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Original article

Fibroblast growth factor 21 and protein energy wasting in hemodialysis patients

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SUMMARY

Introduction: Protein energy wasting (PEW) is the most important risk factor for morbidity and mortality in hemodialysis patients. Inadequate dietary protein intake is a frequent cause of PEW. Recent studies have identified fibroblast growth factor 21 (FGF21) as an endocrine protein sensor. This study aims to investigate the potential of FGF21 as a biomarker for protein intake and PEW and to investigate intradialytic FGF21 changes.

Methods: Plasma FGF21 was measured using an enzyme-linked immunoassay. Complete intradialytic dialysate and interdialytic urinary collections were used to calculate 24-h urea excretion and protein intake. Muscle mass was assessed using the creatinine excretion rate and fatigue was assessed using the Short Form 36 and the Checklist Individual Strength.

Results: Out of 59 hemodialysis patients (65 ± 15 years, 63% male), 39 patients had a low protein intake, defined as a protein intake less than 0.9 g/kg/24-h. Patients with a low protein intake had nearly twofold higher plasma FGF21 compared to those with an adequate protein intake (FGF21 1370 [795–4034] pg/mL versus 709 [405–1077] pg/mL;P < 0.001). Higher plasma FGF21 was associated with higher odds of low protein intake (Odds Ratio: 3.18 [1.62–7.95] per doubling of FGF21; P = 0.004), independent of potential confounders. Higher plasma FGF21 was also associated with lower muscle mass (std β: −0.34 [-0.59;-0.09];P = 0.009), lower vitality (std β: −0.30 [-0.55;-0.05];P = 0.02), and more fatigue (std β: 0.32 [0.07;0.57];P = 0.01). During hemodialysis plasma FGF21 increased by 354 [71–570] pg/mL, corresponding to a 29% increase.

Conclusion: Higher plasma FGF21 is associated with higher odds of low protein intake in hemodialysis patients. Secondarily, plasma FGF21 is also associated with lower muscle mass, less vitality, and more fatigue. Lastly, there is an intradialytic increase in plasma FGF21. FGF21 could be a valuable marker allowing for objective assessment of PEW.

1. Introduction

Protein energy wasting (PEW), a state of decreased body stores of protein and energy fuels, is the most important risk factor for morbidity and mortality in hemodialysis patients [1,2]. Inadequate dietary protein intake is a frequent and important cause of PEW in patients on maintenance hemodialysis [1–5].

Classic dietary assessments have many limitations, including under- and overreporting, illiteracy and motivation requirements, changes in diet due to self-reflections, errors in portion size

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estimates, and socially desirable answers [6–8]. Given the limiting nature of the aforementioned factors and the need to meticulously monitor protein-energy status, biomarkers not subject to these limiting factors are warranted to assess dietary protein intake and PEW.

Fibroblast growth factor 21 (FGF21), a member of the FGF family, is an endocrine factor with a key role in maintenance of protein intake and metabolic homeostasis under metabolic, oxidative, hormonal, environmental, and nutritional stresses [9–13]. Animal studies demonstrated that plasma FGF21 concentrations increase in response to protein restriction [14–16], posing FGF21 as potential biomarker for dietary protein intake and PEW in chronic kidney disease (CKD). Besides low protein intake, PEW also constitutes low muscle mass and contributes to the development of chronic fatigue [3,17,18].

In the current study, we aimed to investigate the potential of FGF21 as a biomarker for protein intake and PEW, and to investigate intradialytic FGF21 changes. To do so, we performed three separate analyses. In our primary analyses, we investigated whether higher plasma FGF21 concentrations are associated with presence of low dietary protein intake in hemodialysis patients. In secondary analyses, we investigated the associations of plasma FGF21 with protein intake, muscle mass, and fatigue expressed as continuous variables in hemodialysis patients. In tertiary analyses, we investigated the intradialytic changes of plasma FGF21 and compared these with changes in markers of hemoconcentration during hemodialysis.

2. Material and methods

2.1. Design and study population

This observational study was performed according to ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments, and was approved by the Medical Ethical Committee of the University Medical Center Groningen, The Netherlands. All participating patients gave written informed consent. Inclusion criteria were twice- or thrice-weekly hemodialysis with 3–5 h per treatment, a hemodialysis vintage of ≥2 months and absence of clinical signs of infection. Patients dialyzing three times per week dialyzed on either Monday-Wednesday-Friday or on Tuesday-Thursday-Saturday. In both cases the mid-week hemodialysis session was used in this study. For patients dialyzing twice-weekly, the last hemodialysis session of the week was used. Hypertension was defined as predialysis systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg. A history of diabetes and cardiovascular disease was obtained from the patients' medical records. Cardiovascular disease was defined as a history of ischemic heart disease, congestive heart failure, coronary artery bypass grafting, percutaneous coronary intervention, stroke, or peripheral vascular disease. Blood pressure and weight were measured before and after hemodialysis. Body mass index (BMI) was defined as body weight after hemodialysis divided by the square of body height.

2.2. Hemodialysis settings

All studies were performed with the Fresenius 5008 hemodialysis apparatus with a low-flux dialyzer (Fresenius Medical Care, Bad Homburg, Germany) using smart*bag* dialysate concentrations (Fresenius Medical Care). Blood flow and dialysate flow were between 200 and 300 mL/min and between 500 and 700 mL/min, respectively. Dialysate temperature was 36.0 or 36.5 °C. Dialysis fluid sodium varied from 136 to 140 mmol/L, potassium from 1 to 3 mmol/L, depending on the plasma potassium concentration, calcium from 1.25 to 1.50 mmol/L and bicarbonate from 34 to 38 mmol/L.

