DR EMMA O BILLINGTON (Orcid ID : 0000-0002-2556-588X)

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Fibroblast growth factor 23 levels decline following sleeve gastrectomy

Running title: FGF23 levels after sleeve gastrectomy

Emma O Billington[1,2]
Rinki Murphy[1]
Greg D Gamble[1]
Karen Callon[1]
Naomi Davies[1]
Lindsay D Plank[3]
Michael Booth[4]
Ian R Reid[1]

[1]Department of Medicine, Faculty of Medical and Health Sciences, University of Auckland, New Zealand
[2]Division of Endocrinology & Metabolism, Cumming School of Medicine, University of Calgary, Alberta, Canada
[3]Department of Surgery, Faculty of Medical and Health Sciences, University of Auckland, New Zealand
[4]Department of Surgery, North Shore Hospital, Waitemata District Health Board

Corresponding Author: Emma Billington

Address: Room 18118
Richmond Road Diagnostic & Treatment Centre
1820 Richmond Road SW
Calgary, Alberta
T2T 5C7
Canada

Email: emma.billington@albertahealthservices.ca

Telephone: +1 403 399 7661

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Data Sharing Statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Summary

Objective: Levels of fibroblast growth factor 23 (FGF23) have been positively associated with measures of adiposity, cardiovascular disease, and mortality. It is unclear whether the relationship of FGF23 with cardiovascular disease and mortality is confounded by obesity. We aimed to determine whether FGF23 concentrations decline following a reduction in adiposity after sleeve gastrectomy (SG).

Design: The effect of SG on FGF23 was evaluated in 22 obese adults (59% male) with type 2 diabetes. Fat mass, weight, BMI, plasma intact FGF23, parathyroid hormone (PTH) and leptin were determined at baseline and at 12 months following SG.

Results: At baseline, median (IQR) age was 51 (43 to 54) years, fat mass 47.8 (41.0 to 59.4) kg, BMI 40.9 (36.9 to 46.9) kg/m^2 and FGF23 66.2 (55.3 to 82.9) pg/mL. Significant changes in median BMI (-10.8 kg/m^2, 95% CI: -12.9 to -7.2, p<0.0001), fat mass (-20.0 kg, 95% CI: -26.7 to -12.4, p <0.0001), and weight (-34.7 kg, 95% CI -40.0 to -23.1, p <0.0001) were observed after SG. FGF23 (-12.4 pg/mL, 95% CI: -19.5 to 2.0, p=0.005), PTH (-1.1 pmol/L, 95% CI: -1.7 to 0.2, p=0.009) and leptin (-1687 pg/mL, 95% CI -4524 to -563, p=0.01) declined following SG. Change in FGF23 was not significantly associated with change in measures of adiposity, PTH or leptin.

Conclusions: FGF23 concentrations decline in the setting of significant weight loss following SG, implying that increased FGF23 concentrations are a downstream consequence of obesity, which may confound its association with cardiometabolic dysfunction. Mediators of the relationship between adiposity and FGF23 require further elucidation.

Keywords

fibroblast growth factor 23, parathyroid hormone, leptin, adiposity, obesity, bone mineral density, bariatric surgery

Introduction

The bone-derived protein fibroblast growth factor 23 (FGF23) is a key regulator of phosphate and vitamin D homeostasis. A number of pieces of evidence link both circulating phosphate and FGF23 concentrations with measures of adiposity, cardiovascular risk, and mortality.[1-7] Our group[8] and others[1-3] have demonstrated positive associations between circulating FGF23 and body mass index (BMI), fat mass, and abdominal circumference in cross-sectional human studies. Furthermore, positive associations between FGF23 concentrations and left ventricular mass index, arterial stiffness, cardiovascular events, and mortality have been reported.[4-6,9] Some evidence suggests that leptin and parathyroid hormone (PTH) could mediate these relationships. Leptin levels are increased in obesity and are positively associated with both PTH and FGF23 in obese women.[10] Administration of leptin has been shown to increase FGF23 concentrations in preclinical studies.[11,12] As with FGF23, PTH is positively correlated with measures of adiposity in humans.[13,14] PTH levels, even within the normal range, are also independently associated with markers of

cardiovascular disease, including blood pressure, dyslipidaemia, left ventricular hypertrophy, and endothelial dysfunction.[14-18]

