Serum FGF21 levels are altered with various factors including lifestyle behaviors

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Abstract

Fibroblast growth factor (FGF) 21 has various functions, including glucose and lipid metabolism, yet the biology of FGF21 remains unclear. This study aimed to investigate specific conditions that might affect the functions of FGF21. Subjects included 398 healthy men who underwent health examinations to obtain information on physical and biochemical parameters and lifestyle behaviors. Associations of serum FGF21 levels with each parameter were assessed in the study. FGF21 levels correlated with age, body mass index, waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure, aspartate aminotransferase, alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ-GTP), uric acid, total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol, fasting plasma glucose, and HbA1c. Moreover, FGF21 levels were significantly associated with lifestyle behaviors, including smoking status and breakfast and alcohol consumption frequency. Multivariable regression analysis showed that age, ALT, γ-GTP, smoking status, and breakfast and alcohol consumption frequency were independent variables for FGF21 levels. Assessment among the non-obese and obese groups showed that FGF21 levels correlated with WC, SBP, and TC only in the non-obese group. Thus, serum FGF21 levels were affected by several factors, including lifestyle behaviors, age, and liver function. To assess the functions of FGF21 in individuals, considering these factors would be essential.

(199 words)

Introduction

Fibroblast growth factor (FGF) 21 is predominantly derived from the liver and is a member of the FGF19 subfamily, which includes FGF15/19 and FGF23. Unlike other FGF subfamilies, members of the FGF19 subfamily exhibit hormone-like functions and require Klotho proteins, namely αKlotho and βKlotho, as co-factors to increase their binding affinity to FGF receptors [1]. FGF21 interacts with βKlotho and plays an important role in glucose and lipid metabolism. It regulates glucose uptake in adipocytes, enhances fatty acid oxidation in the liver, and inhibits lipogenesis [2,3].

FGF21 administration in obese animal models has shown an improvement in insulin sensitivity, a decrease in triglyceride and cholesterol levels, and a reduction in adiposity; therefore, FGF21 is expected to be a potential new therapy for obesity and obesity-related diseases, including type 2 diabetes and nonalcoholic steatohepatitis [4,5]. Paradoxically, serum levels of FGF21 increase in individuals with obesity, type 2 diabetes, and metabolic syndrome [6,7]. While this increase in FGF21 levels is regarded as a compensatory response or the FGF21-resistant state, the precise underlying mechanism for this increase remains unclear [8,9].

We previously reported that FGF21 levels were high in smokers and negatively correlated with the metabolic syndrome-related cytokine, adiponectin. Interestingly, FGF21 levels were differentially associated with liver function and total cholesterol among smokers and never-smokers, suggesting that smoking stress affects the relationship between FGF21 and metabolic parameters [10]. Moreover, we

demonstrated a sex-based difference in the relationship between FGF21 levels and metabolic parameters [11]. These results suggest that there may be some conditions that affect FGF21 functions.

Since FGF21 has various functions in multiple target organs, FGF21 biology is complicated and several uncertainties remain [12]. Therefore, in the present study, we focused on evaluating specific conditions that might affect the functions of FGF21 by assessing the association of serum levels of FGF21 with physical parameters, biochemical parameters, and lifestyle behaviors using cross-sectional data of healthy subjects.

Results

Association of FGF21 levels with each parameter

The characteristics of the study participants are presented in Table 1. The median age and FGF21 levels were 42 (37–49) years and 165 (106–256) pg/mL, respectively.

