Zinc absorption and zinc status are reduced after Roux-en-Y gastric bypass: a randomized study using 2 supplements[1–3]

Manuel Ruz, Fernando Carrasco, Pamela Rojas, Juana Codoceo, Jorge Inostroza, Karen Basfi-fer, Attila Csendes, Karin Papapietro, Fernando Pizarro, Manuel Olivares, Lei Sian, Jamie L Westcott, Leland V Miller, K Michael Hambidge, and Nancy F Krebs

ABSTRACT

Background: Micronutrient deficiencies are common in patients undergoing gastric bypass. The effect of this type of surgery on zinc absorption and zinc status is not well known.

Objective: The objective was to evaluate the effects of Roux-en-Y gastric bypass (RYGBP) on zinc status and zinc absorption at different stages after surgery. We hypothesized that zinc status would be significantly impaired after surgery and that this impairment would be less severe in subjects receiving increased supplemental zinc. We also hypothesized that zinc absorption would be lower after surgery.

Design: Anthropometric and body-composition variables and dietary and biochemical indexes of zinc status and zinc absorption were determined in 67 severe and morbidly obese women [mean (±SD) age: 36.9 ± 9.8 y; BMI (in kg/m²): 45.2 ± 4.7] who underwent RYGBP. The subjects were randomly assigned to 1 of 2 vitamin-mineral supplementation groups. Measurements were made before and 6, 12, and 18 mo after surgery. Fifty-six subjects completed the 18-mo follow-up.

Results: Mean plasma zinc, erythrocyte membrane alkaline phosphatase activity, and the size of the rapidly exchangeable zinc pool decreased after RYGBP. Percentage zinc absorption decreased significantly from 32.3% to 13.6% at 6 mo after RYGBP and to 21% at 18 mo after surgery. No effect of supplement type was observed.

Conclusions: Zinc status is impaired after RYGBP, despite the finding that dietary plus supplemental zinc doubled recommended zinc intakes in healthy persons. Zinc absorption capacity is significantly reduced soon after RYGBP, with no major changes until 18 mo after surgery. *Am J Clin Nutr* 2011;94:1004–11.

INTRODUCTION

Several surgical alternatives are available to treat morbid obesity, such as RYGBP[4], gastric banding, sleeve gastrectomy, and biliopancreatic diversion (1–3). RYGBP has shown highly satisfactory results, including long-term maintained weight loss and improvements in medical conditions and quality of life (3, 4). Surgical complications associated with this procedure include anastomotic leakage (2.5%) and postoperative bleeding of the gastric staple line (0.75%) (4). In terms of adverse nutritional consequences, anemia is relatively common, mainly iron-deficiency anemia, which has been reported in 15–60% of patients who undergo gastric bypass (5–8). Recently, we showed that iron absorption from both a standard diet and from a standard dose of ferrous ascorbate was significantly decreased 6 mo after RYGBP to 32.7% and 40.3% of their initial values, respectively. No further significant modifications were noted until 18 mo after surgery (9).

Knowledge of the effects of gastric bypass on other minerals, zinc for example, is very limited (10, 11). Of the potential causes of zinc deficiency, a reduced zinc absorption capacity has frequently been mentioned, but the experimental evidence to support this is not available. In the current study we determined zinc status and zinc absorption before and 6, 12, and 18 mo after RYGBP. We hypothesized that, compared with presurgical measurements, zinc status would be significantly impaired after surgery and that this impairment would be less severe in subjects who received increased supplemental zinc. A secondary hypothesis was that zinc absorption would be lower after surgery and that the level of absorption would persist.

SUBJECTS AND METHODS

Subjects

Sixty-seven menstruating women were enrolled in this study [mean (±SD) age: 36.9 ± 9.8 y; age range: 18–55 y; mean weight: 115.1 ± 15.6 kg; weight range: 88.4–156.4 kg; mean BMI (in kg/m²): 45.2 ± 4.7]. The patients were enrolled between August 2004 and December 2006. The subjects were evaluated before and 6, 12, and 18 mo after RYGBP. The

inclusion criteria considered women with a BMI ≥40 or a BMI ≥35 with comorbidities and who had a medical indication to undergo RYGBP at the Department of Surgery of the University Clinical Hospital. Fifty-six subjects completed the 18-mo follow up (**Figure 1**). Main initial anthropometric and biochemical characteristics of the individuals who abandoned the study were not significantly different from those who remained (data not shown).

All patients who accepted enrollment into the study signed an authorized consent form. The study was approved by the Ethics Committee for Human Investigation of the Faculty of Medicine of the University of Chile.

Experimental design

Details regarding the experimental design were provided previously while reporting the effects of the protocol on iron status and iron absorption (9). Briefly, the study was intended to produce the minimal interference to the standard medical management of patients undergoing RYGBP while simultaneously testing an improved vitamin and mineral supplement to reduce potential nutritional adverse consequences. All patients followed routine standard procedures after surgery. During the first month, they were fed a 500-mL liquid diet daily, which consisted of chicken breast, egg white, spinach, carrots, and potatoes that provided 2.9 mg Zn. A full medical evaluation was conducted at the end of this month. At this time, 1 unit of vitamins (TOL12; Saval Laboratories SA) was prescribed to all patients to

be administered intramuscularly. This preparation contained 200 mg thiamine chlorhydrate, 100 mg pyridoxine chlorhydrate, and 10 mg hydroxycobalamin. In addition, a solid diet divided into 5 or 6 meals was also prescribed. It provided ∼1000 kcal, 60 g protein, and 6.5 mg Zn. If any nutritional-related signs (including zinc deficiency, such as alopecia or dermic lesions) were present at the medical evaluation conducted 3 mo after surgery, additional supplements of the corresponding nutrient were prescribed and used during distinct periods of treatment. These procedures were repeated 6 mo after surgery and every 6 mo thereafter until 18 mo after surgery.