However, it remains unclear whether elevated FGF23 levels drive the development of adiposity and cardiometabolic dysfunction in humans, or vice versa. The potential causative role of adiposity or other obesity-related parameters, such as cardiometabolic dysfunction or high dietary phosphate intake,[7,19] in increased FGF23 concentrations could be inferred from the effects of weight loss on FGF23. To our knowledge, this has not been comprehensively assessed, although Fernández-Real and colleagues have reported on 10 obese men, who after nonsurgical weight loss of 20kg on average, demonstrated a decline in FGF23 levels of approximately 20%,[20] suggesting that increased FGF23 may be a consequence of obesity or nutritional and metabolic parameters that are associated with excess adiposity.

Elucidating the direction of causality of the relationships between FGF23, measures of adiposity, and cardiovascular disease in humans is of crucial importance. If FGF23 has a causal role in the pathogenesis of obesity and cardiovascular disease, then the development of interventions directly targeted at lowering or inhibiting FGF23 would be warranted. If, on the other hand, high circulating FGF23 levels are a byproduct of cardiometabolic dysfunction rather than a cause, FGF23 could have potential as a surrogate endpoint by which to assess interventions that aim to reduce cardiometabolic risk. Alternately, high dietary phosphate intake has been shown to increase FGF23 concentrations, and it is possible that the elevated FGF23 levels in obesity reflect diets that are high in processed foods with phosphate-based additives,[7] in which case FGF23 may represent a marker of diet quality.

Sleeve gastrectomy (SG) is a restrictive form of bariatric surgery. When compared to malabsorptive bariatric procedures, SG is less likely to cause aberrations in bone mineral homeostasis such as vitamin D deficiency and secondary hyperparathyroidism.[21] To better understand the mechanism(s) underlying the associations between FGF23 levels and adiposity, we assessed changes in FGF23 concentrations, alongside PTH, leptin, 25-hydroxyvitamin D (25OHD), and bone mineral density in a cohort of individuals who underwent SG. It was hypothesized that weight loss following SG would be associated with a decrease in circulating FGF23 concentrations and that changes in FGF23 might be mediated by leptin and PTH.

Materials and methods

FGF23 levels were assessed before and 12 months after SG in a cohort of 22 obese individuals with type 2 diabetes taking part in a double-blind randomized controlled trial comparing outcomes of SG and Roux-en-Y gastric bypass (n=114). This randomized controlled trial commenced in September 2011 and completed recruitment in October 2014. Data collection is ongoing. The trial is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12611000751976) and received approval from the New Zealand regional ethics committee. A detailed protocol has been published elsewhere.[22] Details relevant to the present study are presented here.

Setting

Recruitment and surgery were carried out at a single centre (North Shore Hospital) in New Zealand. Data collection and blood sampling were carried out at the Body Composition

Laboratory at the University of Auckland. Study participants underwent SG between 2011 and 2014.

Patients

Study participants were adults aged 22 to 55 years who were deemed suitable candidates for bariatric surgery. Additional inclusion criteria were: type 2 diabetes of at least six months duration and a BMI of 35 to 65 kg/m^2 for at least five years. Exclusion criteria consisted of: postprandial C peptide concentration <350 pmol/L, current pregnancy, type 1 diabetes or secondary diabetes, chronic pancreatitis, oral steroid therapy, current smoking, unsuitability for general anesthesia. Eligible consenting participants were randomized to receive either Roux-en-Y gastric bypass or laparoscopic SG. The present study includes data from 22 participants randomized to the SG arm who consented to participate in an ancillary mechanistic substudy[22] which involved providing additional data, including fasting blood samples, at baseline and 12 months.