2.3. Sample collection and laboratory measurements

During the hemodialysis session, all dialysate was collected in a 200-Liter tank. The total dialysate volume was measured by calculating the weight difference of the tank before and after the hemodialysis session. At the end of hemodialysis, all dialysate was homogenized, and samples were taken for analysis [8]. Blood was drawn directly from the dialysis line, at the start of hemodialysis and five minutes before the end of the hemodialysis session. Patients with significant residual diuresis, defined as a urine production of more than 200 mL/24-h, were asked to collect two 24-h urine collections before the hemodialysis session during which the dialysate was collected. For patients with a thrice-weekly hemodialysis schedule this was the complete interdialytic urine production. Plasma FGF21 was measured in EDTA plasma samples taken before and after hemodialysis. Unless otherwise stated, analyses are performed using plasma FGF21 concentration before dialysis. FGF21 measurements were performed using an enzyme linked immunoassay according to the manufacturer's instructions (Immuno-Biological Laboratories, Fujioka, Japan). Manufacturer's intra-assay coefficients of variation were 2.7% at 1010 pg/mL, 3.0% at 239 pg/mL and 5.6% at 78 pg/mL. Inter-assay coefficients of variation were 2.9% at 1002 pg/mL, 4.1% at 243 pg/mL and 6.8% at 78 pg/mL. Cross-reactivity for FGF19 was <0.1% and the sensitivity was 29 pg/mL. Urea and high-sensitivity C-reactive protein (hs-CRP) concentrations were measured on Roche routine chemistry analyzers (Modular P/Cobas C, Roche Diagnostics, Mannheim, Germany). Interleukin-6 (IL-6) was measured in a subset of the participants (n = 20) using a Human IL-6 Quantikine HS Elisa kit (R&D systems, Minneapolis, United States). Other laboratory measurements were performed with automated and validated routine methods (Roche Diagnostics, Mannheim, Germany). To determine the combined excretion rate of urea, we combined the intradialytic dialysate and interdialytic urinary urea excretion rate (UUE) [8]:

$$\text{Combined urea excretion rate (mmol/24-h)} = \left(V_{Dialysate} * D_{urea} * n\right) \Big/ 7 + UUE$$

in which $V_{Dialysate}$ = total volume of the spent dialysate (L); D_{urea} = measured urea concentration in the collected dialysate (mmol/L); n = number of hemodialysis sessions per week; UUE = 24-h urinary urea excretion (mmol/24-h), averaged from two 24-h urine collections.

2.4. Dietary protein intake assessed by the biomarker method

The dietary protein intake was calculated based on the combined excretion rate of urea, according to the Maroni formula and indexed to body weight [20]:

$$\text{Protein intake (g/kg/24-h)} = (6.25 * (0.028 * \text{CUER} + 0.031 * \text{BW}) + \text{UPE}) / \text{BW}$$

in which CUER = combined urea excretion rate (mmol/24-h); BW = body weight after dialysis (kg); and UPE = 24-h urine protein excretion (g/24-h), averaged from two 24-h urine collections. A dietary protein intake <0.9 g/kg/24-h was defined as a low protein intake [21–24]. Unless otherwise stated, protein intake refers to protein intake measured by this biomarker method.

In sensitivity analyses, we also aimed to investigate whether plasma FGF21 is associated with protein intake assessed by the normalized protein catabolic rate (nPCR). For these analyses, nPCR was calculated according to formula by Depner and Daugirdas [25]:

$$\text{nPCR(g/kg/24-h)} = C_0/(a + b*kt/V + c/Kt/V) + 0.168$$

in which C_0 is the predialysis blood urea nitrogen (mg/dL) and Kt/V is the single-pool estimate of the dialysis dose. The corresponding coefficients for a, b, and c were 25.8, 1.15 and 56.4 for patients on thrice-weekly hemodialysis and 33.0, 3.60 and 83.2 for patients on twice-weekly hemodialysis [25]. The Kt/V was calculated according to formula of Daugirdas [19]:

$$\text{Kt}/\text{V} = -\ln(\text{R} - 0.008 * \text{t}) + (4 - 3.5 * \text{R})*\text{UF}/\text{W}$$

in which R is the ratio between the post- and predialysis concentration of urea, t is duration of the hemodialysis session (h), UF is the ultrafiltration volume (L) and W the body weight after hemodialysis (kg).

2.5. Protein intake based on dietary diaries

As a sensitivity analysis, we also investigated whether FGF21 is associated with low protein intake based on dietary diaries. Data on dietary diaries were available in 43 out of 59 patients. Participating patients were asked to record all their food and fluid intake in a dietary diary for a period of five days starting five days before the hemodialysis of interest. The number of servings was expressed in natural units (e.g., slice of bread or apple) or household measures (e.g., cup or spoon). The diaries were self-administered and filled out at home. Dietary data were converted into daily protein intake with the use of the Dutch Food Composition Tables (Nevo 2007 and 2011), using EvryDietist calculating software, and are only used in the additional analyses, as explained below.

2.6. Muscle mass

Muscle mass was assessed by calculating the combined intradialytic dialysate and interdialytic urinary creatinine excretion rate (CER):

$$\text{CER}(\mu\text{mol/kg/24-h}) = \left(\left(V_{\text{Dialysate}} * D_{\text{Creat}} * n\right)/7 + \text{UCrE}\right)/\text{BW}$$

in which $V_{\text{Dialysate}}$ = total volume of dialysate (L); D_{Creat} = measured creatinine concentration in the collected dialysate (μmol/L); n = number of dialyses per week; UCrE = 24-h urinary creatinine excretion (μmol/24-h), averaged from two 24-h urine collections; BW = body weight after dialysis (kg).

2.7. Fatigue

Fatigue was assessed using the Short Form 36 (SF-36) and the Checklist Individual Strength (CIS). The SF-36 is a questionnaire that measures quality of life across eight domains [26]. All SF-36 subscales range from 0 to 100 points, with a higher score indicating less disability. In the current study we use the subdomain vitality, which is a measure of fatigue. A lower SF-36 vitality score indicates more fatigue. The CIS is a self-reported multidimensional instrument to assess four qualitatively different aspects of fatigue (fatigue severity, concentration problems, reduced motivation, and reduced activity level) [27,28]. The CIS-questionnaire inquiries about fatigue and fatigue-related behavioral aspects and consists of 20 statements for which the participant indicates on a 7-point Likert-scale to what extent the statement applies to the participant. In the current study we use the subdomain fatigue severity (range 8–56 points) as a measure of self-reported fatigue. A higher CIS score indicates a higher fatigue burden.

