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ORIGINAL ARTICLE

Plasma FGF21 levels in obese patients undergoing energy-restricted diets or bariatric surgery: a marker of metabolic stress?

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BACKGROUND: Fibroblast growth factor 21 (FGF21) has been suggested to be an endocrine signal of nutritional status and an active regulator of metabolism. However, there is no agreement on the effect of weight-loss therapies on circulating levels of FGF21 in humans.

OBJECTIVE: To assess FGF21 circulating levels in adiposity excess and after different weight-loss strategies prescribed in five different groups from four independent centers.

SUBJECTS AND METHODS: Body composition, ketosis, insulin sensitivity and FGF21 were evaluated in 181 excess body weight and 14 normal-weight subjects. From the excess body weight patients, two independent groups (discovery cohort; $n = 20$ and validation cohort; $n = 28$) undertook a very low-calorie ketogenic (VLCK) diet, a third group followed a low-calorie (LC) diet ($n = 84$) and other two groups underwent bariatric surgery (discovery cohort; $n = 24$ and validation cohort; $n = 25$). The follow-up was 4 to 6 or 12 months, respectively.

RESULTS: FGF21 levels were higher in excess body weight patients than in normal-weight subjects. The energy-restriction therapy to lose weight induced a significant decrease, with respect to baseline, in circulating levels of FGF21 (VLCK: -62.5 pg ml^{-1} or -14.8 pg ml^{-1} and LC diet: -67.9 pg ml^{-1}). There were no differences in FGF21 levels between both energy-restriction treatments. On the contrary, after bariatric surgery morbidly obese patients showed a significant increase in FGF21, especially 1 month after surgery (148.8 pg ml^{-1} higher than baseline). The FGF21 differential changes occur concomitantly with a non-induced ketosis situation (0.66 ± 0.56 mM) in bariatric surgery, and an improvement in adiposity and insulin sensitivity induced by the three therapies.

CONCLUSIONS: FGF21 levels were reduced after energy-restricted treatments and severely increased after bariatric surgery, independently of the weight reduction magnitude, insulin sensitivity or ketosis. Therefore, FGF21 appears to be a marker of severe nutritional stress.

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INTRODUCTION

Obesity is a worldwide health problem and is considered as a disease that has reached global epidemic proportions.[1,2] Despite new treatments coming to the field,[3–5] the burden of the disease continues unabated and is expanding.[6] To gain knowledge of the basic biological mechanisms of obesity, which could be translated into targets for treatment of obese patients, worldwide studies are ongoing to evaluate the role of new molecular mechanisms[7,8] and newly discovered molecules that potentially participate in energy homeostasis regulation as well as in appetite control.[9–11] Considerable attention has been devoted to FGF21, a protein previously thought to be a hormone, which is primarily expressed and secreted by the liver[12] but also by muscle, pancreas and adipose tissue,[13] and its production and expression is regulated by fasting and feeding signals.[14–16] A huge interest was generated after reports that pharmacological administration of FGF21 was able to exert positive effects on glucose homeostasis, lipid metabolism, energy expenditure, mitochondrial function and ketogenesis.[17,18] However, some reports have recently challenged previous findings and even proposed that the role of FGF21 in humans is contrary to that observed in rodents,[19–21] therefore, the precise physiological role of FGF21 in humans is still an open question.

Obesity, characterized for impaired glucose tolerance and increased accumulation of lipid in the liver, exhibits elevated

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levels of FGF21.[22,23] Whether such elevated FGF21 plasma levels are a defensive response to improve metabolic disruption or are only a marker of a pathological situation is still debatable, and therefore a better understanding of the physiology and regulatory mechanisms of FGF21 in obesity is needed. Furthermore, contradictory results on the effect of the different obesity therapies on circulating levels of FGF21 have been reported in humans.[24–27]

The aim of the current work was to determine circulating levels of FGF21 in obese patients after three different therapeutic strategies to lose weight; energy-restriction dietary programs, such as very low-calorie ketogenic (VLCK) diet and a low-calorie LC diet, and bariatric surgery.

MATERIALS AND METHODS

Patients

The current study was performed in 195 subjects; 181 obese patients ($n = 108$ women) and 14 normal-weight ($n = 10$ women) volunteers who acted as controls. The obese patients underwent a weight reduction therapy based on two different energy-restriction programs or bariatric surgery. In total, five different studies were performed in four independent centers. Thus, two groups of obese patients completed a treatment with a VLCK diet (Pronokal method) and results were validated by the same method in a different center. A third group was a subsample of the Reduction of the Metabolic Syndrome in Navarra, Spain study (RESMENA) project, a randomized control trial based on a low-calorie (LC) diet. Other two groups underwent a bariatric surgery treatment in two different centers.

Written informed consent to participate in the study was obtained before the start of the study in agreement with the Helsinki Declaration and followed national and European Union guidelines. The study was approved by the respective Institutional Ethics Committee for clinical research of Galicia, Hospital Clinico Universitario de Valladolid, University of Navarra and Hospital Clínico Virgen de la Victoria from Malaga.

