Journal of Trace Elements in Medicine and Biology 48 (2018) 8–15

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Pathobiochemistry

Serum concentrations of trace elements and their relationships with paraoxonase-1 in morbidly obese women

Fedra Luciano-Mateo[a,1], Noemí Cabré[a,1], Martí Nadal[b], Anabel García-Heredia[a], Gerard Baiges-Gaya[a], Anna Hernández-Aguilera[a], Jordi Camps[a,*], Jorge Joven[a], José Luis Domingo[b]

[a] Unitat de Recerca Biomèdica, Hospital Universitari Sant Joan, Institut d'Investigació Sanitària Pere Virgili, Universitat Rovira i Virgili, Carrer Sant Joan s/n, 43201 Reus, Spain

[b] Laboratory of Toxicology and Environmental Health, School of Medicine, Institut d'Investigació Sanitària Pere Virgili, Universitat Rovira i Virgili, Carrer Sant Llorenç s/n, 43201 Reus, Spain

ARTICLE INFO

Keywords:
Diabetes
Minerals
Obesity
Trace elements
Paraoxonase-1

ABSTRACT

The metabolic alterations associated with obesity include mineral dysregulation. Essential trace elements are nutrients with a relevant function in a large number of cellular processes and multiple roles in the correct functioning of metabolic enzymes. Paraoxonase-1 (PON1) is an antioxidant and anti-inflammatory enzyme that is compromised in obesity. In the present study, the potential alterations in trace elements in morbidly obese women were assessed in relation to serum PON1 activity and concentration, as well as to other obesity-related comorbidities such as diabetes mellitus and fatty liver. We recruited 41 morbidly obese women and 51 control individuals. The serum concentrations of 30 elements, PON1 paraoxonase and lactonase activities, and PON1 concentration were measured. We observed significant alterations in the levels of As, Ba, Cu, Ca, Fe, Mg, Na, Se, Sr, and Zn in obese women; some of them (As, Ca, Cr, Cu, Mg, and Se) being significantly correlated with serum PON1 values. The most relevant changes were observed in the concentrations of As, Sr and Mg, the last of which was also significantly associated with diabetes mellitus. The current results raise the possibility that increased ingestion and/or storage of a number of trace elements may be factors predisposing to obesity-related co-morbidities and metabolic alterations.

1. Introduction

Excessive nutrient intake leads to serious clinical and social problems [1]. Obesity is currently a major health concern worldwide, and involves numerous metabolic alterations [2,3]. Obesity is associated with complications such as type II diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), cardiovascular disease, and metabolic syndrome (MetS) [4]. Mineral dysregulation is also prevalent in obesity. Various trace elements are nutrients that function as essential components or cofactors of metabolic enzymes in a large number of cellular processes [5,6], including energy homeostasis [7]. Increased concentrations of trace elements in the circulation often have toxicological consequences, but their relationships with metabolic alterations in obesity are not well documented. Such relationships can be clinically relevant because excessive food intake, the quality of such foods, and environmental pollution by minerals are factors known to be associated with obesity, hyperglycemia and diabetes [8,9].

Many minerals affect the activity of paraoxonase-1 (PON1) [10], an antioxidant and anti-inflammatory enzyme found in the liver, and in the circulation where it is bound to high-density lipoproteins (HDL) [11]. The original function attributed to PON1 is that of a lactonase *i.e.* lipophilic lactones constitute its primary substrates [12]. This catalytic capacity enables PON1 to degrade lipid peroxides not only within the cell but also in the circulating lipoproteins [13]. Additionally, PON1 has an esterase activity deployed in degrading organophosphate xenobiotics such as paraoxon, phenylacetate and nerve agents [11]. A decrease in serum PON1 activity is related to several non-communicable diseases such as obesity, diabetes, and chronic liver disease [14,15]. However,

Abbreviations: BMI, body mass index; DTNB, dithio-bis-2-nitrobenzoic acid; HDL, high-density lipoproteins; HOMA-IR, homeostatic model assessment-insulin resistance index; ICP-MS, inductively coupled plasma mass spectrometry; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; PCA, principal component analysis; PON1, paraoxonase-1; T2DM, type II diabetes mellitus; TBBL, 5-thiobutyl butyrolactone; VIP, variable importance in projection