Table 1
Characteristics of the study subjects.

n	398
Age (years)	42 (37−49)
BMI (kg/m^2)	24.3 (21.9−9.3)
WC (cm)	86.0 (77.5−96.0)
SBP (mmHg)	124 (112−133)
DBP (mmHg)	78 (71−87)
AST (IU/l)	23 (18−32)
ALT (IU/l)	28 (17−47)
γ-GTP (IU/l)	39 (23−69)
Cr (mg/dl)	0.9 ± 0.1
UA (mg/dl)	6.3 ± 1.2
TC (mg/dl)	201 ± 32
TG (mg/dl)	105 (70−158)
HDLC (mg/dl)	52 (44−61)
FPG (mg/dl)	88 (83−94)
HbA1c (%)	5.3 (5.1−5.5)
FGF21 (pg/ml)	165 (106−256)
Data are expressed as means ± SD or medians (interquartile range).	
BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase; Cr, creatinine; UA, uric acid; TC, total cholesterol; TG, triglycerides; HDLC, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; FGF21, fibroblast growth factor 21.	

Table 2 shows the correlations between serum levels of FGF21 and each physical and biochemical parameter. Serum levels of FGF21 significantly correlated with age, Body mass index (BMI: body weight [kg] divided by squared height [m^2]), waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ-GTP), uric acid (UA), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDLC), fasting plasma glucose (FPG), and HbA1c.

Table 2
Correlations of fibroblast growth factor 21 levels with physical and biochemical parameters.

	τ	P
Age	0.24*	< 0.0001
BMI	0.13*	0.0001
WC	0.15*	< 0.0001
SBP	0.20*	< 0.0001
DBP	0.21*	< 0.0001
AST	0.19*	< 0.0001
ALT	0.20*	< 0.0001
γ-GTP	0.31*	< 0.0001
Cr	−0.02	0.571
UA	0.17*	< 0.0001
TC	0.10*	0.004
TG	0.23*	< 0.0001
HDLC	−0.10*	0.003
FPG	0.17*	< 0.0001
HbA1c	0.13*	0.0001
* P < 0.005.		
Abbreviations are as in Table 1.		

Lifestyle behaviors affect serum levels of FGF21

In line with our previous report that serum levels of FGF21 were upregulated in smokers [10,11], FGF21 levels in the current study were significantly higher in smokers [210 (124–353) pg/mL] than in never smokers [147 (101–224) pg/mL, p < 0.0001] and correlated with smoking status (τ = 0.14, p < 0.0001). Moreover, serum levels of FGF21 significantly correlated with breakfast consumption frequency (τ = −0.12, p < 0.0001), alcohol consumption frequency (τ = 0.15, p < 0.0001), and daily alcohol intake (τ = 0.08, p = 0.0237). We further assessed the associations between FGF21 levels and lifestyle behaviors, as shown in Fig. 1. Serum levels of FGF21 were significantly increased in subjects whose breakfast consumption frequency was low (Fig. 1A). In addition, FGF21 levels were significantly increased in subjects whose alcohol consumption frequency was high (Fig. 1B), with marginal increase in subjects

whose daily alcohol intake was high (Fig. 1C). These results suggest that lifestyle behaviors, including breakfast consumption and alcohol consumption affected the serum levels of FGF21 as smoking status.

Age strongly associates with serum levels of FGF21

Multivariable regression analysis showed that age, ALT, γ-GTP, smoking status, and breakfast and alcohol consumption frequency were independent variables for FGF21 levels (Table 3). Correlation and regression analyses showed that age is a strong variable for FGF21 levels; hence, we assessed FGF21 levels in each age group. The median serum levels of FGF21 in participants of age 30–39 years (n = 129), 40–49 years (n = 175), and ≥ 50 years (n = 94) were 119 (81–201) pg/mL, 181 (117–271) pg/mL, 214 (150–359) pg/mL, respectively. Significant differences were observed among age groups (p = 0.0001).

Table 3
Multivariable regression analysis of log-transformed fibroblast growth factor 21.

	β	P
Age	0.0108*	< 0.0001
WC	0.0008	0.634
DBP	0.0011	0.456
ALT	0.0011*	0.042
γ-GTP	0.0006*	0.049
UA	0.0144	0.256
TC	0.0001	0.829
TG	0.0002	0.234
HDLC	−0.0013	0.339
FPG	0.0012	0.378
HbA1c	−0.0326	0.415
Smoking status	0.0678*	0.022
Breakfast consumption frequency	−0.0585*	0.001
Alcohol consumption frequency	0.0795*	< 0.0001
* P < 0.05.		
Abbreviations are as in Table 1.		

