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Assessment of minerals in biological fluids in people with obesity: A pilot study

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ABSTRACT

Background and Aim: Obesity is a multifactorial disease that can lead to nutritional metabolic disorders, in which the general state of trace elements and minerals are altered. Thus, research involving biological fluids to assess the metabolic nutritional status of minerals Manganese (Mn); Selenium (Se); Strontium (Sr); Zinc (Zn); Molybdenum (Mo); Copper (Cu); Iron (Fe); Calcium (Ca); Magnesium (Mg) and Chromium (Cr) are essential in people with obesity. Therefore, the aim of this study was to evaluate the concentrations of these trace elements and minerals in different biological fluids (plasma, erythrocytes, saliva, urine and tear) from people with and without obesity.
Methods: The experimental sample consisted of 28 volunteers divided into two groups: Obesity Group (OG, $n = 14$) and Non-Obese (NO, $n = 14$), who were clinically evaluated by anthropometry, body composition and biochemical tests. Minerals were assessed in different biological fluids (plasma, erythrocyte, saliva, urine and tear) using the ICP-MS methodology. A significance level of 5% ($p < 0.05$) was considered. Data were shown as mean ± standard deviation.
Results: Anthropometric evaluation, body composition and LDL-cholesterol were significantly higher in the OG ($p < 0.05$), as expected. Plasma minerals were significantly lower in OG than the NO (Se = 184±102 vs 229±83 μg/L ($p= = 0.027$); Fe= 578±346 vs 1099±529 μg/L ($p = 0.005$), respectively). Erythrocyte and salivary minerals were significantly higher in, in OG than the NO, (erythrocyte Mg = 5.8 ± 0.8 vs 5.0 ± 0.7 mg/dL ($p = 0.041$) and salivary Ca = 2.7 ± 0.9 vs 1.8 ± 1.0 mg/dL ($p = 0.027$), respectively). Furthermore, urine minerals were significantly lower in OG than the NO, (Se = 17.4 ± 5.6 vs 26.0 ± 12.7 μg/L ($p = 0.034$) and Mo = 27.0 ± 14.3 vs 52±39.2 μg/L ($p = 0.041$), respectively). Regarding tears, there was no difference between groups ($p > 0.05$).
Conclusion: Our study provides a better understanding of minerals concentrations in different biological fluids in people with obesity. Besides, this study may contribute to future identification of potential minerals biomarkers. However, considering our study's major limitation was its small sample size, caution should be taken when interpreting the results.

Abbreviations: WHO, World Health Organization; DM2, type 2 Diabetes mellitus; ICP-MS, inductively coupled plasma mass spectrometry; CAAE, Certificate of Presentation of Ethical Appreciation; OG, obesity group; NO, non-obese; SD, standard deviation; LOD, limit of detection; CI, confidence interval; BMI, body mass index; WC, waist circumference; FFM, fat free mass; FM, fat mass; HDL-cholesterol, high density lipoproteins; LDL-cholesterol, low density lipoproteins; Mn, manganese; Se, selenium; Sr, strontium; Zn, zinc; Mo, molybdenum; Cu, copper; Fe, iron; Ca, calcium; Mg, magnesium; Cr, chromium; Pb, lead; Ba, barium; ROS, reactive oxygen species; GPx, glutathione peroxidase.

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Introduction

Obesity is defined by World Health Organization (WHO) as a multifactorial disease, being a considered worldwide pandemic [1], and this condition can have harmful consequences such as nutritional metabolic disturbance, accumulation of adipose tissue, which harms the subject's health [2]. Micronutrients in people with obesity are explored because of an of excess energy consumption and, therefore, an inadequate intake of trace elements and minerals [3,4]. This imbalance among energy consumption and the intake of trace elements and minerals is known as hidden hunger [4]. Therefore, the main group affected by hidden hunger is the obese population, due to poor food quality intake [5].

Trace elements and minerals requirements vary according to age, sex, metabolic demand and lifestyle [6]. Studies have sought to clarify the relationship between obesity and trace elements and mineral such as Zinc (Zn), Copper (Cu), Manganese (Mn), Selenium (Se), Calcium (Ca), and Iron (Fe) [7,8]. Although some studies have already demonstrated changes in trace elements and minerals in the obese population, the results are still inconclusive, and the variety of fluids (as an example: saliva, tear) analyzed has not been well explored.