At the evaluation scheduled 1 mo after surgery, the subjects were randomly allocated (using a random numbers table and carried out by a member of the Department of Nutrition not related to this study) into 2 groups: group A received the "standard vitamin and mineral supplement" and group B received the "improved vitamin and mineral supplement." The study was blinded to the participants only. The "standard vitamin and mineral supplement" consisted of the routine vitamin and mineral supplement established at the University of Chile Clinical Hospital, which considered the use of a vitamin-mineral supplement (Larotabe; Bayer Laboratories) and 2 tablets daily of a calcium and vitamin D supplement (Elcal D; Andromaco Laboratories) to prevent the development of nutritional deficiencies. The "improved vitamin and mineral supplement" consisted of a specially designed vitamin-mineral supplement that provided at least the RDA of selected nutrients (12, 13) and 2 tablets daily of a calcium and vitamin D supplement (Elcal D-PLUS; Andromaco
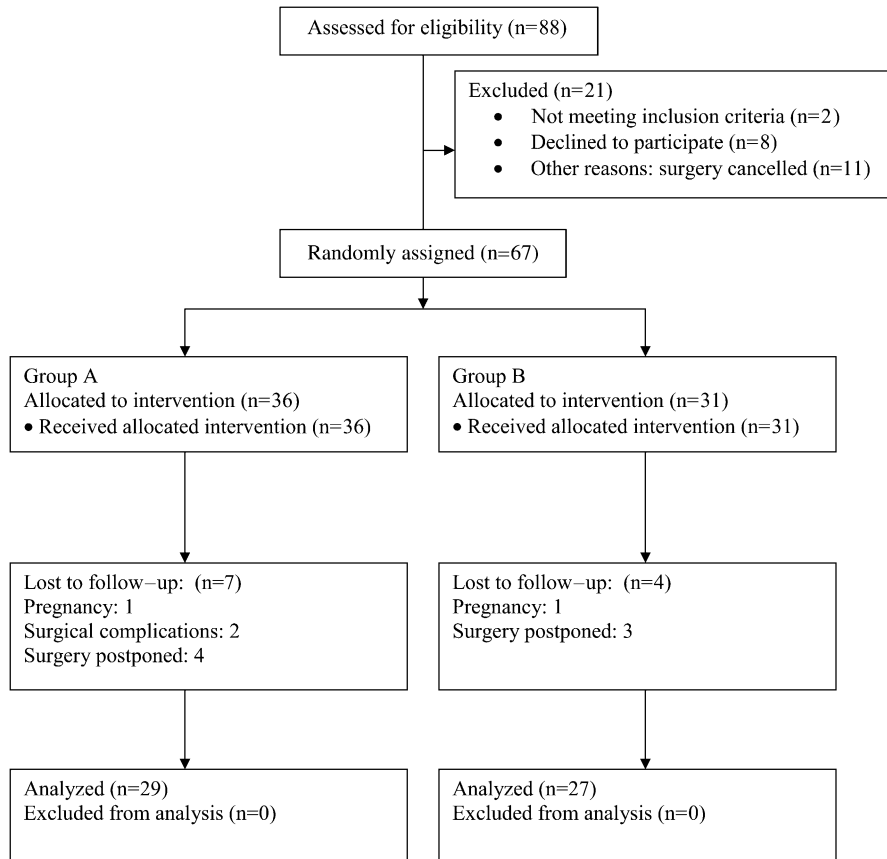
FIGURE 1. Flow diagram of volunteer recruitment and progress throughout the study.

Laboratories). The total contents of the vitamins and minerals supplied by these supplements are described in detail elsewhere (9). In terms of elemental zinc, the standard vitamin and mineral supplement provided 7.5 mg/d and the improved vitamin and mineral supplement provided twice as much, 15 mg/d. A careful record of the amount of zinc-containing supplements consumed was kept throughout the study in a monthly register, and the number of pills remaining in the container was counted by study personnel. Also, a periodic evaluation for any indication of additional zinc supplements registered in the medical record was made. If this situation occurred, the subject was contacted by telephone to determine the extent to which the indicated supplement had effectively been consumed.

Sample size was calculated to detect postsurgical differences between plasma zinc (minimal number required: 21 subjects/group) and zinc absorption (minimal number required: 10 subjects/group). A series of anthropometric, body-composition, dietary, and hematologic evaluations were conducted in all patients before the surgical procedure and 6, 12, and 18 mo after RYGBP. In a subset of 26 individuals randomly selected within each group, zinc absorption tests and the size of the rapidly EZP were carried out during the same evaluation periods.

Surgical procedure

Details of the surgical technique are available elsewhere (4). Briefly, it consisted of a 95% distal gastrectomy and resection of the excluded stomach, which left a small gastric pouch of 15–20 mL. The length of the Roux-en-Y loop was 125–150 cm, in agreement with the current practice of clinical centers in Chile expressed in the 2005 panel of experts' consensus (14).

Anthropometric and body-composition evaluations

Weight (kg) was measured to the nearest 0.1 kg on a digital scale (Seca; Vogel & Halke GMBH & Co), and height (m) was measured to the nearest 0.1 cm with a scale-mounted stadiometer according to standardized procedures (15). From these measurements, BMI (in kg/m^2) was calculated. Body fat mass and fat-free mass were measured by dual-energy X-ray absorptiometry with a Lunar DPX-L densitometer (Lunar).

Dietary evaluation

Before and 6, 12, and 18 mo after RYGBP, the patients were interviewed by a dietitian, and a 3-d record that corresponded to 2 weekdays and 1 weekend day was completed. The data registered was analyzed by using a computer program (Food Processor II; ESHA) to calculate energy and nutrient intakes by using a database that contained locally generated nutrient composition data and information from the literature (16).

