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Research Paper

Circulating levels of fibroblast growth factor-21 increase with age independently of body composition indices among healthy individuals

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ABSTRACT

Background: Circulating FGF21 levels are commonly elevated in disease states. There is limited information regarding concentrations of circulating FGF21 in the absence of disease, as well as age-related differences in body composition that may contribute to FGF21 regulation across groups.
Objective: The objectives of this study were to assess FGF21 levels across age groups (childhood to elder adulthood), and investigate whether body composition indices are associated with age-related differences in circulating FGF21.
Materials and methods: We cross-sectionally analyzed serum concentrations of FGF21 in 184 healthy subjects aged 5–80 y (45% male). Multiple linear regression was performed to assess the independent association of categorical age (children: 5–12 y, young adults: 20–29 y, adults: 30–50 y, older adults: 55–64 y, elder adults: 65–80 y) with FGF21 concentration taking into account DXA-measured body composition indices [bone mineral density (BMD) and percent lean, trunk, and fat mass]. We also stratified analysis by tertile of FGF21.
Results: Incremental increases in FGF21 levels were observed across age groups (youngest to highest). Age group was positively associated with FGF21 level independent of body composition indices (age group variable: $\beta = 0.25, 0.24, 0.24, 0.23$, all $P < 0.0001$, controlling for percent lean, BMD, percent fat, and percent trunk fat, respectively). By FGF21 tertile, age group was associated with FGF21 in the lowest tertile only ($\beta = 13.1, 0.19, 0.18$, all $P \leq 0.01$, accounting for percent lean, fat and trunk fat, respectively), but not when accounting for BMD.
Conclusions: Our findings in a healthy population display an age-related increase in serum FGF21, highlighting a potential age effect in response to metabolic demand over the lifecourse. FGF21 levels increase with age independently of body composition. At lower levels of FGF21, BMD, but not other body composition parameters, attenuates the association between FGF21 level and age, suggesting the metabolic demand of the skeleton may provide a link between FGF21 and energy metabolism.

Abbreviations: FGF-21, Fibroblast growth factor 21; BMD, bone mineral density; BMI, body mass index; DXA, dual-energy x-ray absorptiometry; ELISA, enzyme-linked immunosorbent assay; CV, coefficient of variation.

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Introduction

Fibroblast growth factor 21 (FGF21) has garnered significant interest in recent years, given its emerging role in intermediary metabolism involving glucose and lipid utilization. FGF21 is released into the circulation from the liver, adipose tissue, and skeletal muscle where it is involved in adaptations to energy demand across tissues. FGF21 expression by the liver and adipose tissue is largely controlled by peroxisome proliferator activated receptor (PPAR) α and λ, respectively [1,2], and in skeletal muscle by the insulin/Akt pathway [1]. An insulin-signaling pathway-dependent mechanism has been suggested to be the primary action in skeletal muscle [1], and in adipose tissue, an insulin-independent mechanism promotes glucose uptake by enhancing the expression of GLUT1 [3]. The metabolic effects of FGF21 in adipocytes are also mediated by β-Klotho, a single-pass transmembrane protein induced during adipogenesis [4]. FGF21 has been shown to improve pancreatic β-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways [5]. In the liver, down-regulation of FGF21 leads to fatty liver, dyslipidemia, and reduced serum ketones due to the altered expression of key genes involved in hepatic lipid and ketone metabolism, potentially linking FGF21 to metabolic disease [6]. Moreover, increased production of FGF21 in transgenic mice has been associated with body weight maintenance and longevity [7]. These findings in animal-based studies suggest FGF21 is a potent metabolic regulator with beneficial independent effects on glucose, lipid and overall energy metabolism. However, the extent to which metabolic regulation by FGF21 is modified with aging has not been explored.

Changes in accumulation, degeneration and metabolic activity of various body tissues, well-accepted as part of the aging process, may affect synthesis and release of FGF21. Adipocytes have been shown to be an important source of FGF21 production, whereas the liver has been previously considered as the main source of FGF21 in circulation [8]. More recently, skeletal muscle expression and secretion of FGF21 have been shown to lead to a fivefold increase in circulating FGF21 concentration [9]. Because the aging process is associated with reciprocal modification of body composition – adiposity increases, muscle mass decreases, in general – the synthesis and secretion of FGF21 by these tissues into the circulation may change concomitant to age-related alterations in body composition.

