Obesity Surgery (2019) 29:2144–2150
https://doi.org/10.1007/s11695-019-03797-5

ORIGINAL CONTRIBUTIONS

Bone Metabolism in Adolescents and Adults Undergoing Roux-En-Y Gastric Bypass: a Comparative Study

Débora Santos[1] · Tais Lopes[1] · Patrícia Jesus[1,2] · Sabrina Cruz[1,2] · Adryana Cordeiro[1,2] · Silvia Pereira[3,4] · Carlos Saboya[3,4,5] · Andréa Ramalho[4,6]

Published online: 28 February 2019
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Abstract

Objective To compare the bone metabolism of adolescents and adults with obesity before undergoing a Roux-en-Y gastric bypass (RYGB) and 6 and 12 months after the surgery.

Materials and Methods Adolescents (G1) and adults (G2) with obesity assessed before (T0), six (T1), and 12 months after (T2) RYGB. Sun exposure, serum concentrations of 25(OH)D, calcium, phosphorous, magnesium, zinc, alkaline phosphatase, parathyroid hormone (PTH), and bone mineral density (BMD) were evaluated.

Results Sixty adolescents and 60 adults were assessed. At T0, there was no significant difference between the groups' serum 25(OH)D levels (G1 21.87 ± 7.52 ng/mL, G2 21.73 ± 7.60 ng/mL, $p = 0.94$) or sun exposure (G1 17 ± 2.0 min/day, G2 13.2 ± 5.2 min/day, $p = 0.85$). G1 had high levels of inadequacy of calcium (66.7%), phosphorous (80.0%), and zinc (18.3%) at T0 and had a significant fall in their 25(OH)D ($p < 0.01$) and magnesium ($p < 0.01$) levels from T1 to T2. G2 saw a significant lowering of their serum zinc levels from T0 to T1 and T2 (T1 $p < 0.01$; T2 $p < 0.01$). In both groups, there was a significant rise in PTH from T1 to T2 (G1 $p = 0.04$, G2 $p = 0.02$) and from T0 to T2 (G1 and G2 $p < 0.01$). In G2, 40.4% of individuals with osteopenia and osteoporosis presented inadequacy of 25(OH)D.

Conclusion RYGB was found to worsen the inadequacy of micronutrients related to bone metabolism and was associated with secondary hyperparathyroidism and low BMD values, especially among the adolescents. The irreversible damaging effects of obesity on bone metabolism can occur in adolescence.

Keywords Obesity · Adolescents · Adults · Bone metabolism · Roux-en-Y gastric bypass

✉ Sabrina Cruz
sabrina.cruz.ufrj@gmail.com

Débora Santos
deb.santos.91@gmail.com

Tais Lopes
taislopes@nutricao.ufrj.br

Patrícia Jesus
pcj.nutricao@gmail.com

Adryana Cordeiro
adrynutri@yahoo.com.br

Silvia Pereira
se.pereira@gmail.com

Carlos Saboya
cjsaboya@carlossaboya.com.br

Andréa Ramalho
aramalho.rj@gmail.com

[1] Researcher of the Center for Research on Micronutrients (NPqM) of the Institute of Nutrition Josué de Castro of Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro 21.941-902, Brazil

[2] School of Medicine at Federal University of Rio de Janeiro (UFRJ), Carlos Chagas avenue, 373. Edifício do Centro de Ciências da Saúde, 2° floor, room 49. Cidade Universitária - Ilha do Fundão, Rio de Janeiro 21.941-902, Brazil

[3] Multidisciplinary Center for Bariatric and Metabolic Surgery, School of Medicine of Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

[4] Researcher of the NPqM at Federal University of Rio de Janeiro, (UFRJ), Rio de Janeiro, Brazil

[5] Federal University of São Paulo (UNIFESP), São Paulo, Brazil

[6] Department of Social and Applied Nutrition of the Institute of Nutrition at UFRJ. Coordinator of the Center for Research on Micronutrients (NPqM) of the Institute of Nutrition Josué de Castro of UFRJ, Rio de Janeiro, Brazil

Introduction

Obesity is on the rise in all age groups, but the significant increase among adolescents calls for particular attention [1]. The current concern surrounds adolescent obesity continuing on into adulthood, given that the earlier and more severely the disease occurs, the greater the risk of continuing obesity throughout adulthood and developing associated metabolic alterations [2, 3], such as a decrease in bone mineral density [4].

