



Urinary excretion of sodium and adherence to the Mediterranean  
diet in the Portuguese elderly: the Nutrition UP 65 Study

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Excreção urinária de sódio e aderência à dieta Mediterrânico nos idosos portugueses: o Estudo Nutrition UP 65

Urinary excretion of sodium and adherence to the Mediterranean diet in the Portuguese elderly: the Nutrition UP 65 Study

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## Dedication

*“My mission in life is not merely to survive, but to thrive;  
and to do so with some passion, some compassion,  
some humor, and some style.”*

- Maya Angelou.

*“We know what we are,  
but know not what we may be.”*

- William Shakespeare.

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To my advisors, for the time, patience and guidance.

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To my cousins, for the childhood play.

To my family, for being a home, safe and sound.

To my best friends, for the unending support.

To my friends, for the adventures.

To the friendships I made along the way, for the million little things.

To my dreams, whom I hope to one day be brave enough to chase.

## **Resumo**

**Introdução:** A Dieta Mediterrânea (DM) tem sido recomendada como preventiva para o desenvolvimento de diversas doenças, incluindo a hipertensão no entanto, ainda não está claro como este padrão alimentar se relaciona com a ingestão de sódio.

**Objetivo:** Quantificar a excreção de sódio, caracterizar a adesão à DM, e avaliar a associação entre estas duas condições em idosos.

**Metodologia:** Estudo observacional transversal conduzido em 2015-2016. Utilizou-se uma abordagem de amostragem por *clusters*, representando os idosos portugueses ( $\geq 65$  anos) de acordo com a idade, sexo, educação e região dentro do estudo Nutrition UP 65. De uma amostra de 1500 participantes, 1312 foram elegíveis para a presente análise. Uma amostra de urina de 24h foi recolhida para cada participante e analisada para creatinina e sódio. Ingestão excessiva de sódio foi definida como superior a 2000 mg/dia, que é o limite segundo a OMS. A adesão à DM foi avaliada pelo questionário PREDIMED. Um modelo de regressão logística binária multivariado foi conduzido para avaliar a associação entre a excreção urinária de sódio e a adesão à DM. OR e respetivos 95% CI foram calculados.

**Resultados & Conclusões:** Excreção excessiva de sódio foi observada em 80.0% dos homens e 91.5% das mulheres, sendo que uma boa adesão à DM foi relatada por 42.2% das mulheres e 46.4% dos homens. Após o ajuste para potenciais confundidores, excreção excessiva de sódio foi associada com uma boa adesão à DM em homens (OR=1.94, 95% CI: 1.03-3.65). Tal associação significativa não foi encontrada em mulheres. Estes resultados destacam a necessidade de implementação de estratégias para reduzir a ingestão de sódio, mesmo quando a seguir um padrão de DM.

**Palavras-Chave:** *sódio, excreção urinária de sódio, sal, dieta Mediterrânea, idosos*

## **Abstract**

**Background:** The Mediterranean Diet (MD) has been recommended as preventative for the development of several diseases, including hypertension, however it remains unclear how this dietary pattern may relate to sodium intake.

**Aim:** We aimed to quantify sodium excretion, to characterize the adherence to the MD, and to evaluate the association between those two conditions in elderly.

**Design:** Cross-sectional observational study conducted in 2015-2016.

**Methods:** A cluster sampling approach was used, representing Portuguese older adults ( $\geq 65$  years) according to age, sex, education and region within the Nutrition UP 65 study. From a sample size of 1500 participants, 1312 were eligible for the present analysis. A 24h urine sample was collected for each participant and analyzed for creatinine and sodium. Excessive sodium intake was defined as above 2000 mg/day which is the limit according to the WHO. The adherence to the MD was assessed by the PREDIMED questionnaire. A multivariable binary logistic regression model was conducted to evaluate the association between urinary sodium excretion and the adherence to the MD. Odds Ratio (OR) and respective 95% Confidence Intervals (95% CI) were calculated.

**Results & Conclusions:** Excessive sodium excretion was observed in 80.0% of men and 91.5% of women whereas a good adherence to the MD was reported by 42.2% of women and 46.4% of men. After adjusting for potential confounders, excessive sodium excretion was associated with a good adherence to the MD in men (OR=1.94, 95% CI: 1.03-3.65). No such a significant association was found in women. These results highlight the need for implementing strategies to reduce sodium intake even when following a MD pattern.

