

# Effect of Diet Intervention and Oral Zinc Supplementation on Metabolic Control in Berardinelli-Seip Syndrome

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## Key Words

Diet intervention · Food intake · Zinc supplementation, oral · Venous zinc tolerance test · Berardinelli-Seip syndrome

## Abstract

**Background/Aims:** Berardinelli-Seip syndrome (BSS), also termed congenital generalized lipodystrophy or congenital generalized lipoatropic diabetes, is a rare autosomal recessive disease characterized by the nearly complete absence of metabolically active adipose tissue from birth, extreme insulin resistance, diabetes mellitus, and hepatomegaly. The aim of this study was to evaluate the effect of diet intervention and oral zinc supplementation on the metabolic control of BSS patients. **Methods:** During a 3-month period, 10 BSS patients received individualized diets and oral zinc supplementation. Food intake, clinical laboratory parameters, serum zinc and leptin, and plasma C-peptide concentrations were evaluated at the beginning of the study and after 3 months. **Results:** At the beginning of the study, all patients had elevated energy, protein, total fat, carbohydrate, calcium, iron, and zinc intakes. After 3 months, all of these parameters had decreased. Total fiber intakes remained low before

and after diet intervention and oral zinc supplementation, and plasma levels of fasting glucose remained high. In contrast, glycated hemoglobin decreased significantly. Plasma leptin, C-peptide, and serum zinc levels increased during venous zinc tolerance testing, but there were no significant differences between the 2 curves obtained before and after diet intervention and oral zinc supplementation. **Conclusions:** Diet intervention and oral zinc supplementation were effective at controlling energy consumption, macronutrients, and glycated hemoglobin. Zinc likely acts as an adjunct therapy, thereby improving the effectiveness of leptin.

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## Introduction

Berardinelli-Seip syndrome (BSS) is an extremely rare disorder reported initially by Berardinelli [1] and Seip [2]. Affected patients have a nearly complete absence of metabolically active adipose tissue from birth [3]; nearly 300 cases have been described in the literature [4].

Owing to the absence of functional adipocytes, dietary and endogenously synthesized lipids are stored ab-

errantly in metabolically important tissues such as muscles and the liver. This leads to severe insulin resistance and ultimately diabetes mellitus, which is often difficult to control [5]. Even during infancy, BSS patients show extreme hyperinsulinemia (indicative of insulin resistance), hypertriglyceridemia, and hepatomegaly due to hepatic steatosis. Affected children exhibit acanthosis nigricans, acromegaloid appearance (enlarged hands and feet and prominent mandible), striking muscular appearance, accelerated growth, voracious appetite, increased basal energy expenditure, advanced bone age, as well as markedly low serum leptin and adiponectin levels. Diabetes mellitus develops mostly during pubertal years and is ketosis resistant [6].

The genetic basis of BSS, which has an autosomal recessive inheritance, is still not completely understood [7]. Two loci (*BSCL1* and *BSCL2*) linked to BSS have been mapped on chromosomes 9q34 and 11q13, respectively. Positional cloning of *BSCL1* revealed mutations in *AGPAT2*, which encodes the 1-acylglycerol-3-phosphate O-acyltransferase 2. This 278-amino-acid protein belongs to the family of acyltransferases and catalyzes the acylation of lysophosphatidic acid to form phosphatidic acid, a key intermediate in the biosynthesis of triacylglycerol and glycerophospholipids. Positional cloning of *BSCL2* showed mutations in *Gng3lg* (also called *seipin*), which is homologous to the murine guanine nucleotide-binding protein  $\gamma$ 3-subunit-linked gene, a gene of unknown function [8].

Metabolic abnormalities in patients with BSS develop as a consequence of the mass reduction of adipose tissue and are mainly due to a lack of leptin. Leptin is an adipocyte-derived satiety hormone that plays an important role in regulating glucose and lipid metabolism [9] and acts centrally to reduce food intake and increase energy expenditure [10, 11]. In addition, insulin acts as a major factor in stimulating the synthesis and secretion of leptin [10, 11].