Trace elements and minerals biomarkers monitoring through biological fluids is an alternative to identify possible changes in intake, deficiencies, and diseases, in addition to exposure to chemicals [9]. Samples accessible for analysis are plasma, erythrocytes, whole blood, urine, hair, nails and saliva [10–13]. However, few studies have quantified other unconventional fluids such as tears [14–16], and tissues such as adipose tissue [17].

Therefore, the aim of this study was to evaluate the concentrations of minerals (Mn, Se, Sr, Zn, Mo, Cu, Fe, Cr, Ca and Mg) in different biological fluids (plasma, erythrocytes, saliva, urine and tear) in people with and without obesity. We hypothesize that minerals content in people with obesity differ partially from people without obesity. In this pilot study, we hope to provide valuable information by analyzing the content of minerals in a variety of different physiological fluids that have not yet been well explored.

Methods

Study population

Adults aged between 23 and 52 years participated in this study. The total sample consisted of twenty-eight participants, divided into two groups: Obese Group (OG, $n = 14$) and a control group Non-Obese (NO, $n = 14$). For the OG, the Body Mass Index (BMI) was ≥ 30 kg/m^2, and for the NO between 18.5 – 24.9 kg/m^2 [18]. The exclusion criteria were patients with a diagnosis of Non-Communicable Chronic Diseases including Type 2 Diabetes Mellitus (DM2), metabolic syndrome, hypertension, cancer, and chronic debilitating or disabling diseases; ophthalmological diseases (e.g., Sjögren's Syndrome); previous bariatric surgery; pacemaker use; taking supplement containing trace elements and/or minerals and use of medications that influence digestion and/or intestinal absorption. It is important to highlight that during the selection of participants, they were instructed to maintain their usual activities and food intake, so that there would be no influence on the concentration of minerals in biological fluids. Thus, no volunteer was instructed to modify their daily life pattern.

Venous blood samples were collected at a single time point after an 8-hour fast in the morning (7am – 9am). The study was approved by the Ethics Committee of the Clinical Hospital, Ribeirão Preto Medical School, University of São Paulo, Brazil (HCFMRP/USP) (CAAE: 23996619.6.0000.5440).

Anthropometry, body composition and lipid profile

Weight was measured on a digital scale with a maximum capacity of 150 kg and with an accuracy of 0.1 kg. Height was measured in centimeters, using a stadiometer with precision of 0.5 cm. BMI=weight/height^2 (kg/m^2). To measure the waist circumference (WC) (cm), an inextensible millimeter tape with a precision of 0.1 cm was used. Body composition was assessed by single frequency (50 kHz) tetrapolar electrical bioimpedance device Biodynamics®, Model 450 (Biodynamics Corp., Shoreline, Washington, USA). Regarding the FFM adequacy, the following formulas used by other authors were used: for women from OG Jakicic et al. [19]; for men from OG, Sun et al. [20] and finally for the NO, Kyle et al. [21].

Fat mass (FM) was calculated as the difference between total body weight and FFM. The lipid profile was evaluated in the central laboratory of the HCFMRP/USP using the enzymatic method and posteriorly the results were obtained from the medical records.

Sample collection, preparation and analysis

Plasma

For the trace elements and minerals determination in plasma, a trace-free Vacuette® tube with sodium heparin was used. The samples were centrifuged at 2.058 x g for 12 min to separate the plasma, aliquots were pipetted and stored in an RNAase-free Eppendorf® tube (1.5 ml) in a freezer at −80 ˚C until the moment of analysis. The following trace elements were evaluated: Mn, Se, Sr, Zn, Mo, Cu, Fe, and minerals Ca and Mg.