Zinc status evaluation

Fasting blood (7 mL) was collected by antecubital puncture and maintained in trace element–free containers until analyzed. Blood was obtained immediately before the administration of the stable isotopes used to determine percentage zinc absorption and the size of the rapidly EZP. Plasma was separated within 2 h of sample collection.

Plasma zinc concentrations were measured by atomic absorption spectrophotometry according to the method of Smith et al (17) with a Perkin-Elmer Analyst 100 atomic absorption spectrophotometer (Perkin-Elmer Corporation). Erythrocyte membranes were prepared as indicated by Steck et al (18), and alkaline phosphatase activity (E.C.3.2.1.24) was determined by the method of Ruz et al (19). Enzyme activity was expressed as nmol of product formed per mg membrane protein per min. Protein was determined according to the method of Markwell et al (20). Erythrocyte metallothionein concentrations were measured by using the method of Eaton and Toal (21), and the results were expressed as nmol/g protein. Hair was collected from the proximal centimeter of the occipital scalp. It was washed with nonionic detergent, dried, digested with an HNO$_3$-H$_2$O$_2$ mixture, diluted with deionized water, and analyzed by atomic absorption spectrophotometry (17). The results of the zinc status indexes were also presented as the percentage of cases below selected cutoffs, which were obtained from literature if available, ie, plasma zinc <70 μg/dL (22) and hair zinc <100 μg/g (23). The limit for erythrocyte membrane alkaline phosphatase, (<0.20 U/mg protein) was obtained from previous results obtained in our laboratory. Erythrocyte metallothionein and EZP cutoffs were arbitrarily set at the 5th percentile of the corresponding distribution of cases before surgery.

The accuracy of zinc determinations was assessed by analyzing a reference standard (Huma Trol N, from Human Gesellschaft für Biochemical und Diagnostica GmbH). The values found were between the confidence limits of the reference data. Precision was evaluated by repeated determination of a plasma pool, and the CV was 2.6%.

Zinc absorption and size of the rapidly exchangeable zinc pool

Percentage zinc absorption and EZP were determined by extrinsic labeling with enriched zinc stable-isotope preparations by using a dual isotope tracer ratio technique (24) according to the following protocol: an accurately weighed quantity (~0.45 mg) of ^{70}Zn was administered intravenously (before the oral administration of oral dose of ^{67}Zn) with a 10-mL syringe (via 3-way stopcock and scalp vein needle placed) in a superficial vein in the forearm over a 5–10-min interval. The syringe was flushed twice with normal saline via the 3-way stopcock. An accurately weighed quantity of ^{67}Zn solution (~0.7 mg) was combined with 100 mL of the liquid diet used during the first month after the surgery. The container with the labeled meal was rinsed with deionized water twice, and these rinses were also ingested. Oral and intravenous isotope administration was carried out between 0830 and 0930 under fasting conditions. The subjects suspended taking their assigned vitamin and mineral supplements 3 d before isotope administration. This protocol was repeated at each testing point.

Preparation and administration of zinc stable-isotope solutions

Accurately weighed quantities of enriched zinc oxide (^{67}Zn or ^{70}Zn) preparations (Traces Sciences International) were dissolved in 0.5 mol H$_2$SO$_4$/L to prepare a stock solution. For preparation of the orally administered dose of ^{67}Zn, the stock

solution was diluted with triply deionized water and titrated to pH 3.0 with metal-free ammonium hydroxide. For the intravenously administered ^{70}Zn, the pH of the stock solution was adjusted to 6.0 with ammonium hydroxide, and the stock solution was diluted with sterile isotonic sodium chloride to a zinc concentration of 1.5 mmol/L. Oral and intravenous solutions were filtered through a 0.2-μm filter. The zinc concentrations of these solutions were measured by atomic absorption spectrophotometry with mass correction factors applied (25). The intravenous doses were tested for pyrogens and sterility immediately before use.

Sample collections and analyses

Timed 30–50-mL urine samples were collected daily in acid-washed bottles from 4 to 9 d after administration of the tracers. Urine was transferred to tempered glass beakers, dried at 100°C, and then ashed twice for 24 h in a muffle furnace at 450°C each time. Between the 2 ashings, a few drops of concentrated nitric acid were added to the sample and then the sample was dried on a hot plate. After digestion, each sample was dissolved in 2.5 mL ammonium acetate buffer (pH 5.6). Zinc in the dissolved samples was extracted according to the method of Veillon et al (26). All glass tubes used in the extraction were acid washed in aqua regia and rinsed in deionized water before use. All procedures were conducted at the Department of Nutrition, Faculty of Medicine, University of Chile.

Zinc stable-isotope analyses

^{67}Zn/^{66}Zn and ^{70}Zn/^{66}Zn ratios were determined by inductively coupled plasma mass spectrometry in the Section of Nutrition Laboratory, Department of Pediatrics, School of Medicine, University of Colorado, Denver, according to the following protocol: 8 mL of a 50-μg Zn/L solution in 2% (vol:vol) nitric acid was prepared from each sample. The solution was introduced into an inductively coupled plasma mass spectrometer (PlasmaQuad 3; VG Elemental) for measurement of zinc stable-isotope ratios (^{67}Zn/^{66}Zn and ^{70}Zn/^{66}Zn). Ratios were converted to percentage enrichment by using a mathematical matrix that takes into account the abundance of each isotope in the doses. The samples were introduced through an auto-sampler (ASX-500, model 510; CETAC) with a peri-pump (Perimax 12; CPETEC). Instrument parameters were as follows: argon gas flow, 13 L/min; intermediate gas flow, 1.40 L/min; nebulizer gas flow, 0.82 L/min; forward power, 1350 W; temperature of pneumatic nebulizer, 4°C; sample flow rate, 1 mL/min. Data acquisition parameters were set as follows: dwell times, 3 min for ^{66}Zn, 4 min for ^{67}Zn, and 5 min for ^{70}Zn; one point per peak, 1800 sweeps; 10 replicates; and 50-ns dead time. A natural zinc standard was run every 6 samples and 2% HNO$_3$ every 12 samples. Counts per second of nitric acid were subtracted from counts per second of all samples. The precision of this method with the use of natural abundance standards is <0.3% relative SD for ^{67}Zn/^{66}Zn and <0.6% relative SD for ^{70}Zn/^{66}Zn.

