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# Trace Elements in Saliva as Markers of Type 2 Diabetes Mellitus

Luis Marín Martínez[1] · Diana Molino Pagán[2] · Pía López Jornet[3,4]

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## Abstract

To analyze Mg, Ca, and Zn levels in saliva, comparing patients with type 2 diabetes mellitus and a control group of healthy subjects. This transversal, observational, clinical study included a total sample of 147 patients, 74 with type 2 diabetes mellitus and a control group of 73 healthy subjects. Socio-demographic, anthropometric, diabetological, and metabolic variables were registered. Trace elements in non-stimulated basal saliva were measured using inductively coupled plasma mass spectrometry (ICP-MS): Mg, Ca, and Zn. Concentrations of zinc, calcium, and magnesium were significantly higher in the diabetic group than the control group ($p < 0.001$). A relation was observed between waist circumference and high cardiovascular risk in men (based on two categories: waist circumference < 102 cm; waist circumference ≥ 102 cm), and magnesium levels in saliva ($p = 0.003$). Magnesium, zinc, and calcium levels in saliva could be useful markers for differentiating patients with type 2 diabetes mellitus from non-diabetics. The salivary magnesium could be used as a marker of high cardiovascular risk when associated with abdominal obesity represented by a waist circumference ≥ 102 cm in men. The present results do suggest that salivary zinc levels could act as a good marker of type 2 diabetes mellitus, in light of zinc's well-known role as a co-marker of insulin and its relationship to carbohydrate metabolism.

**Keywords** Trace elements · Saliva · Type 2 diabetes mellitus · Waist circumference · Cardiovascular risk

## Introduction

There is growing interest in identifying biomarkers that might help our understanding of various aspects of different diseases [1]. Some biomarkers can be measured in saliva, a non-invasive alternative to blood, [2–4] which makes it possible to conduct population studies simply and cost-effectively [3].

It has been postulated that both the excess and the lack of trace elements could play an important role in the appearance and evolution of diabetes mellitus [5]. But it is not known whether an imbalance in these elements is the consequence of diabetes or a factor contributing to its development [5, 6].

Previous research suggests a relationship between diabetes mellitus and trace elements; an alteration of copper, zinc, manganese, or chromium levels can produce a failure on the antioxidant capacity, thus affecting the progression of diabetes [3–6].

To date, research based on saliva analysis of diabetics has failed to produce homogeneous results; an alteration in zinc (is essential for growth and development, immunity, and metabolism) levels can produce increased oxidative stress in type 2 diabetes mellitus patients, contributing to resistance to insulin and other complications deriving from diabetes [7]. Mata et al. [8] found increased calcium levels in the saliva of diabetics compared to healthy control subjects, while magnesium, zinc and potassium levels were found to be lower in diabetics than non-diabetics. Lasisi et al. [9] discovered a fall in potassium among diabetics but found no differences in levels of salivary sodium, calcium, chlorine, and bicarbonate between diabetics and non-diabetics.

✉ Luis Marín Martínez
luis.marin.martinez@gmail.com

Diana Molino Pagán
dyana_mp@hotmail.com

Pía López Jornet
majornet@um.es

1 Servicio de Endocrinología y Nutrición, Hospital General Universitario Santa Lucía, Urb. Novo Carthago, 80, 30202 Cartagena, Murcia, Spain

2 Facultad de Medicina y Odontología, Hospital Morales Meseguer, Clínica Odontológica, C / Marqués Vélez s/n, 30008 Murcia, Spain

3 Facultad de Medicina y Odontología, Virgen de la Arrixaca Clinical University Hospital, IMIB-Arrixaca, University of Murcia, Murcia, Spain

4 School of Dentistry, Faculty of Medicine, University of Murcia, Spain C / Marqués Vélez s/n, 30008 Murcia, Spain

Meanwhile, it is a well-known fact that type 2 diabetes mellitus is generally accompanied by other cardiovascular risk factors such as obesity, arterial hypertension, and dyslipidemia, which contribute to the development of cardiovascular diseases such as arteriosclerosis, myocardial infarction, or cerebrovascular disease [10–13]. The prevalence of obesity among populations of type 2 diabetes mellitus sufferers is around 80–95% [14]. The health risks deriving from obesity are caused mainly by abdominal fat deposits, which are considered an independent cardiovascular risk factor [5, 15].