Erythrocytes

After plasma removal, the erythrocyte was washed three times with 10 mL of 0.9% saline solution, and centrifuged at 2058 x g for 12 min, with the supernatant being discarded. After the centrifugation, the erythrocyte aliquots were stored in an RNAase-free Eppendorf® tube (1.5 ml) and kept in a freezer at −80 ˚C until the analysis. The analysis of erythrocytes included a sample of $n = 10$ for the OG and $n = 10$ for the NO. The analysis included 10 samples from each group due to the infeasibility of 8 samples that could not be analyzed. The following trace elements were evaluated: Mn, Se, Sr, Zn, Cu, Mo, and Ca and Mg minerals.

Saliva

For saliva collection, the Salivette® device (Sartedt, Rommelsdorf, Germany) was used. This device contains a cotton wool to stimulate the saliva secretion. The volunteers placed the cotton in their mouths for 2 min, which was soaked in saliva. Subsequently, the Salivette® was centrifuged at 2058 x g, for 10 min, separating the saliva from the cotton, and stored at −80 ˚C [22]. Volunteers were instructed to not consume food, do not drink water, or even to not clean the oral cavity for at least two hours before saliva collection. Before collection, the volunteers rinsed their mouths with 50 ml of ultra-pure water (Milli-q). The following trace elements were evaluated: Mn, Se, Sr, Zn, Cr, Cu, Fe, and Ca and Mg minerals.

Tear

For the tear collection, standardized sterile strips (70 mm 5 mm) were used (Schirmer Test), which is used for diagnosis of dry eye syndrome. The strips (Ophthalmos® Ltda, São Paulo, Brazil) were positioned inside the lower eyelid without anesthetic [23]. The participants remained relaxed with their eyes closed until the strips were filled between 30 mm and 35 mm, which corresponds to approximately 10 µL ± 1.2 µL of tear [14,15]. The strips were removed and placed in 15 ml trace-free Falcon®-type tubes, then 1 ml of 1% nitric acid was added to the test tubes and stored for 24 h at 4 ˚C. Afterward, the liquid was removed and stored at −80 ˚C. In case the volunteer was not able to fill the strips within 10 min (70 mm), after analyzing the trace elements and minerals was performed calculating according to the expected amount [14,15]. For the quantification of tears, the final value of the minerals was considered after subtracting the results generated by the blank material: Sr: 34.4 µg/L; Ca: 638.8 µg/L; Cr: 1.1 µg/L; Fe: 304.5 µg/L; Mn:

Table 1
Limit of Detection (LOD) according to the biological and mineral fluids evaluated.

Sample	Ca	Cr	Cu	Fe	Mg	Mn	Mo	Se	Sr	Zn
Plasma	2.1		0.15	1.30	1.50	0.02	0.005	0.25	0.004	1.8
Erythrocyte	2.5		0.20		1.70	0.05	0.02	0.30	0.006	2.5
Urine	2.0		0.12	1.15		0.01	0.005	0.20	0.008	1.6
Saliva	2.0	0.001	0.10	1.15	1.20	0.01		0.20	0.008	1.5
Tear	2.0	0.002	0.12	1.15	1.20	0.01		0.22	0.008	1.7

Legend:.
Unit of measurement: (µg/L).
LOD: Limit of detection.
Ca: Calcium; Cr: Chromium; Cu: Cooper; Fe: Iron; Mg: Magnesium; Mn: Manganese; Mo: Molybdenum; Se: Selenium; Sr: Strontium; Zn: Zinc.

4.67 µg/L; Cu: 21.4 µg/L; Zn: 72 µg/L; Se: 0.62 µg/L. The following minerals were evaluated: Mn, Se, Sr, Zn, Cr, Cu, Fe, Ca and Mg.

Urine

For urine collection, volunteers collected the first urine of the day (dispensing the first stream) [24,25]. The samples were placed in plastic containers after treatment with 20% nitric acid. After delivery of the urine collected by the volunteer, the aliquots were stored in 2 Eppendorf® tubes (1.5 ml) at a temperature of –80 °C analysis. Two volunteers from the OG were excluded, because urine was stored in an inappropriate pot. The following trace elements were evaluated: Mn, Se, Sr, Zn, Cu, Mo, Fe and mineral Ca.

