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Association between zinc nutritional status and glycemic control in individuals with well-controlled type-2 diabetes

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ABSTRACT

Background/objective: Interest in healthy properties of food and nutrients as co-adjuvant in type-2 diabetes therapy has increased in recent years. Zinc supplementation trials have shown improvements in glycemic control in these patients, although it seems dependent on zinc status of the individuals. The objective of this study was to evaluate the relationship between zinc nutritional status and glucose homeostasis in patients with type-2 diabetes.

Subjects/methods: Eighty patients with well controlled type-2 diabetes were recruited and clinical, anthropometric and dietary evaluations were performed. One week after, insulin sensitivity and beta cell function were assessed by a modified Frequently Sampled Intravenous Glucose Tolerance Test. Zinc status was assessed by plasma zinc and the size of rapidly Exchangeable Zinc Pool (EZP); zinc intake was also determined. Glucagon concentration was evaluated in a subsample of 36 patients.

Results: Patients presented a normal zinc status although zinc intake was lower than recommended. Overall, no associations were observed between zinc status and glycemic control markers. Nevertheless, positive correlations were observed between EZP and fasting insulin concentration ($\rho = 0.393$, $p = 0.021$) and HOMA-IR ($\rho = 0.386$, $p = 0.024$) in women, and between plasma zinc concentration and HbA1c ($\rho = 0.342$, $p = 0.020$) in men.

Conclusions: No significant associations were found between zinc status and glycemic control parameters in patients with well-controlled type 2 diabetes and normal zinc status, although low-degree gender-dependent associations were observed. Further research is required to assess the role of zinc status in zinc deficient patients.

1. Introduction

Type-2 diabetes (T2DM) is a chronic metabolic disease produced by the inability of pancreas to increase insulin secretion to compensate its reduced activity in insulin-sensitive tissues, giving as a result a condition of persistent hyperglycemia, among other features [1]. It is associated with several comorbidities including retinopathy, nephropathy, neuropathy and increased risk of cardio- and cerebro-vascular diseases [1], which increases risk of death up to four times [2].

Treatment of T2DM includes changes in lifestyle as well as pharmacological support. Nutrition therapy is essential to achieve adequate weight reduction and improvement in metabolic parameters [3]. In addition to nutritional counseling, interest in health-promoting properties of foods and nutrients, including zinc, as potential co-adjuvant in treatment for T2DM has markedly increased in recent years [4,5].

Zinc is an essential micronutrient greatly concentrated in endocrine pancreas [6], where it participates in several functions related with blood glucose homeostasis. Adequate or increased amounts of zinc in pancreas may be beneficial in T2DM, favoring assembly of insulin hexamer [7,8] and regulation of insulin and glucagon secretion as well as hepatic insulin clearance [9–11]. In addition, zinc presents insulin-mimetic properties that enhance the activity of key components of insulin signaling pathway [12,13], modulates cytokines expression related with chronic low-grade inflammatory state of T2DM [13,14], and contributes to decrease the oxidative stress generated in T2DM as a consequence of glucose surplus [15].

Several studies have assessed the effect of zinc supplementation in blood glucose control. Two meta-analyses showed modest positive effects of supplemental zinc in glucose homeostasis both in healthy individuals and subjects with diabetes [16,17]. In a recent review

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however, Ruz et al. observed that zinc supplementation improved glycemic control only in those trials where patients had reduced plasma zinc, highlighting the importance of zinc nutritional status of patients at the time of intervention [18]. Although plasma zinc is useful to assess zinc status, it is far from being considered a reliable marker; as a consequence, it is recommended to add other measurements to improve zinc status evaluation [19]. Among these, the size of the rapidly exchangeable zinc pool (EZP), defined as a system of compartments that exchange zinc with the plasma within a 72-hour period, is assumed responsible for most of the physiological functions that requires zinc readily available [20,21]. Variations observed in the size of EZP in response to changes in zinc intake suggests its usefulness as indicator of zinc status [22].

Regarding glucose homeostasis, several approaches have been developed for its evaluation, differing in sensitivity and degree of complexity [23,24]. Bergman's minimal model analysis of Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT) is a combined method to evaluate insulin sensitivity and beta cell function. It presents a high correlation with the euglycemic hyperinsulinemic clamp, the gold standard method to evaluate insulin sensitivity, having the advantage of being less laborious than the latter [25]. In addition, Bergman´s minimal model analysis of FSIVGTT provides results for additional parameters such as glucose effectiveness and first phase insulin secretion that contribute to a comprehensive evaluation of glycemic control.