Saliva is considered a good screening method for inflammatory, metabolic and cardiovascular pathologies. Nevertheless, it is difficult to establish a clear picture of how trace elements in the saliva of patients with diabetes mellitus compare with healthy subjects, and what role they play in its physiopathology and in the metabolic management of the disease. [8, 9, 16–19].

The present study investigates the relation between type 2 diabetes mellitus and trace element levels in saliva, identifying useful markers for differentiating between individuals with and without diabetes, and analyzing the relation between trace elements in saliva and cardiovascular risk associated with large waist circumference.

## Materials and Methods

This transversal, observational, case-control, clinical study analyzed 74 patients of both sexes, aged between 25 and 75 years, diagnosed with type 2 diabetes mellitus, according to criteria established by the *American Diabetes Association* in 2014 [14]. A random, representative sample of diabetic population were drawn from Cartagena (Murcia, Spain) healthcare area II, attending the Endocrinology and Nutrition Service at the Santa Lucia University General Hospital (Cartagena). Study group inclusion criteria were patients over the age of 18 years; patients diagnosed with type 2 diabetes mellitus of over 3 months evolution; patients willing to give their informed consent to take part in the study. Exclusion criteria were pregnancy or lactation; presence of saliva gland pathology (Sjögren's syndrome); dehydration patients in treatment with corticosteroids, radiotherapy, cyclosporine or other immunosuppressants.

The control group consisted of 73 healthy subjects of both sexes aged between 25 and 75 years, without any diagnosis of diabetes mellitus, altered basal glycemia, intolerance to carbohydrates, or saliva gland pathology. These subjects were also recruited from the Cartagena (Murcia, Spain) healthcare area. The study design was approved by the University of Murcia Ethics Committee, and all participants gave their informed consent to take part.

All participants were assessed registering the following: socio-demographic variables (age, sex, smoking, alcohol consumption, and diagnosis of arterial hypertension), as well as anthropometric variables (weight, size, body mass index, and waist circumference). Waist circumference was classified according to sex and waist circumference criteria associated with increased cardiovascular risk established by the *National Cholesterol Education Program-Adult Treatment Panel III* (NCEP-ATP III) in 2001 [20], whereby a waist circumference is considered to constitute cardiovascular risk when it exceeds 88 cm in women and 102 cm in men.

In the diabetic group, patients' diabetological variables and metabolic profiles were registered (duration of diabetes evolution, anti-diabetic treatment, patient following a controlled diabetic diet or not, presence of chronic complications associated with diabetes, patients fulfilling (or not) metabolic syndrome criteria according to the *National Cholesterol Education Program-Adult Treatment Panel III,* 200121.

An oral exam was carried out and active oral pathologies patients (caries and periodontitis) were thus excluded.

### Measurement in Saliva

Non-stimulated whole saliva was collected in the mornings to avoid the effects of compositional fluctuations deriving from circadian rhythms. Patients were asked not to eat for 2 h before saliva collection. Saliva was collected using the passive drool method and the Salivette® device (Sarstedt SA, Granollers, Spain). A cotton wool swab was placed in the mouth, below the tongue, absorbing the saliva sample, which was then placed in the perforated plastic cylinder of the Salivette®. The procedure was conducted applying appropriate asepsis measures.

The samples were then centrifuged at 3500 rpm for 10 min, and stored at a temperature of − 80 °C in an ultrafreezer. Trace element analysis was performed at the Science and Applied Biology Center (CEBAS-CSIC) in Murcia (Spain). The following trace elements were measured by means of inductively coupled plasma mass spectrometry (ICP-MS): Al, S, Be, Bo, Ca, Co, Cu, Cr, Sr, P, Fe, Li, Mg, Mn, Ni, Rb, Ti, and Zn.