Compliance

A member of our team (JC) provided a new container with a known number of vitamin and mineral pills at the beginning of every month throughout the study. At the time of distribution, the number of remaining pills from the previous month was counted. Compliance was assessed by comparing the total pills provided and those consumed during any given period.

Statistical analyses

Two-factor repeated-measures ANOVA with treatment group as a between-subjects factor and time as a within-subjects factor, followed by a Bonferroni test for multiple comparisons, regression and correlation analyses, and the Wilcoxon's signed rank test (27) were performed by using SPSS 15.0 statistical software (SPSS Inc). Because zinc absorption data have skewed distributions, the values were converted to logarithms before performing any statistical analysis. The results were then retransformed into antilogarithms to recover the original units and are expressed as geometric means and range of ±1 SE.

RESULTS

Details on compliance and main anthropometric changes are presented elsewhere (9). Briefly, compliance, defined as the number of vitamin and mineral pills consumed related to the total provided was well above 80% (as median) throughout the study. No adverse effects related to the administration of the vitamin and mineral supplements or to the procedures carried out to determine zinc status and zinc absorption were observed. Body weight decreased by 27.9% 6 mo and by 35.2% 18 mo after RYGBP. Fat mass (in kg) decreased by 38.1% and 52.3% at 6 and 18 mo after surgery, respectively. Fat-free mass (in kg) decreased by 17.7% at 6 mo after RYGBP, after which it remained stable.

The mean supplemental zinc intakes during the 0–6-mo period were 6.5 ± 2.7 mg/d for group A and 11.1 ± 3.9 mg/d for group B, during the 6–12-mo period were 6.9 ± 1.8 mg/d for group A and 13.2 ± 2.7 mg/d for group B, and during the 12–18-mo period were 7.0 ± 2.1 mg/d for group A and 12.7 ± 2.8 mg/d for group B. Dietary intakes of energy and protein were described elsewhere (9). Dietary zinc intakes were 9.1 ± 3.5, 5.4 ± 2.0, 6.4 ± 2.0, and 7.2 ± 2.8 mg/d before and 6, 12, and 18 mo after RYGBP, respectively. All postoperative zinc intakes were significantly different from preoperative intakes (Bonferroni test after repeated-measures ANOVA). No effect of supplementation type was observed.

Two-factor repeated-measures ANOVA was conducted to test the effects of time and type of supplementation (standard compared with improved) on a series of indexes of zinc status: plasma zinc, hair zinc, erythrocyte membrane alkaline phosphatase activity, erythrocyte metallothionein, and EZP (**Table 1**). None of the results indicated an effect of supplementation type. Thus, further analyses are presented and discussed considering both supplementation groups combined. Of the 5 indexes of zinc status, 3 (plasma zinc, erythrocyte membrane alkaline phosphatase activity, and EZP; the latter expressed as total mass and by kg fat-free mass) were significantly lower 12 or 18 mo after RYGBP. Erythrocyte metallothionein remained unchanged during the study. Hair zinc, contrary to expectations, was higher 6 and 12 mo, but not 18 mo after surgery.

The percentages of cases below the selected cutoffs are shown in **Table 2**. With the exception of hair zinc, the percentage of cases below the selected cutoffs significantly increased by the end of the experimental period. Percentage zinc absorption was

TABLE 1
Zinc status indexes in obese women before and after Roux-en-Y gastric bypass[1]

Group and time	Plasma zinc	Hair zinc	Erythrocyte membrane alkaline phosphatase	Erythrocyte metallothionein	EZP[2]
	$\mu g/dL$	$\mu g/g$	U/mg protein	nmol/g protein	mg
0 mo					
A	87.2 ± 10.7 (36)	142.0 ± 42.4 (36)	0.31 ± 0.10 (34)	2.24 ± 0.24 (28)	266.1 ± 51.1 (13)
B	87.2 ± 11.7 (31)	148.8 ± 40.7 (28)	0.31 ± 0.09 (31)	2.21 ± 0.19 (27)	247.1 ± 45.5 (13)
Both	87.2 ± 11.1[a] (67)	145.0 ± 41.5[a] (64)	0.31 ± 0.09[a] (65)	2.23 ± 0.22 (55)	256.6 ± 48.4[a] (26)
6 mo					
A	89.1 ± 17.4 (30)	157.1 ± 26.9 (30)	0.31 ± 0.11 (29)	2.30 ± 0.24 (28)	193.9 ± 51.5 (13)
B	92.9 ± 20.8 (28)	167.7 ± 38.8 (26)	0.33 ± 0.12 (27)	2.19 ± 0.23 (27)	220.3 ± 45.6 (13)
Both	90.9 ± 19.0[a] (58)	162.0 ± 33.1[b] (56)	0.32 ± 0.12[a] (56)	2.25 ± 0.24 (55)	207.1 ± 49.5[b] (26)
12 mo					
A	79.4 ± 15.7 (29)	149.8 ± 41.6 (29)	0.30 ± 0.12 (28)	2.28 ± 0.25 (28)	180.6 ± 40.9 (13)
B	81.4 ± 13.8 (27)	174.8 ± 51.7 (25)	0.29 ± 0.12 (33)	2.34 ± 0.23 (27)	183.4 ± 39.3 (12)
Both	80.3 ± 14.7[b] (56)	161.4 ± 47.8[b] (54)	0.30 ± 0.12[a,b] (51)	2.31 ± 0.24 (55)	181.9 ± 39.4[b,c] (25)
18 mo					
A	80.3 ± 14.9 (29)	146.9 ± 37.0 (27)	0.27 ± 0.07 (25)	2.18 ± 0.37 (22)	177.3 ± 37.8 (13)
B	80.5 ± 13.6 (27)	169.5 ± 52.0 (25)	0.25 ± 0.07 (24)	2.30 ± 0.34 (23)	171.4 ± 27.3 (12)
Both	80.4 ± 14.2[b] (56)	157.8 ± 45.8[a,b] (52)	0.26 ± 0.07[b] (49)	2.24 ± 0.36 (45)	174.4 ± 32.6[c] (25)
P	<0.001	0.003	<0.001	0.65	<0.001