The treatment of lipodystrophies remains challenging. Reduced energy intake and increased physical activity are beneficial in the management of metabolic complications in all patients with lipodystrophies [12].

As all patients are insulin resistant and many have type II diabetes mellitus, we recommended that BSS patients follow a diet based on Nutrition Recommendations and Interventions for Diabetes Mellitus. The goals of nutritional intervention are to attain and maintain optimal metabolic control, prevent and treat the chronic complications of diabetes mellitus, and improve overall health through proper food choices and optimal nutrition [13].

Zinc is an essential micronutrient involved in carbohydrate metabolism. Patients with diabetic complications had significantly lower serum zinc levels than patients without diabetic complications [14]. Zinc plays a key role in the synthesis, storage, and secretion of insulin as well as in the conformational integrity of insulin in the hexameric form [15]. Zinc also plays an important role in appetite regulation, as it alters hypothalamic neurotransmitter metabolism by influencing the leptin system [16, 17].

There are relatively few studies concerning the diet of BSS patients and no information is available on the effects of zinc on this syndrome. This study contributes to the understanding of the interaction between diet, zinc, and nutrition. Accordingly, the aim of this study was to evaluate the usefulness of diet intervention and oral zinc supplementation for the metabolic control of BSS patients with respect to mean energy consumption, specifically the dietary intake of macronutrients, total fiber, calcium, iron, and zinc, as well as how they affected certain measures of overall health: fasting glucose, glycated hemoglobin (HbA1c), total cholesterol, triglycerides, leptin, and C-peptide.

### Patients, Materials, and Methods

Due to its small sample size, this study was not a randomized double-blind trial. The fact that no control group was included may be a limitation of this study. The study group comprised 10 BSS patients treated at the Multidisciplinary Laboratory of Chronic Degenerative Diseases [PPGCSA, Universidade Federal do Rio Grande do Norte (UFRN)]. All of the patients had a generalized form of lipodystrophy, with a near total absence of adipose tissue from birth and muscular hypertrophy. The patients had 2 gene mutations, *AGPAT2* and *Gng3lg* [8]. Two patients took insulin (NPH), 2 took insulin plus metformin, 3 took metformin, and 3 took no medicine. All affected individuals were from the Seridó region, a county in the state of Rio Grande do Norte in northeastern Brazil. There are 32 BSS patients in the state of Rio Grande do Norte. The Seridó region has the highest incidence of this syndrome in the world. The protocol, which was approved by the Medical Ethics Committee of the Universidade Federal do Rio Grande do Norte (No. 006/06), was explained to the volunteers, and written consent was obtained from all the participants. There were several exclusion criteria: age less than 14 years; acute, chronic, infectious, or inflammatory diseases; having undergone surgery or currently taking vitamin-mineral supplements, and not agreeing to take part in the study.

#### *Dietary Protocols and Dietary Intake*

This was a 3-month intervention study. The estimated energy requirement followed Dietary Reference Intake (DRI) recommendations for energy [18]. Macronutrient intake was individualized, and the macronutrient composition of the diet, including fiber, was based on the guidelines developed by the American Di-

abetes Association, with 55% of total energy from carbohydrates, 30% from fat, and 15% from protein. Individuals were encouraged to eat foods containing whole grains and low-glycemic-index foods, and to achieve the fiber intake goal of 14 g/1,000 kcal set for the general population [19–22]. Calcium [23], iron, and zinc [24] requirements were based on DRIs.

Information on food consumption was obtained at the beginning of the study and after 3 months, using food records from 3 nonconsecutive days (2 weekdays and 1 weekend day). The patients were instructed on how to properly record their food intake; they recorded the time of each meal, the foods consumed and their respective amounts on the appropriate form. They were asked about fruit and vegetable size (small, medium, and large); spoon and/or plate size (small, medium, and large); glass or cup size and the amount of liquid contained in them.