Statistical analysis was performed using SPSS® version 15.0 software (SPSS® Inc., Chicago, USA) for Windows. Descriptive statistics were calculated for each variable. The Pearson chi-squared test was applied to qualitative variables, and the Student's $t$ test to two variables. The significance level was established as $p < 0.05$. Explanatory models were generated using logistic regressions to explain different dichotomous variables acting dependently on other variables acting independently. In cases when logistic regression found statistically significant differences/relations, these were represented graphically using ROC (relative operating characteristics) curves, disclosing their sensitivity and specificity.

## Results

The main characteristics of the total sample ($n = 147$), diabetic group ($n = 74$), and control group ($n = 73$) are expressed as descriptive statistics shown in Table 1. The results of saliva sample analysis by means of ICP-MS in the diabetic group, control group, and total sample are shown in Table 2.

Logistic regression analysis was applied to the total sample ($n = 147$subjects). Independent variables were the trace elements measured in saliva, while inclusion in the diabetic group or not was treated as a dependent variable. In this way, explanatory models differentiated the diabetic group from the control group in relation to zinc, magnesium, and calcium levels in the total sample. According to its explanatory model, salivary zinc showed a $p$ value < 0.001, generating the ROC curve shown in Fig. 1. Zinc values over 0.0940 (mg/L) showed greater evidence of belonging to the diabetic group with model sensitivity to correctly classify individuals as belonging to the diabetic group of 82%, and sensitivity to classify individuals as not belonging to the diabetic group of 79.4%. In this way, the zinc levels in saliva were able to differentiate diabetics from healthy subjects non-randomly with a $p$ value of < 0.001.

Magnesium in saliva values over 0.0011 (mg/L) showed greater evidence of belonging to the diabetic group with model sensitivity to correctly classify individuals as belonging to the diabetic group of 79.2% and model sensitivity to classify individuals as not belonging to the diabetic group of 77.8%. So magnesium levels in saliva were able to differentiate diabetics from healthy subjects non-randomly across the total sample.

Salivary calcium showed a constant $p$ value of 0.001. In the logistic regression model, calcium showed a $p$ value of 0.013. Calcium in saliva values over 0.0085 (mg/L) showed greater evidence of belonging to the diabetic group with model sensitivity to correctly classify individuals as belonging to the diabetic group of 80.7% and model sensitivity to classify individuals as not belonging to the diabetic group of 80%. In this way, salivary calcium levels were able to differentiate diabetics from healthy subjects non-randomly across the total sample with a $p$ value < 0.001.

Another dependent variable investigated by logistic regression analysis model of the total sample ($n = 147$) was waist circumference, constituting high cardiovascular risk among male subjects. Individuals were classified as one of two categories: waist circumference < 102 cm or waist circumference ≥ 102 cm, in relation to salivary trace element levels. In this model,