Until now, the reasons found for the relationship between bone metabolism and obesity mainly refer to hormonal alterations with consequent elevation of pro-inflammatory cytokines and oxidative stress, as well as increased mechanical "load." Also, it has been suggested that strategies for reducing body weight can help improve this feature [4].

The increased prevalence of obesity and its negative effects on health, alongside the lack of success of traditional treatments, make bariatric surgery an option for addressing cases of more severe obesity. One of the most widely used procedures worldwide is the Roux-en-Y gastric bypass (RYGB) [5, 6].

Prior to bariatric surgery, nutritional deficiencies are commonly present in patients with obesity and can be aggravated by bariatric surgery, especially after procedures involving malabsorption such as RYGB. Despite the metabolic benefits of the procedure [7–9] RYGB can contribute to alterations to bone metabolism [8, 10–16]. It is worth pointing out that adolescence is a very important time of life for the formation of bone tissue since the skeletal development at this period is crucial for assuring the health of this tissue in adult life [17].

The aim of this study is to evaluate and compare the bone metabolism in adolescents and adults with obesity prior to the surgery and for the 12 months after it.

Methods

Study Population

This longitudinal comparative study investigated two groups: adolescents with severe obesity (G1) and adults with class III obesity (G2) who were receiving treatment at a private metabolic and bariatric surgery clinic in Rio de Janeiro, Brazil. Both groups were assessed on three occasions: 30 days before RYGB (T0), 6 months after the surgery (T1), and 12 months after the surgery (T2). Data were collected between January 2014 and September 2016.

Adolescents with severe obesity at T0 (BMI/age ≥ 99.9th percentile) [18] were included in the study provided the following inclusion criteria also applied: aged between 15 and 19 years 11 months old, stage IV sexual maturity or higher, based on the Tanner scale [19], and a minimum bone age of 13.5 years for the females and 14.5 years for the males. G2was made up of adults from 20 to 49 years of age classified as class III obese (BMI ≥ 40 kg/m^2) [20] who also reported having severe obesity in childhood and/or adolescence.

The exclusion criteria for both groups were the following: previous malabsorptive and/or restrictive surgery; use of hepatotoxic drugs, steroids, multivitamins, or minerals during the preoperative period; the presence of neoplasia, intestinal malabsorption syndrome, renal insufficiency, or liver disease, except for non-alcoholic fatty liver disease; women who were pregnant, going through the menopause; to have lengths of alimentary limb < 1.5 m and lengths of biliopancreatic limb < 1 m; and to have taken less than 80% of the supplements prescribed during the postoperative period.

Both groups followed the standard protocol of daily supplementation established by the surgical clinic, in accordance with the Institute of Medicine (2009), which consisted of 800IU of vitamin D3, 250 mg of calcium carbonate, 125 mg phosphorous, and 100 mg magnesium after the surgery and for an indeterminate period of time. To assess the adherence to supplementation, the study participants were asked to show the containers of the prescribed supplements at every follow-up consultation, when the importance of taking the supplements was reinforced, and they were given educational material explaining their benefits. When inadequacy of vitamin D was found in the preoperative period, all participants consumed 1500 IU of vitamin D, in compliance with the recommendation of the Brazilian Society of Endocrinology and Metabolism [21].

Anthropometric Variables and Biochemical Variables

Weight and height were collected [22] and body mass index calculated (BMI = body mass/height^2) [23].

Blood samples were collected at a specialized laboratory that had a partnership agreement with the clinic in question. The samples (5 mL) were obtained by venipuncture after 12 h of fasting to determine 25(OH)D, ionized calcium, phosphorous, magnesium, ionic zinc, PTH, and alkaline phosphatase concentrations.