Intervention

Prior to SG, participants were prescribed a very low calorie diet for two weeks to reduce hepatic steatosis and thereby liver volume, making laparoscopic abdominal surgery technically easier and safer. The diet consisted of three daily servings of Optifast (Nestle, Vevey, Switzerland), each serving containing approximately 152 calories, plus vegetables. SG was performed as previously described.[22] All pharmacological agents for diabetes were stopped at the time of surgery and restarted postoperatively if mean postoperative capillary glucose exceeded 12 mmol/L. Postoperatively, all participants were prescribed a twice daily multivitamin; either Band Buddies (NutriChew, Brisbane, Australia; each tablet containing

300mg elemental calcium and 500 IU vitamin D3) or Centrum 50+ (Pfizer New Zealand, Auckland, New Zealand; each tablet containing 200mg elemental calcium and 600 IU vitamin D3).

Measurements

Data collection occurred at baseline (approximately 2-4 days prior to SG, while on the very low calorie diet) and at 12 months following SG. Age, sex, and use of diabetes medication(s) were determined at baseline. Use of diabetes medication(s) was reassessed at 12 months following SG.

Body weight was assessed at baseline and at 12 months following SG. Weight was recorded to the nearest 0.1kg using digital scales (SECA, Chino, California, USA). Height was evaluated at both time points using a stadiometer and recorded to the nearest 0.5cm. Percent excess body weight lost was calculated by dividing the difference between body mass index (BMI) at baseline and at 12 months by the difference between BMI at baseline and the upper limit of the normal BMI range (25 mg/kg^2).

Fasting serum blood samples were obtained at baseline and at 12 months following SG. Samples were collected into EDTA separator tubes and BD P800 tubes (BD, Franklin Lakes, New Jersey, USA) containing protease inhibitors. Hemoglobin A1C was measured by high-performance liquid chromatography (Bio-Rad, Hercules, California, USA). Plasma intact FGF23 levels were determined using the Immutopics Human FGF-23 ELISA kit (Immutopics, San Clemente, California, USA; intra-assay CV 2.6-4.4%, inter-assay CV 6.1-6.5%). Leptin was measured from plasma samples using the Milliplex human metabolic hormone magnetic

bead panel (Merck Millipore, Darmstadt, Germany; intra-assay CV <10%, inter-assay CV <15%). Serum 25OHD was measured using an automated competitive immunoassay with chemiluminescence detection performed on the Roche Cobas e601 analyzer (Roche Diagnostics, Mannheim, Germany), and PTH using an automated sandwich immunoassay with chemiluminescence detection, also performed on the Roche Cobas e601 analyzer. Clinical records were reviewed at baseline and 12 months following SG to obtain total calcium, and creatinine levels. Glomerular filtration rate (GFR) was estimated using the CKD-EPI equation.[23]

Total fat mass and lumbar spine (L2-L4) BMD were measured at both time points by dual-energy X-ray absorptiometry (model iDXA, software V.15, GE-Lunar, Madison, Wisconsin, USA).

Statistical analysis

Within-person changes in FGF23 and other variables were evaluated for statistical significance using the Wilcoxon signed-rank test. As this is the first study, to our knowledge, to report on the relationship between FGF23 and weight loss in women, between-sex differences in change in FGF23 were assessed using paired *t*-tests, and sex-by-time interaction examined using mixed models. Associations between change in FGF23, changes in measures of adiposity (weight, BMI, fat mass, % excess body weight lost), PTH, and leptin were explored using Spearman correlation. A mediation analysis was planned to determine whether change in leptin or change in PTH mediated relationships between change in measures of adiposity and change in FGF23.

All data analysis was done with SAS v9.4 (SAS Institute, Cary, North Carolina, USA), and figures were created using Prism v6.0 (GraphPad Software Inc, La Jolla, California, USA). All tests were two-tailed. The threshold for statistical significance was set at $p < 0.05$; the threshold was not adjusted for multiple comparisons in these exploratory analyses.