Energy, protein, total fat, carbohydrates, total fiber, calcium, iron, and zinc dietary intakes were calculated using the nutritional evaluation computer program (NutWin 1.5 software) developed by the Department of Information Technology in Health (Universidade Federal de São Paulo, Brazil), which takes into account the composition of Brazilian foods. Foods not included in the program were inserted based on food chemical composition tables [25, 26] or on nutritional information contained on the labels.

#### *Oral Zinc Supplementation*

Patients received 25 mg/day of elemental zinc orally in the form of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (Merck, Darmstadt, Germany). The syrup was prepared at the Pharmacotechnical Laboratory of the Department of Pharmacy, UFRN. Each drop contained 1 mg of elemental zinc [27]. All of the patients were periodically checked to determine if they were faithfully taking the zinc supplement and keeping food records. Biweekly home visits were conducted to determine if the patients were taking their daily supplementation. They were instructed to consume the zinc supplementation at breakfast.

#### *Venous Zinc Tolerance Test*

The BSS patients rested in supine position throughout the test. An antecubital vein on each forearm was punctured and maintained with physiological saline. A dose of 25 mg elemental zinc (2 ml as heptahydrated zinc sulfate) was injected over the course of 1 min at time 0 min. Blood samples were collected from the contralateral arm at 0, 30, 60, 90, and 120 min [28]. This test was applied because it provides very useful information about the acute effect of zinc on kinetics and hormone secretion [14, 15, 29, 30].

#### *Laboratory Analysis*

The following clinical laboratory parameters were also evaluated at the beginning of the study and after 3 months using standard clinical laboratory methods: fasting glucose, HbA1c (RA-50 Chemistry Analyzer, Bayer Diagnostics Mfg. Ltd., Dublin, Ireland), total cholesterol, and triglycerides (Konelab 60i, Vantaa, Finland). The analyses were performed by the Multidisciplinary Laboratory of Chronic Degenerative Diseases (UFRN). Leptin was measured by fluoroimmunoassay (AutoDELFIA-1235 automatic immunoassay, Perkin Elmer, Mass., USA), and C-peptide was measured using chemiluminescence (IMMULITE, DPC, Los Angeles, Calif., USA).

#### *Statistical Analyses*

Descriptive statistics are presented as mean values and standard error of the mean (mean  $\pm$  SEM). The paired t test was used for statistical assessments. All tests were nondirectional (2-tailed probability) and a level of  $p < 0.05$  was used as the cutoff point for statistical significance. The Graph Pad-Prism statistical package (version 5, Graph-Pad Software, Inc., Calif., USA) was used for statistical analysis of the data.