[1] All values are means ± SDs; number of cases in parentheses. Repeated-measures ANOVA showed no group effect in any of the variables. P values correspond to the time effect. Values (data from both groups at each evaluation period) in a column with different superscript letters are significantly different (Bonferroni correction for multiple comparisons).

[2] EZP, exchangeable zinc pool.

not different between groups and decreased dramatically from 32.3% to 13.6% by 6 mo after RYGBP. Despite a minor recovery observed by the end of the study, zinc absorption (21.0%) remained significantly affected (**Figure 2**).

DISCUSSION

RYGBP has shown greater effectiveness at long-term maintained weight loss than have other surgical procedures (3). The development of micronutrient deficiencies is commonly reported as a side effect. In a previous study carried out in the same group of patients as in the current study, we noted that more than one-third of the subjects became anemic 18 mo after gastric bypass. Iron deficiency, mediated by a dramatic reduction of iron-absorption capacity, was suggested as the main determinant (9). Information about zinc deficiency after RYGBP is limited. For instance, Madan et al (10) observed that the percentage of "abnormal" plasma zinc concentrations increased from 30% before surgery to 36% 1 y after RYGBP. Plasma and urinary zinc, but not erythrocyte zinc, were lower 6 mo after surgery (28). The percentage of plasma zinc concentrations <75 $\mu g/dL$ increased

from 14% before to 37% 12–40 mo after surgery (29). A retrospective study showed that supplementation with 8 mg Zn/d was insufficient to prevent plasma zinc reductions (30). Plasma zinc concentrations <70 $\mu g/dL$ increased from 8.1% before surgery to 34.8% 24 mo after RYGBP, despite supplementation of patients with an average of 20–23 mg Zn/d (11). Thus, the available data, although limited, suggest that zinc deficiency may also be a prevalent condition in patients undergoing this type of bariatric surgery.

The assessment of zinc status remains a difficult task because, despite the large number of indexes proposed, all have problems that affect validity (31, 32). Zinc presents very effective homeostatic mechanisms that respond to zinc intake modifications; in addition, there are no zinc stores (33). Lowe et al (32) carried out a systematic analysis of 32 methods to assess zinc status in humans. Their main conclusions were that plasma zinc may be a useful indicator, despite many limitations, and that kinetic measures (eg, EZP, zinc absorption, and endogenous zinc measured with the use of stable isotopes) have potential as biomarkers. In our study we used a battery of indexes, ie, plasma zinc, hair zinc, erythrocyte membrane alkaline phosphatase

TABLE 2
Subjects with zinc status indexes below selected cutoffs before and after Roux-en-Y gastric bypass

Month	Plasma zinc <70 $\mu g/dL$	Hair zinc <100 $\mu g/g$	Erythrocyte membrane alkaline phosphatase <0.2 U/mg protein	Erythrocyte metallothionein <1.85 nmol/g protein	EZP[1] <155 mg
	n/total n (%)	n/total n (%)	n/total n (%)	n/total n (%)	n/total n (%)
0	3/67 (4.5)	6/64 (9.4)	5/65 (7.7)	2/55 (3.6)	1/26 (3.8)
6	5/58 (8.6)	2/56 (3.6)	7/56 (12.5)	3/55 (5.5)	5/26 (19.2)
12	12/56 (21.4)[2]	3/54 (5.6)	11/51 (21.6)[2]	1/55 (1.8)	4/25 (16.0)
18	12/56 (21.4)[2]	2/52 (3.8)	11/49 (22.4)[2]	9/45 (20.0)[2]	6/25 (24.0)[2]

[1] EZP, exchangeable zinc pool.

[2] Significantly different from month 0, $P < 0.05$ (Wilcoxon's signed rank test).
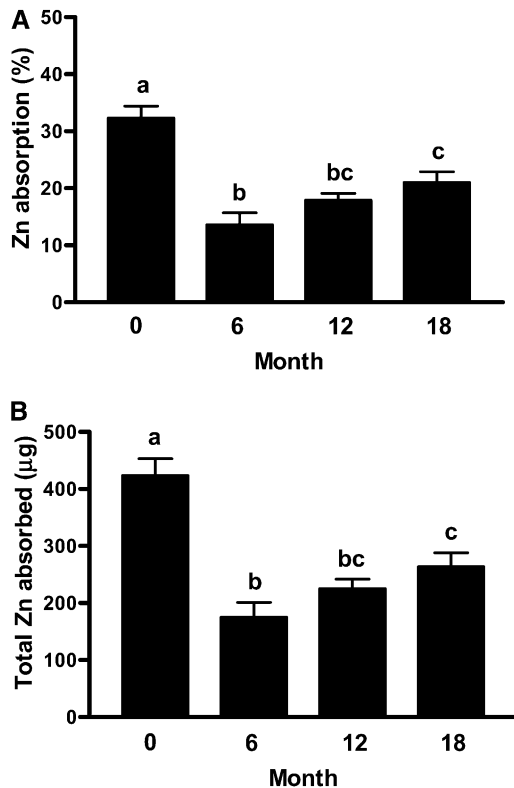
FIGURE 2. Geometric mean (±1 SE) zinc absorption from a standard test meal before and 6, 12, and 18 mo after Roux-en Y gastric bypass (0 mo, $n = 26$; 6 mo, $n = 26$; 12 mo, $n = 25$; 18 mo, $n = 25$). Zinc absorption is expressed as a percentage of the dose (A) and as the total absorbed (zinc from dose plus zinc in test meal). Bars with different lowercase letters were significantly different, $P < 0.05$ (Bonferroni correction for multiple comparisons).