Relationship between FGF21 and each parameter in non-obese and obese group

FGF21 levels show a strong association obesity, whereby FGF21 therapy has been identified as a possible treatment for obesity and obesity-related diseases in recent years [4]. We categorized the study participants into non-obese (BMI < 25 kg/m^2) and obese (BMI ≥ 25 kg/m^2) groups and assessed the relationship between FGF21 levels and each parameter in both groups. The characteristics of each group are shown in Table 4. Serum levels of FGF21 in the non-obese and obese groups were 145 (96−225) pg/mL and 189 (124−300) pg/mL, respectively.

Table 4
Characteristics of subjects in the non-obese and obese groups.

	Non-obese	Obese	P
	BMI < 25 kg/m^2	BMI ≥ 25 kg/m^2	
n	219	179	–
Age (years)	41 (35−48)	43 (39−49) *	0.019
BMI (kg/m^2)	22.2 (20.3−23.5)	29.6 (27.9−31.1) *	< 0.0001
WC (cm)	79.0 (73.0−83.5)	97.0 (93.0−102.0) *	< 0.0001
SBP (mmHg)	117 (108−128)	130 (121−138) *	< 0.0001
DBP (mmHg)	73 (68−81)	85 (77−91) *	< 0.0001
AST (IU/l)	19 (17−24)	30 (24−38) *	< 0.0001
ALT (IU/l)	19 (14−27)	47 (33−72) *	< 0.0001
γ-GTP (IU/l)	27 (19−48)	58 (37−92) *	< 0.0001
Cr (mg/dl)	0.9 ± 0.1	0.9 ± 0.1	–
UA (mg/dl)	5.8 ± 1.2	6.8 ± 1.0*	< 0.0001
TC (mg/dl)	197 ± 30	207 ± 33*	0.002
TG (mg/dl)	80 (58−116)	142 (103−218) *	< 0.0001
HDLC (mg/dl)	58 (50−68)	46 (40−52) *	< 0.0001
FPG (mg/dl)	86 (81−90)	92 (86−101) *	< 0.0001
HbA1c (%)	5.2 (5.0−5.4)	5.4 (5.2−5.8) *	< 0.0001
FGF21 (pg/ml)	145 (96−225)	189 (124−300) *	0.0001
* P < 0.05 versus non-obese group.			
Abbreviations are as in Table 1.			

The correlations between FGF21 levels and each parameter among the non-obese and obese groups are summarized in Table 5. Serum levels of FGF21 significantly correlated with WC, SBP, and TC only in the non-obese group, while the correlation between FGF21 and HbA1c was observed only in the obese group. Thus, it can be suggested that FGF21 effects may differ depending on the BMI of each subject.

Table 5
Correlations of fibroblast growth factor 21 with physical and biochemical parameters in the non-obese and obese groups.

	Non-obese		Obese	
	$BMI < 25 \text{ kg/m}^2$		$BMI \geq 25 \text{ kg/m}^2$	
	τ	P	τ	P
Age	0.27*	< 0.0001	0.17*	0.001
BMI	0.09	0.058	-0.04	0.486
WC	0.14*	0.002	0.00	0.965
SBP	0.21*	< 0.0001	0.05	0.298
DBP	0.21*	< 0.0001	0.12*	0.015
AST	0.16*	0.0004	0.13*	0.011
ALT	0.16*	0.0004	0.12*	0.022
γ-GTP	0.31*	< 0.0001	0.25*	< 0.0001
Cr	-0.04	0.379	0.01	0.911
UA	0.12*	0.011	0.12*	0.018
TC	0.09*	0.040	0.07	0.182
TG	0.14*	0.002	0.24*	< 0.0001
HDLC	-0.02	0.593	-0.04	0.388
FPG	0.11*	0.014	0.16*	0.001
HbA1c	0.04	0.349	0.13*	0.012
* P < 0.05.				
Abbreviations are as in Table 1.				