## **Results**

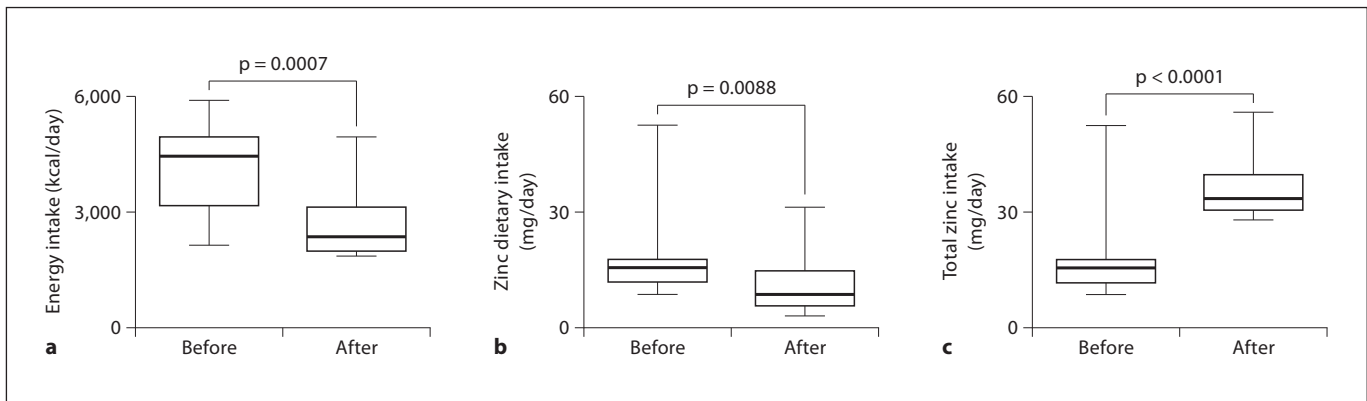
### *Patients*

The sample group of BSS patients comprised 6 women and 4 men aged  $23 \pm 7.9$  years. In this study, body weight loss was not detected before ( $62.66 \pm 2.34$  kg) or after ( $62.41 \pm 2.03$  kg) diet intervention and oral zinc supplementation ( $p = 0.5696$ ). There were no changes in BMI before ( $21.85 \pm 0.53$ ) or after ( $21.78 \pm 0.43$ ) diet intervention and oral zinc supplementation ( $p = 0.6783$ ). No changes in basal metabolic rate before ( $1,577 \pm 52.54$  kcal/day) or after ( $1,583 \pm 50.94$  kcal/day) diet intervention and oral zinc supplementation ( $p = 0.3320$ ) were observed.

### *Dietary Protocols and Dietary Intake*

The median energy intakes are shown in figure 1a. There was a decrease in energy intake after diet intervention and oral zinc supplementation. Protein, total fat, and carbohydrate intakes also decreased after diet intervention and oral zinc supplementation (table 1). However, there were no differences in percentage distribution of macronutrients in the diet consumed before and after oral zinc supplementation (table 1).

There were no significant alterations in total fiber intake, which remained below American Diabetes Association recommendations (14 g/1,000 kcal or 36 g/day). At the beginning of the study, fiber intake was  $22.73 \pm 4.33$  g/day, and decreased slightly (though not significantly) over the 3-month period to  $18.48 \pm 4.37$  g/day ( $p = 0.1545$ ). Compared to diets at the beginning of the study ( $1,637 \pm 280.20$  mg/day), calcium intake decreased after diet intervention and oral zinc supplementation ( $852.7 \pm 103.30$  mg/day) ( $p = 0.0219$ ); this value decreased below DRI recommendations (1,000 mg/day). Iron intake decreased from  $31.95 \pm 4.02$  to  $22.03 \pm 3.14$  mg/day by the end of the study ( $p = 0.0058$ ); this value remained above DRI recommendations (8 mg/day for males and 18 mg/day for females). Zinc dietary intake (obtained from the food records) decreased from  $18.41 \pm 3.92$  to  $11.30 \pm 2.69$  mg/day (fig. 1b). Even so, the median value remained



**Fig. 1.** Values of energy intake (a), zinc dietary intake (b), and total zinc intake (c) before and after 3 months of diet intervention and oral zinc supplementation in 10 patients with BSS. Energy intake and zinc dietary intake decreased significantly after diet intervention and oral zinc supplementation. Conversely, total zinc

intake increased significantly after diet intervention and oral zinc supplementation in the same BSS patients. All values are expressed as minimum, median, and maximum.  $p < 0.05$  was considered significant.

**Table 1.** Recommendations and actual intake of energy, protein, total fat, and carbohydrates, before and after 3 months of diet intervention and oral zinc supplementation in 10 patients with BSS

	DRI recommendations	Before (mean $\pm$ SEM)	After (mean $\pm$ SEM)	p value
Energy intake, kcal/day	2,605	4,138 $\pm$ 363	2,663 $\pm$ 302	0.0007
Protein (% of total energy), g/day	98 (15)	190.4 $\pm$ 24.5 (18)	123.5 $\pm$ 19.2 (18)	0.0340
Total fat (% of total energy), g/day	87 (30)	101.3 $\pm$ 11.4 (22)	64.1 $\pm$ 11.9 (21)	0.0005
Carbohydrates (% of total energy), g/day	358 (55)	624.1 $\pm$ 53.6 (60)	407.4 $\pm$ 38.0 (61)	0.0014

All of these parameters decreased significantly after diet intervention and oral zinc supplementation. Actual intake values are expressed as means  $\pm$  SEM.  $p < 0.05$  was considered significant.

close to DRI recommendations (11 mg/day for males and 8 mg/day for females). However, oral zinc supplementation increased total zinc intake significantly from 18.41  $\pm$  3.92 to 36.30  $\pm$  2.69 mg/day (fig. 1c).