activity, erythrocyte metallothionein, and size of the EZP. Despite the host of factors that can affect plasma zinc concentrations (31, 32), a consistent and significant decreasing trend was observed in our subjects. Similarly, erythrocyte membrane alkaline phosphatase activity, which had responded accordingly in a previous zinc depletion-repletion study (19), also decreased after surgery. EZP represents the mass of zinc that interchanges with plasma in a relatively short period of time (34). EZP was reduced in cases of severe acute zinc deficiency (35) but not in cases of moderate zinc deficiency (36). EZP at birth was smaller in small-for-gestational-age than in appropriate-for-gestational-age preterm infants (37). EZP increased after 6 mo of supplementation with 15 and 30 mg Zn/d in adult men (38). In our study, EZP values expressed both as total mass and relative to kg fat-free mass (data not shown) were consistently decreased after RYGBP. In contrast, erythrocyte metallothionein, which had been shown to respond to dietary zinc variations in previous studies, was almost unaffected in ours (39, 40). Hair zinc showed a trend contrary to expectations. Hair zinc is not considered to be a very sensitive index of zinc status (31, 32). Besides, despite care being taken to avoid zinc contamination from shampoo, it could not be completely ruled out. The results of zinc indexes expressed by the proportion of values below selected cutoffs showed that, with the exception of hair zinc, percentages increased during the study up to 20–24%. We interpreted our results to indicate that subjects had (or were at risk of having) a mild zinc deficiency after RYGBP. Additional

research is needed to determine the potential functional effects of this finding. Mild zinc deficiency in adults has been associated with alterations in immune function (41), dermic lesions (42), and cognitive function (43). Presumably, without at least the level of supplementation used in this study, the patients would have been at risk of even further compromising their zinc status. When this study was designed, no evidence indicated the need for preventive zinc supplementation greater than 1–2 times the RDA. One group, the standard vitamin and mineral supplementation group, received the routine supplementation being used by the bariatric surgery team from the University Clinical Hospital at that time; this value was approximately equivalent to the RDA for zinc, the "improved vitamin and mineral supplementation" doubled it. These differences in the supplementation level were not accompanied by changes in the indexes of zinc status. Thus, data suggest justification for routine supplementation, but at a higher dose, probably in the range of 40 to 60 mg/d.

Three major factors can determine zinc status in humans: reduced zinc intake, reduced zinc absorption, and increased intestinal losses. Gastric capacity is dramatically reduced in RYGBP; as a consequence, dietary nutrient intake is also reduced. In our study, total dietary zinc was significantly reduced after surgery. In addition to a decrease in total zinc intake, zinc intake from red meat—a highly available zinc source—also decreased. The magnitude of the reduction of red meat in our patients was nearly 50% after surgery (9). Low tolerance to read meat is frequently observed after gastric bypass (5). In addition to dietary zinc, the patients received an average of ~9.5 mg Zn/d as supplements during the entire experimental period.

Zinc absorption takes place mainly in the duodenum and initial portions of the jejunum (44). These portions of the intestine are bypassed during RYGBP. The main consequence is a substantial reduction in intestinal absorption surface and, in turn, less absorption capacity. To the best of our knowledge, no previous studies have evaluated zinc absorption after RYGBP. We assessed zinc absorption using a widely accepted method involving stable isotopes (45). In our study we found a significant reduction in zinc absorption from a standard diet. The level of zinc supplementation did not have a differential effect on the magnitude of absorption. Zinc absorption is a complex process and involves the participation of many transporters from both the ZIP (solute-linked carrier SLC39) and the ZnT (solute-linked carrier SLC30) families (46–48), the most prominent of which are ZIP4 and ZnT1. ZIP4 is the primary regulator of zinc uptake at the intestine, and ZnT1 is crucial to the zinc efflux from the enterocyte (46–49). Zinc transporter expression is regulated by cytokines, hormones, and zinc itself (46). Changes in the expression of zinc transporters have important effects on zinc homeostasis. For instance, in ileostomy patients, supplementation with 25 mg Zn/d for 14 d resulted in decreased ZnT1, ZnT5, and ZIP4 proteins in the intestinal mucosa (47). This may explain, at least in part, why we did not observe differences in zinc absorption between our 2 groups.

Zinc is mainly excreted by the intestine through intestinal and pancreatic secretions (33). This component is vital to maintain zinc homeostasis (50). Whereas endogenous zinc losses increase with diarrhea (51), they are rapidly reduced when facing dietary zinc restrictions (33, 52). We did not determine intestinal zinc losses in our subjects. Because we did not observe major changes in transit time, zinc losses presumably did not increase. In addition,

EZP has been shown to be directly related to endogenous fecal zinc, among other variables (53). Thus, far from being increased, endogenous zinc lost by the intestine probably decreased.

In conclusion, we found that zinc deficiency occurred in many patients after RYGBP. According to our data, average supplemental zinc of ∼9.5 mg/d would not be enough to prevent an impairment of zinc status. Zinc absorption capacity is markedly reduced 6 mo after RYGBP, and this impairment persists at least until 18 mo after surgery.