Study design

VLCK diet. Two cohorts of patients following a VLCK diet were enrolled in this study. The first group included 20 obese patients (body mass index (BMI): 35.5 ± 4.4) attending the Obesity Unit at the Complejo Hospitalario Universitario of Santiago de Compostela, Spain, as a discovery cohort, in addition, an independent cohort of 28 patients (BMI: 33.2 ± 1.6) attending the Endocrinology and Nutrition Department at the Hospital Clinico Universitario of Valladolid received the same treatment, as a validation cohort. In both cohorts, participants followed a VLCK diet according to a commercial weight-loss program (Pronokal method), which includes lifestyle and behavioral modification support as described elsewhere.[28–31] This method is based on high-biological-value protein preparations obtained from cow's milk, soya, avian eggs, green peas and cereals. Each preparation contained 15 g protein, 4 g carbohydrates, 3 g fat and 50 mg docosahexaenoic acid, and provided 90–100 kcal (http://www.pronokal.com). The weight-loss program has three phases. The first phase consists of a VLCK diet (600–800 kcal day^{-1}), low in carbohydrates (< 50 g day^{-1} from vegetables) and lipids (10 g day^{-1} of olive oil) and 0.8–1.2 g kg^{-1} of ideal body weight. Throughout this ketogenic phase, supplements of vitamins and minerals such as K, Na, Mg, Ca and omega-3 fatty acids were provided. When the target amount of weight was lost, the ketogenic phase ended and the patients started a low-calorie diet (800–1500 kcal day^{-1}) followed by a maintenance diet of 1500–2000 kcal day^{-1}. The weight-loss program has five steps[29] and adheres to the most recent 2015 guidelines of EFSA on total carbohydrate intake.[32]

Patients followed the different steps of the method for up to a maximum of 4–6 months of follow-up, although patients remained under medical supervision for the following 12 months.[28–31] The intervention included an evaluation by the specialist physician conducting the study and an assessment by an expert dietician. All patients underwent a structured program of physical exercise, with external supervision (http://www.pronokal.com).

Throughout the study the patients completed a maximum of 10 visits with the research team (every 15 ± 2 days), of which 4 visits were for a complete physical, anthropometric and biochemical assessment, whereas the remaining visits were to control adherence and evaluation of potential side effects. The four assessment visits were made according to the evolution of each patient through the steps of ketosis as follows: normal level of ketone bodies (baseline); maximum ketosis (1–2 months); reduction of the ketotic approach because of partial reintroduction of normal nutrition (around 3 months); no ketosis (4–6 months; end of the study).[29] The total ketosis state lasted for 60–90 days. In all the visits, patients received dietary instructions, individual supportive counseling and encouragement to exercise on a regular basis using a formal exercise program.

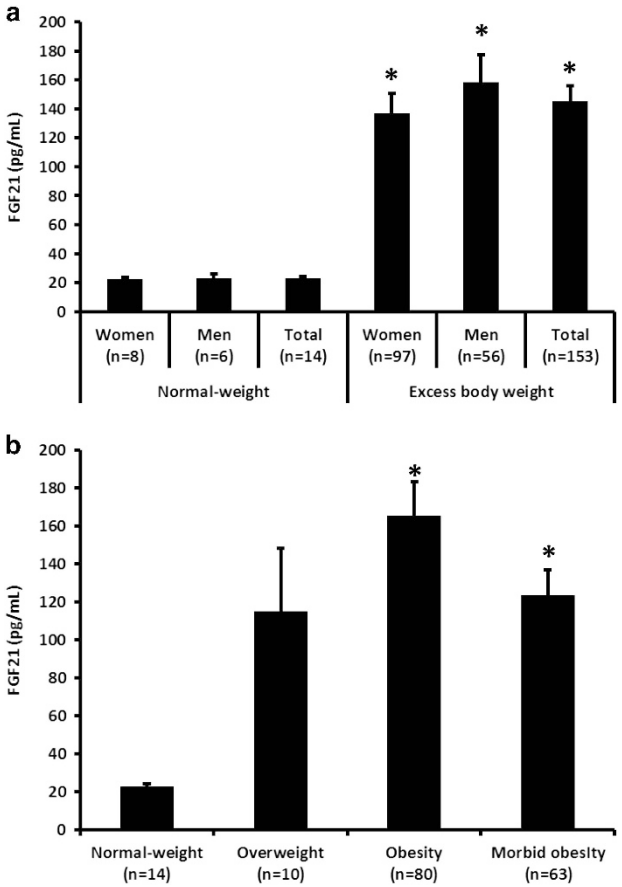

Figure 1. Comparison of circulating levels of FGF21 according to adiposity. (**a**) Differences in FGF21 levels between normal weight healthy subjects and patients with excess body weight. (**b**) Differences among the excess body weight patients classified according to BMI as overweight (25–29.9), obese (30–39.9) and morbidly obese (⩾40). The data are presented as the mean (s.e.). Asterisk (*) denotes statistical significant differences ($P < 0.05$) respect to normal weight subjects evaluated using analysis of covariance (ANCOVA) adjusted by age and gender.

LC diet. A third group of obese patients ($n = 84$; BMI 35.8 ± 4.5) followed a therapy program based on a nutritional intervention controlled by trained dieticians from the Department of Nutrition, Food Sciences, and Physiology of the University of Navarra, Spain. The RESMENA-based therapy program was described in detail elsewhere.[33–35] Briefly, the study lasted 6 months in two sequential periods: one intervention period of 2 months (−30% energy restriction, that is, a reduction of 600–800 kcal day^{-1}), 40–55% of energy intake from carbohydrates, 30% from lipids and 15–30% from proteins) in which subjects received nutritional assessment every 15 days followed by a self-control period of 4 months, in which subjects were advised to follow the lifestyle learned in the first period. Two energy-restricted diets were prescribed and compared. The control diet was based on the American Heart Association (AHA) guidelines and included 3–5 meals per day and a macronutrient distribution of 50–55% total caloric value from carbohydrates, 15% from proteins and 30% from lipids. The RESMENA diet was composed of 7 meals per day with a 40% total caloric value from

carbohydrates, 30% from proteins and 30% from lipids. Moreover, all participants were asked to maintain their normal physical activity during the study. Anthropometric measurements and venous blood samples were collected at baseline (week 0), at the end of the diet intervention (endpoint, week 8), and 4 months after ending the treatment (follow-up, week 24). At the end of the follow-up, patients were allocated to two groups, those who regained ⩾ 10% of the weight loss (regainers) and those who maintained the weight loss (non-regainers).[36]