The objective of this work was to evaluate the relationships between zinc nutritional status and glucose homeostasis in patients with well-controlled T2DM.

2. Materials/subjects and methods

2.1. Subjects

Eighty subjects with T2DM (46 men and 34 women) were recruited in Santiago, Chile. Advertisements in metropolitan newspapers, institutional emails (University of Chile) and invitation to participate in health centers were implemented. Fig. 1 presents a flow chart describing the number of subjects contacted, enrolled and analyzed. Inclusion criteria were: age between 30–65 years old, T2DM with less than 10 years since time of diagnosis, body mass index (BMI) between 20–40 kg/m^2, stable body weight (weight variation less than 5% in last three months), glycated hemoglobin (HbA1c) less than 9% (75 mmol/mol), and fasting glycemia less than 180 mg/dL. Exclusion criteria were: use of insulin therapy, history of ketoacidosis or hyperosmolar hyperglycemic non-ketotic syndrome in the previous 6 months, estimated glomerular filtration rate less than 60 mL/min by Modification of Diet in Renal Disease (MDRD) Study equation [26], alanine aminotransferase or aspartate aminotransferase higher than 2.5 times the upper limit, congestive heart failure (grade III or IV according to the New York Heart Association criteria) [27], presence of uncontrolled hypertension, history of stroke, transient ischemic attack or acute myocardial infarction in previous 5 years, antecedents of surgery or acute infection in previous 3 months, use of antipsychotic medication, systemic use of glucocorticoid steroids in previous 6 weeks, alcohol intake equal or over 2 drinks/day, cancer diagnosis or treatment in the past five years with the exception of those who have been cured and carried good prognosis, subjects HIV positive, pregnant or lactating women, and use of vitamins and/or mineral supplements in previous 3 months. Serum glucagon was measured in a subsample. For this purpose, samples were collected with aprotinin, and individuals had to present total cholesterol less than 240 mg/dL and plasma triglycerides less than 200 mg/dL, and avoid using dipeptidyl peptidase 4 inhibitors (DPP4i) or glucagon-like peptide 1 (GLP-1) agonists. This was possible to carry out in 36 subjects.

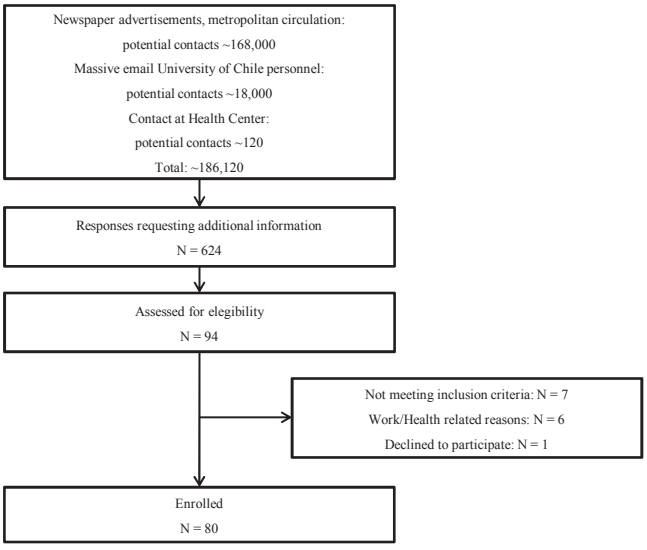

Fig. 1. Flow chart of enrollment of participants.