In humans, serum FGF21 concentrations appear to vary between individuals and across age groups, but to-date there is no single dataset across age groups. In separate studies, higher circulating values have been reported in adults compared to children, but an underlying explanation has not been elucidated. Further, values have been reported to vary widely (e.g., 250-fold) among healthy normal-weight adults ages 20–80 y, ranging from 21 to 5300 pg/ml [10]. While less studied in children, a recent investigation in a healthy, non-obese pediatric Danish cohort, including subjects ages 8–16 y, reported a range from below level of detection (30 pg/ml) to 1715.1 pg/ml [11]. To our knowledge no studies have examined FGF21 across age groups in healthy individuals. Thus, the two-fold objective of this study was to assess fasting FGF21 concentrations across age groups, ranging from 5 to 70 y, and to assess whether body composition indices (fat, lean or bone mass) may account for any age-related differences.

Materials and methods

Subjects

Fasting blood samples were obtained from the compilation of several pediatric and two adult studies conducted at University of Alabama at Birmingham (UAB). Specific study inclusion criteria have been published elsewhere for pediatric [12–15] and adult cohorts [16,17]. While the cited studies include interventional components, the current investigation is limited to cross-sectional analyses of baseline data for all participants. For each of these studies, subjects were recruited using newspaper advertisements, posted flyers, by word-of-mouth and through local radio advertisements. All measurements were performed at the Clinical Research Unit (CRU), UAB Center for Exercise Medicine and the Human Physiology Core at UAB. All study protocols were approved by the Institutional Review Boards (IRBs) of UAB and/or the Birmingham Veterans Affairs Medical Center, and all subjects provided written, informed consent and assent (where appropriate) prior to participation to utilize samples collected for future research.

Children/adolescents

The pediatric population included participants enrolled in a variety of clinical studies conducted at UAB investigating metabolic changes during growth and maturation [12–15]. The total pediatric sample included 69 healthy children ages 5–12 y (Tanner stage < 4), who underwent DXA scans and fasting morning venipuncture over the period of 2009–2014. Exclusionary criteria for each of the studies were medical diagnoses and/or current use of medications known to affect body composition, lipid or glucose metabolism, or blood pressure (e.g., diabetes; impaired fasting glucose; use of thyroid medication, diuretics, beta-blockers, thiazolidinediones, etc.); an allergy to lidocaine (used for topical anesthesia prior to venipuncture in study participants); and history of an eating disorder(s). The study physician conducted an overall health assessment for each of the participants to rule out medical diagnoses.

Adults

The de-identified data and samples from adults $20–80^{+}$ yr derived from fasting morning venipuncture encompassing the baseline assessments in one of two studies [16,17]. For each of these studies, subjects were free of any musculoskeletal or other disorders that could potentially affect their ability to complete testing. Subjects were non-obese (BMI < 30) and none of the participants were treated with pharmacological interventions thought to influence body composition or glucose metabolism.

Body composition and fat distribution

Body composition indices [percent lean mass, bone mineral density (BMD), total percent fat mass, and percent trunk fat] were determined using dual energy x-ray absorptiometry (DXA) scans (GE Lunar Corporation, Madison, WI, USA) and encore 2002 software (version 6.10.029) according to manufacturer's instructions (pediatric version where appropriate). Subjects were scanned in light clothing, lying flat on their back with arms at their sides.

Serum assay

For all participants, blood was drawn in the morning after an overnight fast. Serum concentrations of FGF21 were measured using a commercially-available, enzyme-linked immunosorbent assay (ELISA; Millipore Corporation, Billerica, MA). All samples were processed immediately upon completion of blood draw to extract serum samples and subsequently stored at -80° until measurement of the analytes of interest in batched assays in accordance with assay specifications. The inter-assay coefficient of variation (CV) was $<11\%$ and intra-assay CV was $<5\%$. The minimum sensitivity was 31.3 pg/ml. Four participants (all children)

had FGF21 levels less than minimum level of detection and were set to 29 pg/ml.