Discussion

FGF21 has multiple functions, and recent studies have shown its various protective effects. Our previous study showed that FGF21 levels correlated with adiponectin, a metabolic syndrome-related cytokine that also has protective effects, including anti-inflammatory, anti-atherogenic, and anti-diabetic effects [10,13]. Since FGF21 regulates oxidative stress and attenuates inflammation, it is considered a stress-response factor [14,15]. In the present study, we analyzed the association of FGF21 levels with metabolic parameters and lifestyle behaviors. We previously reported that smoking status affects the serum levels of FGF21 [10]. In this study, we demonstrated that not only smoking, but also breakfast and alcohol consumption frequency affected the FGF21 levels. Breakfast is considered the most important meal of the day and affects physical and mental health [16−18]. Skipping breakfast has been reported to be associated with inflammation and cardiovascular disease risk, related to an increase in WC and low-density lipoprotein cholesterol [19−22]. We found FGF21 levels to be increased in subjects with a low breakfast consumption frequency and a high alcohol consumption frequency. FGF21 has a protective effect against liver damage induced by alcohol. In animal models, FGF21 administration reduces alcohol intake via the central nervous system, which interacts with βKlotho [14,23]. An increase in FGF21 levels in a stressed condition, including smoking, skipping breakfast, and alcohol ingestion, is suggested to be a protective response of FGF21.

While serum levels of FGF21 were related to many parameters, we found that age was a strong factor affecting FGF21 levels. Evaluation of serum levels of FGF21 in each age group showed that FGF21 levels were significantly increased along with aging. Aging is associated with various diseases, including cardiovascular diseases, cancer, and pulmonary diseases, which are known to be the leading causes of death worldwide [24]. Moreover, obesity and obesity-related diseases are associated with aging [25]. Previous studies have reported that FGF21 extended the lifespan of mice and showed a protective effect on age-related changes [26,27]. Since FGF21 can ameliorate both aging and metabolism, FGF21 is regarded as a key factor linking aging and metabolism [28]. In the present study, although the subjects were healthy individuals who did not present any signs of disease, FGF21 levels were upregulated in the aged groups. Considering the anti-aging effects of FGF21, these increased FGF21 levels might be a compensatory response toward progress in aging and age-related changes.

Individuals with obesity, in whom protective effects are not seen with increased serum levels of FGF21, are considered FGF21-resistant [6,8]. To evaluate differential functions of FGF21 between non-obese and obese subjects, we assessed the association of FGF21 with metabolic parameters among both groups. As previously reported, FGF21 levels were significantly high in the obese group. Serum levels of FGF21 correlated with more variables in the non-obese group than in the obese group. Moreover, WC, SBP, and TC correlated with FGF21 only in the non-obese group. WC is a known parameter for the definition of metabolic syndrome and is strongly associated with visceral fat accumulation [29]. Although both WC and FGF21 levels were high in obese subjects, there was no correlation between WC and FGF21. This disrupted relationship between FGF21 and WC suggests that the protective effects of FGF21 are attenuated in obese subjects. We previously reported that serum levels of FGF21 correlated with metabolic parameters differently among smokers and never-smokers [10]. We also confirmed that there

were sex differences in the relationship between FGF21 levels and metabolic parameters [11]. These results show a variable relationship between FGF21 levels and metabolic parameters and that the effect of FGF21 may vary depending on the individual background. Since FGF21 is expected as a new therapy for obesity and obesity-related diseases [4], effective conditions are needed to be evaluated.

FGF21 is reported to induce angiotensin-converting enzyme 2, which has recently been focused on as a key mechanism against SARS-coronavirus 2 (SARS-CoV-2) infection, and prevent hypertension and vascular damage in mice [30,31]. Since FGF21 upregulating factors, including smoking, type 2 diabetes, and obesity, are also known risk factors for SARS-CoV-2-related mortality [32], it is intriguing to speculate the protective effects of FGF21 on SARS-CoV-2 infection.