2.2. Study protocol

During an initial examination, a series of clinical, anthropometric and dietary evaluations were carried out. Clinical evaluation included measurement of blood pressure and a thorough skin and foot examination for detection of lesions; anthropometric evaluation included measurement and registration of weight, height and waist circumference by standardized methods; and dietary evaluation included determination of food and nutrient intake by food history and three-day record questionnaires (two weekdays and one weekend day). At this moment, and after an overnight fast, a blood sample was drawn to assess metabolic status (fasting blood glucose, HbA1c, total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol and plasma triglycerides) and for plasma zinc measurement. All subjects meeting inclusion and ruling out exclusion criteria were scheduled for a second examination one week later to assess beta cell function and insulin sensitivity by a modified Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT) [25] and to administer ^{67}Zn stable isotope to measure EZP. For this purpose, patients were admitted after 12-h fast and a catheter was placed in the forearm vein of each arm for blood sampling and intravenous glucose and insulin infusion. Blood samples were taken 15 and 5 min prior to the beginning of the procedure to determine basal plasma glucose and insulin. At time 0, a bolus of glucose (0.3 g/kg BW as 50% glucose in saline solution) was infused within two minutes and blood samples were taken at 2–6, 8, 10, 12, 14 and 19 min. Twenty minutes after glucose infusion, a bolus of insulin (0.05 IU/kg BW) was infused within 30 s and blood samples were taken at 22, 24, 27, 30, 40, 50, 70, 90, 120, 150 and 180 min. All samples were analyzed for blood glucose and insulin. In addition, at −5, 10 and 30 min, blood samples were obtained in ice chilled tubes with 500 KIU bovine lung aprotinin/mL of whole blood (Merck KGaA, Darmstadt, Germany) for glucagon measurement. After the last blood sample was taken, 0.5 mg of the stable isotope ^{67}Zn was infused in approximately 1 mL of sterile saline solution to determine the size of the rapidly exchangeable zinc pool (EZP). Urine samples were collected daily between days 3 and 8 after FSIVGTT to determine urine ^{67}Zn enrichment by ICP-MS in a PlasmaQuad III (VG Elemental, Winsford, UK.). Details regarding the method to measure ^{67}Zn enrichment have been provided elsewhere [28]. Isotope measurements were carried out at the Pediatric Nutrition laboratory, University of Colorado, USA.

2.3. Measurements

Insulin and glucagon were measured by radioimmunoassay

(Siemens Healthcare Diagnostics Inc., Los Angeles, CA, USA). Glucose, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were determined by an endpoint colorimetric assay (DIALAB GmbH, Wr. Neudorf, Austria). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamil transpeptidase (GGT), albumin, and creatinine were determined using the dry chemistry system Vitros 4600 (Ortho Clinical Diagnostics, Rochester, New York, USA). Precision and accuracy of measurements were assessed by internal controls of kits; results were in agreement with those values provided by the manufacturers. Data on modified FSIVGTT were analyzed according to Bergman's minimal model using MINMOD Millenium 6.02 software to calculate the Acute Insulin Response to glucose (AIRg), Disposition Index (DI), Insulin Sensitivity (Si) and Glucose Effectiveness (Sg). Homeostasis model assessment- insulin resistance (HOMA-IR) was also calculated from glucose and insulin results. Data on food and nutrient intake were analyzed using the software Food Processor FP2 (ESHA Research, Salem, OR, USA). Plasma zinc was determined according to Smith et al. [29] in a Perkin-Elmer AAnalyst 100 spectrophotometer (Perkin-Elmer Corp., Waltham, MA, USA). Quality control of zinc determinations was carried out using Huma Trol N reference material (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany). Coefficient of variation of plasma Zn measurements was 2.3%. EZP was determined according to the method described by Miller et al [20].

2.4. Statistical analysis

Data were analyzed using SPSS 23.0 (IBM Corp., Armonk, NY, USA). Descriptives are presented as mean ± SD, median (Q1–Q3), or n (%). Because main variables related to glycemic control parameters were not normally distributed, according to the Shapiro Wilk test, Spearman's correlation coefficient was used to evaluate associations between zinc status and these variables; no correction for multiple tests was performed. A p-value < 0.05 was considered statistically significant. Multiple regression analyses were performed to search for potential associations between anthropometric, demographic, biochemical and metabolic variables and zinc status.

2.5. Ethical approval

The study was carried out in accordance with the Helsinki Declaration of 1964, as revised in 2008. The study protocol was approved by the Ethics committee for research in human subjects of the Faculty of Medicine, University of Chile.

Informed consent was obtained from all individual participants included in the study and a modest monetary compensation (equivalent to approximately USD 30) was provided to each participant to cover transportation costs and time dedicated to examination.

This study is part of trial registered at ISRCTN registry as ISRCTN95262434.