2.8. Statistical analysis

Data analyses and computations were performed with SPSS 24.0 software (IBM, Armonk, NY, USA) and R version 3.6.1 software (The R-Foundation for Statistical Computing, Vienna, Austria). Baseline data are presented as mean ± standard deviation for normally distributed data, median [interquartile range] for non-normally distributed data, and as numbers (percentages) for nominal data. A two-sided $P < 0.05$ was considered to indicate statistical significance. Primary data are shown according to tertiles of FGF21 to facilitate interpretation. Because it is recommended to use continuous variables rather than tertiles for statistical analyses in studies with a relatively small sample size [29], further statistical analyses were performed using $\log_2$-transformed plasma FGF21 concentration as a continuous variable.

2.9. Primary analyses

Primary analyses consist of logistic regression analyses of plasma FGF21 concentration with a low protein intake, defined as a protein intake <0.9 g/kg/24-h. Models were adjusted for *a priori* selected variables and variables identified in the baseline table by a P_{trend} <0.05. The following models were used: Model 1: crude; Model 2: adjusted for age and sex; Model 3: as model 2, additionally adjusted for BMI; Model 4: as model 3, additionally adjusted for dialysis vintage; Model 5: as model 3, additionally adjusted for hours of dialysis per week; Model 6: as model 3, additionally adjusted for C-reactive protein; Model 7: as model 3, additionally adjusted for diabetes and history of cardiovascular disease. To visualize the continuous associations of plasma FGF21 with low protein intake, $\log_2$-transformed plasma FGF21, as a continuous variable, was individually plotted against the odds of low protein intake.

Several sensitivity analyses were performed for the primary analyses. First, we performed similar analyses using different cut-offs for a low protein intake, i.e. 0.8 g/kg/24-h and 1.0 g/kg/24-h. Second, we performed analyses after exclusion of outliers in plasma FGF21. Outliers in $\log_2$ FGF21 were defined as values deviating more than two standard deviations from the mean. Based on this, four participants were excluded, leaving 55 participants for analysis. Similarly, we performed analyses after exclusion of outliers in protein intake. Outliers in protein intake were defined as values deviating more than two standard deviations from the mean. Based

on this, two participants were excluded, leaving 57 participants for analysis. Since FGF21 has been shown to be related to glucose and insulin homeostasis [30], we also performed sensitivity analyses in which we excluded participants with diabetes. Furthermore, to compare the association of plasma FGF21 with low protein intake assessed by either the biomarker method or 5-day dietary diaries, we performed similar analyses in a subset of the data, including only patients that filled in the 5-day dietary diaries (43 out of 59 patients). Finally, we investigated whether plasma FGF21 is also associated with a low protein intake assessed by the protein catabolic rate.

2.10. Secondary analyses

Secondary analyses consist of linear regression analyses of plasma FGF21 with protein intake, muscle mass, vitality, and fatigue. Plasma FGF21 was $\log_2$-transformed for analyses and creatinine excretion rate, SF-36 vitality score, and CIS fatigue severity score were used to as markers for muscle mass, vitality, and fatigue, respectively. The linear regression analyses models were adjusted for the same potential confounders as the logistic regression models mentioned above. For these analyses, regression coefficients were given as standardized beta values, referring to the number of standard deviations a dependent variable changes per standard deviation increase in the independent variable, thereby allowing for comparison of the strength of the associations of different variables. To visualize the linear associations, continuous $\log_2$ plasma FGF21, as a continuous variable, was individually plotted against protein intake, muscle mass, vitality, and fatigue. We performed one sensitivity analysis for the secondary analyses, in which we investigated whether plasma FGF21 was also associated with protein intake assessed using the normalized protein catabolic rate.

2.11. Tertiary analyses

Tertiary analyses compare the intradialytic changes in FGF21 with the intradialytic changes of hemoglobin, hematocrit, albumin, urea, and creatinine. The absolute change was calculated as the concentration after dialysis minus the concentration before dialysis. The relative change was calculated as the absolute change divided by the concentration before dialysis, multiplied by 100%. Differences in concentrations before and after dialysis were tested using the paired sample t-test and the Wilcoxon signed-rank test, for normally distributed variables and non-normally distributed variables, respectively. Correlation analyses were employed to investigate the Pearson correlation coefficient between plasma FGF21 before dialysis and plasma FGF21 after dialysis. Lastly, we investigated whether there are intradialytic changes in inflammation parameters hs-CRP and IL-6.

3. Results

3.1. Baseline data

A total of 59 hemodialysis patients were included in the study, of whom 37 (63%) were male. Mean age at inclusion was 65 ± 15 years with a median [interquartile range] hemodialysis vintage of 15 [6–41] months. Nearly all (95%) patients dialyzed thrice-weekly and most patients (81%) dialyzed four hours per session. A total of 26 (44%) patients had residual diuresis, with a mean urinary volume of 0.90 ± 0.63 L. Mean BMI was 25.5 ± 4.3 kg/m^2. Systolic blood pressure was 147 ± 21 mmHg and diastolic blood pressure was 70 ± 12 mmHg. Hypertension, cardiovascular disease, and diabetes mellitus were prevalent in 33 (58%), 25 (42%) and 15 (25%) of the patients, respectively. Plasma FGF21 concentration before hemodialysis was 1026 [692–2997] pg/mL. Differences in baseline characteristics amongst tertiles of plasma FGF21 are shown in Table 1. Compared to patients in the lowest tertile of plasma of FGF21, patients in the highest tertile had higher age, body weight, BMI, hs-CRP, and more fatigue (P_{trend} for all <0.05). Patients in the highest tertile had lower plasma urea, urea excretion, protein intake, creatinine excretion rate, and lower vitality (all P_{trend} <0.05).