Results

Stored, paired blood samples were available from a total of 22 adults (9 females, 13 males) and assessed for FGF23. Baseline characteristics are set out in Table 1. Although median baseline 25-hydroxyvitamin D level was <50 nmol/L, the cohort did not demonstrate evidence of secondary hyperparathyroidism, with a median (interquartile range [IQR]) PTH of 4.9 (3.8 to 6.3) pmol/L.

Over the 12 months following SG, four participants (18%) were taking a Band Buddies supplement twice a day, providing a daily total of 600mg elemental calcium and 1000 IU vitamin D3, while 16 (73%) were taking Centrum 50+ twice a day, providing a daily total of 400mg elemental calcium and 1200 IU vitamin D3. The remaining two (9%) participants were treated with vitamin D 50,000 IU monthly for the first six months following SG.

Change in measures of adiposity from baseline to 12 months following SG are laid out in Table 2. Fat mass, BMI, and weight declined significantly in the year following SG. Median (95% CI) percent excess body weight lost was 68.1 (58.4 to 74.6). Hemoglobin A1C also declined significantly following SG (Table 2). At 12 months after SG, 6 (27%) participants

required pharmacologic diabetes therapy, including 2 (9%) who required treatment with insulin.

As shown in Table 2, FGF23 levels decreased significantly following SG (p=0.012); changes were not different for males and females (p=0.67) and no significant sex-by-time interaction was observed (p=0.63). Leptin, and PTH levels decreased significantly and 25-hydroxyvitamin D levels increased significantly after SG (Table 2). Relationship between change in FGF23 and change in fat mass is shown in Figure 1. In univariate analyses, change in FGF23 was not significantly associated with changes in measures of adiposity (fat mass, BMI, weight, percent excess body weight lost), or with changes in leptin, PTH, or 25-hydroxyvitamin D (data not shown); further mediation analyses were therefore not undertaken.

Discussion

FGF23 correlates positively with measures of adiposity, with levels being increased in obese individuals.[1-3,8] FGF23 concentrations are also associated with metabolic syndrome, vascular dysfunction, left ventricular hypertrophy, cardiovascular events, and mortality.[4-6,9] However, the bulk of existing data regarding these associations is from observational studies, in which interventions to modulate FGF23 levels and/or adiposity were not assessed.[1-6,9] As such, the mechanisms underpinning these relationships are not known, and the direction of causation remains unclear. To our knowledge, no controlled trials in humans have assessed the ability of interventions that lower FGF23 concentrations to improve cardiometabolic parameters, nor have any controlled trials evaluated the ability of weight loss interventions to lower

circulating FGF23. The results of the present pre-post study are a novel addition to the existing literature; they demonstrate that SG, an intervention that causes significant weight loss, is also effective in lowering FGF23 levels. Our findings provide support for the hypothesis that high FGF23 concentrations are a by-product of excess adiposity or other obesity-related factors (such as high dietary phosphate intake, metabolic disturbances, increased inflammation) rather than necessarily being pathogenic. While further study is required to identify the mediators of this process, our results suggest that factors other than leptin, PTH, and 25OHD may play a significant role.

Multiple studies have confirmed a positive relationship between FGF23 and measures of adiposity. In the community-based Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort, which included 946 adults aged 70 years (50% female), correlations of FGF23 with body weight ($r=0.07$, $p<0.05$) and fat mass ($r=0.07$, $p<0.05$) were demonstrated.[1] In 2134 middle-aged men and women from the EPIC-Germany cohort, waist circumference and BMI increased across quartiles of FGF23.[2] In the Health ABC study, a similar significant relationship between quartiles of FGF23 and BMI was observed, with mean BMI being 26.6 kg/m^2 in quartile 1 and 28.0 kg/m^2 in quartile 4.[3] In the population-based male MrOS Sweden cohort (n=964), body weight was positively correlated with FGF23 after adjustment for age ($r=0.18$, $P<0.0001$) and after further adjustment for indices of mineral metabolism ($r=0.20$, $P<0.0001$).[1] In individuals from the Multi-Ethic Study of Atherosclerosis cohort[24] with eGFR ≥60 mL/min (n=5610, 52.8% women), FGF23 levels were positively associated with BMI, with mean FGF23 measuring 37.7 pg/mL in individuals with a BMI <25 kg/m^2 and 40.9 pg/mL in those with BMI ≥40 kg/m^2. FGF23 also correlated with abdominal adiposity in a subgroup (n=1313) who underwent abdominal computed