**Table 1** General characteristics of sample

| Variable | Diabetic group $N = 74$ | Control group $N = 73$- | $P$ value |
|---|---|---|---|
| Age (years) | 59.00 ± 9.07 | 41.12 ± 11.68 | $P < 0.001$ |
| Sex | | | |
| Men | 37 (50%) | 20 (27.4%) | |
| Women | 37 (50%) | 53 (72.6%) | |
| Weight (kg) | 96.05 ± 19.10 | 66.83 ± 13.93 | $P < 0.001$ |
| BMI (kg/m$^2$) | 35.98 ± 6.89 | 24.06 ± 3.96 | $P < 0.001$ |
| Waist circumference (cm) | 117.14 ± 14.55 | 84.23 ± 12.52 | $P < 0.001$ |
| Women | | | |
| Waist circumference < 88 cm | 2 (4%) | 49 (96%) | $P < 0.001$ |
| Waist circumference ≥ 88 cm | 72 (75%) | 24 (25%) | $P < 0.001$ |
| Men | | | |
| Waist circumference < 102 cm | 13 (16%) | 68 (84%) | $P < 0.001$ |
| Waist circumference ≥ 102 cm | 61 (92%) | 5 (8%) | $P < 0.001$ |
| Alcohol consumption | | | |
| < 1.5 g/day | 42 (56.8%) | 55 (75.3%) | $P = 0.190$ |
| 1.5–11.5 g/day | 27 (36.5%) | 18 (24.7%) | $P = 0.186$ |
| > 11,5 g/day | 5 (6.8%) | 0 | $P < 0.001$ |
| Smoking | | | |
| Smoker | 12 (16.2%) | 8 (11%) | $P = 0.378$ |
| Non-smoker | 26 (35.1%) | 55 (75.3%) | $P = 0.001$ |
| Ex-smoker | 36 (48.6%) | 9 (12.3%) | $P < 0.001$ |
| Arterial hypertension | | | |
| Yes | 59 (79.7%) | 4 (5.5%) | $P < 0.001$ |
| No | 15 (20.3%) | 69 (94.5%) | $P < 0.001$ |

**Table 2** Mean values of salivary trace elements in diabetic group, control group, and total sample

| Trace elements in saliva | Mean trace elements in saliva: diabetic group(mg/L) | Mean trace elements in saliva: control group (mg/L) | *P* value |
|---|---|---|---|
| Aluminum | 0.4771 ± 0.3473 | 0.2139 ± 0.2589 | *p* < *0.001* |
| Sulfur | 0.0096 ± 0.0057 | 0.0042 ± 0.0031 | *p* < *0.001* |
| Beryllium | 0.0011 ± 0.0008 | 0.0009 ± 0.000 | *p* = *0.015* |
| Boron | 0.1167 ± 0.1125 | 0.0723 ± 0.0591 | *p* < *0.001* |
| Calcium | 0.0168 ± 0.0094 | 0.0065 ± 0.0038 | *p* < *0.001* |
| Cobalt | 0.0011 ± 0.0009 | 0.0009 ± 0.0001 | *p* = *0.035* |
| Copper | 0.0488 ± 0.0365 | 0.0276 ± 0.0249 | *p* < *0.001* |
| Chromium | 0.0129 ± 0.0130 | 0.0068 ± 0.0115 | *p* = *0.001* |
| Strontium | 0.4480 ± 0.3001 | 0.1210 ± 0.1112 | *p* < *0.001* |
| Phosphorous | 0.0332 ± 0.0151 | 0.0183 ± 0.0101 | *p* < *0.001* |
| Iron | 0.7878 ± 0.0722 | 0.3232 ± 0.3447 | *p* < *0.001* |
| Lithium | 0.0036 ± 0.0068 | 0.0020 ± 0.0053 | *p* = *0.016* |
| Magnesium | 0.0026 ± 0.0017 | 0.0007 ± 0.0005 | *p* < *0.001* |
| Manganese | 0.4089 ± 0.2574 | 0.1689 ± 0.1094 | *p* < *0.001* |
| Nickel | 0.0722 ± 0.0477 | 0.0331 ± 0.0287 | *p* < *0.001* |
| Rubidium | 0.9018 ± 0.3605 | 0.5493 ± 0.1596 | *p* < *0.001* |
| Titanium | 0.0519 ± 0.0452 | 0.0263 ± 0.0271 | *p* < *0.001* |
| Vanadium | 0.0066 ± 0.0060 | 0.0027 ± 0.0035 | *p* < *0.001* |
| Zinc | 0.1906 ± 0.1347 | 0.0711 ± 0.0554 | *p* < *0.001* |

magnesium presented a *p* value of 0.003, generating the ROC curve shown in Fig. 2. Magnesium levels over 0.0012 (mg/L) showed greater evidence of corresponding to a waist circumference ≥ 102 cm, with model sensitivity of 84% and specificity of 81.8% to correctly classify men with waist circumferences ≥ 102 cm on the basis of salivary magnesium levels.