3.2. Primary analyses of plasma FGF21 with low protein intake

A total of 39 (66%) patients had a low protein intake, defined as a protein intake less than 0.9 g/kg/24-h. Patients with a low protein intake had nearly twofold higher plasma FGF21 (1370 [795–4034] pg/mL versus 709 [405–1077] pg/mL; P < 0.001) as compared to those with an adequate protein intake. Logistic regression analyses between plasma FGF21 and low protein intake are shown in Table 2. Higher plasma FGF21 was associated with higher odds of low protein intake (Odds Ratio (OR): 2.88 [1.61–6.41] per doubling of FGF21; P = 0.002). After adjustment for age, sex and BMI, higher plasma FGF21 remained associated with higher odds of low protein intake (OR: 3.18 [1.62–7.95]; P = 0.005). Further adjustment for the potential confounders dialysis vintage, hours of dialysis per week, C-reactive protein, diabetes, and history of cardiovascular disease, did not materially change the association. A visual representation of the association of plasma FGF21 with low protein intake is displayed in Fig. 1. We performed several sensitivity analyses for the primary analyses. Logistic regression analyses of plasma FGF21 with low protein intake defined by a cutoff of 0.8 g/kg/24-h or 1.0 g/kg/24-h are displayed in Table S1 and Table S2, respectively. Regardless of the used cutoff value for defining low protein intake, higher plasma FGF21 remained significantly associated with low protein intake. Logistic regression analyses of plasma FGF21 with low protein intake also remained significant after exclusion of outliers in plasma FGF21 and protein intake, Table S3 and Table S4, respectively. Logistic regression analyses between plasma FGF21 and low protein intake also remained significant after exclusion of patients with diabetes, Table S5. To determine whether similar associations would be found if we use protein intake based on dietary diaries, we performed additional analyses in patients with data on dietary diaries available. A comparison of the association of plasma FGF21 with low protein intake, assessed by either the biomarker method or by dietary diaries is shown in Table S6. In this subset of the data (n = 43), plasma FGF21 remained significantly associated with low protein intake assessed by the biomarker method (OR: 2.78 [1.47–7.01]; P = 0.008). However, no association was found between plasma FGF21 and low protein intake assessed by dietary diaries (OR: 1.32 [0.87–2.14]; P = 0.21). After adjustment for potential confounders, the association of plasma FGF21 with low protein intake assessed by the biomarker method remained significant, whereas the association of plasma FGF21 with low protein intake assessed by dietary diaries did not reach significance in any of the models. Lastly, we investigated whether plasma FGF21 was also associated with the normalized protein catabolic rate. Plasma FGF21 appeared significantly and inversely associated with the normalized protein catabolic rate, independent of potential confounders, Table S7.

3.3. Secondary analyses with protein intake, muscle mass, fatigue and vitality as continuous variables

Linear regression analyses of plasma FGF21 with protein intake, muscle mass, fatigue and vitality expressed as continuous variables are shown in Table 3. Plasma FGF21 was inversely associated with protein intake (std β: −0.54 [-0.76; −0.32]; P < 0.001), muscle mass

Table 1
Hemodialysis patient characteristics according to tertiles of plasma Fibroblast Growth Factor 21.

	Full cohort $n = 59$ 94–26,912 pg/mL	Tertile 1 $n = 19$ <771 pg/mL	Tertile 2 $n = 20$ 771–1623 pg/mL	Tertile 3 $n = 20$ >1623 pg/mL	*P* value[a]
Plasma FGF21 concentration					
Before dialysis, pg/mL	1026 [692–2297]	599 [340–692]	1021 [841–1360]	3888 [2230–6148]	-
After dialysis, pg/mL	1444 [954–2874]	723 [395–1042]	1444 [1046–1620]	3502 [2748–8881]	**<0.001**
Demographics					
Age, years	65 ± 15	60 ± 16	67 ± 15	67 ± 13	**0.03**
Sex, n (%) male	37 (63)	13 (68)	12 (60)	12 (60)	0.98
Height, m	1.75 ± 0.09	1.74 ± 0.08	1.75 ± 0.09	1.77 ± 0.10	0.39
Body weight[a], kg	80 ± 16	74 ± 13	80 ± 19	85 ± 13	**0.03**
BMI, kg/m^2	25.5 ± 4.3	23.9 ± 3.8	25.6 ± 4.9	26.9 ± 3.8	**0.03**
Dialysis related					
Amount of dialysis, n (%)					
Two sessions per week	3 (5)	3 (16)	0 (0)	0 (0)	0.14
Three sessions per week	56 (95)	16 (84)	20 (100)	20 (100)	
Hours per hemodialysis, n (%)					
3–3.5 h	6 (10)	1 (05)	5 (25)	0 (0)	0.44
4 h	48 (81)	17 (89)	13 (65)	18 (90)	0.32
4.5–5 h	5 (8)	1 (5)	2 (10)	2 (10)	05.9
Hemodialysis vintage, months	15 [6–41]	13 [4–21]	10 [4–49]	19 [13–40]	0.70
Ultrafiltration volume, mL	1962 ± 901	1860 ± 1059	2112 ± 875	1895 ± 823	0.84
Residual diuresis, n (%)	26 (44)	6 (32)	10 (50)	10 (50)	0.66
Cardiovascular parameters					
Systolic blood pressure, mmHg	147 ± 21	144 ± 20	152 ± 20	145 ± 22	0.38
Diastolic blood pressure, mmHg	70 ± 12	71 ± 9	71 ± 15	67 ± 11	0.05
Heart rate, bpm	74 ± 14	73 ± 12	72 ± 15	77 ± 14	0.29
Hypertension[b], n (%)	33 (58)	10 (56)	13 (65)	10 (53)	0.54
Cardiovascular disease, n (%)	25 (42)	8 (42)	9 (45)	8 (40)	0.45
Diabetes mellitus, n (%)	15 (25)	4 (21)	7 (35)	4 (20)	0.98
Laboratory parameters					
Hemoglobin, mmol/L	6.9 ± 0.7	7.0 ± 0.6	7.0 ± 0.5	6.8 ± 0.9	0.26
Hematocrit, v/v	0.35 ± 0.04	0.35 ± 0.03	0.35 ± 0.03	0.34 ± 0.05	0.28
Urea, mmol/L	19 ± 5	22 ± 5	20 ± 4	17 ± 5	**<0.001**
Creatinine, μmol/L	689 ± 207	710 ± 246	649 ± 174	707 ± 201	0.57
Albumin, g/L	40 [37–42]	41 [38–43]	39 [36–41]	40 [36–42]	0.14
Hs-CRP, mg/L	4.9 [1.6–14.0]	2.1 [1.3–9.0]	3.1 [1.4–11.0]	10.0 [5–26]	**0.03**
Protein intake					
Urea excretion, mmol/24-h	274 ± 107	305 ± 76	285 ± 148	234 ± 71	**0.004**
Protein intake, g/kg/24-h	0.82 ± 0.23	0.95 ± 0.20	0.83 ± 0.25	0.69 ± 0.14	**<0.001**
Muscle mass					
Creatinine excretion, μmol/kg/24-h	105 ± 36	124 ± 35	96 ± 38	97 ± 29	**0.009**
Fatigue measures					
CIS Fatigue severity score	30 [15–39]	22 [13–36]	22 [13–38]	36 [28–48]	**0.01**
SF-36 Vitality score	60 [47–80]	73 [48–86]	70 [58–85]	50 [35–65]	**0.02**