Bariatric surgery. A fourth group of morbidly obese patients (discovery cohort; $n = 24$; BMI > 35) underwent bariatric surgery, performed by laparoscopic techniques such as Roux-en-Y gastric bypass ($n = 13$; 54.2%), biliopancreatic diversion ($n = 9$; 37.5%), and sleeve gastrectomy ($n = 2$; 8.3%) in the Complejo Hospitalario Universitario de Santiago de Compostela (CHUS), Spain. The fifth group, another independent cohort of morbidly obese patients ($n = 25$; BMI > 35) underwent bariatric surgery by laparoscopic techniques such as Roux-en-Y gastric bypass ($n = 2$; 8%), biopancreatic diversion ($n = 6$; 24%), sleeve gastrectomy ($n = 17$; 68%) in the Hospital Clínico Virgen de la Victoria from Málaga, Spain, was evaluated as a validation cohort.

After the surgical intervention, the patients were advised to follow a special diet that consisted of 1 month of liquid diet, 1 month of soft diet, and then a normal consistency diet providing about 800 kcal day^{-1}. The patients who received bariatric surgery were provided with a daily vitamin-mineral supplement, beginning on the day of the surgical procedure, to reduce the risk of developing nutritional deficiencies.[37] Patients within this study returned to the clinic for all follow-up visits. The follow-up regimen included visits at 1, 3, 6 and 12 months where anthropometric measurements and venous blood samples were collected.

Anthropometric and body composition measurements. All anthropometric measurements were undertaken with patients wearing only underwear and after an overnight fast (8–12 h) following validated procedures. Body weight and height measurements were performed using a wall-mounted stadiometer (Seca 220 scale, Medical Resources, EPI, Lewis Center, OH, USA). The BMI was calculated by dividing body weight by the square of height (kg m^{-2}). Total body composition was measured by dual-energy X-ray Absortiometry (DXA; GE Healthcare Lunar, Madison, WI, USA) as described elsewhere.[29]

Biochemical analysis

Venous blood samples were drawn after a 12 h overnight fast, and EDTA-plasma and serum were separated from whole blood and immediately frozen at − 80°C until assay. The quantitative measurement of total human circulating FGF21 was analyzed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Biovendor, Brno, Czech Republic) according to the manufacturer's instruction.[23–27] Circulating FGF21 levels were evaluated in plasma samples for all the studies except for the validation cohort of the VLCK diet where serum samples were employed. Absorbance from each sample was measured in duplicate using a spectrophotometric microplate reader at a wavelength of 450 nm (Versamax Microplate Reader; Associates of Cape Cod, East Falmouth, MA, USA). Circulating ketone bodies, specifically β-hydroxybutyrate (β-OHB), were measured using a portable meter (GlucoMen LX Sensor, A Menarini Diagnostics, Neuss, Germany). Glucose and total cholesterol serum concentrations were measured in an autoanalyzer Pentra C-200 (HORIBA ABX, Madrid, Spain) with specific kits. Insulin concentrations were assessed using an ELISA kit available from Mercodia, AB (Uppsala, Sweden) in a Triturus autoanalyzer (Grifols, Barcelona, Spain). Insulin resistance was indirectly determined by the homeostatic model assessment index (HOMA-IR), which was calculated following the formula (fasting plasma glucose (mg ml^{-1}) × fasting plasma insulin (mU l^{-1})/405), as described elsewhere.[38]

Statistical analysis

The sample size of the current study was calculated to detect differences for FGF21 taking into account published values of circulating FGF21 and standard deviation.[23–27] The normal distribution of variables was explored using the Kolmogorov–Smirnov and Shapiro–Wilk tests. An analysis of variance (ANOVA) was used to study differences between groups adjusted for gender and age. A repeated-measures analysis of variance was used to study the effects of time course of the nutritional therapy program and groupings of body composition, biochemical parameters and FGF21 levels in obese patients. The potential association between body composition and biochemical parameters with FGF21 levels was evaluated using the Spearman coefficient test.

Statistical analyses were performed using SPSS version 22.0 software (SPSS, Chicago, IL, USA) for Windows XP (Microsoft, Redmond, WA, USA). $P \leqslant 0.05$ was considered statistically significant.

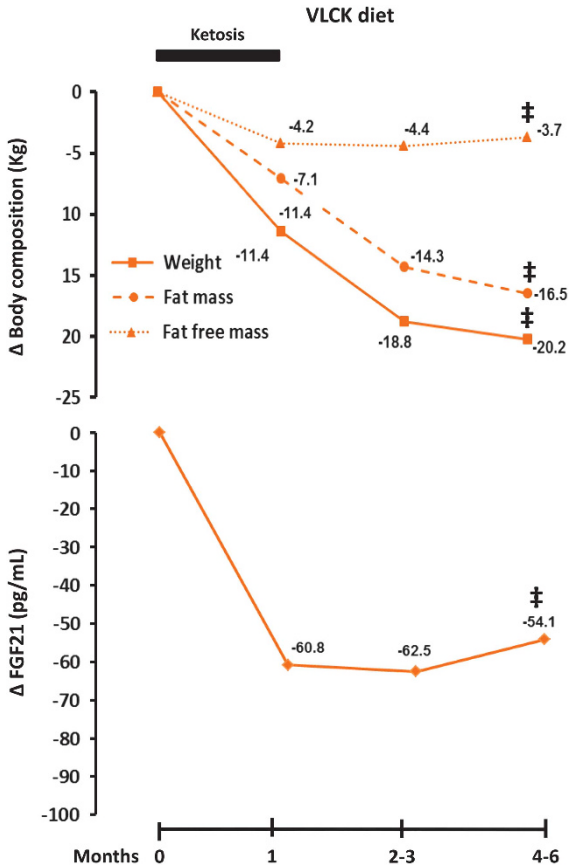

Figure 2. Effect of a VLCK diet on body composition and FGF21 circulating levels during a follow-up of 4–6 months. Data show mean of differences from baseline on body composition, and FGF21 levels after a period of 60–90 days of ketosis ended and at 4–6 months of intervention. Asterisk (*) denotes statistically significant ($P < 0.05$) changes across time evaluated by means of a repeated-measures ANOVA.