tomography.[24] Our group[8] has previously demonstrated positive correlations between FGF23 and both weight (r=0.60, p=0.007) and BMI (r=0.49, p=0.03) in healthy postmenopausal women (n=20) with mean BMI of 26.9 kg/m^2. To our knowledge, no studies have assessed change in FGF23 following restrictive bariatric surgery. However, our findings are largely consistent with the one existing study that has reported on change in FGF23 following non-surgical weight loss. Fernández-Real assessed 10 obese men (mean BMI 33.8 kg/m^2) who participated in a weight loss program, finding that a mean weight loss of 20kg resulted in an average decline in FGF23 of 6.3 ng/mL.[20]

Vitamin D, PTH, and leptin are three potential mediators of circulating FGF23 concentrations. FGF23 is a negative regulator of vitamin D activity, but the effects of vitamin D supplementation on FGF23 concentrations are not clearly established and may depend on baseline vitamin D status. For example, while a decline in FGF23 levels was observed following vitamin D supplementation in a small cohort (n=19) of vitamin D deficient women,[25] Burnett-Bowie and colleagues observed that treatment with ergocalciferol 50,000 IU weekly for 12 weeks resulted in significant increases in FGF23 levels in young adults (n=90) who had 25OHD levels <50 nmol/L at baseline.[26] A larger randomized controlled trial (n=181) subsequently reported no effect on FGF23 with vitamin D supplementation unless baseline 25-hydroxyvitamin D was <50 nmol/L, in which case FGF23 levels increased with supplementation.[27] In the MrOS cohort, baseline 25OHD levels were comparable across quartiles of FGF23.[28] In bone cells, PTH has been shown to increase the transcription of FGF23 via activation of the nuclear orphan receptor nuclear receptor-associated protein 1 (Nurr1).[29] In the *ob/ob* (leptin-deficient) mouse, administration of leptin increases FGF23 expression in bone.[12] Grethen *et al* [10] conducted a cross-sectional

analysis of obese women (n=20) and healthy controls (n=20) and reported that leptin was positively associated with PTH and FGF23 in both groups. In the PIVUS and MrOS cohorts, FGF23 was positively associated with leptin, although the relationship became non-significant in a multivariate-adjusted model that included eGFR, phosphate and PTH.[1] Of relevance, associations between FGF23 and fat mass in the PIVUS and MrOS cohorts were attenuated when leptin was included in a multivariate-adjusted model, suggesting a role for leptin as a mediator.[1] In the present cohort, we observed declines in PTH and leptin following SG, as has been previously reported.[30] We also found a significant increase in 25OHD concentrations after SG, which was not surprising given that all participants received postoperative vitamin D supplementation;[31] this supplementation also likely contributed to the observed decrease in PTH. However, change in FGF23 following SG was not significantly related to changes in 25OHD, PTH or leptin; this may relate in part to the modest sample size, which limited our ability to identify potential mediators.