Analysis of samples

The trace elements and minerals concentrations in plasma, erythrocytes, urine, saliva, and tear were determined by the inductively coupled plasma mass spectrometry method (ICP-MS Perkin Elmer DCR II) according to Batista et al. [26]. For this, 100 μL of the samples were diluted at 1:25 for plasma, 1:50 for erythrocytes, 1:25 for urine in a demineralized polypropylene tube, containing a 0.01% Triton X-100 solution, and 0.5% (v/v) nitric acid. For saliva and tear fluid, the dilution was 200 μL/4 mL HNO_3 2%. Limit of Detection (LOD) (µg/L) are described in Table 1.

Statistical analysis

Statistical analyses were performed with SPSS software (version 24.0, Inc. Chicago.IL). Results are expressed as mean and standard deviation. Categorical variables were analyzed using Fisher's exact test or Pearson's chi-square test and values are expressed in absolute and relative frequency. Numerical variables were submitted to the Shapiro-Wilk test to verify data distribution. Comparison between groups was performed using the T test for independent samples or Mann-Whitney, for parametric and non-parametric data, respectively. Concentrations of minerals below the LOD were replaced by a value equal to the LOD divided by the square root of 2 [27,28]. A significance level of 5% ($p < 0.05$) was considered. For the figure, the GraphPadTM Prism version 9 program (GraphPad Software Inc., La Jolla, CA, USA) was used.

Results

Age, sex, anthropometric data, body composition and biochemical tests are described in Table 2. There was no significant difference between the two groups with respect to age ($p > 0.05$). Pearson's chi-square showed no association between the groups and sex of volunteers. As expected, it was observed some differences in the variables weight, BMI, and WC, in which the OG had higher means compared to the NO ($p < 0.001$). This was taking as appropriated because we want to compare data OG against NO participants with similar age and sex.

Concerning body composition, the OG had higher means of FFM, FM ($p < 0.001$). Both groups had biochemical test values within the normal range for total cholesterol (< 190 mg/dL), HDL-cholesterol (> 40 mg/dL), LDL-cholesterol (< 130 mg/dL) and triglycerides (< 150 mg/dL). However, the OG had lower HDL-cholesterol values when compared to the NO ($p = 0.005$). On the other hand, LDL-cholesterol was increased in the OG ($p = 0.022$).

The results of the comparison of trace elements and minerals in biological fluids between OG and NO with significant differences are shown in Fig. 1. All results of minerals in biological fluids are found in the Table 3.

The results of nine plasma minerals were evaluated. The OG presented lower means of plasma Se (µg/L) and Fe (µg/L) when compared to the NO as follows, respectively: 184±102 vs 229±83 ($p = 0.027$) for Se and 578±346 vs 1099±529 ($p = 0.005$) for Fe **(**Fig. 1**A and B)**.

Regarding erythrocytes, eight minerals were evaluated, but only the mineral Mg (mg/dL) showed a difference between the groups, with the mean of the OG being higher than that of the NO, 5.8 ± 0.8 vs 5.0 ± 0.7 respectively ($p = 0.041$) **(**Fig. 1**C)**.

For the analysis of saliva, among the nine elements evaluated, the OG had a higher mean mineral Ca (mg/dL) than NO, 2.7 ± 0.9 vs 1.8 ± 1.0 respectively ($p = 0.027$) **(**Fig. 1**D)**.

Regarding the analysis of urine, eight elements were evaluated. It was possible to identify lower excretion of Se (µg/L) and Mo (µg/L) in the OG when compared with NO as follows: 17.4 ± 5.6 vs 26.0 ± 12.7 ($p = 0.034$) for Se and 27±14.3 vs 52±39.2 ($p = 0.041$) for Mo respectively **(**Fig. 1**E).**

Finally, regarding the tear fluid, there was no difference between the elements evaluated ($p > 0.05$).

Discussion

This limited, pilot study evaluated the concentrations of 7 trace elements and 2 minerals in different biological fluids in a small group of people with obesity ($n = 14$) compared to non-obese people ($n = 14$). The data collection, anthropometric, FM, and biochemical, shows that OG had significant markers of obese participants. Therefore, it is suggested that the following results may be due to nutritional metabolic disorders associated with obesity. In the present study, as expected, the OG showed increased anthropometric values such as weight, BMI and WC compared to the NO. Similar results were also observed by Morais et al. [29]. It is noteworthy that these results are indicative of abdominal obesity and may be associated with the development of comorbidities such as hypertension and DM2, in addition to being a predictor of dyslipidemia and metabolic syndrome [30]. It was observed that the OG had low HDL-cholesterol levels and higher LDL-cholesterol levels compared to the NO. In agreement with our results, other studies have also found lower HDL levels in obese individuals [31,32].