* Corresponding author.
E-mail address: jcamps@grupsagessa.com (J. Camps).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jtemb.2018.02.023
Received 15 December 2017; Received in revised form 7 February 2018; Accepted 26 February 2018

the interactions between trace elements, PON1 and obesity, as well as the metabolic consequences have not been extensively investigated, to date. The aims of the present study were to assess potential alterations in serum concentrations of several trace elements in women with morbid obesity and to seek relationships with serum PON1 activity and concentration, as well as T2DM and NAFLD.

2. Subjects and methods

2.1. Subjects

We recruited 42 women from among those receiving attention in the Surgery Department of the *Hospital Universitari de Sant Joan* (Reus, Spain) for the assessment of morbid obesity [body mass index (BMI) ≥ 40 kg/m^2]. All the patients fulfilled the indications for laparoscopic sleeve gastrectomy. We chose to study women because they represent > 70% of the morbidly obese patients attending our Hospital. A blood sample was extracted 2 h pre-surgery. A small portion of the liver for histology examination was obtained during the surgical procedure. Exclusion criteria were age < 25 years, systematic alcohol abuse, hepatic disease of non-metabolic origin, infectious or chronic inflammatory diseases, or cancer. The control group contained 51 healthy women participating in a population-based study conducted in our geographical area. A detailed description of this population has been published recently [16]. The patients were instructed to adopt a very low calorie diet 10 days before surgery. None of the patients or control subjects were taking vitamin or mineral supplements. Venous blood samples were obtained after an overnight fast. Sera were collected after centrifugation, and stored at −80 °C until the day of analysis. The procedures were approved by the Ethics Committee of our Hospital (Institutional Review Board, project code: INFLAMED/15-04-30/4prog7), and written informed consent was obtained from all participants.

2.2. Trace elements

The concentrations of trace elements were determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer Sciex-Elan 6000, Wellesley, MA, USA). Prior to analysis, samples (1.2 mL) were digested with 1 mL of HNO_3 (65% Suprapur, Merck, Darmstadt, Germany) in a Milestone Start D Microwave Digestion System (Sorisole, Italy) for 35 min at 190 °C. After cooling, samples were diluted to final volume of 10 mL with ultrapure water, and kept at −20 °C until batched analysis. The levels of the following 30 elements were analyzed in each extract: Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Sc, Se, Sm, Sn, Sr, Ti, Tl, V, and Zn [17,18]. Blank and reference materials (Seronorm™ Trace Elements Whole Blood L-2, SERO, Billingstad, Norway) were used to monitor the accuracy of the instruments, methods and procedures. The percentages of recovery for the different trace elements ranged between 87 and 139%. The detection limits of the assays (mg/L) were as follows: Al, 0.0385; As, 0,0038; B, 0.0192; Ba, 0.0019; Be, 0.0019; Bi, 0.0010; Ca, 0.9615; Cd, 0.0010; Co, 0.0019; Cr, 0.0041; Cu, 0.0041; Fe, 0.1923; Hg, 0.0039; K, 9.6154; Mg, 1.9230; Mn, 0.0041; Mo, 0.0041; Na, 19.2307; Ni, 0.0096; Pb, 0.0019; Sb, 0.0038; Sc, 0.0192; Se, 0.0384; Sm, 0.0019; Sn, 0.0019; Sr, 0.0038; Ti, 0.0192; Tl, 0.0010; V, 0.0192; Zn, 0.0385.

2.3. PON1-related variables

Serum PON1 paraoxonase activity was determined as the rate of hydrolysis of paraoxon at 410 nm and 37 °C in a 0.05 mM glycine buffer (pH 10.5) with 1 mM $CaCl_2$ [19]. Activities were expressed as U/L (1 U = 1 μmol of paraoxon hydrolyzed per minute). Serum PON1 lactonase activity was measured in an assay reagent containing 1 mmol/L $CaCl_2$, 0.25 mmol/L 5-thiobutyl butyrolactone (TBBL) and 0.5 mmol/L 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) in 0.05 mmol/L Tris-HCl buffer (pH 8.0). The change in absorbance was monitored at 412 nm. Activities were expressed as U/L (1 U = 1 mmol of TBBL hydrolyzed per minute) [20]. Serum PON1 concentration was determined by an in-house ELISA with rabbit polyclonal antibodies generated against the synthetic peptide CRNHQSSYQTRLNALREVQ, which is a sequence specific for mature PON1 [21,22].