The 25(OH)D levels were determined by high-performance liquid chromatography with UV detection. Deficiency was set at < 20 ng/mL, insufficiency at 20–29.9 ng/mL, and adequacy at ≥ 30.0 ng/mL and < 100.0 ng/m) [24, 25]. Ionized calcium was measured directly by ion-selective electrode, with inadequate levels being <1.20 mmol/L for G1 and < 1.0 mmol/L for G2 [26, 27]. Phosphorous, magnesium, and alkaline phosphatase concentrations were assessed by a colorimetric method. Phosphorous levels were considered inadequate when they were < 4.0 mg/dL for G1 and < 2.5 mg/dL for G2; inadequate magnesium levels were < 1.6 mg/dL for G1 and < 1.7 mg/dL for G2; and inadequate alkaline phosphatase levels were > 128.0 U/L for

G1, > 104.0 U/L for the women from G2, and > 129.0 U/L for the men from G2 [26, 28–30].

Zinc was analyzed by atomic absorption spectrophotometry and zinc deficiency was set at < 70.0 mcg/dL [26, 31]. Chemiluminescent immunoenzymatic assays were used to measure PTH, and the level for secondary hyperparathyroidism was set at > 53.0 pg/mL [32].

Sun exposure

Sun exposure to evaluate vitamin D status at T0 was assessed following the protocol validated by Hanwell et al. [33]. Sun exposure was assessed according to the number of minutes per day, and adequate exposure was set at ≥ 20 min/day [25].

Bone Mineral Density

Bone mineral density (BMD) in the lumbar spine (vertebrae L1-L4) and in the femoral neck was assessed by dual energy x-ray absorptiometry. The criteria recommended by the WHO (2004) were used to classify bone mass [34].

Statistical Analyses

The symmetry of the distribution of the continuous variables was verified by the Kolmogorov-Smirnov test. The Mann-Whitney test was used to compare the continuous variables and the chi-squared test was applied to compare the categorical variables. The correlation analysis was conducted using Spearman's test. Results were considered statistically significant using an alpha level of 0.05. All statistical analyses were performed using SPSS, Version 17 (SPSS Inc., Chicago, IL, USA).

Ethical Considerations

Patients were included in the study upon receipt of an informed consent form signed by the individuals themselves (from G2) or their parent/guardian (for G1), as well as an informed consent agreement filled out by the individuals from G2, as required by the National Health Council Resolution No. 196 [34]. The study was approved by the Research Ethics Committee of the Clementino Fraga Filho University Hospital of the Federal University of Rio de Janeiro (protocol 011/06).

Results

Sixty adolescents (G1) and 60 adults (G2) were assessed. There was a majority of female individuals (G1 63.7%, $n = 38$; G2 68.3%, $n = 41$), and the mean age of each group was 17.1 ± 0.8 years and 39.4 ± 5.5 years, respectively. No difference was found between the sexes ($p = 0.08$), for which reason, the analysis was conducted of all the subjects irrespective of gender, which was designed to make the results more robust. The mean sun exposure of the individuals prior to surgery was 17.1 ± 2.0 min/day for G1 and 13.2 ± 5.2 min/day for G2, both of which are below the recommended daily average, with no statistical difference.

The mean BMI of the individuals from G1 was higher than that of the individuals from G2 at T0, but no significant differences were observed between the groups ($p = 0.12$). A significant reduction in the mean BMIs was seen throughout the study period for both groups (Table 1).

The vitamin D ($p < 0.01$) and magnesium ($p < 0.01$) concentrations fell only for the adolescent group from T1 to T2. Meanwhile, calcium levels for both groups rose from T0 to T1 ($p < 0.01$), then declined from T1 to T2 ($p < 0.01$).

The mean zinc concentrations among the adolescents were significantly lower at T0 and 12 months after surgery ($p = 0.01$). For the adults, a significant difference was found between the concentrations of zinc from T0 to T1 ($p < 0.01$) and from T1 to T2 ($p < 0.01$). No variations were observed in serum phosphorous concentrations for either group throughout the study (Table 1).

PTH concentrations were higher at T2 than at T1 (G1 $p = 0.04$, G2 $p = 0.02$) and higher at T2 than at T0 ($p < 0.01$) for both groups. A fall in alkaline phosphatase levels was found from T0 to T1 but only among the adults ($p = 0.03$; Table 1).

The adolescents were found to have a higher frequency of inadequate calcium ($p < 0.01$) and phosphorous ($p < 0.01$) than the adults at all three times assessed, and their frequency of inadequate zinc ($p = 0.02$) was also higher than the adults at T0 (Table 2).