## Discussion

The results of the present study concur with previous research findings, which have pointed to differences in the saliva composition of patients with diabetes mellitus compared with healthy subjects [21], although it is difficult to establish just how these

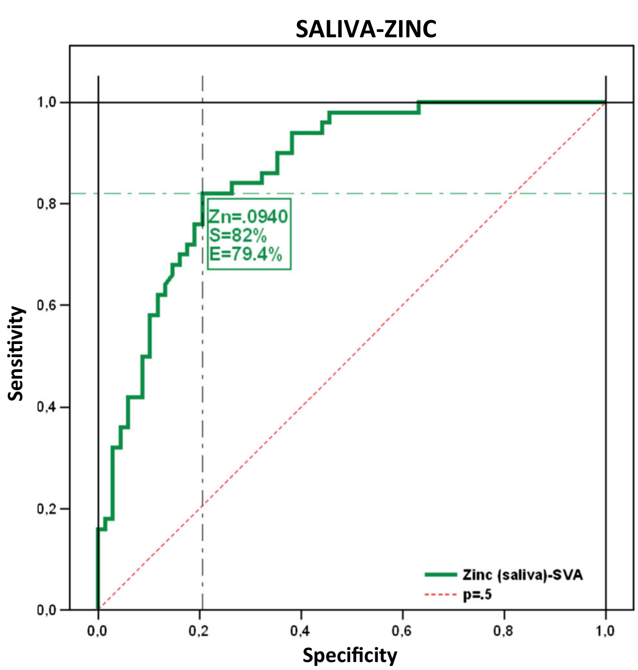


**Fig. 1** ROC curve of dependent variable "diabetic group" in relation to salivary zinc across total sample

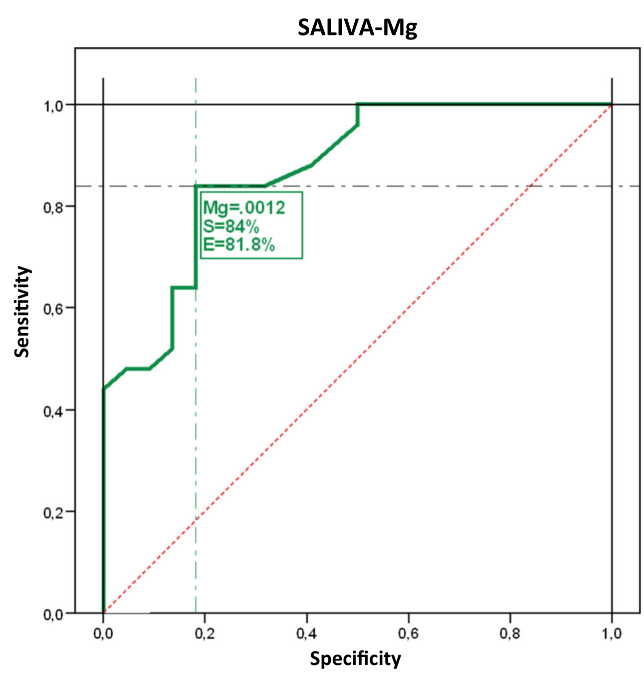


**Fig. 2** ROC curve of waist circumference in relation to salivary magnesium across total sample

differences relate to the disease's physiopathology and metabolic management [8, 9]. Nevertheless, the present results do suggest that salivary zinc levels could act as a good marker of type 2 diabetes mellitus, in light of zinc's well-known role as a co-marker of insulin and its relationship to carbohydrate metabolism. Changes in body zinc status have been associated with insulin resistance, intolerance to carbohydrates, diabetes mellitus, and cardiovascular disease [22–24].