Alternately, it is possible that additional factors not directly evaluated in the present study are involved in mediating the FGF23 response to SG. For example, while the present study is limited by a lack of comprehensive data regarding phosphate intake and serum phosphate concentrations, our group has previously shown that measures of adiposity are inversely associated with circulating phosphate levels.[8] In the Multi-Ethnic Study of Atherosclerosis cohort, abdominal adiposity (assessed with computed tomography) was inversely associated with serum phosphate and positively correlated with fractional excretion of phosphate.[24] Oral phosphate intake has also been positively associated with FGF23 concentrations.[7,19] As inorganic phosphate has been shown to stimulate calcification of vascular smooth muscle cells[32] and serum phosphate levels correlate with the development of cardiovascular

disease and death,[33,34] it is possible that the higher circulating levels of phosphatonins (e.g. FGF23 and PTH) in obese states reflect an adaptive mechanism to reduce the phosphate burden in these individuals, who are already at increased risk of cardiovascular dysfunction on the basis of their obesity.[35]

Other potential mediators of the decline in FGF23 after SG include inflammation and insulin. Proinflammatory cytokines such as interleukin-6 and tumor necrosis factor-alpha, may stimulate FGF23 expression directly, or indirectly via 1,25-hydroxyvitamin D.[11] Insulin has recently been shown to suppress FGF23 transcription in osteoblast-like cells;[36] in healthy volunteers, FGF23 levels decreased as insulin levels increased following an oral glucose tolerance test.[36] SG is associated with decreases in circulating inflammatory markers[37] and improvements in insulin sensitivity,[38] both of which may result in decreased FGF23 production. Our results clearly demonstrate that FGF23 levels decline following SG, indicating that the increased FGF23 levels of obesity are responsive to this weight-reducing intervention. However, we cannot exclude the possibility that the observed reduction in FGF23 levels was the direct result of changes in hormonal, metabolic, or inflammatory parameters, rather than changes in adiposity per se.

Finally, as FGF23 is an osteocyte-derived hormone, it is plausible that the reductions in mechanical loading of the skeleton that accompany weight loss could influence FGF23 expression. However, this possibility has not been borne out in animal models. In mice, increased mechanical loading was not associated with increased skeletal expression of FGF23 or increased circulating FGF23 levels,[39] and endurance and power training has not been shown to change FGF23 expression in rats.[40] To our knowledge, human data regarding

the effects of mechanical loading on FGF23 production are limited, although a recently registered clinical trial[41] may help to answer this question.

Conclusions

Our results demonstrate that FGF23 levels decline with weight loss in obese individuals undergoing restrictive bariatric surgery, implying that FGF23 is a by-product of obesity and/or other adiposity-related parameters rather than being a causative factor in the development of obesity and other adiposity-associated conditions. Previously reported relationships between FGF23 and adverse cardiometabolic outcomes may be confounded by the presence of excess adiposity, which itself is an established risk factor for cardiovascular disease and mortality. Our findings do not support the development of interventions that would directly lower or inhibit FGF23 with the goal of promoting weight loss or reducing cardiometabolic risk. While FGF23 may have utility as a surrogate marker to assess the effectiveness of other treatments to reduce cardiometabolic risk, this will require a better understanding of the mediators of the relationship between adiposity, cardiovascular disease and FGF23.

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Conflict of Interest Statement: The authors declare no conflicts of interest.

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Table 1. Baseline characteristics of individuals undergoing sleeve gastrectomy

Characteristic	All participants (n=22)
Age (y)	51 (43-54)
Height (cm)	174.5 (163.5-182.4)
Weight (kg)	122.6 (110.4-139.4)
BMI (kg/m^2)	40.9 (36.9-46.9)
Fat mass (kg)	47.8 (41.0-59.4)
Hemoglobin A1C (%)	7.5 (7.3-8.5)
Diabetes Medications, n (%)	20 (91)
Metformin	18 (82)
Sulfonylurea	5 (23)
DPP-4 inhibitor	2 (9)
Thiazolidinedione	1 (5)
Insulin	7 (32)
Vitamin D supplement, n (%)*	7 (32)
FGF23 (pg/mL)	66.2 (55.3-82.9)
PTH (pmol/L)	4.9 (3.8-6.3)
Calcium (mmol/L)	2.33 (2.25-2.40)
Leptin (pg/mL)	7731 (3334-16081)
25OHD (nmol/L)	34 (25-46)
eGFR (mL/min/1.73^2)	107 (100-111)
Spine BMD (g/cm^2)	1.28 (1.22-1.41)