RESULTS

Different FGF21 circulating levels between normal weight and obese patients

A total of 195 subjects (14 normal weight and 181 obese patients) were included in this study. The general auxological characteristics of the subjects participating in the study are shown in Supplementary Table 1.

Participants including 14 normal weight and 153 obese patients of both genders were evaluated for the baseline levels of circulating FGF21. Obese patients from the VLCK diet validation cohort ($n = 28$) were excluded in this analysis because the FGF21 levels were quantified in serum and values were lower than those observed in plasma. Thus, no gender-dependent component ($P = 0.768$) was detected when 105 women (128.4 ± 12.7 pg ml^{-1}) and 62 men (145.5 ± 17.9 mg ml^{-1}) were compared, and no statistically significant interaction ($P = 0.776$) between obesity and gender was observed (Figure 1a). FGF21 concentrations were significantly ($P = 0.001$) higher in patients with excess body weight

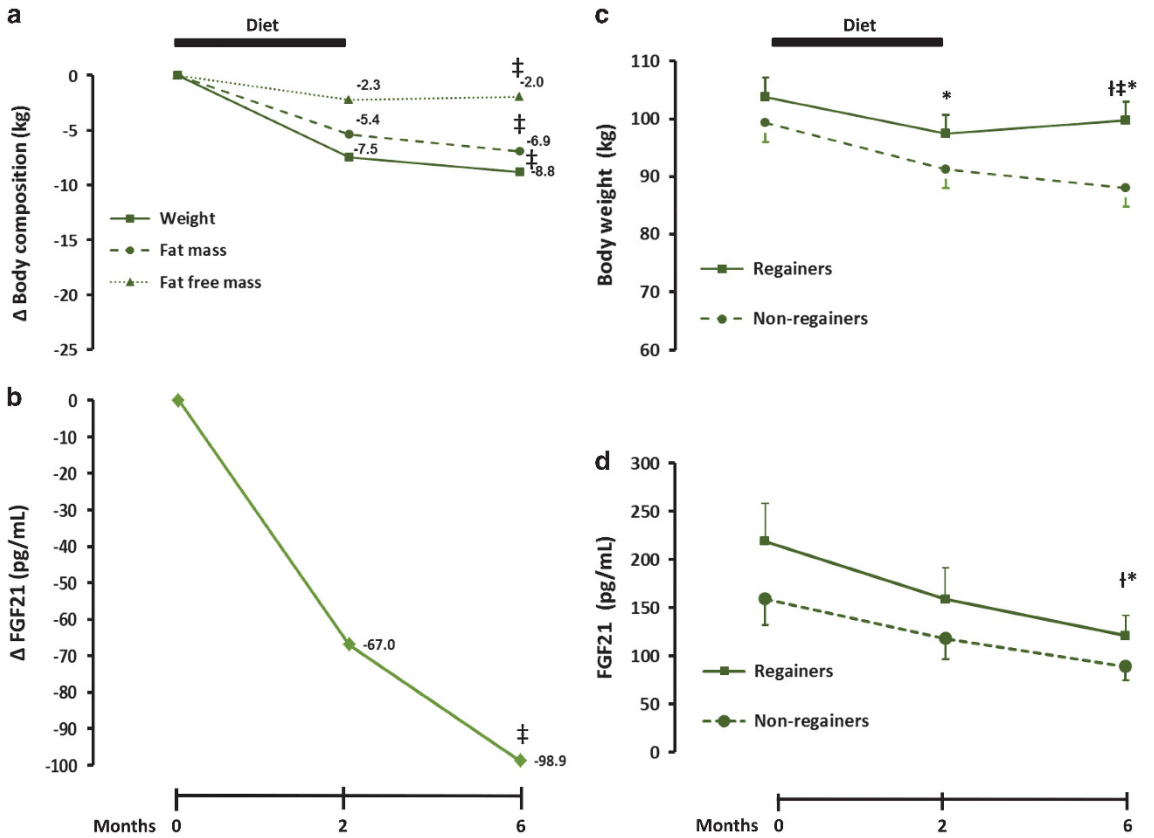

Figure 3. Effect of a LC diet (RESMENA study). (**a**) Changes from baseline in body weight and body composition through the LC diet treatment. (**b**) Changes from baseline in FGF21 circulating levels through the LC diet treatment. (**c**) Evolution of body weight during the intervention (2 months) and follow-up (4 months) according to the weight maintenance success after the LC diet. (**d**) Time course of FGF21 plasma levels during the LC diet intervention (2 months) and follow-up (4 months) according to the weight maintenance success. Data show differences from baseline in the time course of the intervention or mean (s.e.), respectively. Statistically significant differences were evaluated by means of a repeated-measures ANOVA. [‡]Statistically significant differences over the duration of the nutritional program (from 0 to 6 months). [†]Statistical significance for the time-weight maintenance group (regainers, non-regainers) interaction and *statistically significant differences between regainers and non-regainers evaluated by Student's *t*-test.

than in normal weight subjects (Figure 1a). Notably, when patients with excess body weight were classified according to BMI as overweight (25–29.9), obese (30–9.9) and morbidly obese (⩾40), no statistically significant ($P=0.771$) differences were observed between groups (Figure 1b).