The frequency of 25(OH)D deficiency and insufficiency was similar for both groups studied at T0 43.3% ($n = 26$) and 41.7% ($n = 25$) for G1 and 45.0% ($n = 27$) and 41.7% ($n = 25$) for G2 ($p = 0.95$).

Six months after surgery, these levels had altered, respectively, to 25.0% ($n = 15$) and 55.0% ($n = 33$) for G1 and 23.3% ($n = 14$) and 53.3% ($n = 32$) for G2 ($p = 0.90$). By 12 months after surgery, 25(OH)D deficiency was found among 55.0% ($n = 33$) of the adolescents and 36.7% ($n = 22$) of the adults, while insufficient levels were found in 33.3% ($n = 20$) of G1 and 36.7% ($n = 22$) of G2 ($p = 0.103$). The variations in the frequency of 25(OH)D inadequacy were found to be significant between T0 and T1 (88.4 vs 80.0; $p = 0.02$) and between T1 and T2 (80.0 vs 88.3; $p < 0.01$) for the adolescents, and also between T0 and T1 (88.3 vs 76.6; $p < 0.01$) for the adults.

A negative correlation was found between 25(OH)D and alkaline phosphatase levels among the adults ($r = -0.372$, $p < 0.01$) at T1. At T2, a positive correlation was found with calcium ($r = 0.329$, $p = 0.01$) and magnesium (r = 0.291, $p = 0.02$). Positive correlations were found between insufficient

Table 1 Variations in the means of the anthropometric and biochemical variables assessed in G1 and G2 at times T0, T1, and T2

	T0 Mean (SD) **	T1 Mean (SD) **	T2 Mean (SD) **	*p* value T0-T1*	*p* value T1-T2*	*p* value T0-T2*
BMI (Kg/m^2)						
G1 ($n = 60$)	46.11 (± 7.30)	31.60 (± 4.86)	27.20 (± 5.47)	< 0.01	< 0.01	< 0.01
G2 ($n = 60$)	43.52 (± 4.39)	30.80 (± 3.38)	28.20 (± 3.34)	< 0.01	< 0.01	< 0.01
25(OH)D (ng/mL)						
G1 ($n = 60$)	21.87 (± 7.52)	25.58 (± 7.74)	21.45 (± 6.76)	< 0.01	< 0.01	0.92
G2 ($n = 60$)	21.73 (± 7.60)	25.89 (± 7.16)	25.58 (± 11.85)	< 0.01	0.53	0.06
Ionic calcium (nmol/L)						
G1 ($n = 60$)	1.13 (± 0.12)	1.29 (± 0.29)	1.12 (± 0.10)	< 0.01	< 0.01	0.90
G2 ($n = 60$)	1.18 (± 0.20)	1.33 (± 0.31)	1.18 (± 0.22)	< 0.01	< 0.01	0.73
Phosphorous (mg/mL)						
G1 ($n = 60$)	3.30 (± 0.66)	3.60 (± 4.22)	3.48 (± 0.62)	0.05	0.14	0.11
G2 ($n = 60$)	3.35 (± 0.65)	3.37 (± 0.64)	3.31 (± 0.71)	0.63	0.68	0.85
Magnesium (mg/mL)						
G1 ($n = 60$)	1.93 (± 0.16)	2.31 (± 0.70)	1.96 (± 0.16)	< 0.01	< 0.01	0.44
G2 ($n = 60$)	1.95 (± 0.16)	2.34 (± 0.72)	3.24 (± 5.49)	<0.01	0.53	0.05
Ionic zinc (μg/dL)						
G1 ($n = 60$)	87.61 (± 24.58)	87.72 (± 22.40)	93.77 (± 20.73)	0.97	< 0.01	0.04
G2 ($n = 60$)	118.93 (± 77.10)	92.04 (± 15.75)	93.13 (± 15.70)	< 0.01	0.65	0.01
PTH (pg/mL)						
G1 ($n = 60$)	47.32 (± 25.15)	54.90 (± 22.07)	63.17 (± 21.73)	0.05	0.04	< 0.01
G2 ($n = 60$)	45.19 (± 24.92)	49.43 (± 21.10)	56.36 (± 24.94)	0.13	0.02	< 0.01
Alkaline phosphatase (U/L)						
G1 ($n = 60$)	77.25 (± 12.69)	77.98 (± 13.58)	78.90 (± 13.50)	0.77	0.91	0.45
G2 ($n = 60$)	77.33 (± 15.67)	72.58 (± 15.04)	76.55 (± 16.47)	0.03	0.05	0.81