3. Results

The general characteristics of the 80 subjects studied are presented in Table 1 (demographic, anthropometric, prescribed drugs use and nutrient intake) and Table 2 (biochemical markers). Overweight (body mass index, BMI, 25.0–29.9 kg/m^2) and obesity (BMI ≥30 kg/m^2) accounted for almost 80% of the subjects, 42.5% of individuals presented waist circumference considered normal (< 102 cm and < 88 cm for men and women, respectively) [30]. Metformin alone or in combination was the drug more extensively used among the patients. In terms of nutrient intake values varied in a wide range. Mean zinc intake in the group was 7.4 mg/d; in men, zinc intake was 8.4 ± 4.4 mg/d and in women 6.0 ± 2.1 mg/d. These represent 76.4% and 75.0% of adequacy with respect to the Institute of Medicine of the National Academy of Sciences of USA (11 mg/d for males, 8 mg/d for females) [31], for men and women, respectively. Mean total cholesterol and triglycerides concentrations were below the cutoff point considered high (200 mg/dL and 150 mg/dL, respectively) [32]. Mean plasma zinc concentration was in the normal range. No women and only three men had values below the respective cutoff value (74 µg/dL for males and 70 µg/dL for females) [33].

Table 1
Demographic and anthropometric characteristics, prescribed drugs and nutrient intake in 80 subjects with type 2 diabetes.

	Mean ± SD or n (%)	Range
Demographic and anthropometric parameters		
Age (years)	55.0 ± 6.7	35.0–65.0
Weight (kg)	79.9 ± 14.0	52.4–119.3
BMI (kg/m^2)	29.3 ± 3.8	21.1–37.9
- Normal	11 (13.8)	–
- Overweight	35 (43.8)	–
- Obese	34 (42.5)	–
WC (cm)	98.2 ± 9.2	78.0–122.0
WC < 102 cm men or < 88 cm women[a]	34 (42.5)	–
Fat mass (%)	26.6 ± 9.2	11.7–49.5
Diabetes and Metabolic Syndrome Drugs		
Metformin	52 (65.0)	–
SUs	2 (2.5)	–
Metformin + SUs	22 (27.5)	–
Metformin + DPP4i	3 (3.8)	–
Metformin + SUs + DPP4i	1 (1.3)	–
Statins	36 (45.0)	–
Fibrates	9 (11.3)	–
ACEi/ARBs	25 (31.3)	–
Nutrient Intakes		
Energy (kcal/d)	1866 ± 423	1080–3062
Protein (g/d)	80.8 ± 21.1	37.7–142.3
Carbohydrates (g/d)	240.2 ± 67.2	92.8–400.0
Lipids (g/d)	67.1 ± 21.2	28.3–133.4
Saturated (g/d)	19.8 ± 8.3	5.6–46.3
Monounsaturated (g/d)	17.3 ± 8.2	5.2–48.1
Polyunsaturated (g/d)	13.1 ± 4.9	4.8–30.5
Zinc (mg/d)	7.4 ± 3.8	2.6–21.8
Fiber (g/d)	23.4 ± 10.5	8.5–78.6

Body mass index (BMI) Normal = 18.5–24.9 kg/m^2, Overweight = 25.0–29.9 kg/m^2, Obese ≥30.0 kg/m^2; ACEi: Angiotensin Converting Enzyme inhibitors; ARBs: Angiotensin Receptor Blockers; BMI: Body Mass Index; DPP4i: Dipeptidyl Peptidases 4 inhibitors (saxagliptin, sitagliptin); SUs: Sulphonylureas (glibenclamide, gliclazide); WC: Waist Circumference.

[a] Waist circumference cutoff values established by the Third Report of the National Cholesterol Education Program (NCEP) Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) for high risk of coronary heart disease [30].

Table 2
Biochemical markers in 80 subjects with type 2 diabetes.

	Mean ± SD	Range
Total cholesterol (mg/dL)	171.1 ± 33.6	111.2–274.5
HDL cholesterol (mg/dL)	36.5 ± 10.3	15.0–67.0
LDL cholesterol (mg/dL)	107.4 ± 30.2	56.9–193.7
Plasma Triglycerides (mg/dL)	136.4 ± 60.5	50.4–334.6
ALT (U/L)	31.4 ± 13.9	7.0–82.3
AST (U/L)	22.3 ± 8.1	6.1–51.2
GGT (U/L)	29.5 ± 19.6	9.0–118.0
Alkaline phosphatase (U/L)	189.6 ± 53.1	81.0–386.0
eGFR (mL/min/1.73 m^2)	83.3 ± 13.0	51.8–120.4
ACR (mg/g)	19.9 ± 54.5	0.88–455.0
Plasma zinc (mg/dL)	90.1 ± 12.5	65.5–122.7
Exchangeable zinc pool (mg)	166.3 ± 33.6	113.4–288.4

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; GGT: Gamma Glutamil Transpeptidase; eGFR: estimated Glomerular Filtration Rate by *Modification of Diet in Renal Disease* (MDRD) equation [26]; ACR: Albumin-Creatinine ratio.