Table 1
Clinical characteristics and selected biochemical variables in the obese and control group of participants.

	Control group n = 51	Obese patients n = 42	*P*-value
Demographic and clinical characteristics			
Age, years	28 (22–24)	55 (45–55)	< 0.001
BMI, kg/m^2	21.2 (20.5–22.2)	44.8 (41.8–47.8)	< 0.001
T2DM, n (%)	0 (0)	17 (40.55)	< 0.001
Diabetic neuropathy	0 (0)	0 (0)	–
Hypertension, n (%)	0 (0)	21 (50.0)	< 0.001
Medication, %			
Metformin	0 (0)	15 (35.7)	< 0.001
Insulin	0 (0)	4 (9.5)	0.038
Sulfonylureas	0 (0)	1 (2.4)	0.452
ACEI + ARA-II	0 (0)	14 (33.3)	< 0.001
Diuretics	0 (0)	4 (9.5)	0.038
Statins	0 (0)	11 (26.2)	< 0.001
Biochemical variables			
Total cholesterol, mmol/L	4.8 (4.4–5.2)	4.3 (2.6–5.6)	0.029
HDL-cholesterol, mmol/L	1.7 (1.5–1.9)	1.4 (1.1–1.7)	< 0.002
LDL-cholesterol, mmol/L	2.7 (2.3–3.0)	3.1 (2.5–3.5)	0.054
Triglycerides, mmol/L	0.7 (0.6–1.1)	1.8 (1.0–2.3)	< 0.001
Glucose, mmol/L	4.3 (4.0–4.5)	7.0 (6.1–8.3)	< 0.001
Insulin, pmol/L	38.3 (25.6–53.7)	69.1 (29.7–112.0)	0.001
HOMA-IR	1.01 (0.7–1.5)	2.8 (1.6–4.8)	< 0.001
Albumin, g/L	43.8 (41.5–45.0)	44.0 (42.0–45.0)	0.678
Liver histology			
Steatosis, %	–	21 (50.0)	–
Lobular inflammation, %	–	24 (57.1)	–
Intracellular ballooning, %	–	13 (30.9)	–
NAS score			
< 5	–	25 (83.3)	–
≥5	–	5 (16.7)	–

Values are presented as percentage or median (interquartile range).
ACEI: angiotensin-converting enzyme inhibitors; ARA-II: angiotensin II receptor antagonists; BMI: body mass index; HDL: high-density lipoprotein; HOMA-IR: homeostatic model assessment of insulin resistance; LDL: low-density lipoprotein; LDH: lactate dehydrogenase; T2DM: type 2 diabetes mellitus

2.4. Other biochemical variables

Serum cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, albumin, and insulin concentrations were analyzed by standard tests in a Roche Modular Analytics P800 system (Roche Diagnostics, Basel, Switzerland). Low-density lipoprotein (LDL) concentration was estimated by the Friedewald formula [23]. The homeostatic model assessment-insulin resistance index (HOMA-IR) was calculated as described [24].

2.5. Histology evaluations

Histological alterations in the liver biopsies were assessed in sections stained with hematoxylin and eosin. The degree of steatosis was measured using an image analysis software and was expressed as percentage (AnalySIS image software system, Soft Imaging System, Munster, Germany). Patients were considered free of steatosis when values were < 5% [25,26]. The NAFLD Activity Score (NAS) was employed to estimate the presence of non-alcoholic steatohepatitis (NASH). This score is the unweighted sum of the separate scores for steatosis (0–3), hepatocellular ballooning (0–2) and lobular inflammation (0–3). Patients with a NAS score of ≥5 were considered as likely to