Changes in FGF21 circulating levels after weight-loss interventions

A group of 20 obese patients was treated with a VLCK diet (discovery cohort). After a period of 60–90 days ketosis ended and at 4–6 months, the VLCK diet induced a 20 kg body weight reduction (Figure 2). These changes in body composition were mainly achieved at the expense of fat mass (−16.5 kg), whereas only 3.7 kg were lost in fat-free mass (Figure 2a). At baseline, this group of patients presented circulating levels of FGF21 of 102.6 ± 110.8 pg ml^{-1}. A remarkable decrease in FGF21 circulating levels (after 1 month 41.8 ± 8.5 pg ml^{-1}; 2–3 months 40.1 ± 9.5 pg ml^{-1}; and 4–6 months 48.5 ± 31.5 pg ml^{-1}; $P=0.040$) was observed (Figure 2). This decrease was especially noticeable at 1 month at the time of the maximum ketosis level (1 ± 0.6 mmol l^{-1} of β-OHB), and a weight loss of 11.4 kg. The effect of the VLCK diet on the plasma FGF21 levels was further corroborated in an independent, validation cohort of 28 patients in which patients lost a mean of 20.1 kg of body weight and serum FGF21 levels decreased from baseline 48.8 ± 42.1 pg ml^{-1}; after 1 month 33.8 ± 16.3 pg ml^{-1}; 2–3 months 34.4 ± 23 pg ml^{-1} and 4–6 months 41.8 ± 24.9 pg ml^{-1} ($P=0.049$); that is, a maximal decrease from baseline of 14.8 pg ml^{-1} of FGF21 levels.

A third group of 84 obese patients underwent a LC diet along 2 months with a total duration of 6 months of follow-up (Figure 3). The nutritional treatment consisted of two diets with different compositions (AHA and RESMENA), and no statistically significant differences were observed between these dietary groups regarding the effect on body composition.[39] The effect of the LC diet induced a statistically significant weight loss over 2 months (−7.5 kg), mainly due to a decrease in fat mass (−5.4 kg) with a small but statistically significant decrease in fat-free mass (−2.3 kg). This decreasing trend continued during the last 4 months of follow-up (Figure 2b). The evaluations of the circulating FGF21 levels in all patients following the nutritional treatment revealed a statistically significant decrease after the energy restriction and subsequent follow-up period (Figure 3b).

To correlate the FGF21 circulating levels with success in weight maintenance after the LC diet, patients were categorized according to the 10% of weight regain classification criterion.[36] Thus, a total of 51 patients maintained the weight lost (non-regainers) and 26 regained at least 10% of the lost weight (regainers). After the follow-up period (Figure 3c), the regainers group presented statistically ($P<0.01$) higher body weight (99.2 ± 14.1 kg) than non-regainers (89.1 ± 18.6 kg). Interestingly, FGF21 concentrations were higher in regainers than non-regainers during the whole LC diet program, reaching statistical significance at 6 months (Figure 3d).