REFERENCES

1. Buchwald H, Avidor Y, Braunwald E, Jensen MD, Pories W, Fahrbach K, Schoelles K. Bariatric surgery: a systematic review and meta-analysis. JAMA 2004;292:1724–37.
2. Maggard MA, Shugarman LR, Suttorp M, Maglione M, Sugerman HJ, Livingston EH, Nguyen NT, Li Z, Mojica WA, Hilton L, et al. Meta-analysis: surgical treatment of obesity. Ann Intern Med 2005;142:547–59.
3. Sjöström L, Lindroos AK, Peltonen M, Torgerson J, Bouchard C, Carlsson B, Dahlgren S, Larsson B, Narbro K, Sjöström CD, et al. Swedish Obese Subjects Study Scientific Group. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. N Engl J Med 2004;351:2683–93.
4. Csendes A, Burdiles P, Papapietro K, Diaz JC, Maluenda F, Burgos A, Rojas J. Results of gastric bypass plus resection of the distal excluded gastric segment in patients with morbid obesity. J Gastrointest Surg 2005;9:121–31.
5. Kushner RF. Micronutrient deficiencies and bariatric surgery. Curr Opin Endocrinol Diabetes 2006;13:405–11.
6. Parkes E. Nutritional management of patients after bariatric surgery. Am J Med Sci 2006;331:207–13.
7. Skroubis G, Sakellaropoulos G, Pouggouras K, Mead N, Nikiforidis G, Kalfarentzos F. Comparison of nutritional deficiencies after Roux en-Y gastric bypass and after biliopancreatic diversion with Roux-en-Y gastric bypass. Obes Surg 2002;12:551–8.
8. Poitou Bernert C, Ciangura C, Coupaye M, Czernichow S, Bouillot JL, Basdevant A. Nutritional deficiency after gastric bypass: diagnosis, prevention and treatment. Diabetes Metab 2007;33:13–24.
9. Ruz M, Carrasco F, Rojas P, Codoceo J, Inostroza J, Rebolledo A, Basfi-fer K, Csendes A, Papapietro K, Pizarro F, et al. Iron absorption and iron status are reduced after Roux-en-Y gastric bypass. Am J Clin Nutr 2009;90:527–32.
10. Madan AK, Orth WS, Tichansky DS, Ternovits CA. Vitamin and trace mineral levels after laparoscopic gastric bypass. Obes Surg 2006;16:603–6.
11. Sallé A, Demarsy D, Poirier AL, Lelièvre B, Topart P, Guilloteau G, Bècouarn G, Rohmer V. Zinc deficiency: a frequent and underestimated complication after bariatric surgery. Obes Surg 2010;20:1660–70.
12. Food and Nutrition Board, Institute of Medicine. Dietary references for calcium, phosphorus, magnesium, vitamin D and fluoride. Washington, DC: National Academy Press, 1977.
13. Food and Nutrition Board, Institute of Medicine. Dietary references for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington, DC: National Academy Press, 2001.
14. Carrasco F, Klaassen J, Papapietro K, Reyes E, Rodríguez L, Csendes A, Guzmán S, Hernández F, Pizarro T, Sepúlveda A. Propuesta y fundamentos para una norma de manejo quirúrgico del paciente obeso. (A proposal of guidelines for surgical management of obesity.) Rev Med Chil 2005;133:699–706 (in Spanish).
15. Gibson. RS. Principles of nutritional assessment. 2nd ed. New York, NY: Oxford University Press, 2005.
16. Schmidt-Hebbel H, Pennacchiotti I. Tabla de composición química de alimentos Chilenos. Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. (Food composition table of Chilean foods.) Santiago, Chile: Faculty of Chemical and Pharmaceutical Sciences, University of Chile, 1985 (in Spanish).
17. Smith JC Jr, Butrimovitz GP, Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectroscopy. Clin Chem 1979;25:1487–91.
18. Steck TL, Weinstein RS, Straus JH, Wallach DFH. Inside-out red cell membrane vesicles: preparation and purification. Science 1970;168:255–7.
19. Ruz M, Cavan KR, Bettger WJ, Gibson RS. Erythrocytes, erythrocyte membranes, neutrophils, and platelets as biopsy materials for the assessment of zinc status in humans. Br J Nutr 1992;68:515–27.
20. Markwell MA, Hass SM, Bieber LL, Tolbert NE. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 1978;87:206–10.
21. Eaton DL, Toal BF. Evaluation of the Cd/hemoglobin affinity assay for the rapid determination of metallothionein in biological tissues. Toxicol Appl Pharmacol 1982;66:134–42.
22. International Zinc Nutrition Consultative Group (IZiNCG), Brown KH, Rivera JA, Bhutta Z, Gibson RS, King JC, Lönnerdal B, Ruel MT, Sandtröm B, Wasantwisut E, Hotz C. International Zinc Nutrition Consultative Group (IZiNCG) Technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 2004;25(suppl 2):S99–S203.
23. Takyi EEK. Hair zinc status and its correlation with height indicator in pre-school and school children from a mixed income, low density (mild) community in southern Ghana. East Afr Med J 2004;81:42–6.
24. Friel JK, Naake VL, Miller JV, Fennessey PV, Hambidge KM. The analysis of stable isotopes in urine to determine the fractional absorption of zinc. Am J Clin Nutr 1992;55:473–7.
25. Peirce PL, Hambidge KM, Goss CH, Miller LV, Fennessey PV. Fast atom bombardment mass spectrometry for the determination of zinc stable isotopes in biological samples. Anal Chem 1987;59:2034–7.
26. Veillon C, Patterson KY, Moser-Veillon PB. Digestion and extraction of biological materials for zinc stable isotope determinations by inductively coupled plasma mass spectrometry. J Anal Atom Spectrom 1996;11:727–30.
27. Snedecor GW, Cochran WG. Statistical methods. 8th ed. Ames, IA: The Iowa State University Press, 1989.
28. Pires LV, Martins LM, Geloneze B, Tambascia MA, Hadad do Monte SJ, do Nascimento Nogueira N, Santos de Sousa G, Marreiro DN. The effect of Roux-en-Y-gastric bypass on zinc nutritional status. Obes Surg 2007;17:617–21.
29. Gehrer S, Kern B, Peters T, Christoffel-Courtin C, Peterli R. Fewer nutrient deficiencies after laparoscopic sleeve gastrectomy (LSG) than after laparoscopic Roux-en-Y gastric bypass (LRYGB)—a prospective study. Obes Surg 2010;20:447–53.
30. Gasteyger C, Suter M, Gaillard RC, Giusti V. Nutritional deficiencies after Roux-en-Y gastric bypass for morbid obesity often cannot be prevented by standard multivitamin supplementation. Am J Clin Nutr 2008;87:1128–33.
31. Ruz M, Cavan KR, Bettger WJ, Thompson L, Berry M, Gibson RS. Development of a dietary model for the study of mild zinc deficiency in humans and evaluation of some biochemical and functional indices of zinc status. Am J Clin Nutr 1991;53:1295–303.
32. Lowe NM, Fekete K, Decsi T. Methods of assessment of zinc status in humans: a systematic review. Am J Clin Nutr 2009;89(suppl):2040S–51S.
33. Hambidge KM, Miller LV, Westcott JE, Sheng X, Krebs NF. Zinc bioavailability and homeostasis. Am J Clin Nutr 2010;91(suppl):1478S–83S.
34. Miller LV, Hambidge KM, Naake VL, Hong Z, Westcott JL, Fennessey PV. Size of the zinc pools that exchange rapidly with plasma zinc in humans: alternative techniques for measuring and relation to dietary zinc intake. J Nutr 1994;124:268–76.

