumor necrosis factor-α or high glucose in endothelial cells: role of cAMP and AMP kinase signaling. *Am J Physiol*. 2007;293:E1836–E1844.
52. Larsson T, Nisbeth U, Ljunggren O, Juppner H, Jonsson KB. Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not change in response to variation in phosphate intake in healthy volunteers. *Kidney Int*. 2003;64:2272–2279.
53. Gutierrez O, Isakova T, Rhee E, Shah A, Holmes J, Collerone G, Juppner H, Wolf M. Fibroblast growth factor-23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. *J Am Soc Nephrol*. 2005;16:2205–2215.
54. Westerberg PA, Linde T, Wikstrom B, Ljunggren O, Stridsberg M, Larsson TE. Regulation of fibroblast growth factor-23 in chronic kidney disease. *Nephrol Dial Transplant*. 2007;22:3202–3207.
55. Frankenfield DL, Rocco MV, Roman SH, McClellan WM. Survival advantage for adult hispanic hemodialysis patients? Findings from the end-stage renal disease clinical performance measures project. *J Am Soc Nephrol*. 2003;14:180–186.
56. Robinson BM, Joffe MM, Pisoni RL, Port FK, Feldman HI. Revisiting survival differences by race and ethnicity among hemodialysis patients: the dialysis outcomes and practice patterns study. *J Am Soc Nephrol*. 2006;17:2910–2918.
57. Wolf M, Shah A, Gutierrez O, Ankers E, Monroy M, Tamez H, Steele D, Chang Y, Camargo CA Jr, Tonelli M, Thadhani R. Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney Int*. 2007;72:1004–1013.
58. Wolf M, Betancourt J, Chang Y, Shah A, Teng M, Tamez H, Gutierrez O, Camargo CA Jr, Melamed M, Norris K, Stampfer MJ, Powe NR, Thadhani R. Impact of activated vitamin D and race on survival among hemodialysis patients. *J Am Soc Nephrol*. 2008;19:1379–1388.

Arteriosclerosis, Thrombosis, and Vascular Biology

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Circulating Fibroblast Growth Factor-23 Is Associated With Fat Mass and Dyslipidemia in Two Independent Cohorts of Elderly Individuals

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Supplemental Table I. Weight, BMI, serum biochemistries and body composition over FGF23 tertiles in MrOS.

	FGF23 Tertile 1 (≤ 33.1 pg/mL)	FGF23 Tertile 2 (33.1 - 53.7 pg/mL)	FGF23 Tertile 3 (> 53.7 pg/mL)
Clinical measurements			
Weight (kg)	**78.4 (12.2)**	**81.2 (11.5)***	**82.9 (12.5)***
Body mass index (kg/m^2)	25.7 (3.5)	26.2 (3.4)	**26.7 (3.5)***
Serum lipids			
Cholesterol (mmol/L)	5.52 (0.97)	5.39 (1.06)	5.34 (1.05)
Low density lipoprotein (mmol/L)	3.59 (0.93)	3.50 (0.99)	3.46 (0.97)
High density lipoprotein (mmol/L)	**1.34 (0.39)**	**1.25 (0.33)***	**1.22 (0.32)***
Triglycerides (mmol/L)	**1.32 (0.57)**	**1.44 (0.64)***	**1.48 (0.67)***
Leptin (ng/mL)	**19.81 (19.15)**	20.89 (17.13)	**26.72 (24.86)*,†**
Apolipoprotein A1 (mmol/L)	**1.68 (0.33)**	**1.60 (0.30)***	**1.58 (0.31)***
Apolipoprotein B (mmol/L)	1.07 (0.23)	1.06 (0.26)	1.06 (0.25)
Body composition			
Total fat mass (kg)	**17.4 (5.7)**	**18.5 (5.6)***	**19.6 (5.8)***
Total lean mass (kg)	**58.1 (6.5)**	**59.8 (6.8)***	**59.9 (6.9)***
Trunk fat (kg)	**8.9 (3.5)**	**9.6 (3.5)***	**10.4 (3.6)*,†**
Percent body fat (%)	**21.9 (4.8)**	**22.6 (4.7)***	**23.5 (4.8)***

Values are mean (standard deviation) for normally distributed continuous variables, median (10th — 90th percentiles) for non-normally distributed variables. * p<0.05 for the difference relative to Tertile 1. † p<0.05 for the difference relative to Tertile 2. Statistically significant results are in bold.

Supplemental Table II. Weight, BMI, serum biochemistries and body composition over FGF23 tertiles in PIVUS.

	FGF23 Tertile 1 (≤ 36.0 pg/mL)	FGF23 Tertile 2 (36.0 - 49.5 pg/mL)	FGF23 Tertile 3 (> 49.5 pg/mL)
Clinical measurements			
Weight (kg)	75.4 (14.3)	77.1 (13.9)	**79.5 (15.1)***
Body mass index (kg/m^2)	26.7 (4.4)	27.0 (4.3)	**27.5 (4.2)***
Waist circumference (cm)	89.5 (11.7)	90.9 (11.6)	**93.1 (11.4)*, †**
Waist-to-hip ratio	0.9 (0.1)	0.9 (0.1)	**0.9 (0.1) *, †**
Serum lipids			
Cholesterol (mmol/L)	5.42 (1.00)	5.55 (1.03)	**5.28 (1.00) †**
Low density lipoprotein (mmol/L)	3.34 (0.86)	3.49 (0.90)	**3.26 (0.87) †**
High density lipoprotein (mmol/L)	1.50 (1.10 - 2.10)	1.40 (1.00 - 2.00)	**1.40 (1.00 - 1.90)***
Triglycerides (mmol/L)	1.04 (0.64 - 1.84)	**1.16 (0.72 - 2.09)***	**1.24 (0.74 - 2.22)***
Leptin (ng/mL)	9.80 (3.10 - 28.30)	10.50 (3.40 - 27.90)	11.35 (3.70 - 27.00)
Apolipoprotein A1 (mmol/L)	1.69 (0.31)	1.65 (0.30)	**1.60 (0.31)***
Apolipoprotein B (mmol/L)	1.01 (0.23)	**1.07 (0.24)***	1.03 (0.23)
Body composition			
Total fat mass (kg)	24.5 (8.8)	**26.3 (9.3)***	26.1 (9.0)
Total lean mass (kg)	46.8 (10.2)	47.5 (10.1)	48.7 (10.1)
Trunk fat (kg)	13.3 (4.9)	**14.4 (5.3)***	**14.4 (5.0)***
Percent body fat (%)	32.9 (9.7)	33.9 (8.9)	33.2 (8.6)

Values are mean (standard deviation) for normally distributed continuous variables, median (10th — 90th percentiles) for non-normally distributed variables. * p<0.05 for the difference relative to Tertile 1. † p<0.05 for the difference relative to Tertile 2. Statistically significant results are in bold.