The OG presented significant data of some trace elements and minerals compared to the NO. These results show that the nutritional status of obesity can impact the values of trace elements and minerals evaluated in different biological samples.

Table 2
Demographic data, anthropometry, body composition and lipid profile of OG and NO.

Variables	OG (n = 14) Mean (SD)	NO (n = 14) Mean (SD)	p-value
Age (years)	34±9	32±7	0.352
Female n (%)	10 (71)	9 (64)	1.000
Weight (kg)	109±23	65±8	<0.001*
Height (cm)	165±10	168±9	0.476
BMI (kg/m^2)	40±7	23±2	<0.001*
WC (cm)	104±16	76±5	<0.001*
FFP (kg)	60±15	34±5	<0.001*
FM (kg)	49±15	30±5	<0.001*
Total cholesterol (mg/dL)	183±35	167±27	0.174
HDL (mg/dL)	42±8	53±11	0.005**
LDL (mg/dL)	120±32	95±21	0.022**
Triglycerides (mg/dL)	106±35	92±35	0.178

Legend:.
OG: Obese Group; NO: Non-Obese;.
n= sample number; SD: standard deviation;.
BMI: Body Mass Index;.
WC: Waist circumference;.
FFM: Fat-Free Mass; FM: Fat mass;.
HDL: High-Density Lipoproteins; LDL: Low-Density Lipoproteins;.
* : $p < 0.001$;.
** $p < 0.05$;.