#### Oral Zinc Supplementation

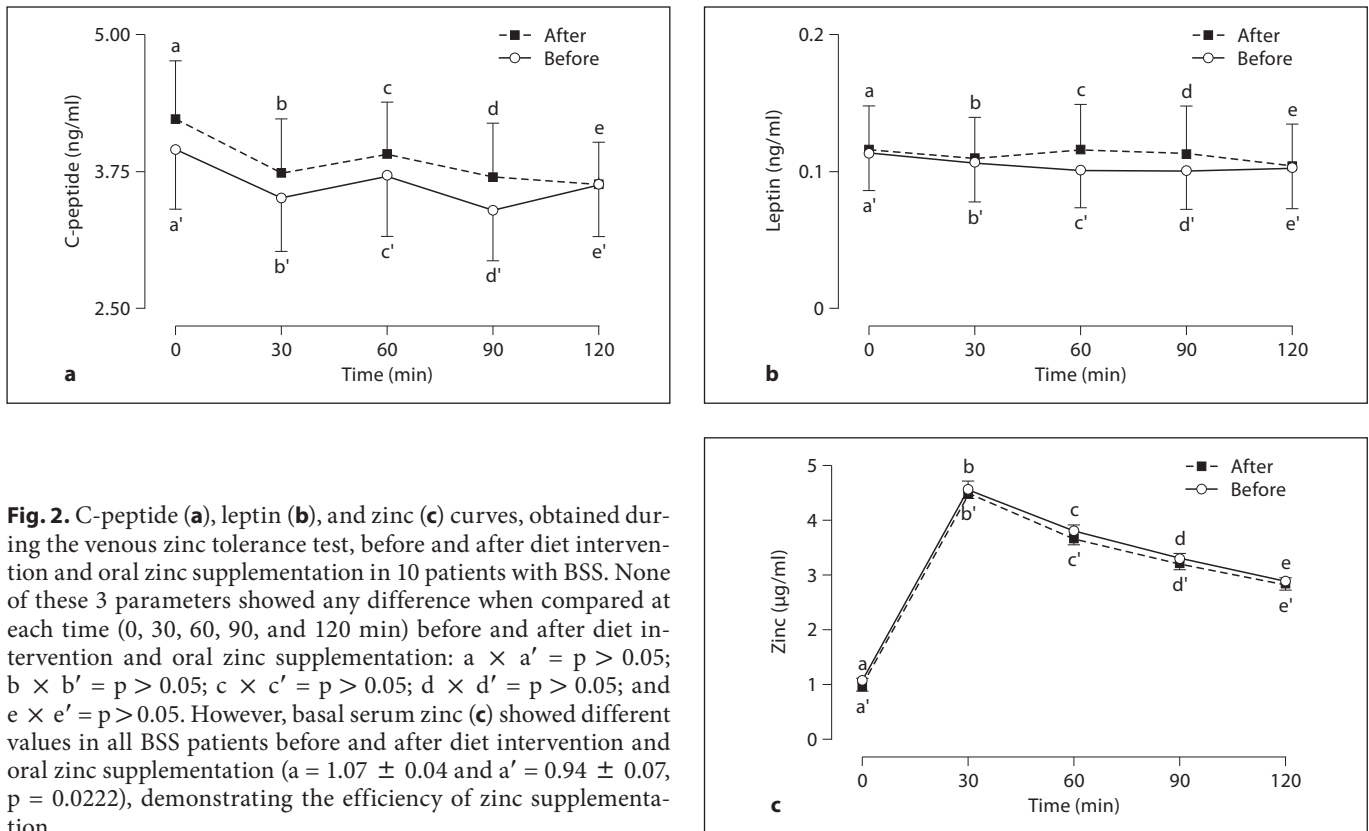
All BSS patients used the zinc drops faithfully throughout the study. No side effects were reported after their administration.