*Mann-Whitney test (statistical significance $p < 0.05$); **Comparison of groups, $p > 0.05$; T0, 30 days before RYGB; T1, 6 months after RYGB; T2, 12 months after RYGB; G1, adolescents; G2, adults; BMI, body mass index; PTH, parathyroid hormone

and deficient 25(OH)D and magnesium ($r = 0.341$, $p = 0.01$) at T0 and between insufficient and deficient 25(OH)D and calcium ($r = 0.298$, $p = 0.04$) at T2, and a negative correlation was found between insufficient and deficient 25(OH)D and alkaline phosphatase at T1.

With respect to BMD, similar frequencies were observed between the analyzed groups showing a high percentage of osteopenia in the assessed times, particularly in the site of the femoral neck (T0 G1 = 38.3% and G2 = 38.3%, $p = 0.94$; T0:G1 = 36.7% and G2 = 38.3%, $p = 0.98$). In G2, an

Table 2 – Frequency of inadequate biochemical variables as measured against reference values for adolescents and adults at times T0, T1, and T2

	T0			T1			T2		
	G1 *n* (%)	G2 *n* (%)	*p* value*	G1 *n* (%)	G2 *n* (%)	*p* value*	G1 *n* (%)	G2 *n* (%)	*p* value*
25(OH)D	53 (88.4)	53 (88.3)	0.95	48 (80.0)	46 (76.6)	0.90	53 (88.3)	47 (78.4)	0.10
Ionic calcium	40 (66.7)	1 (1.7)	< 0.01	14 (23.3)	0 (0)	< 0.01	45 (75.0)	1 (1.7)	< 0.01
Phosphorous	48 (80.0)	3 (5.0)	< 0.01	42 (70.0)	3 (5.0)	< 0.01	46 (76.7)	6 (10.0)	< 0.01
Magnesium	0 (0)	0 (0)	**	0 (0)	0 (0)	**	0 (0)	0 (0)	**
Ionic zinc	11 (18.3)	2 (3.3)	0.02	9 (15.0)	3 (5.0)	0.12	7 (11.7)	3 (5.0)	0.32
PTH	21 (35.0)	20 (33.3)	0.85	29 (48.3)	21 (35.0)	0.14	42 (70.0)	33 (55.0)	0.09
Alkaline phosphatase	0 (0)	0 (0)	**	0 (0)	0 (0)	**	0 (0)	0 (0)	**

*Chi-square test (statistical significance $p < 0.05$); **Not possible to calculate; T0, 30 days before RYGB; T1, 6 months after RYGB; T2, 12 months after RYGB; G1, adolescents; G2, adults; PTH, parathyroid hormone

association was found between the adequacies of 25(OH)D and BMD of the femoral neck and lumbar spine in T2 ($p < 0.01$). It is worth highlighting that 40.4% of individuals with classification of osteopenia and osteoporosis in those sites, in both groups, had insufficiency or deficiency of 25(OH)D. In addition, in adolescents, the frequency of inadequacy of calcium and phosphorus in T0 was associated with the frequency of osteopenia and osteoporosis in the site of the femoral neck in T2 ($p = 0.03$; $p = 0.02$).

Discussion

In adolescence, there is an increase in bone formation that can be four times higher in this phase of life in which the peak of bone mass can be reached until the age of 23, depending on gender [35]. Factors such as increased adiposity or bariatric surgery itself can contribute to its reduction. Thus, possibly markers and nutrients such as vitamin D, calcium, phosphorus, magnesium, zinc, as well as hormones involved in bone metabolism as alkaline phosphatase and PTH may have increased demands in adolescence, and consequently be more harmful to bone mineral density. However, to our knowledge, there has been no study in the literature that compares bone metabolism between adults and adolescents.

This study shows that significant nutritional deficits were found among both adolescents and adults prior to surgery, especially vitamin D inadequacy. However, the adolescents were more compromised in view of the prevalence of the cases of calcium, phosphorous, and zinc deficiencies prior to the RYGB.