Data are presented as medians (interquartile ranges) except where otherwise specified
*Dose of 50,000 IU/month
BMI = body mass index, DPP-4 = dipeptidyl peptidase-4, FGF23 = fibroblast growth factor 23, PTH = parathyroid hormone, 25OHD = 25-hydroxyvitamin D, eGFR = estimated GFR, BMD = bone mineral density

Table 2. Changes in parameters between baseline and 12 months following sleeve gastrectomy

Parameter	**Change at 12 months following SG (n=22)**	
	Median (95% CI)	*p-value**
Weight (kg)	-34.7 (-40.04, -23.1)	<0.0001
BMI (kg/m^2)	-10.8 (-12.9, -7.2)	<0.0001
Excess body weight lost (%)	68.1 (58.4, 74.6)	<0.0001
Fat mass (kg)	-20.0 (-26.7, -12.4)	<0.0001
Hemoglobin A1C (%)	-1.4 (-1.9, -1.1)	<0.0001
FGF23 (pg/mL)	-12.4 (-19.4,2.0)	0.005
PTH (pmol/L)	-1.1 (-1.7, 0.2)	0.009
Calcium (mmol/L)	0.05 (-0.10, 0.10)	0.62
Leptin (pg/mL)	-1687 (-4524, -563)	0.01
25OHD (nmol/L)	43.5 (23, 69)	<0.0001
eGFR (mL/min/1.73^2)	-0.2 (-5.3, 4.5)	0.83
Spine BMD (g/cm^2)	-0.04 (-0.06, -0.02)	<0.0001

Data are presented as medians (95% confidence intervals).
*p-value for change in each parameter determined using the Wilcoxon signed-rank test
BMI = body mass index, FGF23 = fibroblast growth factor 23, PTH = parathyroid hormone, 25OHD = 25-hydroxyvitamin D, eGFR = estimated GFR, BMD = bone mineral density

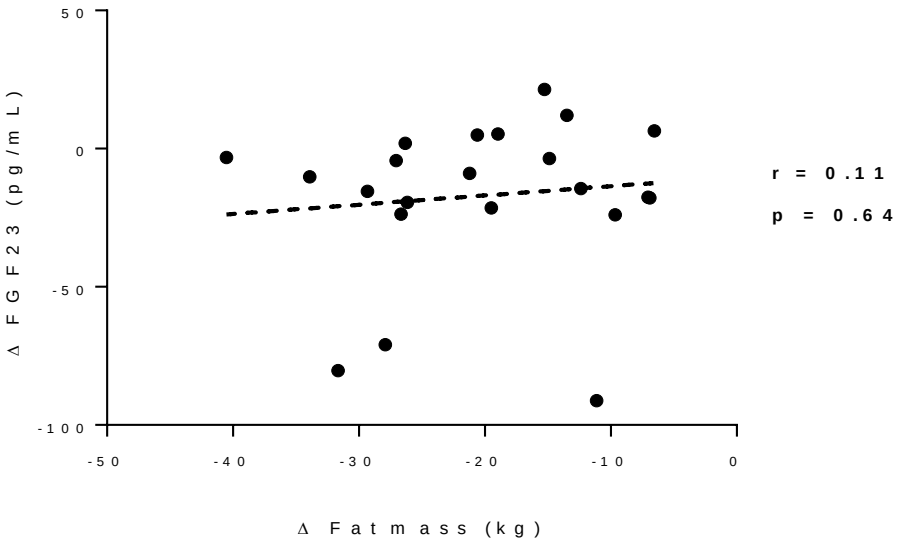

Figure 1. Association between change in fibroblast growth factor 23 (FGF23) and change in fat mass before and 12 months following sleeve gastrectomy in 22 adults. r = Spearman correlation coefficient.
Dashed line represents line of best fit.