The present study also found that calcium levels could be used to discern between individuals with type 2 diabetes mellitus and healthy subjects. Authors such as Pittas et al. [25] have already established a relation between calcium and type 2 diabetes mellitus. They suggested that alterations in the homeostasis of calcium and vitamin D could play an important role in the development of type 2 diabetes mellitus. Calcium is essential to insulin mediation in intracellular processes and insulin response in tissues. Changes in calcium concentrations could contribute to peripheral insulin resistance and increase the risk of developing type 2 diabetes mellitus. As a consequence, low calcium ingestion has been associated with the incidence of type 2 diabetes mellitus and metabolic syndrome, whereby patients with diabetes present lower calcium levels than healthy subjects [25]. In this context, the present calcium results point to the potential role of salivary calcium as a marker of type 2 diabetes mellitus.

Some trace elements—magnesium, zinc or calcium—show a special association with diabetes and cardiovascular risk [22–29]. For this reason, attempts have been made to clarify the relationship between trace elements and obesity. A recent literature review looked into zinc, copper, iron, calcium, and selenium as elements involved in the evolution of obesity. Other nutrients such as vitamin D also appear to be related to obesity [30].

Chrome is an essential nutrient that boosts insulin action: it has an effect on carbohydrate metabolism, lipids and proteins. Therefore, it could be used as a complement in order to ease weight loss and to improve blood glucose control on type 2 diabetic people [3–6].

Cu was a crucial component of a variety of metalloenzymes and plays an important role in the redox reaction. Our results were in accordance with Badran et al. [31]. We found that serum Cu increased statistically in type 2 diabetes mellitus.

With regard to magnesium in saliva and its relationship with type 2 diabetes mellitus, it is known that magnesium in blood is a cofactor in many of the organism's reactions relating to carbohydrate metabolism and glycemic control. In fact, hypomagnesemia has been associated with prediabetic states, insulin resistance, type 2 diabetes mellitus, and even the progression of chronic complications associated with diabetes [26, 27]. In view of the present results, salivary magnesium levels could be regarded as another marker of type 2 diabetes mellitus.

Magnesium has also been associated with abdominal or visceral obesity. States of low serum magnesium have been associated with increased chronic inflammatory activity and oxidative stress, found in patients with abdominal obesity who usually present a magnesium deficiency. This deficiency has also been associated with other chronic inflammatory diseases such as arterial hypertension, artherosclerosis, diabetes mellitus, or cancer [32–34]. The results obtained in the present study suggest that salivary magnesium could be used as a marker of high cardiovascular risk when associated with abdominal obesity represented by a waist circumference ≥ 102 cm in men. In the case of women, no significant relation was found between trace elements in saliva and waist circumferences ≥ 88 cm, considered at cardiovascular risk, but this could be related to the low incidence of abdominal obesity among the women in the sample.

The present work suffered some limitations derived from its status as a transversal study, and from the possible risk of bias in the selection of patients as these were drawn from patients attending a hospital, a fact that makes it difficult to extrapolate the findings to other populations. Another possible source of bias derives from the influence on saliva composition of the possibly varying oral health status of each individual [35].

Few studies have assessed the levels of trace elements in saliva that can be considered to constitute a normal range, a fact that explains the wide variability between different studies. Nevertheless, there are clear differences between salivary levels of trace elements between individuals with type 2 diabetes mellitus and non-diabetics.

## Conclusions

Trace elements are essential for the organism. The present study identified salivary magnesium, zinc, and calcium levels as good markers for differentiating between individuals with type 2 diabetes mellitus and non-diabetics. The salivary magnesium could be used as a marker of high cardiovascular risk when associated with abdominal obesity represented by a waist circumference ≥ 102 cm in men.

More studies are needed to look into the mechanisms by which mineral metabolism is related to carbohydrate metabolism and cardiovascular risk. This could identify biomarkers that would facilitate early detection of diabetes and cardiovascular disease, as well as possible therapeutic approaches based on trace elements.

### Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no competing interests.

**Ethical Approval** The study protocol was approved by the Ethics Committee of University Of Murcia (Spain) (16/10/2014) and all procedures performed in this study were in accordance with the ethical standards of the institutional research committee.