#### Venous Zinc Tolerance Test

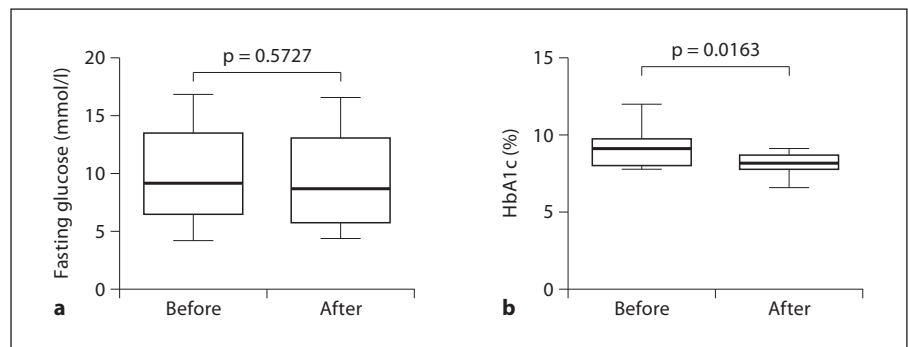
Serum zinc increased during the venous zinc tolerance test. However, the curves are similar, without any significant difference in the patients (fig. 2c). The normal zinc reference range was 0.7–1.2  $\mu$ g/ml. No side effects were observed during this test.

#### Laboratory Analysis

Fasting glucose remained elevated and unchanged between the beginning (9.75  $\pm$  1.29 mmol/l) and the end (9.39  $\pm$  1.29 mmol/l) of the study (fig. 3a) (reference range 3.9–5.6 mmol/l). However, HbA1c decreased significantly over the 3-month period from 9.20  $\pm$  0.42 to 8.16  $\pm$  0.22% (fig. 3b) (reference range 5.3–8%). Total cholesterol levels were in the normal range at the beginning and end of the study (4.63  $\pm$  0.38 and 4.53  $\pm$  0.25 mmol/l, respectively;  $p = 0.6193$ ) (reference range 1.3–5.2 mmol/l). Triglycerides remained elevated (3.62  $\pm$  0.65 and 3.56  $\pm$  0.61 mmol/l,  $p = 0.8197$ ) (reference range  $\leq$  1.7 mmol/l). Plasma C-peptide levels increased during the venous zinc tolerance test, although not significantly (fig. 2a) (reference range 1.1–5 ng/ml). The same was observed with leptin (fig. 2b) [reference ranges: adolescent



**Fig. 3.** Values of fasting glucose (a) and HbA1c (b) before and after 3 months of diet intervention and oral zinc supplementation in 10 patients with BSS. Fasting glucose remained increased, while HbA1c decreased after diet intervention and oral zinc supplementation. All values are expressed as minimum, median, and maximum.  $p < 0.05$  was considered significant.



females = 1–24 ng/ml (BMI 18–23); adolescent males = 0.2–14 ng/ml (BMI 18–23); adults with BMI 20–25 = 0.4–14 ng/ml (males) and 1.5–59 ng/ml (females)].

Hepatic function was evaluated by monitoring alanine aminotransferase, aspartate aminotransferase,  $\gamma$ -glutamyl transferase, total bilirubin, alkaline phosphatase, and total protein. All values remained within the normal reference ranges throughout the study (data not shown).

## Discussion

Zinc may have beneficial effects on insulin secretion and sensitivity [27]. Insulin action is a major factor in the stimulation of the synthesis and secretion of leptin [10, 11]. All BSS patients have insulin resistance that is associated with leptin resistance, and leptin treatment has been shown to reverse insulin resistance, which demonstrates the direct relationship between the two [31, 32].