Data are presented as mean ± SD, number (percentage), or median [IQR]. P for trend was assessed using linear regression analyses, in which plasma FGF21 was $\log_2$-transformed for analyses.
[a] Body weight before hemodialysis.
[b] Hypertension defined as systolic blood pressure >140 and/or diastolic blood pressure >90.

(std β: −0.34 [-0.59; −0.09]; P = 0.009) and SF-36 vitality score (std β: −0.30 [-0.55; −0.05]; P = 0.02), while plasma FGF21 was positively associated with CIS fatigue severity score (std β: 0.32 [0.07; 0.57]; P = 0.01). After adjustment for age, sex, and BMI, higher plasma FGF21 remained significantly associated with lower protein intake, lower muscle mass, lower vitality, and more fatigue. Further adjustment for potential confounders, including dialysis vintage, hours of dialysis per week, C-reactive protein, diabetes, and history of cardiovascular disease, did not materially change the associations. A visual representation of the associations of plasma FGF21 with protein intake, muscle mass, vitality, and fatigue severity is displayed in Fig. 2. As a sensitivity analysis, we investigated whether plasma FGF21 was also associated with the normalized protein catabolic rate. Plasma FGF21 was significantly and inversely associated with the normalized protein catabolic rate, independent of potential confounders, Table S8. A visual representation of the associations of plasma FGF21 with normalized protein catabolic rate is shown in Fig. S1.

Table 2
Logistic regression analyses of plasma FGF21 with low protein intake.

Model	Adequate protein intake (>0.9 g/kg/24-h)		Low protein intake (<0.9 g/kg24-h)		
	Odds ratio	P-value	Odds ratio	95% CI	P-value
Model 1	Reference	(–)	2.88	1.61–6.41	0.003
Model 2	Reference	(–)	2.83	1.52–6.56	0.006
Model 3	Reference	(–)	3.18	1.62–7.95	0.005
Model 4	Reference	(–)	2.95	1.54–7.26	0.005
Model 5	Reference	(–)	3.22	1.63–7.99	0.003
Model 6	Reference	(–)	3.13	1.56–7.98	0.005
Model 7	Reference	(–)	3.29	1.67–8.27	0.03

Odds ratios are expressed per doubling of plasma FGF21. Model 1: crude; Model 2: adjusted for age and sex; Model 3: as model 2, additionally adjusted for BMI; Model 4: as model 3, additionally adjusted for dialysis vintage; Model 5: as model 3, additionally adjusted for hours of dialysis per week; Model 6: as model 3, additionally adjusted for C-reactive protein; Model 7: as model 3, additionally adjusted for diabetes and history of cardiovascular disease.

3.4. Tertiary analyses of intradialytic changes in plasma FGF21

During dialysis plasma FGF21 increased from 1026 [667–2253] pg/mL before to 1444 [954–2874] pg/mL after dialysis (P < 0.001), corresponding with an absolute increase of 354 [71–570] pg/mL.

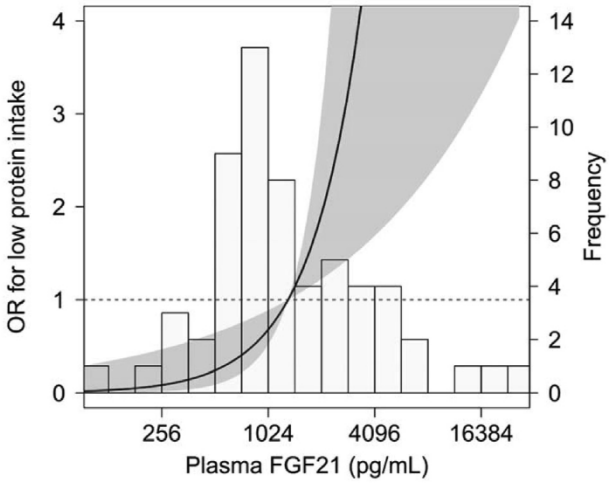

Fig. 1. Visual representation of the association of plasma FGF21 concentration with low protein intake, defined as a weight indexed protein intake below 0.9 g/kg/24-h. Plasma FGF21 concentration was log_2-transformed for analysis. The histogram depicts the distribution of log_2 transformed plasma FGF21 concentration. The black line shows the adjusted odds ratio (OR) and the gray area corresponds to the 95% pointwise confidence interval (CI). The model is adjusted for age and sex.

