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Trace elements in saliva and plasma of patients with type 2 diabetes: Association to metabolic control and complications

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A B S T R A C T

Aim: An analysis is made of the saliva and plasma levels of trace elements in patients with type 2 diabetes mellitus and their association to metabolic control and the presence of chronic complications.

Methods: A cross-sectional observational clinical study was carried out in 74 patients with type 2 diabetes mellitus. Mass spectrometry (ICP-MS) was used to determine the following trace elements in plasma and unstimulated basal saliva: ^{13}Al, ^{16}S, ^{4}Be, ^{5}B, ^{20}Ca, ^{27}Co, ^{29}Cu, ^{24}Cr, ^{38}Sr, ^{15}P, ^{3}Li, ^{12}Mg, ^{25}Mn, ^{28}Ni, ^{82}Pb, ^{37}Rb, ^{22}Ti, ^{23}V and ^{30}Zn.

Results: The levels of cobalt ($p = 0.048$) in saliva and of strontium ($p = 0.001$) in plasma were related to the presence of chronic complications. Significant differences with respect to metabolic control were observed for beryllium ($p = 0.038$), boron ($p = 0.023$) and phosphorus in saliva ($p = 0.046$), and for rubidium ($p = 0.005$), titanium ($p = 0.016$) and zinc in plasma ($p = 0.013$). A significant correlation ($p < 0.001$) was found between boron in plasma and boron in unstimulated basal saliva.

Conclusions: The determination of trace elements in plasma and saliva constitutes a complementary tool for the assessment of metabolic control and for predicting chronic complications associated to type 2 diabetes mellitus. Further studies involving the biomonitoring of trace elements in saliva and plasma are needed.

1. Introduction

Type 2 diabetes mellitus is characterized by chronic hyperglycemia secondary to a defect in insulin synthesis or action [1,2]. It has been suggested that both an excess and a defect in trace elements may play an important role in the development and evolution of diabetes mellitus [3]. However, it is not fully clear whether the alteration of such elements is a consequence of diabetes or rather a factor contributing to development of the disease [4]. Prolonged hyperglycemia has an impact upon both the microvasculature and the larger blood vessels, giving rise to microvascular (diabetic nephropa-

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thy, diabetic retinopathy, diabetic neuropathy, diabetic foot) and macrovascular complications (peripheral arterial disease, cerebrovascular disease, cardiovascular disease) [5,6]. The prevalence of the different chronic complications varies according to the type of diabetes, the duration of the disease, and the degree of metabolic control. The information about trace elements and metabolic control and chronic complications in type 2 diabetes is poor [7]. The present study was therefore carried out to analyze the saliva and plasma levels of trace elements in patients with type 2 diabetes mellitus and their association to metabolic control and the presence of chronic complications.

2. Material and methods

A cross-sectional observational clinical study was carried out in 74 patients of either gender and aged between 25 and 75 years, with a diagnosis of type 2 diabetes mellitus according to the American Diabetes Association (2014) [2]. The patients pertained to Healthcare Area II (Cartagena, Murcia, Spain) and were seen in the Department of Endocrinology and Nutrition of Hospital General Universitario Santa Lucia (Cartagena). The inclusion criteria were: patients over 18 years of age, a diagnosis of type 2 diabetes mellitus at least three months before the study, and the obtainment of written informed consent prior to participation in the study. The exclusion criteria were: pregnancy or nursing, salivary gland disease (Sjögren syndrome), removable dental metal protheses, corticosteroid treatment, radiotherapy, and treatment with cyclosporine or other immunosuppressors. The study was approved by the Bioethics Committee of the University of Murcia.

The study sample ($n = 74$) was divided into two groups according to the degree of metabolic control, based on the international consensus guidelines referred to therapeutic objectives in diabetes (American Association of Clinical Endocrinologists [8,9] American College of Endocrinology and the American Diabetes Association [2015] American Diabetes Association. Standards of Medical Care in Diabetes—2015): good metabolic control (glycosylated hemoglobin [HbA1c] < 7%) (35 patients) and poor metabolic control (HbA1c $\geq$ 7%) (39 patients). The subjects likewise were divided into those with chronic complications associated to diabetes and those without.