Before this study, all participating BSS patients were only instructed about food items they should consume and those to be avoided. Initial adherence to the diet plan was limited by the voracious appetite that was found in all of the patients at the beginning of the study. Analysis of the collected food records showed that the patients had second helpings at breakfast, lunch, and dinner, and nearly always consumed the same amount at each meal. We therefore decided to introduce oral zinc supplementation to determine if zinc had any beneficial effect on food ingestion. At the end of the study, the BSS patients had not changed the quality of the food they were eating according to the proposed diet. However, we observed that all patients had a reduced appetite and were taking their meals at regular times. It is possible that zinc intake could affect appetite regulation and the metabolism by influencing the leptin system [16]. Leptin is a protein with hormonal action that is produced in adipocytes and acts to increase energy expenditure and decrease the appetite [11, 33].

It should be noted that patient weights remained roughly the same throughout the study. Despite reduced energy intake, there was no decrease in the body weight of these patients. It was also found that the estimate of energetic expenditure remained unchanged. A reduction in basal metabolic rate along with a decrease in thermogenesis and greater metabolism control may have occurred. Thus, at the end of the study the energy intake of the patients was sufficient to offset energetic expenditure and maintain body weight. It should be pointed out that energetic restriction contributed to maintaining normal hepatic function at the beginning and at the end of the study.

However, we did observe calcium dietary intake below recommended levels at the end of the study, which was possibly due to an overall reduction in food consumption (fig. 1a; table 1). The dietary consumption of iron and zinc (fig. 1b) was also reduced but did not fall below recommended levels. Total fiber dietary intake was low at the beginning of the study and remained so after 3 months. With oral zinc supplementation, total zinc ingestion increased, but it did not exceed the tolerable upper intake level value, which is 40 mg/day. It is essential to know the tolerable upper intake level value, which has been established for several different nutrients, as it is the highest amount of a specific nutrient that a healthy person can safely consume. It also focuses on the risks of excessive nutrient intake [34, 35]. Increased zinc may contribute to decreased calcium and iron absorption through chemical competition [36]. In our study, zinc bioavailability may

have been improved by the low dietary consumption of protein from animal sources, calcium, iron, and total fiber.

Adequate dietary calcium intake is essential to maintain the optimal functioning of many body systems. There is a persuasive body of recent evidence suggesting that low calcium intake contributes to, or aggravates, the disease burden of disorders as varied as osteoporosis, kidney stones, hypertension, colon cancer, and obesity [37]. Initially, patients had adequate calcium ingestion and we recommended a mean calcium intake of 1,120 mg/day, but at the end of the study, the low calcium consumption may have been a result of reduced appetite (fig. 1a). At the beginning of the study and after 3 months, the primary sources of dietary calcium intake were skimmed milk, soy, and cheese.

There are few studies in scientific literature that have examined proper nutritional guidelines for BSS patients. Montenegro et al. [38] reported a case of BSS that was treated with a normocaloric diet, consisting of 30% lipids (medium-chain triglycerides), 15% proteins, 53% carbohydrates, and insulin therapy. After 1 month of treatment there was a reduction in fasting serum triglycerides and glucose levels with later normalization that led to discontinuation of the insulin therapy. The patient has maintained good control over his or her diet ever since. On the other hand, Kazlauskaite et al. [39] reported a case of BSS in which the patient received intensive insulin therapy and 4 dietary regimens varying in energy and fat content. In regimen 1, energy restriction was limited because of undesirable weight loss and persistent hunger. Hence, the other regimens were hypercaloric with a moderate-fat (regimen 2), very-low-fat (regimen 3), and low-fat (regimen 4) diet. They observed that insulin sensitivity and hyperglycemia improved with regimens 3 and 4.

Diet plays an important role in the development and control of type 2 diabetes mellitus [40]. Our patients received an individualized diet that aimed to improve their overall health through proper food choices and enhanced metabolic control. They were encouraged to choose foods with a low glycemic index and those containing a variety of fibers, such as whole grains, fruits, and vegetables, which provide fiber, minerals, vitamins, and other substances that are recognized as important for good health. In addition, they were instructed to avoid foods rich in refined carbohydrates, saturated fats, and trans fats. A number of factors were considered during diet preparation, such as age, sex, metabolic abnormalities, physical or occupational activities, food habits, sociocultural habits, food availability, the financial situation of the family,

and the use of medication. The families of these patients had a mean monthly income of USD 97.76, which is below the poverty level of USD 105.55 established by the United Nations Development Program (UNDP) in 2002 [41].