Data analysis

Descriptive statistics (mean ± standard deviation) were determined to describe age- and FGF21 tertile-specific categories. FGF21 was not normally distributed, thus median and interquartile ranges were reported. Natural log transformation was used FGF21 to achieve normal distribution for and was subsequently used in this form for analyses. General linear models were used to analyze differences in parameters by age as a categorical variable and FGF21 tertile with Tukey's test to evaluate *post hoc* group-wise differences, all controlled for sex [except for when determining differences in age, sex and BMI percentile (sex-specific) by FGF21 tertile]. In addition to sex, weight was also adjusted for when evaluating differences in weight by age category and FGF21 tertile.

Partial Pearson correlations were analyzed using log-transformed FGF21 and absolute body composition indices (i.e., percent lean mass, BMD, percent fat mass, and percent trunk fat) controlling for sex. Multiple linear regression analyses were performed in the overall sample and by FGF21 tertile, with log FGF21 as the dependent variable and the categorical variable, age group, as the independent variable. All models were controlled for sex, with individual addition of body composition indices to models. Statistical significance was accepted at $P \leq 0.05$ for all tests using SAS software (version 9.4, SAS Institute Inc., Cary NC).

Results

Participant characteristics

Participant ($n = 184$; 45% male) descriptive characteristics are presented by age category as follows: children, 5–12 y; young adults, 20–29 y; adults, 30–50 y; older adults, 55–64 y; elder adults, 65–80 y (Table 1). Adults were all non-obese (BMI <30 kg/m^2), while 44.9% of the children were obese based on CDC sex- and age-specific criteria (≥95th BMI percentile) [18]. FGF21 levels increased as age group increased (lower in children relative to adults, older adults and elder adults, and lower in young adults and adults relative to elder adults all $P < 0.01$). Sex-adjusted body composition variable comparisons revealed no differences in BMD among adult categories but greater BMD in all adult groups relative to children ($P \leq 0.05$). Percent total fat and percent lean mass were highest and lowest, respectively, in elder adults ($P \leq 0.05$) compared to all except older adults. Percent trunk fat was highest in elder adults and older adults ($P \leq 0.05$), who did not differ from each other.

Table 2 presents sample characteristics by FGF21 tertile. There were age, height, weight, BMD, and percentage trunk fat differences when analyzed by tertile of FGF21. Mean age significantly increased across ascending tertiles of FGF21. Height, weight and BMD were greatest in the highest FGF21 tertile relative to the lowest tertile, and percent trunk fat was greatest in the highest FGF21 tertile relative to lowest and mid-tertiles (all $P \leq 0.05$). There were no sex differences by FGF21 tertile, nor were there differences in BMI, BMI percentile, lean mass percent or total fat mass percent.

Partial Pearson correlations between log FGF21 and body composition indices

FGF21 was correlated positively with percent trunk fat ($r = 0.25$, $P = 0.001$) and BMD ($r = 0.30$, $P < 0.0001$), but not with percent total fat ($r = 0.10$, $P = 0.187$) or percent lean mass ($r = -0.07$, $P = 0.339$).

Multivariable associations

Table 3 presents multiple linear regression analyses in the overall sample and by tertile of FGF21, with age group as the independent variable, and FGF21 as the dependent variable (controlling for sex). FGF21 was positively associated with age group (categorical variable) independent of individual body composition indices (parameter estimate/β for age group including the following covariates in individual models: percent lean mass, $\beta = 0.25$; BMD, $\beta = 0.24$; percent total fat mass, $\beta = 0.24$; percent trunk fat, $\beta = 0.23$; all $P < 0.0001$). By FGF21 tertile, age group was associated with log FGF21 in the lowest tertile only (parameter estimate/β for age group including the following covariates in individual models: percent lean mass, $\beta = 13.1$; percent total fat mass, $\beta = 0.19$; and percent trunk fat, $\beta = 0.18$; all $P \leq 0.01$), but not when accounting for BMD.