2.1. Determinations in saliva

Unstimulated whole saliva samples were collected using a previously published protocol used to successfully collect saliva samples [7]. Patients were instructed not to eat, brush their teeth for at least 2 h before saliva collection. All patients previously rinsed their mouth with distilled wáter. Whole saliva samples were passively collected using the Salivette® device (Sarstedt, S.A., Granollers, Spain). A cotton swab was placed in the mouth for 5 min and then placed in the mentioned device. The samples were centrifuged at 3500 rpm for 10 min and then stored at −80 °C. The unstimulated saliva samples were collected early in the morning in order to avoid circadian variations in composition.

2.2. Determinations in plasma

The blood samples were collected early in the morning after a fasting period of at least 8 h. The samples were identified and coded in tubes and stored at −80 °C in the clinical analyses laboratory of Hospital General Universitario Santa Lucia (Cartagena).

Mass spectrometry (ICP-MS) was used to determine the following trace elements in plasma: aluminum (mg/kg), sulfur (g/100 g), beryllium (mg/kg), boron (mg/kg), calcium (mg/dl), cobalt (mg/kg), copper (mg/kg), chrome (mg/kg), strontium (mg/kg), phosphorus (mg/dl), lithium (mg/kg), magnesium (mg/dl), manganese (mg/kg), nickel (mg/kg), lead (mg/kg), rubidium (mg/kg), titanium (mg/kg), vanadium (mg/kg) and zinc (mg/kg).

On the other hand, the biochemical parameters albumin, ferritin, C-reactive protein and erythrocyte sedimentation rate (ESR) (grouped as "acute phase reactants") were determined in the plasma samples, with a view to evaluating plasma proteins that undergo variations during inflammation [7,10].

A descriptive analysis was made of the different study variables. Comparisons of qualitative variables were carried out with the Pearson chi-squared test, while quantitative data were contrasted by analysis of variance (ANOVA) for more than two variables and the Student *t*-test in the case of two variables. The SPSS® version 15.0 statistical package (SPSS® Inc, Chicago, IL, USA) was used throughout. Statistical significance was considered for $p < 0.05$.

3. Results

The main characteristics of the study sample are described in Table 1. Thirty-one of the 74 patients presented chronic complications of diabetes (41.9%), while 43 did not (58.1%). The complications were microvascular ($n = 22$) (29.7%), macrovascular ($n = 4$) (5.4%), or both ($n = 5$) (6.8%).

The values corresponding to the acute phase reactants were: ferritin 59.96 ± 54.136 (ng/ml), C-reactive protein 0.66 ± 0.48 (mg/dl), and ESR 27.39 ± 18.22 (mm/h). On analyzing the groups according to the degree of metabolic control (good or poor), as determined from the HbA1c concentration, no significant differences were observed referred to ferritin ($p = 0.884$), C-reactive protein ($p = 0.300$) or ESR ($p = 0.282$).

Table 2 shows the results referred to the statistical analysis of the levels of trace elements in plasma and saliva corresponding to the total patient sample ($n = 74$). Of all the trace elements analyzed, only the boron concentrations were seen to be significantly correlated in plasma and saliva ($p < 0.001$).

Significant differences were recorded regarding the presence of chronic complications in relation to the levels of the trace elements in saliva, with the cobalt levels being significantly lower among the diabetic patients with chronic complications ($p = 0.048$) (Table 3). Likewise, significant differences were observed in the plasma levels of strontium ($p = 0.001$) in the diabetic patients with chronic complications (Table 4).

Table 1 – Principal characteristics of the study sample.

General characteristics of the global sample (n = 74)	
Age	59.00 ± 9.07 years
Gender	Males (n = 37) (50%) Females (n = 37) (50%)
Body mass index (BMI)	35.98 ± 6.89 kg/m^2
Diabetes, years of evolution	11.50 ± 7.67 years
Smoking habit	Smokers (n = 12) (16.2%) Non-smokers (n = 26) (35.1%) Ex-smokers (n = 36) (48.6%)
Alcohol consumption	<5 g/day (n = 42) (56.8%) 5–11.5 g/day (n = 27) (36.5%) >11.5 g/day (n = 5) (6.8%)
Arterial hypertension	Yes (n = 59) (79.7%) No (n = 15) (20.3%)
Waist circumference	117.14 ± 14.55 cm
Physical activity	Never (n = 41) (55.4%) Occasional (n = 18) (24.3%) Regular (n = 15) (20.3%)
Diabetic diet	Yes (n = 24) (32.4%) No (n = 50) (67.6%)
Antidiabetic treatment	Oral antidiabetics (n = 17) (23%) Oral antidiabetics + insulin (n = 28) (37.8%) Only insulin (n = 1) (1.4%) Only GLP-1 agonists (n = 1) (1.4%) GLP-1 agonists + oral antidiabetics (n = 10) (13.5%) Insulin + GLP-1 agonists (n = 1) (1.4%) Insulin + oral antidiabetics + GLP-1 agonists (n = 16) (21.6%)