This study was carried out according to the principles of the Declaration of Helsinki, 2013. Informed consent was obtained from all individual participants included in the study.

## References


1. Strimbu K, Tavel JA (2010) What are biomarkers? Curr Opin HIV AIDS 5:463–466. 9. https://doi.org/10.1097/COH.0b013e32833ed177
2. Desai GS, Mathews ST (2014) Saliva as a non-invasive diagnostic tool for inflammation and insulin-resistance. World J Diabetes 5: 730–738. https://doi.org/10.4239/wjd.v5.i6.730
3. Srinivasan M, Blackburn C, Mohamed M, Sivagami AV, Blum J (2015) Literature-based discovery of salivary biomarkers for type 2 diabetes mellitus. Biomark Insights 10:39–45. https://doi.org/10.4137/BMI.S22177.
4. Zhang CZ, Cheng XQ, Li JY, Zhang P, Yi P, Xin X, Zhou XD (2016) Saliva in the diagnosis of diseases. Int J Oral Sci 8:133–137. https://doi.org/10.1038/ijos.2016.38
5. Walter RM, Uriu-Hare JY, Lewis Olin K, Oster MH, Anawalt BD, Critchfield JW, Keen CL (1991) Copper, zinc, manganese, and magnesium status and complications of diabetes mellitus. Diabetes Care 14:1050–1056. https://doi.org/10.1080/03630260701727077.
6. Zheng Y, Li XK, Wang Y, Cai L (2008) The role of zinc, copper and Iron in the pathogenesis of diabetes and diabetic complications: therapeutic effects by chelators. Hemoglobin 32:135–145. https://doi.org/10.1080/03630260701727077
7. Norouzi S, Adulcikas J, Sohal SS, Myers S (2017) Zinc transporters and insulin resistance: therapeutic implications for type 2 diabetes and metabolic disease. J Biomed Sci 24(1):87. https://doi.org/10.1186/s12929-017-0394-0
8. Mata AD, Marqués D, Rocha S, Francisco H, Santos C, Mesquita MF, Singh J (2004) Effects of diabetes mellitus on salivary secretion and its composition in the human. Mol Cell Biochem 261(1–2): 137–142
9. Lasisi TJ, Fasanmade AA (2012) Salivary flow and composition in diabetic and nondiabetic subjects. Niger J Physiol Sci 27:79–78
10. American Diabetes Association (2014) Diagnosis and classification of diabetes mellitus. Diabetes Care 37:81–90. https://doi.org/10.2337/dc14-S081
11. Goday A (2002) Epidemiología de la diabetes y sus complicaciones no coronarias. Rev Esp Cardiol 55:657–670
12. Bosch X, Alfonso F, Bermejo (2002) Diabetes y enfermedad cardiovascular. Una mirada hacia la nueva epidemia del siglo XXI. Rev Esp Cardiol 55:525–527
13. Soriano Perera P, De Pablos Velasco PL (2007) Epidemiología de la diabetes mellitus. Endocrinol Nutr 54:2–7
14. Mafong D, Henry R (2008) Exenatide as a treatment for diabetes and obesity: implications for cardiovascular risk reduction. Curr Atheroscler Rep 10:55–60
15. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH (2006) Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. Arterioscler Thromb Vasc Biol 26(5):968–976. https://doi.org/10.1161/01.ATV.0000216787.85457.f3
16. Carda C, Mosquera-Lloreda N, Salom L, Gómez de Ferraris ME, Peydró A (2006) Structural and functional salivary disorders in type 2 diabetic patients. Med Oral Patol Oral Cir Bucal 11:309–314
17. Borges BC, Fulco GM, Souza AJ, De Lima KC (2010) Xerostomia and hyposalivation: a preliminary report of their prevalence and associated factors in Brazilian elderly diabetic patients. Oral Health Prev Dent 8(2):153–158
18. Aitken-Saavedra J, Rojas-Alcayaga G, Maturana-Ramírez A, Escobar-Álvarez A, Cortés-Coloma A, Reyes-Rojas M, Viera-Sapiain V, Villablanca-Martínez C, Morales-Bozo I (2015) Salivary gland dysfunction markers in type 2 diabetes mellitus patients. J Clin Exp Dent 7:501–505