Table 3
Fasting glucose, insulin, glucagon, and glycemic control parameters in 80 subjects with type 2 diabetes.

	Mean ± SD or median [Q1–Q3] or n (%)	Range
Fasting glucose (mg/dL)	132.1 ± 21.8	90.5–181.3
Fasting glucose < 130 mg/dL[a]	37 (46.3)	–
HbA1c (%) (mmol/mol)	6.3 [5.8–7.0]	4.3–9.9
HbA1c < 7.0% (53 mmol/mol)	60 (75.0)	–
Fasting insulin (μIU/mL)	8.8 [5.8–13.7]	3.2–45.2
Fasting glucagon (pg/mL)[b]	64.4 ± 33.7	0.0–132.9
HOMA-IR (mM mU L^{-1})	2.8 [1.9–4.7]	0.8–14.4
AIR_g (mU L^{-1} min^{-1})	48.9 [17.2–161.2]	−30.6–1080.9
S_I (mU L^{-1} min^{-1})	1.9 [1.2–2.6]	0.0 – 28.1
DI (SI x AIR)	101.0 [27.4–293.2]	−76.8–1441.9
S_G (10^{-2} min^{-1})	13.7×10^{-3} [10.8×10^{-3}–18.1×10^{-3}]	1.2×10^{-3}–36.3×10^{-3}

HOMA-IR: Homeostasis model assessment- insulin resistance. AIR_g: Acute Insulin Response to glucose; S_I: Insulin Sensitivity index; DI: Disposition Index; S_G: Glucose effectiveness.
[a] Goals established by American Diabetes Association (ADA) 2017 for blood glucose control in type 2 diabetes patients [36].
[b] N = 36.

Table 3 shows results of circulating glucose, insulin and glucagon concentrations, and glycemic control parameters in the subjects studied. As expected, HOMA-IR was increased and AIRg reduced in subjects with diabetes compared to values reported in literature for healthy individuals [34,35]. According to the proportion of individuals exceeding the cutoff points suggested by the American Diabetes Association as therapeutic targets (fasting glucose < 130 mg/dL and HbA1c < 7% or 53 mmol/mol) [36], diabetes condition of the group can be classified as well controlled. Also, descriptive results of parameters obtained from the use of Bergman's minimal model analysis of Frequently Sampled Intravenous Glucose Tolerance Test are presented. Among these: AIRg or first-phase insulin release that reflects β-cell functionality; Si, Insulin Sensitivity index that reflects the ability of insulin to enhance effect of glucose to normalize its own concentration; DI, Disposition Index that reflects insulin secretion normalized to the degree of insulin resistance; and Sg, Glucose Effectiveness that represents the effect of glucose to enhance glucose disappearance at basal insulin.

Table 4 shows all bi-variate Spearman's correlation coefficients (identified as *rho "ρ")* between fasting glucose, insulin and glucagon concentrations and a series of glycemic control parameters with zinc intake, and two markers of zinc status, plasma zinc and EZP. No significant correlations were observed. Exploratory analysis of these associations by gender showed mild positive correlations between EZP and fasting insulin (ρ = 0.393, p = 0.021) and HOMA-IR (ρ = 0.386, p = 0.024) in women only. In turn, a mild positive correlation between plasma zinc and HbA1c (ρ = 0.342, p = 0.020) and zinc intake with fasting glucagon concentration (ρ = 0.549, p = 0.022) were observed in men but not women.

Table 4
Spearman's correlation coefficients between glycemic control parameters and hormones with zinc intake and zinc status biomarkers in 80 subjects with type 2 diabetes.