Metabolic control in turn showed significant differences in relation to the plasma concentrations of rubidium (p = 0.005), titanium (p = 0.016) and zinc (p = 0.013). Lastly, metabolic control showed significant differences in relation to the salivary concentrations of beryllium (p = 0.038), boron (p = 0.023) and phosphorus (p = 0.046) (Table 5).

4. Discussion

The present study sought to establish correlations between the trace elements measured in saliva and in plasma in the global sample of diabetic patients. The results revealed a significant correlation between the boron levels in saliva and plasma (p < 0.001). Boron is necessary for the conversion of vitamin D into its active form, which intervenes in normal growth, the consolidation of fractures, and in adequate calcification. Boron deficiency is related to defective growth and to osteoporosis (fractures), osteoarthritis and calcification problems. Although the precise role of boron in carbohydrate metabolism is not fully clear, an association has been found between the prevalence of type 2 diabetes mellitus and boron levels [11].

With regard to other trace elements such as zinc, our findings coincide with those of other investigators such as Greger et al. [12]and Bales et al. [13], who reported no clear correlation between the zinc levels in saliva and plasma [14]. On the other hand, Ladgotra et al., in 2016 [10], described a good correlation between the plasma and saliva concentrations referred to calcium and phosphorus. But precisely in this study it is not significant for calcium; p = 0.314 or phosphorus p = 0.758 Perhaps it could be due to the determination method and the patients were type I and II diabetics.

4.1. *Determinations in plasma*

The patients with good metabolic control (HbA1c < 7%) presented levels of rubidium (p = 0.005), zinc (p = 0.013) and titanium (p = 0.016) in plasma. The results obtained indicate significant differences in plasma trace elements in relation to metabolic control. According to Ekmekcioglu et al. (2001) [15], however, the plasma concentrations of trace elements are not correlated to the degree of glycemic control as assessed from the HbA1c levels. This difference may be explained by the complexity of standardizing the HbA1c concentrations that define good or poor metabolic control, because of the great variability of the clinical contexts found in the different studies [9]. In effect, choosing one cut-off point or other as the objective for metabolic control can vary according to the type of population studied, and the existing clinical guides likewise offer no clear consensus regarding the definition of a single cut-off point based on HbA1c concentration, since there are many conditioning factors such as the age of the patient, the presence or not of chronic complications, the hypoglycemia rate, patient life expectancy and the existence of other comorbidities.

According to the consulted consensus guides regarding therapeutic objectives [3,8,9,16–18] (the American Association of Clinical Endocrinologists, the American College of Endocrinology and the American Diabetes Association (2015), glycemic control is established as HbA1c 6.5% in patients with serious concomitant diseases and a risk of hypoglycemia. This definition of metabolic control is based on the ACCORD[16] (Action to Control Cardiovascular Risk in Diabetes 2008), ADVANCE (Action in Diabetes and Vascular Disease Preterax and Diamicron MR Controlled Evaluation)

Table 2 – Correlations between plasma and saliva trace elements in the global sample.

	Al (saliva)	S (saliva)	B (saliva)	Ca (saliva)	Cu (saliva)	Cr (saliva)	Sr (saliva)	P (saliva)	Li (saliva)	Mg (saliva)	Mn (saliva)	Ni (saliva)	Pb (saliva)	Rb (saliva)	Ti (saliva)	V (saliva)	Zn (saliva)
Al (plasma)	Rho 0.234 P value 0.076																
S (plasma)		Rho 0.022 p 0.862															
B (plasma)			Rho 0.447 p 0.000														
Ca (plasma)				Rho −0.126 p 0.314													
Cu (plasma)					Rho 0.132 p 0.313												
Cr (plasma)						Rho 0.083 p 0.551											
Sr (plasma)							Rho 0.040 p 0.750										
P (plasma)								Rho −0.039 p 0.758									
Li (plasma)									Rho −0.092 p 0.543								
Mg (plasma)										Rho −0.008 p 0.948							
Mn (plasma)											Rho −0.040 p 0.748						
Ni (plasma)												Rho 0.003 p 0.982					
Pb (plasma)													Rho 0.215 p 0.093				
Rb (plasma)														Rho 0.224 p 0.068			
Ti (plasma)															Rho 0.139 p 0.310		
V (plasma)																Rho 0.078 p 0.540	
Zn (plasma)																	Rho −0.119 p 0.409
Simple size (N)	N 58	N 66	N 62	N 66	N 60	N 54	N 66	N 66	N 46	N 67	N 66	N 61	N 62	N 67	N 55	N 64	N 50