Plasma FGF21 before hemodialysis is closely correlated with plasma FGF21 after hemodialysis (Pearson correlation coefficient $r = 0.961$; $P < 0.001$). A scatterplot of plasma FGF21 before hemodialysis and plasma FGF21 after dialysis is shown in Fig. S2. Percentage wise, plasma FGF21 increased 29% during hemodialysis. In comparison, the relative increases in hemoglobin, hematocrit and albumin were 8%, 6% and 10%, respectively. In contrast, urea and creatinine decreased by 70% and 63%, respectively. An overview of the intradialytic changes in biochemical parameters are shown in Table 4. There were no significant intradialytic changes in inflammation parameters, with concentrations of hs-CRP of 4.9 [1.7–13.5] mg/L and 4.6 [1.6–14.5] mg/L before and after hemodialysis respectively ($P = 0.13$) and concentrations of IL-6 of 6.4 [3.5–12.9] pg/mL and 5.5 [3.5–17.5 pg/mL before and after hemodialysis respectively ($P = 0.87$).

4. Discussion

In a cohort of hemodialysis patients, we demonstrated that higher plasma FGF21 is associated with higher odds of low protein intake, defined by a cutoff of 0.9 g/kg/24-h. This association was independent of confounders and was also found using cutoffs of 0.8 g/kg/24-h and 1.0 g/kg/24-h to define low protein intake. Secondarily, in linear regression analyses, plasma FGF21 is associated with lower protein intake, lower muscle mass, less vitality, and more fatigue. Lastly, during dialysis, plasma FGF21 increases by an average amount of 29%, which is more than can be accounted for by hemoconcentration. Our findings implicate FGF21 as a potential marker for assessing protein intake and protein energy wasting in hemodialysis patients. The increase during hemodialysis suggests that it may also indicate a metabolic impact of loss of amino acids during this treatment.

FGF21, a member of the FGF family, is a hormone involved in glucose, lipid, and amino acid metabolism as well as in the response to several stresses [9–13]. Although FGF21 is expressed in multiple tissues, plasma FGF21 is primarily derived from the liver and adipose tissues, with smaller contributions from the gut, brain, muscle, and pancreas [31,32]. In 2014, Laeger et al. demonstrated that hepatic FGF21 expression is induced by dietary protein restriction, but not energy restriction, with plasma FGF21 increasing up to 10-fold in rodents on a low protein diet [14]. In addition, they demonstrated that FGF21 knockout mice neither increased their food intake compared with wild-type mice, nor showed changes in energy expenditure when challenged with low-protein diets. These findings implicate FGF21 as an endocrine signal of protein restriction as well as a key regulator coordinating the metabolic response to protein restriction. Similarly, in humans, a low protein diet for 28 days also increased FGF21 in healthy volunteers [14]. In line with these findings, our study demonstrates that higher plasma FGF21 is associated with higher odds of low protein intake in hemodialysis patients. The exact cutoff for defining low protein intake in hemodialysis patients remains a matter of debate and suggest cutoffs vary between 0.8 and 1.0 g/kg/24-h [21–24,33]. To account for this, we performed sensitivity analyses for the cutoffs 0.8 g/kg/24-h and 1.0 g/kg/24-h, and in both cases higher plasma FGF21 remained associated with higher odds of low protein intake. In another sensitivity analysis, we investigated whether the same association would be found when protein intake was assessed using dietary diaries instead of the biomarker method. Interestingly, no significant associations between plasma FGF21 and low protein intake were found when protein intake was based on the 5-day dietary diaries. These findings underscore the need for reliable biomarkers to assess dietary protein intake.

Observational studies in the general population demonstrated that plasma FGF21 concentrations increase with age, varying from

Table 3
Linear regression analyses of plasma FGF21 concentration on protein intake, muscle mass, fatigue score and energy score.

Model	Protein intake[a]		Muscle mass[b]		Fatigue score (CIS)[c]		Vitality score (SF-36)[d]	
	Std. β (95% CI)	P	Std. β (95% CI)	P	Std. β (95% CI)	P	Std. β (95% CI)	P
Model 1	−0.54 (−0.76; −0.32)	<0.001	−0.34 (−0.59; −0.09)	0.009	0.32 (0.07; 0.57)	0.01	−0.30 (−0.55; −0.05)	0.02
Model 2	−0.49 (−0.72; −0.26)	<0.001	−0.24 (−0.46; −0.01)	0.04	0.37 (0.10; 0.63)	0.008	−0.37 (−0.63; −0.11)	0.007
Model 3	−0.49 (−0.73; −0.24)	<0.001	−0.26 (−0.50; −0.02)	0.04	0.36 (0.08; 0.65)	0.01	−0.28 (−0.55;-0.01)	0.04
Model 4	−0.48 (−0.72; −0.24)	<0.001	−0.26 (−0.50; −0.01)	0.04	0.36 (0.08; 0.64)	0.01	−0.27 (−0.54;-0.01)	0.04
Model 5	−0.50 (−0.75; −0.25)	<0.001	−0.27 (−0.51; −0.03)	0.03	0.36 (0.07; 0.65)	0.02	−0.27 (−0.54; −0.01)	0.04
Model 6	−0.46 (−0.71; −0.22)	<0.001	−0.25 (−0.49; −0.01)	0.05	0.35 (0.06; 0.63)	0.02	−0.27 (−0.54; −0.00)	0.05
Model 7	−0.51 (−0.76; −0.26)	<0.001	−0.27 (−0.52; −0.02)	0.03	0.36 (0.06; 0.65)	0.02	−0.29 (−0.56; −0.01)	0.04

The independent variables in the analyses are log_2 transformed plasma FGF21 concentration and additional variables adjusted for. Model 1: crude; Model 2: adjusted for age and sex. Model 3: as model 2, additionally adjusted for BMI. Model 4: as model 3, additionally adjusted for dialysis vintage. Model 5: as model 3, additionally adjusted for hours of dialysis per week. Model 6: as model 3, additionally adjusted for C-reactive protein. Model 7: as model 3, additionally adjusted for diabetes and history of cardiovascular disease.