	Zinc intake (mg/day)	Plasma Zinc (μg/dL)	Exchangeable zinc pool (mg)
Fasting glucose (mg/dL)	−0.029	0.204	0.118
	P = 0.806	P = 0.070	P = 0.299
HbA1c (%)	−0.112	0.167	0.007
	P = 0.336	P = 0.139	P = 0.948
Fasting insulin (μIU/mL)	−0.007	0.095	0.071
	P = 0.954	P = 0.403	P = 0.533
Fasting glucagon (pg/mL)[a]	0.315	0.066	−0.019
	P = 0.069	P = 0.701	P = 0.915
HOMA-IR (mM mU L^{-1})	0.011	0.131	0.125
	P = 0.926	P = 0.245	P = 0.269
AIR_g(mU L^{-1} min^{-1})	−0.054	−0.183	−0.064
	P = 0.640	P = 0.105	P = 0.570
S_I (mU L^{-1} min^{-1})	−0.015	−0.023	−0.131
	P = 0.898	P = 0.839	P = 0.246
DI (SI x AIR)	−0.084	−0.206	−0.107
	P = 0.472	P = 0.067	P = 0.344
S_G (10^{-2} min^{-1})	−0.078	0.050	−0.071
	P = 0.501	P = 0.662	P = 0.531

HOMA-IR: Homeostasis model assessment- insulin resistance. AIR_g: Acute Insulin Response to glucose; S_I: Insulin Sensitivity index; DI: Disposition Index; S_G: Glucose effectiveness.
[a] N = 36.

Multiple regression analyses considering plasma zinc concentration and EZP as markers of zinc status as dependent variables were carried out. Independent variables incorporated included: gender, years since T2DM diagnosis, BMI, zinc intake, fasting blood glucose, HbA1c, AIRg, Si, Sg, total cholesterol, HDL-cholesterol and plasma triglycerides. EZP, zinc intake, HbA1c, total cholesterol and plasma triglycerides were previously normalized by log-transformation while gender, years since T2DM diagnosis, AIRg, Si and Sg were included as dichotomic variables using the median as cut-off point. Multiple regression for plasma zinc as dependent variable was not significant (p = 0.792). When ln-EZP was used as dependent variable, three independent variables were retained in the model: gender (B = 0.139 for men, p = 0.005) and BMI (B = 0.013, p = 0.035) were positively associated, whereas years since T2DM diagnosis were negatively associated (B = −0.088 for a value equal to or above 4.0, p = 0.047) (adjusted R^2 = 0.229, p = 0.003).

4. Discussion

Interest on the association between zinc and diabetes has been present during a long time. Early studies noted the dramatic pancreas zinc content decrease in this condition [37], later the crucial work by Chimienti et al. identifying the zinc transporter ZnT8 as a key element in insulin synthesis, packing and secretion [38]. In addition, a series of functions of zinc that are able to interact with glucose metabolism and insulin/glucagon action, namely, zinc as a membrane stabilizer, antioxidant, anti-inflammatory and more recently, as a signaling ion [39–41]. Two meta-analyses have searched into potential effects of zinc supplementation on diabetes control reporting modest positive effects of zinc intervention. Nevertheless, a close analysis of available information shows high variation in results suggesting that additional factors have to be considered for a proper explanation. Thus, in a review from our group we observed that positive effects of zinc supplementation were related to a prior subnormal zinc status condition [18]. In this study we were interested in exploring the potential associations between zinc intake and zinc status with glycemic control parameters in a group of individuals with well controlled diabetes.

As result of the strict inclusion/exclusion criteria the group of selected individuals fit into the category of well-controlled diabetes

according to the American Diabetes Association therapeutic goals [36]. In terms of glycemic control parameters, we analyzed the more commonly used biomarkers (fasting glucose, HOMA-IR), and hormones involved (insulin and glucagon) but we also included a series of parameters obtained after using Bergman's minimal model analysis of Frequently Sampled Intravenous Glucose Tolerance Test (AIRg, Si, DI and Sg). With respect to zinc, we analyzed zinc intake and two zinc status markers, plasma zinc and EZP.