FGF21 levels have been reported to be altered in various diseases [6,7,33]. However, as demonstrated in the present study, the serum levels of FGF21 are affected by several factors. Therefore, to assess the functions of FGF21 in subjects with such diseases, considering these factors and matching the conditions among the subjects is essential. Furthermore, as the mechanisms of change in FGF21 levels are still unclear, we believe that our findings might help elucidate the complicated biology of FGF21.

In conclusion, we evaluated the parameters and lifestyle behaviors that may affect FGF21 functions in the present study. Serum levels of FGF21 were affected by several lifestyle behaviors, including smoking status, breakfast consumption, and alcohol consumption. Moreover, in the FGF21 relating parameters, age was strongly associated with FGF21 levels. Serum levels of FGF21 are associated with metabolic parameters differently in non-obese and obese subjects.

Methods

Study subjects

This study included cross-sectional data obtained from employees at Osaka University. The subjects were randomly selected from among those who underwent an annual health checkup at the Osaka University Health and Counseling Center. A total of 398 healthy Japanese men who had not taken any chronic or frequent medication for at least 1 year before their health checkup and had not experienced any acute illness within the previous 2 weeks were enrolled in the study. This study was carried out in accordance with the Declaration of Helsinki and the Ethics Guidelines for Clinical Research from the Ministry of Health, Labour and Welfare and the Ministry of Education, Culture, Sports, Science and Technology. All experimental protocols in this study were approved by the Ethics Committee of Health and Counseling Center, Osaka University, and written informed consent was obtained from all subjects prior to participation in the study.

Physical and biochemical parameters

BMI, WC at the umbilical level, SBP, DBP were measured as physical parameters.

Serum was collected from subjects between 9 and 11 AM after an overnight fast and stored at $\leq -20°C$ until assayed. Serum levels of FGF21 were measured using a sandwich enzyme-linked immunoassay system according to the manufacturer's instructions (R&D Systems Inc., Minneapolis, USA).

Lifestyle assessments

Information on the medical history, current treatments, smoking status, and lifestyle behaviors of the study participants were obtained via questionnaires. Each piece of information was reconfirmed through expert interviews by trained nurses. Smoking status was semi-quantified as 0 = never smoker and 1 = smoker. Lifestyle behaviors, including breakfast and alcohol consumption frequency, and daily alcohol intake, were asked as follows, and each answer was semi-quantified using the following scales. Breakfast consumption frequency: "How many days a week do you eat breakfast?" on three scales: 1 = 0−2 days a week, 2 = 3−6 days a week, 3 = everyday; alcohol consumption frequency: "How many days a week do you drink alcohol?" on three scales: 1 = 0−2 days a week, 2 = 3−6 days a week, 3 = everyday; daily alcohol intake: "How many amounts of pure alcohol do you have on a typical day when you are drinking?" on three scales: 1 = < 20 g, 2 = 20−40 g, 3 = ≥ 40 g.

Statistical analyses

All statistical analyses were performed using STATA 14 (STATA Corp LLC, College Station, TX, USA). The distribution of continuous variables was tested using the Shapiro−Wilk test. Normally distributed variables are presented as means ± standard deviation; non-normally distributed variables are reported as medians with interquartile ranges. Student's t-test or the Mann−Whitney U test was used to compare differences between the two groups. Kendall's rank correlation coefficient and multiple regression analysis were used to analyze the variables. For multi-group comparisons, the Kruskal−Wallis test with Dunn's post-hoc test was used. Statistical significance was set at $P < 0.05$.

Declarations

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

KN, IN, and KY-T designed the study. KN performed the experiments, analyzed the data, and wrote the manuscript. CI, IN, and KY-T provided appropriate suggestions during the development of this study.