Discussion

Prior investigations of FGF21 have focused on its ability to improve glucose regulation and lipid handling in disordered metabolic states (e.g., obesity, T2DM, cardiovascular disease). While a regulatory role in various metabolic functions has become apparent, the targeted actions of FGF21 in humans are likely influenced by changing metabolic demands over the life course. We found an increase in circulating FGF21 levels with age in healthy individuals independent of body composition indices. When examined across FGF21 tertiles, older age category was associated with greater concentrations of FGF21 in the lowest tertile only, independent from measures of lean and fat mass, but not when

Table 1
Descriptive statistics [mean ± SD or median (interquartile range)] categorized by age group

	Children,[d] $n = 69$ (5–12 y, 38% male)	Young adults, $n = 21$ (20–29 y, 48% male)	Adults, $n = 27$ (30–50 y, 44% male)	Older adults, $n = 31$ (55–64 y, 48% male)	Elder adults, $n = 36$ (65–80 y, 58% male)
Height (cm)	**139.5 ± 12.4**	167.6 ± 8.1	173.3 ± 10.4	171.4 ± 10.2	170.1 ± 11.2
Weight (kg)[e]	**44.1 ± 17.0**	67.3 ± 11.7[a]	75.5 ± 11.7[a,b]	74.5 ± 14.0[a,b]	78.1 ± 14.6[b]
BMI	**75.7th ± 29.8[f]**	23.8 ± 3.0[a]	25.1 ± 2.2[a]	25.2 ± 3.1[a]	26.8 ± 2.9[b]
FGF21 (median, IQR)	156.0 (59.0, 254.0)[a]	211.0 (140.0, 302.0)[a,b]	267.0 (156.0, 411.0)[b,c]	292.0 (177.0, 499.0)[b,c]	358.5 (238.5, 481.0)[c]
FGF21 (min–max)	29.0–595.0	34.0–615.0	47.0–666.0	55.0–766.0	129.0–822.0
Total lean (%)	63.3 ± 9.2[a]	67.0 ± 9.9[a]	64.9 ± 8.2[a,b]	63.2 ± 6.7[a,b]	59.0 ± 6.7[b]
BMD (g/cm^3)	0.9 ± 0.1[a]	1.2 ± 0.1[b]	1.2 ± 0.1[b]	1.2 ± 0.1[b]	1.2 ± 0.1[b]
Total fat (%)	32.6 ± 9.7[a]	29.4 ± 10.7[a]	31.6 ± 8.2[a,b]	33.0 ± 7.1[a,b]	37.5 ± 6.1[b]
Trunk fat (%)	13.4 ± 6.1[a]	12.9 ± 4.8[a]	15.0 ± 3.4[a,b]	17.0 ± 3.9[b,c]	19.2 ± 3.3[c]

Fibroblast growth factor 21 (FGF21), bone mineral density (BMD).
[a,b,c]represents age group differences (all adjusted for sex, [d]Tanner 1 (51%), Tanner 2 (33%), Tanner 3 (10%), Tanner 4 (6%); [e]variable also adjusted for height; all $P \leq 0.05$); variables in bold are not compared with adults; [f]BMI percentile.

Table 2
Sample characteristics [mean ± SD or median (interquartile range)] by FGF21 tertile

	Tertile 1, $n = 60$	Tertile 2, $n = 63$	Tertile 3 ($n = 61$)
Age	23.3 ± 20.5[a]	36.9 ± 24.5[b]	49.4 ± 23.8[c]
% Male	41.7	46.0	45.9
Height[c]	152.6 ± 17.3[a]	159.9 ± 20.2[a,b]	164.6 ± 17.0[b]
Weight[d]	56.2 ± 19.9[a]	63.1 ± 22.6[a,b]	70.1 ± 18.4[b]
BMI[c,e]	24.6 ± 3.3	25.3 ± 3.0	25.9 ± 2.8
BMI percentile[e]	78.5 ± 28.7	68.7 ± 32.1	80.4 ± 28.6
Total lean (%)[c]	63.3 ± 9.5	64.3 ± 8.0	61.6 ± 7.9
BMD (g/cm^3)[c]	1.0 ± 0.2[a]	1.1 ± 0.2[a,b]	1.1 ± 0.2[b]
Total fat (%)[c]	32.5 ± 10.2	32.2 ± 8.4	34.8 ± 7.8
Total trunk fat (%)[c]	14.0 ± 5.7[a]	14.8 ± 5.4[a]	17.2 ± 4.2[b]

Fibroblast growth factor 21 (FGF21), bone mineral density (BMD).
[a,b]represents differences by FGF21 tertile ($P \leq 0.05$), [c]adjusted for sex, [d]adjusted for height and sex, [e]BMI includes adults only, where BMI percentile includes only children.

accounting for bone density. This poses a compelling question regarding a potential differential role of FGF21 during anabolic (as with childhood growth) versus catabolic states (as with aging) at variable physiological levels. The considerable inter-individual variability of circulating FGF21 level in humans as they age may reside in temporal changes in tissue distribution and responsiveness to metabolic demand.