[a] Protein intake expressed as g/kg/24-h.
[b] Muscle mass was assessed using creatinine excretion rate (μmol/kg/24-h).
[c] A higher CIS fatigue severity score indicates more fatigue.
[d] A lower SF-36 vitality scores implicates more fatigue.

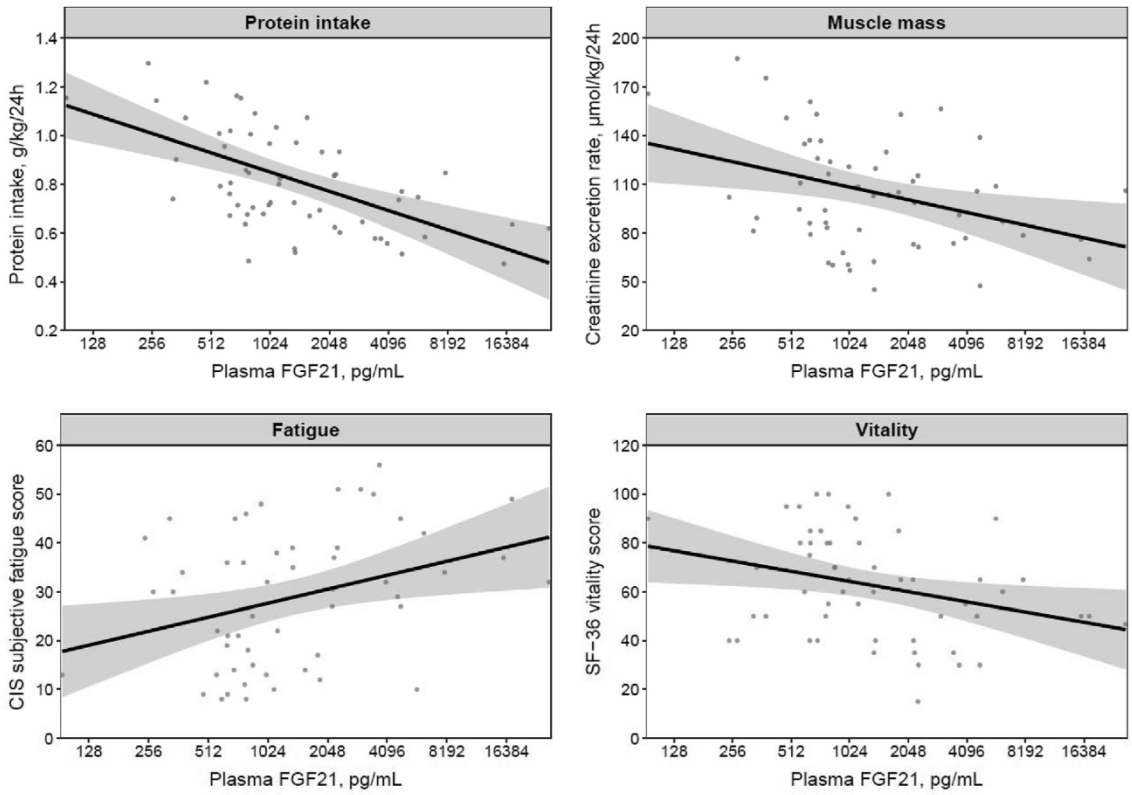

Fig. 2. Visual representation of the linear regression analyses of plasma FGF21 with protein intake, muscle mass, fatigue and vitality. Muscle mass was assessed using creatinine excretion rate (µmol/kg/24-h). A higher CIS fatigue severity score indicates more fatigue. A lower SF-36 vitality scores implicates more fatigue.

Table 4
Intradialytic changes in biochemical parameters.

	Before dialysis	After dialysis	Pearson correlation	Absolute change	Percentage change (%)	P[a]
FGF21, pg/mL	1026 [667–2253]	1444 [954–2874]	0.96	354 [71–570]	29 ± 40	<0.001
Hemoglobin, mmol/L	6.9 ± 0.7	7.4 ± 0.9	0.52	0.5 ± 0.8	8 ± 5	<0.001
Hematocrit, v/v	0.35 ± 0.04	0.37 ± 0.04	0.48	0.02 ± 0.04	6 ± 3	0.001
Albumin, g/L	39 ± 5	42 ± 5	0.54	4 ± 5	10 ± 6	<0.001
Urea, mmol/L	19 ± 5	6 ± 2	0.68	−14 ± 4	−70 ± 8	<0.001
Creatinine, µmol/L	689 ± 207	257 ± 92	0.79	−432 ± 146	−63 ± 8	<0.001

Absolute change = After hemodialysis − Before hemodialysis.
Percentage change (%) = (After hemodialysis − Before hemodialysis)/Before hemodialysis * 100%.
[a] Differences in concentrations before and after dialysis were tested using the paired sample t-test and the Wilcoxon signed-rank test, for normally distributed variables and non-normally distributed variables, respectively.