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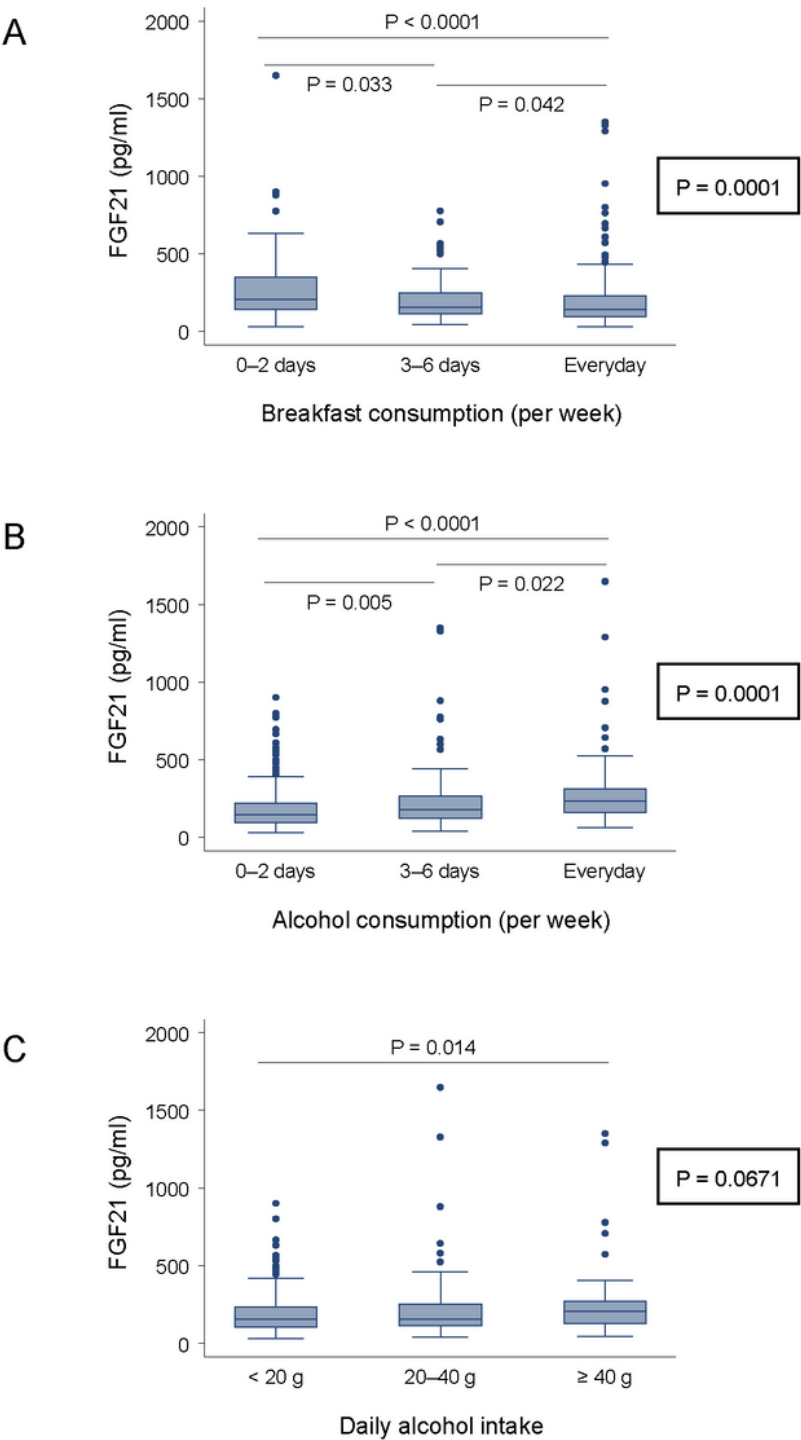
Figures

Figure 1

Figure 1

Serum levels of fibroblast growth factor (FGF) 21 are affected by lifestyle behaviors. Associations between serum levels of FGF21 and breakfast consumption (A), alcohol consumption (B), and daily alcohol intake (C). Data are shown as the sample minimum, lower quartile, median, upper quartile, and sample maximum.