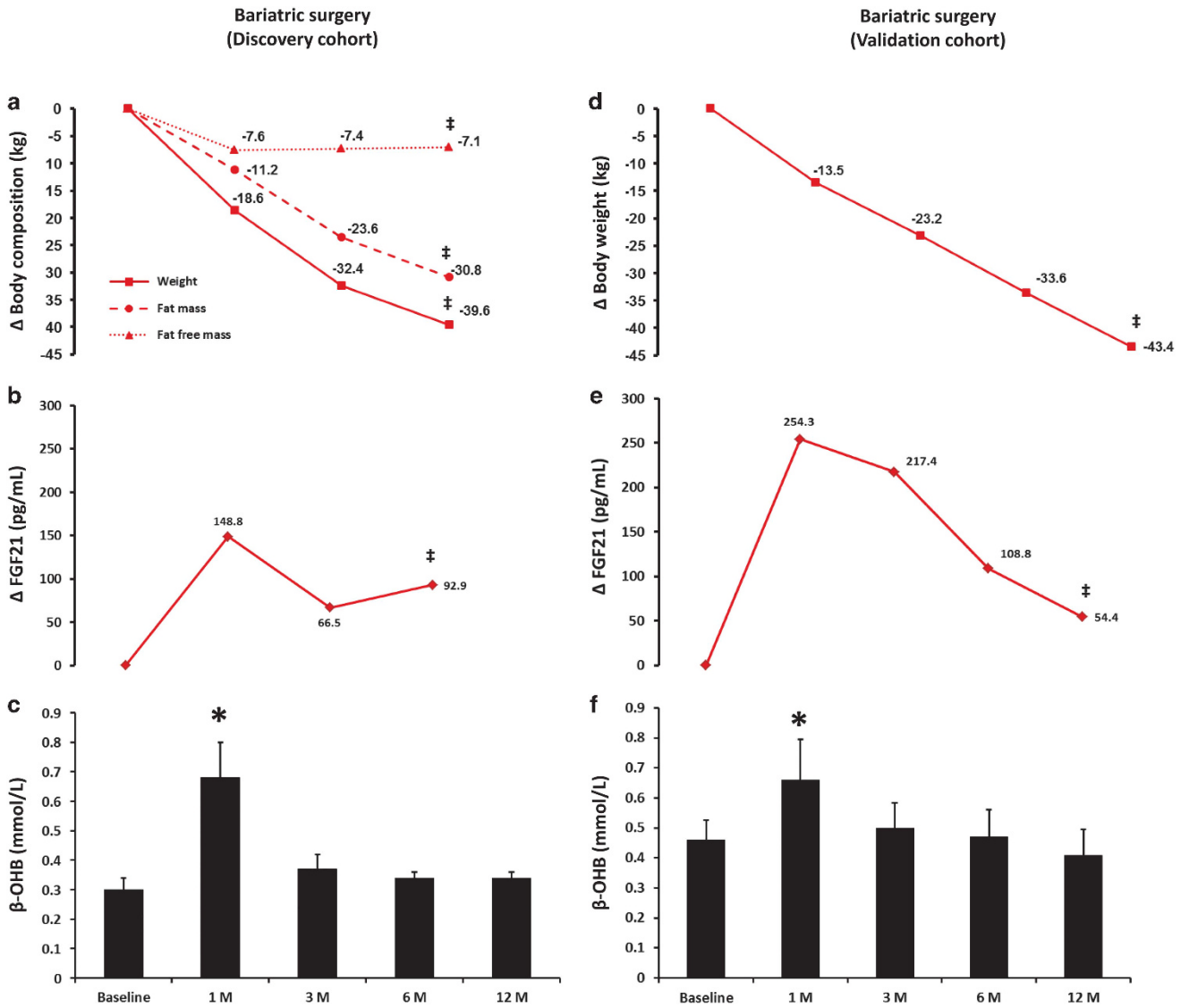

Figure 4. Effect of bariatric surgery on body composition, FGF21 and β-OHB circulating levels during a follow-up of 6–12 months. (**a**–**c**) Data from the discovery cohort of bariatric surgery ($n = 24$). (**d**–**f**) Data from the validation cohort of bariatric surgery ($n = 25$). Data show mean of differences from baseline in body composition and FGF21 or mean (s.e.) for β-OHB. ‡Statistically significant ($P < 0.05$) changes across time evaluated by a repeated-measures ANOVA. *Statistically significant differences respect to baseline evaluated by Student's *t*-test.

The fourth group of patients, who underwent bariatric surgery, exhibited a relevant weight loss, reaching a maximum of 39.6 kg of loss at 6 months (Figure 4a). This reduction in body weight was explained by a statistically significant reduction in fat mass of 30.8 kg and a decrease of 9 kg in fat-free mass (Figure 4a). Contrary to that observed in the energy-restriction treatment, 1 month after bariatric surgery the circulating levels of FGF21 increased with statistical significance (Figure 4b). Notably, patients underwent bariatric surgery exhibited an increase in β-OHB 1 month after the intervention, this increase was ameliorating in the subsequent 3, 6 and 12 months (Figure 4c). The results were somehow surprising then, a fifth group of patients underwent bariatric surgery in an independent center was evaluated as a validation cohort (Figure 4d). This further analysis reinforced the remarkable increase in FGF21 especially 1 month after bariatric surgery from baseline 104.8 ± 77.9–359.1 ± 360.3 pg ml^{-1} ($P < 0.010$; Figure 4e), and an increase in β-OHB was also observed 1 month after the intervention (Figure 4f). In spite of differences on the laparoscopic techniques distribution, no differences were observed in the time course of FGF21 levels between both bariatric surgery cohort ($P = 0.763$).

The β-OHB levels also differed with different weight-loss therapies. As expected by design, the VLCK diet induced a very large increase in β-OHB, whereas no ketosis was observed after the LC diet. On the other hand, bariatric surgery also induced a statistically significant increase in β-OHB levels (Figure 5a). In addition, the three therapeutic approaches induced a significant improvement in the HOMA-IR (Figure 5b).

The intervention-induced changes in FGF21, body composition, β-OBH and HOMA were compared between the patients from the three different treatments at the point of 1 or 2 months, depending on the intervention (Figure 6). Bariatric surgery induced an increase in FGF21 circulating levels that was statistically different ($P < 0.001$) with respect to levels found after both energy-restriction treatments. However, no statistically significant differences were observed between the LC and VLCK diet studies. Concomitantly, bariatric surgery induced a higher and more rapid decrease in body composition than both dietary

interventions. Regarding changes in HOMA and ketosis, the VLCK diet was the approach that achieved the highest decrease in insulin resistance (−3.5 ± 0.4) compared with the LC diet (−1.9 ± 0.3) or bariatric surgery (−1.3 ± 0.5) and reaching insulin sensitivity (HOMA < 2).

Finally, to elucidate if the changes in FGF21 levels observed were associated with changes in body composition, a correlation analysis was performed using data from all patients. Intervention-induced changes in FGF21 were inversely associated with changes in body weight ($r = -0.23$; $P = 0.011$), fat mass ($r = -0.23$; $P = 0.010$) and fat-free mass ($r = -0.21$; $P = 0.020$). However, when the correlation analysis was adjusted by the intervention group, the statistical significance was lost. No correlation was observed between FGF21 levels and β-OHB or HOMA-IR changes evaluating all patients together.