Although longitudinal studies in humans have not been conducted, cross-sectional median serum FGF21 values reported among adult populations have been typically higher relative to pediatric populations. In our sample, characterized by healthy individuals across a wide spectrum of ages, FGF21 levels were likewise lowest among children/adolescents. We report concentrations within a more narrow range than that previously reported – from below level of detection (31.3 pg/ml) to 595 in healthy boys and girls of varied body habitus (normal weight, overweight and obese) whose mean age was 9.5 y ± 1.8 [11], with a similar median value of 156 pg/ml (59, 254). Based on an in vitro study of primary human chondrocytes, FGF21 may inhibit linear growth by induction of growth hormone resistance [19]. Given our lack of information on individual growth velocities, we were unable to correlate this with serum FGF21. We posit that the balance of anabolic and catabolic processes supporting growth and tissue maintenance may underlie the difference in adults and children.

We further examined FGF21 levels after excluding children and found a fairly wide range of absolute FGF21 levels (34–822 pg/ml).

Table 3
Association between FGF21 (dependent variable) and age group[a] (independent variable), controlling for body composition indices in individual models in the overall sample and by FGF21 tertile (all models controlled for sex)

	Overall sample	Tertile 1 (29–160 pg/ml)	Tertile 2 (163–312 pg/ml)	Tertile 3 (318–822 pg/ml)
n	*184*	*61*	*62*	*61*
Variable	β			
Age group	**0.25**a	**13.1**b	−0.73	−4.49
% lean mass	7.8×10^{-4}	0.13	−0.38	−1.94
Age group	**0.24**a	0.12	−0.01	−0.01
BMD	0.09	0.72	0.17	0.04
Age group	**0.24**a	**0.19**b	1.6×10^{-3}	−0.01
% fat mass	1.6×10^{-3}	1.7×10^{-3}	1.8×10^{-3}	3.7×10^{-3}
Age group	**0.23**a	**0.18**b	8.6×10^{-4}	−0.02
% trunk fat	0.01	0.01	6.2×10^{-4}	0.1

Bone mineral density (BMD). Bolded values indicate significant associations (a$P < 0.0001$; b$P \leq 0.01$).
[a] Children, 5–12 y; young adults, 20–29 y; adults, 30–50 y; older adults, 55–64; elder adults, 65–80.

Median values in young adults were 211 pg/ml, adults 267 pg/ml, older adults 292 pg/ml, and elder adults 359 pg/ml, values similar to some but not all reports. Elder adults had the greatest FGF21 levels, ranging from 129 to 822 pg/ml. A study of healthy adults similar in age to our cohort of older adults ($n = 50$, mean age 65.3 y) found a fasting serum FGF21 concentration of 468 pg/ml (295, 520) [20], yet another study ($n = 539$, mean age 61.9 y) reported a value two-fold lower of 210 pg/ml (114, 335) [21]. Notably, both studies had similar demographic characteristics (e.g., sex, BMI) as ours. Although the adults in comparative studies were presumed healthy based on their identification as "controls," a potential influence of metabolic health (i.e., presence of morbidities) could explain the discordant values. The cohort with higher values included a small percentage of participants with clinical characteristics indicative of metabolic derangement known to be associated with elevated FGF21 concentrations (e.g., hypertension, cardiovascular events, cancer, osteoarthritis, chronic kidney disease) [8,22,23]. In both reference cohorts, relative to controls, circulating FGF21 was greater among individuals with impairments in glucose regulation (individuals with overt T2DM and indication of pre-diabetes), which has also been found in other studies [2,8,24]. Another recent study reported a mean concentration of FGF21 of 254 pg/ml ± 113, with a range from 17 to 629 pg/ml [25] in men ($n = 160$; 30–79 y; mean BMI 24.1 kg/m^2) with no history of chronic diseases, Of note, in that study two outliers displaying extremely high concentrations of FGF21were excluded from the analysis (1914 and 2411 pg/ml). Inclusion of the outliers would have markedly increased the reported mean value. Also noteworthy, ELISA assay kits differed across studies (*Jian*: BioVendor Laboratory Medicine, Modrice, Czech Republic; *Semba and Taniguchi*: R&D Systems, Minneapolis, MN; *Hanks*: Millipore Corporation, Billerica, MA), which may certainly contribute to reported discrepancies. As interest continues to generate regarding the role of FGF21 and metabolic health outcomes, particularly in context of dynamic periods of growth, further evaluation into physiologically 'normal' levels is warranted.