Table 3 – Trace elements in saliva in relation to the presence of chronic complications of diabetes.

Trace elements in saliva	Average valor ± standard deviations No Complications of diabetes	Mean ± standard deviation Complications	P-value
Aluminum	0.4234 ± 0.3044 (mg/kg)	0.5529 ± 0.3938 (mg/kg)	p = 0.146
Sulfur	0.0098 ± 0.0058 (g/100 g)	0.0094 ± 0.0056 (g/100 g)	p = 0.907
Beryllium	0.0010 ± 0.0006 (mg/kg)	0.0012 ± 0.0010 (mg/kg)	p = 0.336
Boron	0.0886 ± 0.0974 (mg/kg)	0.1509 ± 0.1217 (mg/kg)	p = 0.052
Calcium	0.0174 ± 0.0100 (g/100 g)	0.0161 ± 0.0087 (g/100 g)	p = 0.726
Cobalt	0.0012 ± 0.0011 (mg/kg)	0.0009 ± 0.0000 (mg/kg)	**p = 0.048**
Copper	0.0516 ± 0.0444 (mg/kg)	0.0451 ± 0.0226 (mg/kg)	p = 0.644
Chrome	0.0139 ± 0.0124 (mg/kg)	0.0116 ± 0.0140 (mg/kg)	p = 0.349
Strontium	0.4590 ± 0.3312 (mg/kg)	0.4330 ± 0.2570 (mg/kg)	p = 0.990
Phosphorus	0.0338 ± 0.0161 (g/100 g)	0.0324 ± 0.1389 (g/100 g)	p = 0.953
Lithium	0.0029 ± 0.0035 (mg/kg)	0.0044 ± 0.0093 (mg/kg)	p = 0.875
Magnesium	0.0026 ± 0.0017 (g/100 g)	0.0026 ± 0.0016 (g/100 g)	p = 0.896
Manganese	0.4224 ± 0.2765 (mg/kg)	0.3906 ± 0.2327 (mg/kg)	p = 0.866
Nickel	0.0767 ± 0.0547 (mg/kg)	0.0658 ± 0.0356 (mg/kg)	p = 0.743
Rubidium	0.9203 ± 0.3223 (mg/kg)	0.8743 ± 0.4156 (mg/kg)	p = 0.371
Titanium	0.0510 ± 0.0439 (mg/kg)	0.0532 ± 0.0479 (mg/kg)	p = 0.688
Vanadium	0.0067 ± 0.0061 (mg/kg)	0.0064 ± 0.0059 (mg/kg)	p = 0.752
Zinc	0.1770 ± 0.1396 (mg/kg)	0.2072 ± 0.1297 (mg/kg)	p = 0.164

The bold means statistical significance p value.

Table 4 – Trace elements in plasma in relation to the presence of chronic complications of diabetes.