The 3-month fasting glucose measurement may not have reflected the previous glucose metabolism. We observed good control of HbA1c after 3 months (fig. 3b). HbA1c is more representative of glucose metabolism than fasting glucose, given that it reflects glycemia over 120 days. Curiously, energy restriction did not promote body weight loss but contributed to the amelioration of HbA1c [42]. A decrease in dietary fat intake has also been shown to enhance insulin sensitivity [43]. Soluble fiber has a hypocholesterolemic and hypoglycemic effect [44] and, in our study, the patients consumed soluble fiber, which may have contributed to the reduction in HbA1c. With respect to the lipid profile, total cholesterol remained in the normal range and triglyceride concentrations remained high, either because hypertriglyceridemia is a marked parameter of BSS [6] or owing to the short duration of this study (3 months).

This paper focuses on the nature of 2 rare pathologies that are common in BSS patients, the scarceness of adipose cells [1] and decreased serum leptin levels [45]. In our study, the patients displayed hypoleptinemia (fig. 2b) at the beginning and after 3 months of following the diet, due to a lack of subcutaneous adipose tissue. However, this result did not rule out the possibility of leptin sensitization by zinc [16, 17].

Additionally, zinc supplementation increases the production of TNF- $\alpha$  and IL-2, which could also increase the production of leptin [16]. In this regard, hyperleptinemia induces the production of the anorexigenic neurotransmitters melanocortin, pro-opiomelanocortin, and cocaine-amphetamine-regulated transcript and inhibits the production of orexigenic neuropeptide Y and agouti-related protein [46]. Some studies reported that zinc deficiency decreases appetite, whereas zinc supplementation increases appetite. In our study, we observed appetite regulation that was likely caused by the action of zinc on leptin sensitivity. Nevertheless, for ethical reasons, we did not continue this study after the cessation of oral zinc supplementation, because all of the BSS patients opted to maintain zinc supplementation.

In diabetic patients, oral zinc supplementation has been shown to improve glucose tolerance, as well as the clinical features associated with zinc deficiency [47]. Zinc is involved in insulin physiology: it is found in the insulin molecule, and in secretory granules, and has been shown to participate in insulin synthesis, stabilization of proin-

sulin and insulin hexamers, insulin secretion, insulin sensitivity, and insulin degradation [27]. Zinc has also been reported to have insulin-like effects, strengthening the mitogenic signaling of insulin and activating the extracellular-signal-regulated kinases 1 and 2 [48, 49]. C-peptide is released from the  $\beta$ -cells in equimolar amounts with insulin. In this sense, zinc kinetics demonstrated a significant increase in serum zinc, and plasma C-peptide levels increased during the venous zinc tolerance test, but not significantly, probably because of the small number of patients (fig. 2a).

This is the first time that both diet intervention and oral zinc supplementation have been shown to be important in the metabolic control of BSS patients. However, there are limitations to this study: no control group was included and the number of BSS patients was small. Nevertheless, the number of patients studied was relatively high, given the rarity of this pathology.

## Conclusion

Diet intervention and oral zinc supplementation decreased energy consumption, macronutrients, and calcium and iron intake. There was no effect on total cholesterol or triglyceride levels. However, zinc was effective at decreasing HbA1c. Zinc likely acts as an adjunct therapy and probably enhances the effectiveness of insulin and leptin. We therefore suggest the need for complementary studies to standardize the nutritional guidelines for this high-risk group so that we may better understand the pathophysiological pathways by which zinc, insulin, and leptin regulate dietary energy intake. This is the first time that this phenomenon has been described. This result is unique and will certainly have considerable impact on this advanced field of nutrition.

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