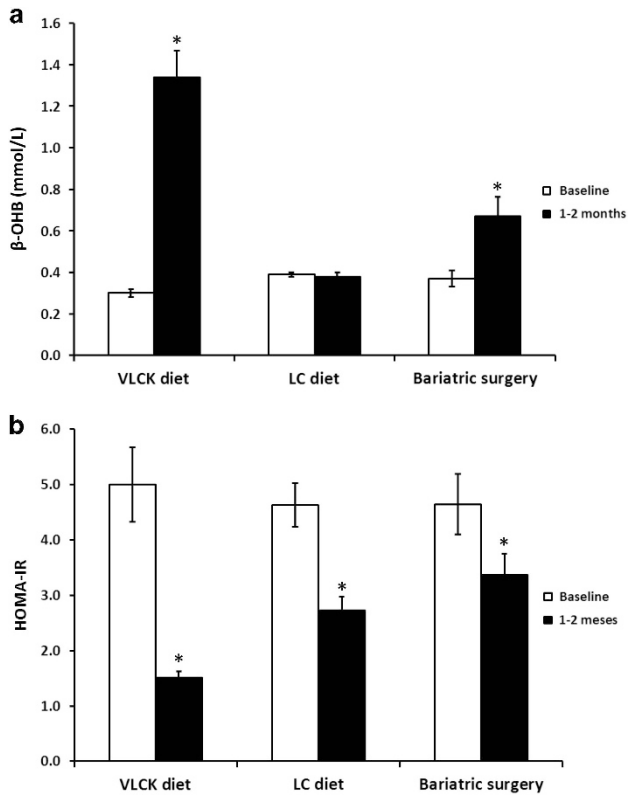

Figure 5. Differences from baseline according to the three weight-loss therapies. (**a**) β-OHB plasma levels. (**b**) The homeostatic model assessment of insulin resistance (HOMA-IR). Data show mean (s.e.). Asterisk (*) denotes statistically significant ($P < 0.05$) differences at the highest point of FGF21 changes (1 month in VLCK diet, 2 months in LC diet and 1 month in bariatric surgery) with respect to baseline. LC, low-calorie diet (RESMENA study); VLCK, very low-calorie ketogenic diet (Pronokal method).

DISCUSSION

The main findings of this work were as follows: (a) obesity reduction by two different treatments of a hypocaloric diet markedly reduced FGF21 levels; (b) bariatric surgery induced a very large increase in FGF21 levels; (c) these variations in FGF21 levels were not translated into any measured effects on metabolism such as weight reduction, insulin sensitivity or ketogenesis.

FGF21 was proposed as a promising therapeutic target to counteract obesity and type 2 diabetes because, at pharmacological doses, FGF21 induced weight loss and improved glucose tolerance and circulating lipid profile.[18] However, obesity is associated with elevated levels of FGF21, and the expected beneficial effects of this protein are absent, suggesting the existence of a FGF21-resistant state in obesity.[22] The results of the current study corroborated that obese patients exhibited elevated circulating levels of FGF21, although these increased levels were not dependent on the grade of obesity. Moreover, this study clearly showed that weight and adiposity reduction by two hypocaloric methods performed in three different hospitals reduced FGF21 levels, and that weight recovery showed a tendency to increase FGF21 or at least lessen its reduction. As changes in fat-free mass are small, mostly water, and the true muscle mass reduction is around 1 kg in 20 kg weight lost,[29] it could be concluded that FGF21 levels strictly follow adiposity levels. However, the results obtained in this study after bariatric surgery suggest that the circulating levels of FGF21 are associated with other obesity-related factors rather than adiposity itself.

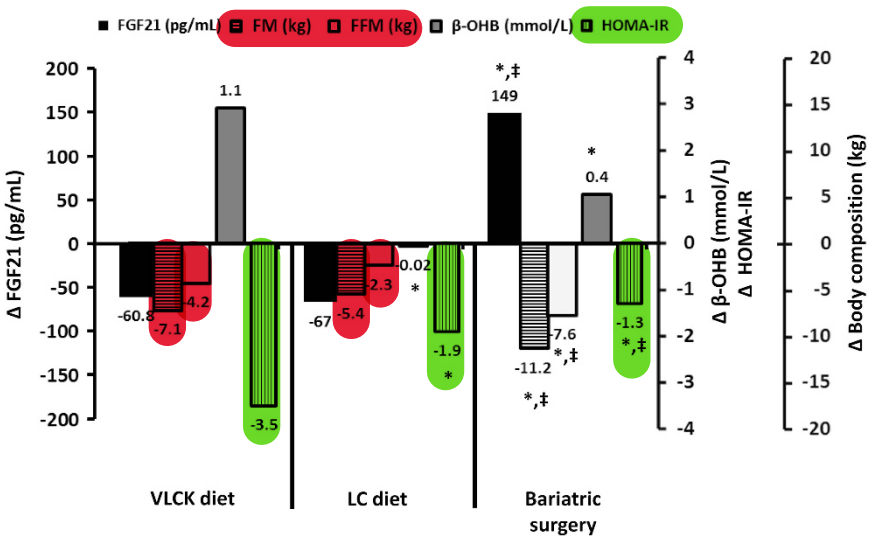

Figure 6. Comparison of the changes from baseline between the three weight-loss therapies on FGF21, body composition, insulin sensitivity and β-OHB. Data show mean of differences from baseline at the highest point of FGF21 changes (1 month in VLCK diet, 2 months in LC diet and 1 month in bariatric surgery). *Statistically significant differences with respect to VLCK diet and ‡statistically significant differences with respect to LC diet evaluated by Student's *t*-test.

It has also been proposed that FGF21 had a role in ketogenesis[40] and increased after a low-carbohydrate, high-fat ketogenic diet,[41] which suggested a role in metabolic states that require increased fatty acid oxidation.[42] However, FGF21 changes reported here were only related to levels of ketone bodies in the group of bariatric surgery, being β-OHB levels too high (VLCK diet), mild (bariatric surgery) or normally low (LC diet) concomitantly with low, high and low FGF21 levels, respectively. Therefore, the physiological relevance, if any, of FGF21 in a ketosis situation is still an open question. In line with this fact, a recent study found evidence that FGF21, after being initially proposed as a novel fasting-induced hormone in murine models, was increased only after a prolonged period (10 days) of fasting in healthy volunteers; however, the protein exhibited a decline after a short fasting period.[19] Moreover, fasting-induced ketosis was induced when FGF21 circulating levels decreased from baseline values.[19] Therefore, in humans, FGF21 seems not regulated by, nor contributes to ketosis in states of energy deprivation.