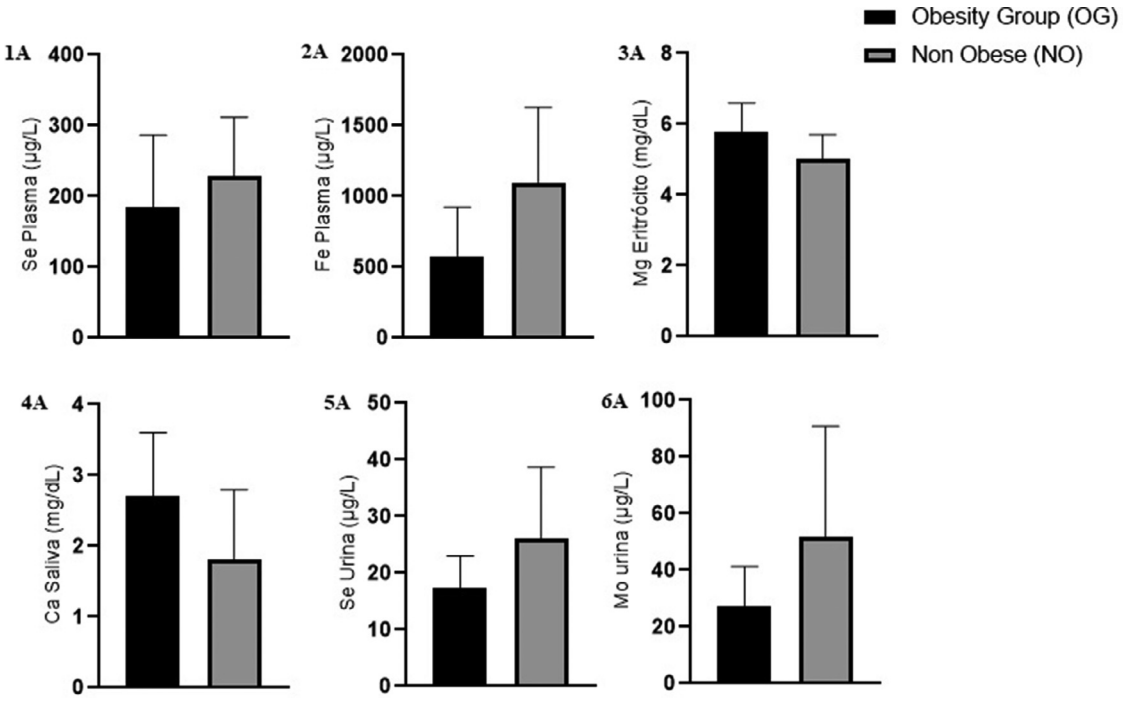

Fig. 1. Significant concentration of trace elements and minerals in the Obese Group (OG) and Non-Obese (NO) of biological fluids (plasma, erythrocytes, saliva and urine).
* $p < 0.05$. **1A** Se plasma OG (n = 14) 184±102 µg/L and NO (n = 14) 229±83 µg/L (p = 0.027);**1B** Fe plasma OG (n = 14) 578±346 µg/L and NO (n = 14) 1099±529 µg/L (p = 0.005); **1C** Mg erythocyte OG (n = 10) 5.8 ± 0.8 mg/dL and NO (n = 10) 5.0 ± 0.7 mg/dL (p = 0.041); **1D** Ca saliva OG (n = 14) 2.7 ± 0.9 mg/dL and NO (n = 14) 1.8 ± 1.0 mg/dL (p = 0.027); **1E** Se urine OG (n = 12) 17.4 ± 5.6 µg/L and NO (n = 14) 26.0 ± 12.7 µg/L (p = 0.034); **1F** Mo urine OG (n = 12) 27.0 ± 14.3 µg/L and NO (n = 14) 52±39.2 µg/L (p = 0.041).

Our results indicated lower plasma concentrations for Se in OG. Previous studies corroborate our findings [32,33,34]. On the other hand, others observed divergent results [24,35]. The low plasma concentrations of Se in the OG compared to NO can be explained by the high condition of oxidative stress and chronic inflammation [36]. The increase in adipose tissue induces oxidative stress and the synthesis of reactive oxygen species (ROS) [37]. Se is an essential mineral that can act as a cofactor for the functioning of the enzyme glutathione peroxidase (GPx) enzyme, so it is believed that the demand for Se in people with obesity may be increased [36].

Lower plasma Fe values in obese people have also been identified in other studies [32,35]. Fe homeostasis depends on the relationship between absorption, utilization and storage of this mineral [38]. The main hormone responsible for this communication is hepcidin which, when elevated, can interfere with its absorption, reducing the availability of Fe, which has been identified in people with obesity [39].

Table 3
Concentration values of minerals evaluated in plasma, erythrocyte, saliva, tear and urine. .