19. López-Pintor RM, Casañas E, González-Serrano J, Serrano J, Ramírez L, de Arriba L, Hernández G (2016) Xerostomia, hyposalivation, and salivary flow in diabetes patients. J Diabetes Res 2016:1–15. https://doi.org/10.1155/2016/4372852
20. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001) Executive Summary of 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). JAMA 285(19):2486–2497
21. Panchbhai AS, Deqwekar SS, Bhowte RR (2010) Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India. J Oral Sci 52:359–368
22. Wolide AD, Zawdie B, Alemayehu T, Tadesse S (2017) Association of trace metal elements with lipid profiles in type 2 diabetes mellitus patients: a cross sectional study. BMC Endocr Disord 17(1):64. https://doi.org/10.1186/s12902-017-0217-z
23. Ranasinghe P, Wathurapatha WS, Ishara MH, Jayawardana R, Galappatthy P, Katulanda P, Constantine GR (2015) Effects of zinc supplementation on serum lipids: a systematic review and meta-analysis. Nutr Metab (Lond) 12:26. https://doi.org/10.1186/s12986-015-0023-4
24. Jansen J, Karges W, Rink L (2009) Zinc and diabetes. Clinical links and molecular mechanisms. J Nutr Biochem 20:399–417. https://doi.org/10.1016/j.jnutbio.2009.01.009
25. Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, Hu FB (2006) Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care 29:650–656
26. Mooren FC (2015) Magnesium and disturbances in carbohydrate metabolism. Diabetes Obes Metab 17:813–823. https://doi.org/10.1111/dom.12492
27. Bertinato J, Wang KC, Hayward S (2017) Serum magnesium concentrations in the Canadian population and associations with diabetes, glycemic regulation, and insulin resistance. Nutrients 9:1–13. https://doi.org/10.3390/nu9030296
28. Simić A, Hansen AF, Åsvold BO, Romundstad PR, Midthjell K, Syversen T, Flaten TP (2017) Trace element status in patients with type 2 diabetes in Norway: the HUNT3 survey. J Trace Elem Med Biol 41:91–98. https://doi.org/10.1016/j.jtemb.2017.03.001
29. Navas-Acien A, Guallar E, Silbergeld EK, Rothenberg S (2007) Lead exposure and cardiovascular disease: a systematic review. Environ Health Perspect 115:472–482. https://doi.org/10.1289/ehp.9785
30. Karatela S, Paterson J, Ward NI (2017) Trace elements and human obesity: an overview. J Trace Elem Med Biol 41:10–15. https://doi.org/10.1016/j.jtemb.2017.01.003
31. Badran M, Morsy R, Soliman H, Elnimr T (2016) Assessment of trace elements levels in patients with type 2 diabetes using multivariate statistical analysis. J Trace Elem Med Biol 33:114–119. https://doi.org/10.1016/j.jtemb.2015.10.006
32. Laires MJ, Moreira H, Monteiro CP, Sardinha L, Limão F, Veiga L, Gonçalves A, Ferreira A, Bicho M (2004) Magnesium, insulin resistance and body composition in healthy postmenopausal women. J Am Coll Nutr 23:510–513

33. King DE, Mainous AG, Geesey ME, Woolson RF (2005) Dietary magnesium and C reactive protein levels. J Am Coll Nutr 24:166–171
34. Nielsen FH (2010) Magnesium, inflammation, and obesity in chronic disease. Nutr Rev 68(6):333–340. https://doi.org/10.1111/j.1753-4887.2010.00293.x
35. Tóthová L, Kamodyová N, Červenka T, Celec P (2015) Salivary markers of oxidative stress in oral diseases. Front Cell Infect Microbiol 5:73. https://doi.org/10.3389/fcimb.2015.00073