To our knowledge, this is the first time that a mild ketosis is observed after bariatric surgery in obese patients. Perhaps this contributed to the poor appetite and energy intake of these patients in the first month after surgery. It seems that this situation has been overlooked in the past, and no appropriate measures were taken despite the fact that isolated episodes of diabetic ketoacidosis had already been reported.[43] Thus, one possibility why bariatric surgery has different effects compared with other therapies could be the occurrence of metabolic disruptors, perhaps nutritional deficiencies. The effect of bariatric surgery is characterized by a rapid and massive weight loss, which mainly occurs during the first postoperative year after bariatric surgery.[44,45] This intervention induces the loss of muscle mass and fat-free mass that could lead to malnutrition post surgery because of the occurrence of oral protein intolerance and obesity-associated proteinuria.[46–48] In fact, several data demonstrated that FGF21 is rapidly and robustly induced by dietary-protein restriction in rodents and humans, and that this hormone is required for behavioral and metabolic responses to protein restriction.[15,49] Accordingly, the decrease observed after the energy-restricted dietary programs evaluated in the current work was observed under a condition of slightly dietary-protein enrichment. Diets enriched in proteins induce consistent beneficial effects on reduction and maintenance of weight loss and obesity comorbidities.[50]

Even the physiologically positive effects of FGF21 on metabolism previously reported, using pharmacological doses,[14] are not sustained. In fact, body weight changes as well as peripheral insulin sensitivity were not related to FGF21 levels. Following the three approaches performed, the insulin sensitivity was always improved and not correlated with FGF21. It was notable that the VLCK diet was more potent than any other intervention in ameliorating insulin sensitivity.

In line with the current findings, previous studies have demonstrated opposing effects on FGF21 of caloric restriction and Roux-en-Y gastric bypass in morbidly obese[24] and type 2 diabetes.[25] In addition, a post surgery increase in fasting plasma FGF21 levels was observed in adolescents who underwent vertical sleeve gastrectomy,[51] which suggested a contribution to the beneficial effect of the intervention.[24] However, in obesity, both VLCK and LC diets exert a beneficial effect on weight loss and metabolic disturbances associated to obesity,[29,31,34,52] and both situations were associated in the present work with a strong reduction in FGF21.

Therefore, the role of FGF21 observed in preclinical models and with pharmacological doses does not appear to be translated to humans; in addition, FGF21 increases in adiposity excess and decreases with weight loss induced by energy-restricted diets in obese patients. Regarding its role in ketogenesis or in the improvement of insulin sensitivity, no parallel was observed between FGF21 levels and changes in ketone bodies or the HOMA index. In addition, FGF21 increases in any situation of metabolic stress, that is, after prolonged fasting[19,53] or after the severe muscle loss induced by bariatric surgery. Thus, at present FGF21 could be only considered a biomarker of metabolic stress. This hypothesis is reinforced by the current results and others observed in HIV-1-infected patients with lipodystrophy[54] or liver steatosis.[55–57] Moreover, the sustained beneficial effects of systemically administered FGF21 are observed in a state of metabolic imbalance, as FGF21 does not appear to otherwise alter metabolic parameters in the healthy state.[20]

The strength of this study is its longitudinal design, which allows the evaluation of the time course of changes of FGF21 induced by three different therapeutic strategies designed to lose weight. Two of them were performed separately in two different centers with the same final results. However, this work was not able to demonstrate causality because it is an observational association study. Moreover, biomarkers of nutritional status in addition to fat-free mass were not evaluated to further demonstrate the association between the intervention-related changes in FGF21 and nutritional status after the weight-loss treatment. Long-term prospective studies are needed to confirm the association between FGF21 circulating levels and nutritional status after a therapy designed to lose weight. On the other hand, it was recently demonstrated that FGF21 is proteolytically inactivated by cleaving off 10 amino acid residues at the C terminus, and both active and inactive forms circulate in human blood.[58,59] The proteolytic activity is mediated by a site-specific endopeptidase for FGF21 named fibroblast activation protein FAP-α whose inactivation was postulated as a potential therapeutic approach to increase endogenous FGF21 activity for the treatment of obesity and related metabolic disorders in humans.[60,61] In the current work, the employed ELISA kit determined total FGF21, although it was demonstrated that this kit detects active FGF21 form with twofold better efficiency than the inactive form.[61] Moreover, the plasma levels of FAP-α was not quantified. Elevated circulating total FGF21 associated with obesity and co-diseases could be related to an increase in plasma FAP-α that could be negatively regulating the protective activity of FGF21, this hypothesis as well as the biological relevance of FAP-α were not elucidated in humans until today. Thus, the determination of the ratio active/total FGF21 protein as well as the plasma FAP-α levels would add valuable information to elucidate the physiological relevance of human FGF21 in different nutritional status.

In conclusion, FGF21 levels are reduced after energy-restricted treatments and severely increase after bariatric surgery. These results reinforce the hypothesis that FGF21 is dependent on nutritional status, highlighting the role of individual macronutrients in regulating FGF21, with particular sensitivity to protein malnutrition. Therefore, FGF21 circulating levels could be used as a biomarker for monitoring nutritional status and preventing the potential metabolic stress induced in obesity and/or after a therapy program for weight loss in obese patients.

CONFLICT OF INTEREST

ABC, DB and FFC received advisory board fees and or research grants from Pronokal Protein Supplies Spain. IS is Medical Director of Pronokal Spain. The remaining authors declare no conflict of interest.

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DISCLAIMER

The funding source had no involvement in the study design, recruitment of patients, study interventions, data collection or interpretation of the results. The Pronokal personnel (IS) was involved in the study design and revised the final version of the manuscript, without intervention in the analysis of data, statistical evaluation and final interpretation of the results of this study.

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