Minerals	Obese Group (OG)		Non-Obese (NO)		*p*-Value
	Mean ± SD	CI (95%)	Mean ± SD	CI (95%)	
Plasma					
Mn (μg/L)	1.5 ± 0.5	1.2–1.8	1.9 ± 1.2	1.2–2.6	0.265
Se (μg/L)	184±102	125–243	229±83	181–276	0.027*
Sr (μg/L)	30.5 ± 8.3	25.7–35.3	31.6 ± 15.8	22.5–40.7	0.769
Zn (μg/L)	872±79	826–918	943±123	872–1014	0.081
Mo (μg/L)	1.7 ± 0.6	1.4–2.0	1.7 ± 0.8	1.3–2.2	0.910
Cu (μg/L)	1262±264	1110–1415	1162±433	912–1412	0.077
Fe (μg/L)	578±346	378–778	1099±529	794–1405	0.005*
Ca (mg/dL)	9.7 ± 0.9	9.2–10.2	10.2 ± 1.5	9.3–11.0	0.363
Mg (mg/dL)	2.1 ± 0.2	2.0–2.2	2.2 ± 0.1	2.1–2.2	0.694
Erythrocyte					
Mn (μg/L)	35.5 ± 77	0.03–90.2	10.0 ± 3.8	7.3–12.8	0.247
Se (μg/L)	73±10.9	65–81	78±17.5	65–90	0.480
Sr (μg/L)	1.3 ± 1.5	0.2–2.3	0.6 ± 1.3	0.004–1.5	0.105
Zn (μg/L)	26821±3776	24120–29523	27414±4100	24482–30347	0.740
Cu (μg/L)	488±39.2	460–516	480±79	423–537	0.775
Mo (μg/L)	1.4 ± 2.0	0.02–2.8	0.4 ± 0.7	0.01–1.0	0.247
Ca (mg/dL)	0.5 ± 0.2	0.4–0.7	0.5 ± 0.1	0.4–0.6	0.529
Mg mg/dL)	5.8 ± 0.8	5.2–6.3	5.0 ± 0.7	4.5–5.5	0.041*
Saliva					
Mn (μg/L)	81±52	50–111	56±49	27.8–84	0.194
Se (μg/L)	14.2 ± 4.4	11.7–16.8	14.0 ± 11.0	7.6–20.3	0.114
Sr (μg/L)	459±269	303–614	297±188	188–405	0.069
Zn (μg/L)	93±178	1.06–195	47.4 ± 48.8	19.2–76	0.482
Cr (μg/L)	23.9 ± 7.2	19.8–28.1	21.2 ± 8.9	16.2–26.4	0.352
Cu (μg/L)	24.6 ± 6.0	21.1–28.1	24.0 ± 10.7	17.8–30.2	0.306
Fe (μg/L)	704±205	586–823	560±167	464–657	0.052
Ca (mg/dL)	2.7 ± 0.9	2.2–3.2	1.8 ± 1.0	1.2–2.3	0.027*
Mg (mg/dL)	1.2 ± 0.4	0.9–1.5	1.0 ± 0.5	0.6–1.3	0.104
Tear					
Mn (μg/L)	1.9 ± 2	0.007–3.9	–	–	–
Se (μg/L)	0.8 ± 0.5	0.5–1.1	0.8 ± 0.3	0.6–1.0	0.616
Sr (μg/L)	15.1 ± 1.7	0.006–30.2	9.7 ± 10.3	0.006–35.5	0.800
Zn (μg/L)	76±61	29.0–123	50±38.6	20.4–80	0.298
Cr (μg/L)	0.8 ± 0.6	0.2–1.4	–	–	–
Cu (μg/L)	10.2 ± 11.1	3.7–16.5	3.8 ± 2.1	0,5–7.0	0.233
Fe (μg/L)	153±211	0.8–315	145±149	7.2–283	1.000
Ca (μg/L)	790±472	205–1377	531±420	142–920	0.338
Mg (μg/L)	355±333	99–611	206±220	58–353	0.331
Urine					
Mn (μg/L)	5.2 ± 9.5	0.007–11.2	1.1 ± 0.5	0.8–1.4	0.494
Se (μg/L)	17.4 ± 5.6	13.8–20.9	26.0 ± 12.7	18.7–33.3	0.034*
Sr (μg/L)	179±108	110–247	135±105	74–196	0.322
Zn (μg/L)	282±141	192 –372	423±306	246–600	0.347
Cu (μg/L)	132±73	85–178	112±47	85–139	0.415
Mo (μg/L)	27.0 ± 14.3	17.8–36.0	52±39.2	29–74	0.041*
Fe (μg/L)	191±91	133–249	217±181	112–322	1.000
Ca (mg/dL)	18.9 ± 13.7	0.2–27.7	17.5 ± 16.9	7.8–27.3	0.595

Legend: OG: Obese Group; NO: Non-Obese; SD: standard deviation; CI: confidence interval; *: $p < 0.05$;.
Plasmatic concentration in OG ($n = 14$) and NO ($n = 14$);.
Erythocyte concentration in OG ($n = 10$ and NO ($n = 10$);.
Saliva concentration in OG ($n = 14$) and NO ($n = 14$);.
Tear concentration: Mn: OG ($n = 06$) and NO ($n = 01$); Se: OG ($n = 13$) and NO ($n = 14$); Sr: OG ($n = 02$) NO ($n = 03$); Zn: OG ($n = 09$) and NO ($n = 09$); Cu: OG ($n = 14$) and NO ($n = 04$); Fe: OG ($n = 09$) NO ($n = 07$); Ca: OG ($n = 05$) and NO ($n = 07$); Mg: OG ($n = 09$) and NO ($n = 11$).
Urine concentration in OG ($n = 12$) and NO ($n = 14$);.