It is noteworthy that even in the first postoperative year, both groups showed significant reductions in serum calcium concentrations and increase in PTH when comparing T1 and T2. The occurrence can suggest that calcium can present intense reductions in its concentrations in the period of 6–12 months postoperatively due to the restrictive and disabsorptive character of RYGB. Moreover, the increase in PTH could have contributed to even more intense reductions of calcium concentrations in the adipose tissue and, consequently, activation of lipolysis and fat oxidation pathways [36].

As far as the size of the deficiencies encountered and their impact on bone health is concerned, it should be stressed that the process of bone remodeling is continuous, and the balance between resorption and formation alters at different stages of life. Bone formation is intense during childhood and adolescence, while the two processes balance out in adulthood, then, later in life, there is a predominance of bone resorption [37]. The development of bone mass can also be affected by environmental factors, especially nutrition. Bone mass acquired late in the growth period is more important than bone loss in adulthood. As such, nutritional deficiencies at such an important stage of growth could hamper bone tissue development [38].

Secondary hyperparathyroidism appeared late in both groups studied, in line with the findings of Sakhaee et al. [39], who found that serum PTH values tended to increase or remain normal but were higher after surgery. Meanwhile, in an evaluation of Swedish women before RYGB and 2 and 5 years after the surgery, Raoof et al. [40] also identified the late appearance of secondary hyperparathyroidism in association with the occurrence of bone remodeling.

The results here corroborate other studies that have identified nutritional deficiencies in individuals with obesity. Sánchez et al. [41] found a high prevalence of inadequate vitamin D and PTH levels (71.7% and 66%, respectively) prior to bariatric surgery, while Lefebvre et al. [42] found that micronutrient deficiency, including vitamin D deficiency (67.9%), was common in the different age groups studied.

Data on sun exposure demonstrate that both groups were below the recommended level prior to surgery. This is consistent with studies that have assessed the association between vitamin D deficiency, obesity, and sun exposure [43, 44]. It is suggested that the relationship between obesity and low serum vitamin D is influenced by behavioral factors, insofar as individuals with obesity, especially severe obesity, are less exposed to the sun because of their limited mobility, which causes them to do less outdoor activity, and because they wear more clothing [45].

The insufficient ingestion of these nutrients plus the malabsorptive component of RYGB [46] yields nutritional deficiencies that could be even more serious if we consider the degree of vulnerability and increased nutritional demands of adolescence [47]. This could explain the high frequencies of inadequate calcium and phosphorous levels found for this group at all the times assessed and the fall in 25(OH)D and magnesium concentrations 6 and 12 months after surgery.

The high inadequacy of serum micronutrient levels related to bone metabolism and the low BMD values encountered in the adolescents are causes for concern and draw attention to potential metabolic alterations associated with longer exposure to obesity. In addition, the negative effects of bariatric surgery on bone tissue can also be associated with changes in body composition [9]. Although studies indicate an increased risk of osteoporosis associated with RYGB after 2–5 years after the surgery [48, 49], the present study finds results in a period less than 12 months, with a high percentage of osteopenia in both groups at the times assessed, particularly in the femoral neck.

In these circumstances, the interventions for maintaining bone health are more effective and necessary in the phases of life with higher bone turnover, which include adolescence. Furthermore, adequate supplementation in adolescents and adults who undergo RYGB is important for assuring bone formation and preventing bone disease at more advanced stages of life.

While the study does not investigate sun exposure and BMD at all the times under study, so far this is the first study that compares alterations in bone metabolism between adolescents and adults in a 12-month period after RYGB.

Conclusion

RYGB was found to worsen the nutritional status of micronutrients related to bone metabolism in adolescents and adults with obesity and was associated with secondary hyperparathyroidism, especially in the adolescents, even when they were taking micronutrient supplements.

This study draws attention to the metabolic alterations associated to longer exposure to obesity and indicates that the irreversible damaging effects this disease has on bone metabolism can occur in adolescence. Further longitudinal studies evaluating the long-term effects of obesity on bone metabolism are needed and may provide a better understanding of the most effective control measures for a greater control of bone health in the presence of obesity.

The present study suggests that actions as adequacy of serum concentrations of vitamin D in the preoperative period, adherence to supplementation after surgery, and observation of studies that investigate different protocols of supplementation and administration pathways can minimize the impact of this nutritional issue.

Funding Information The different stages of this study are funded by the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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