Zinc status is hard to assess, in fact there is still a lot of debate regarding appropriate biomarkers. This is in part because zinc is required for multiple general metabolic functions, there are no body stores and also as result of the existence of slow and rapid exchangeable zinc pools. Besides, progression of deficiency is different to other nutrients. These aspects are discussed more in depth by King et al. [42]. Plasma zinc is the most common marker of zinc status although a number of limitations suggest using and interpreting it with caution. Despite this, some studies evaluating the effect of T2DM in zinc homeostasis had found inverse correlations between HbA1c and plasma zinc [43,44]. In this study no associations between plasma zinc and glycemic control parameters were observed when analyzed the entire group. In terms of plasma zinc and HbA1c despite no association in the entire group was noted, a modest positive correlation between both parameters in men but not women was observed. This discrepancy with respect to literature may be, at least in part, explained by the degree of control of T2DM. While inverse correlations reported in the literature involved values of HbA1c largely above 8% as well as a poor zinc status, in our study values of HbA1c slightly above 8% were presented only in 3 out of 46 men evaluated. In addition, only 3 out of 46 men had plasma zinc values less than 74 μg/mL. Multiple regression analysis considering plasma zinc as dependent variable and 12 independent variables was not significant, suggesting lack of sensitivity of this marker in this context.

Kinetic studies allowed to quantify the size of the rapidly exchangeable zinc pool (EZP), which represents approximately 10% of total body zinc and it has been suggested as a useful zinc marker [21]. Although the lack of standardized cutoff values for EZP hampers identifying the degree of subnormal values in a given group, it is indeed valuable to evaluate associations with variables of interest, such as those provided by the Bergman's minimal model. However, as with plasma zinc, caution must be taken when interpreting the results of EZP because it is also affected by other factors beyond zinc nutrition like age, weight and sex [21]. As far as we know, this is the first study assessing the association between EZP and glycemic control parameters in diabetes. Similarly to observed in plasma zinc, no significant bivariate associations between EZP and glycemic control parameters were noted in the whole group, although mild positive correlations between EZP and fasting insulin ($\rho = 0.393$, $p = 0.021$) and HOMA-IR ($\rho = 0.386$, $p = 0.024$) were noted in women only. Multiple regression analysis showed that EZP as zinc marker was determined by gender, BMI and duration of diabetes. The latter is particularly relevant because in our subjects, despite they presented an adequate control of diabetes; zinc homeostasis was progressively impaired according to duration of this disease.

In addition to defects in insulin secretion and action, disturbances in glucagon regulation have also a role in the pathophysiology of T2DM. Absolute or relative hyperglucagonemia produces a chronically elevated hepatic glucose output contributing to both fasting glucose impairment and glucose intolerance [45]. We found a non-significant trend ($p = 0.069$) to a positive association between zinc intake and fasting glucagon concentration in the whole group that becomes significant only in men ($p = 0.022$). This observation deserves attention because the study of diet and hormones related to diabetes is mainly focused on insulin overlooking glucagon.

The associations between hormones related to diabetes and glycemic control parameters with zinc intake and zinc status observed in our study are minor in this group of subjects with well controlled diabetes and adequate zinc status. This may reflect that associations reported in individuals with more advanced degrees of diabetes and/or compromised zinc status are not evident until a given threshold of deviation from normality is reached. Such threshold remains to be determined. While selection of our subjects following the inclusion/exclusion criteria can be considered a strength of our study, in terms of adequate characterization of the group, sample size of 80 individuals may be a limitation. Results of a sensitivity analysis showed that with 80 individuals, 0.05 alpha error and 0.8 power we can detect bi-variate correlations of 0.3 and higher. Although we can lack of enough power to detect correlations lower than 0.3, the biological relevance of such correlations is probably negligible.

In conclusion, overall, no significant associations were found between zinc intake and status and glycemic control parameters in patients with well-controlled type 2 diabetes and normal zinc status, although some gender-dependent low-degree associations were apparent after subgroup analysis. Also, after multiple regression analyses EZP suggests being more sensitive to the effect of some variables of interest than plasma zinc in this type of patients. Future research should be directed towards evaluate the influence of mild and/or severe zinc deficiency in patients with T2DM and also in more advanced stages of the disease. In these conditions, a stronger association between zinc status and glucose metabolism may be speculated, however this issue should be further studied. Additionally, to address potential gender differences in the relationship between zinc nutritional status and T2DM seems worthy.

Conflict of interest

Manuel Ruz, Pamela Rojas, Fernando Carrasco, Karen Basfi-fer, Francisco Perez-Bravo, and Jorge Inostroza were coauthors of the research project Fondecyt 1120323 that funded this study. AP was recipient of National Commission for Research in Science and Technology (CONICYT) National Doctoral Fellowship No. 21110424. The rest of authors declare that they have no conflict of interest.

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