Due to differences in metabolic activity, body composition compartments vary in energy requirements [26]. The dynamic interaction between tissue partitioning and bioenergetics due to metabolic demand over the life course may contribute to differences in FGF21 with age. The characteristic amassment of body fat and reduction in fat-free mass in adulthood differs from tissue partitioning in childhood when increased metabolic demand leads to gains in both fat and fat-free mass. As might be anticipated with changes in tissue compartments, percent total fat and trunk fat increased with increasing age categories, whereas mean BMD remained stable across adult categories (albeit expectedly higher relative to children). Lean mass was numerically highest among young adults, which was a significantly higher value relative to elder adults. To assess whether the observed differences in body composition may contribute to the association between FGF21 and age group, the influence of the various indices of body composition was assessed. The age category variable remained a significant predictor of FGF21 levels in statistical models independent of each body composition parameter. This suggests that, among healthy individuals, quantitative differences in fat and fat-free mass do not influence age-related levels of circulating FGF21.

Because we hypothesized FGF21 levels in circulation may be in response to metabolic demand, we also evaluated whether age and/or body composition may differentially influence FGF21 level at lower, middle or relatively high concentrations of FGF21. By tertile of FGF21, age group explained the variance in FGF21 concentration in only the lowest tertile independently of percent lean, total fat, and trunk fat, but not BMD. While not apparent in overall models, this finding suggests that the metabolically active, energy demanding skeleton may exert greater influence on metabolic

regulation by FGF21 and supports potential lifecourse dependence. At lower tertiles of FGF21, age, BMD and trunk fat were lower relative to higher tertiles. FGF21 synthesis and release may be a compensatory response to (re)establish metabolic control.

Not only does body composition undergo changes with age, so too does a multitude of hormones and factors involved in growth, maturation, and maintenance of systemic physiologic requirements. PPARs, which influence the contribution by the liver and adipose tissue to FGF21 appearance in circulation, are key regulators in various age-associated pathophysiological processes related to energy metabolism and oxidative stress [27]. PPARs are also associated with bone resorptive (catabolic) processes [28,29]. In addition, several studies have demonstrated that PPARα and PPARγ inhibit the expression of inflammatory genes, such as cytokines, metalloproteases, and acute phase proteins [30]. In future studies, the governance of FGF21 expression and secretion by specific hormones and transcription factors warrant consideration. Age may have more of an influence on FGF21 levels in youngest individuals, which could be explained by differential underlying regulatory factors and pathways which also undergo aged-related differences. Whether maintenance of bone mass in individuals with lower physiologic FGF21 levels may attenuate FGF21 secretion into the circulation warrants further investigation.

Our study had several notable strengths. Our cohort included a wide age range (i.e., childhood through late adulthood) and the method used to assess body composition (DXA) is notably robust. Despite the strengths of this study, there were some limitations. Although participants were considered to be overall healthy, specific measures of glucose, insulin and lipid homeostasis would have provided further insight. While the adult cohort included only non-obese individuals, the pediatric population included a wider range of body habitus based on BMI centiles. The inclusion of body composition parameters analyzed by the sophisticated technique, DXA, limited this potential for confounding. This study was cross-sectional in nature, which disallowed the ability to assess longitudinal changes.

In conclusion, our findings in a healthy population display an age-related increase in circulating FGF21, highlighting a potential differential age effect in response to metabolic demand over the lifecourse. At lower levels of FGF21, however, bone density may explain the age-related association. Given the role of FGF21 in cellular energy metabolism as a regulator of lipid and glucose utilization in animal models and our findings presented herein, at this stage it is unclear whether the age-related increases of FGF21 are a consequence of body composition or, alternatively, causative.

Conflicts of interest

The authors declare they have no conflicts of interest.

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