35. Lowe NM, Woodhouse LR, Sutherland B, Shames DM, Burri BJ, Abrams SA, Turnlund JR, Jackson MJ, King JC. Kinetic parameters and plasma zinc concentration correlate well with net loss and gain of zinc from men. J Nutr 2004;134:2178–81.

36. Pinna K, Woodhouse LR, Sutherland B, Shames DM, King JC. Exchangeable zinc pool masses and turnover are maintained in healthy men with low zinc intakes. J Nutr 2001;131:2288–94.

37. Krebs NF, Westcott JL, Rodden DJ, Ferguson KW, Miller LV, Hambidge KM. Exchangeable zinc pool size at birth is smaller in small-for-gestational-age than in appropriate-for-gestational-age preterm infants. Am J Clin Nutr 2006;84:1340–3.

38. Feillet-Coudray C, Meunier N, Rambeau M, Brandolini-Bunlon M, Tressol JC, Andriollo M, Mazur A, Cashman KD, Coudray C. Long-term moderate zinc supplementation increases exchangeable zinc pool masses in late-middle-aged men: the Zenith study. Am J Clin Nutr 2005;82:103–10.

39. Grider A, Bailey EB, Cousins RJ. Erythrocyte metallothionein as an index of zinc status in humans. Proc Natl Acad Sci USA 1990;87:1259–62.

40. Thomas EA, Bailey EB, Kauwell GA, Lee DY, Cousins RJ. Erythrocyte metallothionein response to dietary zinc in humans. J Nutr 1992;122:2408–14.

41. Prasad AS. Zinc: role in immunity, oxidative stress and chronic inflammation. Curr Opin Clin Nutr Metab Care 2009;12:646–52.

42. Haase H, Overbeck S, Rink L. Zinc supplementation for the treatment or prevention of disease: current status and future perspectives. Exp Gerontol 2008;43:394–408.

43. Tupe RP, Chiplonkar SA. Zinc supplementation improved cognitive performance and taste acuity in Indian adolescent girls. J Am Coll Nutr 2009;28:388–96.

44. Krebs NF, Hambidge KM. Zinc metabolism and homeostasis: the application of tracer techniques to human zinc physiology. Biometals 2001;14:397–412.

45. Hambidge KM, Abebe Y, Gibson RS, Westcott JE, Miller LV, Lei S, Stoecker BJ, Arbide I, Teshome A, Bailey KB, et al. Zinc absorption during late pregnancy in rural southern Ethiopia. Am J Clin Nutr 2006;84:1102–6.

46. Cousins RJ, Liuzzi JP, Lichten LA. Mammalian zinc transport, trafficking, and signals. J Biol Chem 2006;281:24085–9.

47. Cragg RA, Phillips SR, Piper JM, Varma JS, Campbell FC, Mathers JC, Ford D. Homeostatic regulation of zinc transporters in the human small intestine by dietary zinc supplementation. Gut 2005;54:469–78.

48. Lichten LA, Cousins RJ. Mammalian zinc transporters: nutritional and physiologic regulation. Annu Rev Nutr 2009;29:153–76.

49. Wang K, Zhou B, Kuo YM, Zemansky J, Gitschier J. A novel member of a zinc transporter family is defective in acrodermatitis enteropathica. Am J Hum Genet 2002;71:66–73.

50. Sian L, Mingyan X, Miller LV, Tong L, Krebs NF, Hambidge KM. Zinc absorption and intestinal losses of endogenous zinc in young Chinese women with marginal zinc intakes. Am J Clin Nutr 1996;63:348–53.

51. Ruz M, Solomons NW. Fecal excretion of endogenous zinc during oral rehydration therapy: nutritional implications. J Trace Elem Exp Med 1995;7:89–100.

52. King JC, Cousins RJ. Zinc. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ, eds. Modern nutrition in health and disease. 10th ed. Philadelphia, PA: Lippincott Williams&Wilkins, 2006:271–85.

53. Krebs NF, Hambidge KM, Westcott JE, Miller LV, Sian L, Bell M, Grunwald G. Exchangeable zinc pool size in infants is related to key variables of zinc homeostasis. J Nutr 2003;133:1498S–501S.