Plasma	No complications	Complications	P-value
Aluminum	0.2312 ± 0.1661 (mg/kg)	0.2164 ± 0.1563 (mg/kg)	p = 0.688
Sulfur	0.0977 ± 0.0066 (g/100 g)	0.1005 ± 0.0083 (g/100 g)	p = 0.106
Boron	0.0420 ± 0.0598 (mg/kg)	0.0659 ± 0.0743 (mg/kg)	p = 0.172
Calcium	9.474 ± 0.4938 (mg/dl)	9.503 ± 0.4834 (mg/dl)	p = 0.803
Copper	1.2835 ± 0.2972 (mg/kg)	1.3031 ± 0.1942 (mg/kg)	p = 0.734
Chrome	0.0072 ± 0.0073 (mg/kg)	0.0072 ± 0.0074 (mg/kg)	p = 0.911
Strontium	0.0459 ± 0.0195 (mg/kg)	0.0661 ± 0.0284 (mg/kg)	**p = 0.001**
Phosphorus	2.767 ± 1.7135 (mg/dl)	2.365 ± 1.7142 (mg/dl)	p = 0.183
Lithium	5.5357 ± 1.5477 (mg/kg)	5.6890 ± 1.0858 (mg/kg)	p = 0.331
Magnesium	1.944 ± 0.2363 (mg/dl)	1.923 ± 0.1924 (mg/dl)	p = 0.691
Manganese	0.1619 ± 0.0262 (mg/kg)	0.1651 ± 0.0190 (mg/kg)	p = 0.930
Rubidium	0.1707 ± 0.0545 (mg/kg)	0.1627 ± 0.0430 (mg/kg)	p = 0.887
Titanium	0.0351 ± 0.0266 (mg/kg)	0.0300 ± 0.0179 (mg/kg)	p = 0.607
Vanadium	0.0045 ± 0.0026 (mg/kg)	0.0037 ± 0.0022 (mg/kg)	p = 0.166
Zinc	1.2475 ± 0.2400 (mg/kg)	1.2218 ± 0.1582 (mg/kg)	p = 0.545

The bold means statistical significance p value.

and VADT studies (Veterans Affairs Diabetes Trials), which concluded that strict metabolic control in middle-aged patients with advanced type 2 diabetes mellitus and high vascular risk does not reduce cardiovascular mortality. Rather, such control was found to be associated to an increase in the frequency of hypoglycemia, which is regarded as a cardiovascular and overall mortality indicator in type 2 diabetics. Consequently, in our sample of patients we chose HbA1c 7% as the cut-off point distinguishing between good and poor metabolic control [19–27].

Most studies report significantly lower plasma zinc concentrations in patients with type 2 diabetes mellitus compared with healthy controls [19,23,26,28]. This may be related to the observed alterations in insulin secretion and action that could be associated to a decrease in plasma zinc levels. This relationship between insulin and zinc is due to the fact that the latter acts as a cofactor of insulin [29,30]. Furthermore, approximately 0.5% of the hexameric structure of crystalline insulin is composed of zinc. Nevertheless, the mechanism by which zinc intervenes in carbohydrate metabolism has not been fully established [28]. With regard to the plasma trace elements measured in the group of patients with type 2 diabetes mellitus and the chronic complications associated to diabetes – whether macrovascular or microvascular in nature – our study found the plasma strontium levels to be significantly higher among the diabetic patients with complications (p = 0.001). Little is known of the possible role of strontium in relation to the chronic complications of diabetes, or of whether it may act as a biomarker in identifying patients at high risk of suffering complications of the disease. A review of the literature yielded no studies capable of corroborating our findings; further investigations are therefore needed, addressing the different types of macrovascular or microvascular complications, e.g., ischemic heart disease,

Table 5 – Trace elements in saliva and plasma in relation to metabolic control of diabetes.