When comparing erythrocyte concentrations, only Mg showed a significant difference between groups. The OG had higher values compared to the NO. Similar results were observed in a study with children and adolescents [40]. Morais et al. [29] found similar values to our data, however, with no difference between groups. Fan et al. [40] noted that increased blood Mg concentration was associated with obesity. The concentration of Mg in erythrocytes is higher when compared to plasma [41]. The increase in erythrocyte Mg in obese people can be explained by its homeostatic mechanism. When this mineral is deficient, other tissues such as muscle and bone will supply Mg to restore its blood level by increasing its concentration [42]. However, further investigations are needed.

In salivary fluid, it was identified that the OG had higher levels of Ca. In agreement with our findings, studies have identified higher salivary

Ca concentrations in individuals with DM2 when compared to healthy controls [43,44]. Changes in Ca concentrations can contribute to peripheral insulin resistance, and salivary Ca is a biomarker that can help identify the risk of developing DM2 [43]. It is worth to consider that none of the obese participants of our sample had DM2.

When comparing the trace elements and minerals concentration in the tear, there was no significant difference between groups. In contrast, Cancarine et al. [14] evaluated people with and without DM2 and found higher values of Zn, Cr, Co, Mn, Ba, and Pb in the DM2 group.

Regarding urine, Soares de Oliveira et al. [33] found higher urinary Se concentrations in obese women, diverging from our findings. In contrast, the findings by Błażewicz et al. [41] corroborate our results by identifying lower urinary Se concentrations in obese children. The authors suggest that the low Se intake, concomitant with the reduction in serum values, may lead to a decrease in the urinary concentration [45], which is in agreement with our findings, since plasma Se was reduced in OG. The relationship of Mo with the pathophysiology of obesity is little explored in the literature. Its main route of excretion is urine, which may reflect the ingestion of this mineral, in which the greater its consumption, the greater its urinary concentration [46]

Although the literature suggests associations of Zn with obesity indicators [40,47,48], we did not observe significant differences for this mineral. Furthermore, significant results found in our study, such as Se, Fe, Mg, and Ca, have also been investigated in the pathophysiology of obese individuals [33,40,47].

To the best of our knowledge, this is the first study to analyze trace elements and minerals in tears in people with obesity and few studies have investigated their concentration in the salivary fluid.

The small sample size as well as the non-assessment of food intake in this study may be a limitation, but it represents a possible data collection for a future investigation and correlation with obesity data patients. Coupled with the very small sample size, this is a pilot study designed to test the feasibility of using multiple body fluid to assess minerals. Also noteworthy is the lack of a creatinine adjustment for urine mineral levels, which might greatly minimize the variability of metal excretion data. Most studies that included the analysis of minerals through saliva and tear fluids evaluated people with DM2 compared to controls. Therefore, data from people with obesity may help to elucidate metabolic nutritional understanding of obesity.

Conclusion

We found significant differences in the trace elements Se, Fe, Mo, and Mg, and Ca minerals between participants with obesity compared to a control group in the analyzed biological fluids. Our study provides a better understanding of the trace elements and minerals concentrations in different biological fluids in people with obesity, as well as assisting in future research on the development and validation of the new biomarkers.

We would like to highlight that this is a pilot study, and for it to be conclusive, it would need to be replicated in a larger study with adequate statistical power. Thus, further investigations with a larger sample size are needed to elucidate the pathophysiological aspects involved in the mechanism of absorption, secretion, and excretion of essential minerals in people with obesity.

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Declaration of Competing Interest

The authors declare no conflict of interests.

CRediT authorship contribution statement

Gizela Pedroso Junqueira: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Márcia Varella Morandi Junqueira-Franco:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Rocio San Martin:** Investigation, Writing – review & editing. **Camila Fernanda Cunha Brandão:** Conceptualization, Writing – review & editing. **Fernando Barbosa Júnior:** Resources, Writing – review & editing. **Eduardo Melani Rocha:** Conceptualization, Methodology. **Julio Sergio Marchini:** Conceptualization, Methodology, Writing – review & editing, Supervision.

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