156 [59–254] pg/mL in children to 359 [239–481] pg/mL in adults [34]. While most participants in our study were elderly, we too found a positive association between plasma FGF21 concentration and age. Besides age, observational studies also found positive association of FGF21 with creatinine, blood urea nitrogen and cystatin C [35]. A study in 499 patients with CKD demonstrated kidney function as a primary independent predictor of serum FGF21 levels, with a more than 20-fold increase in serum FGF21 levels from CKD stage 1 to 5, showing a linear association between CKD stage and FGF21 levels [36]. Similarly, a study in 200 patients with CKD and 40 controls found that plasma FGF21 increased with progressive worsening of CKD [37]. Plasma FGF21 was 128 [86–218] pg/mL in healthy controls, 317 [210–733] in CKD stage 2 (60–90 mL/min/1.73 m^2), 517 [220–912] pg/mL in CKD stage 3 (30–60 mL/min/1.73 m^2) and 1099 [523–2468] pg/mL in stage 4 and 5 (<30 mL/min/1.73 m^2) [37]. These results of the latter group are in accordance with the plasma FGF21 concentrations in our study, namely 1026 [692–2297] pg/mL.

Besides low protein intake, muscle mass represents an important diagnostic criterium for PEW. A relatively simple, non-invasive method to estimate muscle mass is to measure the creatinine excretion rate (CER). Creatinine is produced at a constant rate, depending on the quantity of muscle mass, as creatinine is formed by the non-enzymatic conversion of creatine to creatinine in muscles [38,39]. Therefore, CER is an established method to assess total body muscle mass in both healthy populations and patient populations, including patients with chronic kidney disease [8,40–44]. An advantage of assessment of CER in hemodialysis patients is that CER by its biochemical nature is insensitive to hydration status, intramuscular fat, and edema and thereby provides a direct reflection of muscle mass [45,46]. However, only a few patient population-specific reference values are available and no cutoffs of CER for low muscle mass have been defined for hemodialysis patients. For these reasons, we only investigated the association of FGF21 with CER expressed as a continuous variable. Interestingly, we found that higher plasma FGF21 is associated with

lower muscle mass, suggesting a role for FGF21 as a marker for PEW.

Similarly, a strong association between plasma FGF21 and fatigue was found. In contrast to protein intake and muscle mass, fatigue is not among the criteria of PEW. However, it is in the Society on Sarcopenia, Cachexia and Wasting Disorders criteria of cachexia [47]. The differences between PEW and cachexia are very limited and hardly justified and it has recently been proposed that PEW is cachexia, a continuum with PEW first, followed by cachexia [48]. We included fatigue in our study because fatigue is an under-recognized and under-treated symptom and from a patient's perspective this is one of the most debilitating symptoms experienced in the context of hemodialysis. Indeed, this is highlighted by a study demonstrating that 94% of the patients would accept more intense hemodialysis if it would increase their energy level, whereas only 19% would do so for an increase in survival by 3 years [49]. To assess fatigue in our study, we used the Short Form 36 and the Checklist Individual Strength (CIS). The Short Form 36 is very popular instrument for evaluating Health-Related Quality of Life, of which the subdomain vitality can be used as an valid measure of fatigue [50,51]. CIS is a 20-item fatigue questionnaire developed in the Netherlands in 1994 for a dimensional assessment of chronic fatigue syndrome [52], which been well-validated and is frequently used in research in patients with various illnesses [27,28,52–54], including dialysis patients [55,56]. The found association between plasma FGF21 and fatigue supports a role of protein undernutrition in the pathophysiology of chronic fatigue in hemodialysis.

FGF21 is a protein of roughly 21 kilodalton, making it small enough to pass the glomerular filtration barrier, but its clearance by most low-flux dialyzers is negligible [35]. Based on this, an intradialytic increase in plasma FGF21 is to be expected. A study in hemodialysis patients indeed found somewhat higher FGF21 concentrations at the venous side of the dialyzer compared to the arterial site [30]. However, they were unable to determine whether this increase was due to hemoconcentration. In our study, we compared blood drawn at the beginning of the dialysis session with blood drawn at the end of the dialysis session and found a significant increase in plasma FGF21. Plasma FGF21 increased 29%, whereas hemoglobin, hematocrit, and albumin merely increased with 8%, 6% and 10%, respectively. Thus, plasma levels of FGF21 increased during hemodialysis beyond what is to be expected by hemoconcentration, implicating that hemodialysis treatment itself stimulates the release of FGF21 into the circulation. This increase in FGF21 could not be explained by a change in inflammation parameters. Another possible explanation could be the intradialytic losses of amino acids to the dialysate, leading to low plasma amino acid concentrations that could stimulate FGF21 secretion [57].

The strength of this study is that we used a biomarker-based method to assess dietary protein intake, thereby avoiding potential biases of classic dietary assessments. In addition, we collected the total dialysate instead of taking several samples during the hemodialysis sessions, thereby increasing the accuracy of protein intake assessment. However, we acknowledge that our study has limitations, primarily the relatively small sample size of this study, making our study unfit for determining optimal cut-off values for FGF21. In addition, we did not have data on insulin concentrations, which are known to associate with FGF21 [30]. Furthermore, we did not assess muscle mass by other methods, including magnetic resonance imaging, computed tomography, dual-energy X-ray absorptiometry and bioelectric impedance analysis. Lastly, because our participants were largely Caucasian, our results cannot be extrapolated to other ethnic groups.

In conclusion, we demonstrated that higher plasma FGF21 is associated with higher odds of low protein intake in hemodialysis patients. This association was independent of confounders. Secondarily, in linear regression analyses, plasma FGF21 is associated with lower protein intake, lower muscle mass, less vitality, and more fatigue. Lastly, there is an intradialytic increase in plasma FGF21 that exceeds beyond hemoconcentration. Combined, these findings implicate FGF21 as a potential marker for assessing protein intake and protein energy wasting in hemodialysis patients.

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Data share statement

Data described in the manuscript, code book, and analytic code will be made available upon request of the editor.

Conflicts of interest

Adrian Post, Dion Groothof, Joëlle C. Schutten, Dylan Kelly, J. Casper Swarte, Jose L. Flores-Guerrero, Yvonne van der Veen, Ido P. Kema, Akin Ozyilmaz, Ralf Westerhuis, Stephan J.L. Bakker and Casper F.M. Franssen declare that they have no conflict of interest.

Anyano Enya is employed by Immuno-Biological Laboratories Co.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2021.01.036.

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