	Blood Mean ± standard deviation HbA1c < 7%	Blood Mean ± standard deviation HbA1c ≥ 7%	P-value	Saliva Mean ± standard deviation HbA1c < 7%	Saliva Mean ± standard deviation HbA1c ≥ 7%	P-value
Aluminum	0.2424 ± 0.1878 (mg/kg)	0.2136 ± 0.1423 (mg/kg)	p = 0.646	0.4723 ± 0.3384 (mg/kg)	0.4803 ± 0.3568 (mg/kg)	p = 0.896
Sulfur	0.09817 ± 0.0063 (g/100 g)	0.0994 ± 0.0082 (g/100 g)	p = 0.464	0.0090 ± 0.0051 (g/100 g)	0.0100 ± 0.0061 (g/100 g)	p = 0.526
Boron	0.1610 ± 0.1283 (mg/kg)	0.0907 ± 0.0944 (mg/kg)	**p = 0.023**	0.1610 ± 0.1283 (mg/kg)	0.0907 ± 0.0944 (mg/kg)	**p = 0.023**
Calcium	0.0182 ± 0.0104 (g/100 g)	0.0160 ± 0.0088 (g/100 g)	p = 0.398	0.0182 ± 0.0104 (g/100 g)	0.0160 ± 0.0088 (g/100 g)	p = 0.398
Cobalt	0.0012 ± 0.0011 (mg/kg)	0.0010 ± 0.0007 (mg/kg)	p = 0.754	0.0012 ± 0.0011 (mg/kg)	0.0010 ± 0.0007 (mg/kg)	p = 0.754
Copper	0.0597 ± 0.0525 (mg/kg)	0.0425 ± 0.0211 (mg/kg)	p = 0.690	0.0597 ± 0.0525 (mg/kg)	0.0425 ± 0.0211 (mg/kg)	p = 0.690
Chrome	0.0151 ± 0.0171 (mg/kg)	0.0118 ± 0.0103 (mg/kg)	p = 0.807	0.0151 ± 0.0171 (mg/kg)	0.0118 ± 0.0103 (mg/kg)	p = 0.807
Strontium	0.4461 ± 0.2905 (mg/kg)	0.4491 ± 0.3089 (mg/kg)	p = 0.947	0.4461 ± 0.2905 (mg/kg)	0.4491 ± 0.3089 (mg/kg)	p = 0.947
Phosphorus	0.0384 ± 0.0162 (g/100 g)	0.0301 ± 0.0136 (g/100 g)	**p = 0.046**	0.0384 ± 0.0162 (g/100 g)	0.0301 ± 0.0136 (g/100 g)	**p = 0.046**
Lithium	0.0036 ± 0.0052 (mg/kg)	0.0036 ± 0.0075 (mg/kg)	p = 0.748	0.0036 ± 0.0052 (mg/kg)	0.0036 ± 0.0075 (mg/kg)	p = 0.748
Magnesium	0.0025 ± 0.0015 (g/100 g)	0.0027 ± 0.0018 (g/100 g)	p = 0.944	0.0025 ± 0.0015 (g/100 g)	0.0027 ± 0.0018 (g/100 g)	p = 0.944
Manganese	0.4464 ± 0.2705 (mg/kg)	0.3875 ± 0.2504 (mg/kg)	p = 0.432	0.4464 ± 0.2705 (mg/kg)	0.3875 ± 0.2504 (mg/kg)	p = 0.432
Nickel	0.0789 ± 0.0582 (mg/kg)	0.0687 ± 0.0414 (mg/kg)	p = 0.702	0.0789 ± 0.0582 (mg/kg)	0.0687 ± 0.0414 (mg/kg)	p = 0.702
Rubidium	0.0789 ± 0.0582 (mg/kg)	0.0687 ± 0.0414 (mg/kg)	p = 0.702	0.9228 ± 0.3649 (mg/kg)	0.8884 ± 0.3615 (mg/kg)	p = 0.690
Titanium	0.0789 ± 0.0582 (mg/kg)	0.0687 ± 0.0414 (mg/kg)	p = 0.702	0.0555 ± 0.0486 (mg/kg)	0.0496 ± 0.0433 (mg/kg)	p = 0.489
Vanadium	0.0789 ± 0.0582 (mg/kg)	0.0687 ± 0.0414 (mg/kg)	p = 0.702	0.0077 ± 0.0072 (mg/kg)	0.0059 ± 0.0051 (mg/kg)	p = 0.587
Zinc	0.0789 ± 0.0582 (mg/kg)	0.0687 ± 0.0414 (mg/kg)	p = 0.702	0.2061 ± 0.1871 (mg/kg)	0.1814 ± 0.0934 (mg/kg)	p = 0.626

The bold means statistical significance p value.

cerebrovascular accidents, peripheral arterial disease, diabetic foot, diabetic nephropathy or diabetic retinopathy, among others.

4.2. Determinations in saliva

Saliva was the other biological fluid analyzed in our study. Since saliva allows direct and noninvasive sampling, it may prove to be an ideal alternative or complement to the analysis of plasma. Determinations in saliva have a series of advantages, since the technique is easy to perform. However, this fluid shows variations in composition before, during and after meals. Furthermore, the composition of partial or whole stimulated or unstimulated saliva samples is influenced by circadian rhythms [7]. We standardized saliva collection in our study in order to avoid such fluctuations.

The present study is limited by its cross-sectional design, with patient screening based on the consecutive sampling technique. Another possible source of bias is the influence of dental status and diet [31–33].

The lack of specific trace element biomarkers for metabolic control and complications of type 2 diabetes mellitus is an additional limitation.

The determination of trace elements in plasma and saliva constitutes a complementary tool for the assessment of metabolic control and for predicting chronic complications associated to type 2 diabetes mellitus. Further studies involving the biomonitoring of trace elements in saliva and plasma are needed.

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Declaration of Competing Interest

We declare no conflict of interest in this manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2019.107871.

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