**Key-words:** *sodium, urinary sodium excretion; salt; Mediterranean diet; elderly*

## **Index**

Dedication .....	page III
Acknowledgements .....	page IV
Resumo .....	page V
Abstract .....	page VI
List of Abbreviations .....	page IX
List of Tables .....	page X
Introduction .....	page 1
Aims .....	page 6
Methods .....	page 7
Study Population .....	page 7
Data Collection .....	page 8
Socio-demographic Data .....	page 8
Clinical Data .....	page 9
Anthropometric Assessment .....	page 9
Physical Activity Assessment .....	page 10
Mediterranean Diet Pattern Assessment .....	page 11
Biological Samples .....	page 11
Bioethics .....	page 13
Statistical Analysis .....	page 13
Results .....	page 14
The Portuguese Elderly, Sodium Excretion and Mediterranean Diet Adherence .....	page 14
Association between the Adherence to the Mediterranean Diet and Sodium Excretion .....	page 20

Discussion .....	page 21
Conclusions .....	page 25
Limitations .....	page 26
References .....	page 27

## **List of Abbreviations**

BMI – Body Mass Index

CI – Confidence Intervals

FCNAUP – Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto

FMUP – Faculdade de Medicina da Universidade do Porto

FWR – Free Water Reserve

HGS – Hand Grip Strength

MD – Mediterranean Diet

MMSE – Mini Mental State Examination

MNA – Mini-Nutritional Assessment

OR – Odds Ratio

PREDIMED – PRevención com dieta Mediterránea

UNESCO – United Nations Educational, Scientific and Cultural Organization

Uosm – Urine Osmolality

WHO – World Health Organization

## **List of Tables**

Table 1 – Socio-demographic, anthropometric and lifestyle characteristics of female participants, by sodium excretion and Mediterranean Diet adherence

Table 2 – Socio-demographic, anthropometric and lifestyle characteristics of male participants, by sodium excretion and Mediterranean Diet adherence

Table 3 – Odds ratios for the association between adherence to the Mediterranean Diet and adequacy of sodium excretion in Portuguese elderly

## Introduction

Demographic projections show that the European population will continue to grow old, being estimated that the population of 65 years or more, will suffer an increase of 17% (in 2008), to more than 25% in 2035 and 30% in 2060 <sup>[1]</sup>. More specifically in the Portuguese population, in 2011 19% of the population was  $\geq 65$  years and there was an increase of 18.7% in the older population between 2001 and 2011<sup>[2]</sup>.

These projections are of major concern, because ageing is linked to cognitive and functional decline, emotional changes and depressive symptoms <sup>[3]</sup>, and frail elders living in the community, institutionalized in nursing homes or in hospitals are at increased risk of nutritional disorders <sup>[4]</sup>.

Nowadays, most of the elderly Portuguese population passes through financial constraints, which translates a direct impact on food safety, leading to nutritional implications <sup>[3]</sup>.

The worldwide prevalence of hypertension increased 5.2% from 2000 to 2010, affecting 31% of all adults in 2010 <sup>[5,6]</sup>. In Portugal, the overall prevalence of hypertension in adults was 42.2% although the estimate is much higher among subjects older than 64 years for whom a prevalence of 74.9% was described in 2012 <sup>[7]</sup>.

The World Health Organization (WHO) recommends no more than two grams of sodium per day [equivalent to five grams of salt (sodium chloride)/day] in adults, in order to reduce the burden of non-communicable diseases <sup>[8]</sup>. However, it has been reported consumptions much higher than the recommendations in several countries around the world <sup>[9]</sup>, including Portugal, where a mean intake of 10.7g/day has been reported in 2001/2012 <sup>[7]</sup>.

The "hidden" salt in food represents most of the daily intake of this mineral, being that salt from unprocessed food represents 12% of the total intake, while processed foods contribute with more than 75% <sup>[10]</sup>.

Excessive sodium intake has been repeatedly associated with hypertension <sup>[10-17]</sup>, but it is not the only factor involved, having possible sensitivity to sodium <sup>[11]</sup> and handicapped sodium excretion at renal level <sup>[11,12]</sup> been described in the literature.

High blood pressure is a major risk factor for cardiovascular disease, contributing to almost half of the deaths <sup>[10,13,18]</sup>, promoting vascular abnormalities and rigidity, vasoconstriction and peripheral resistance neurologically mediated <sup>[10,19]</sup>. Recently it was demonstrated that in hypertensive patients the reduction of salt intake for just two weeks is enough to reduce the global cardiovascular risk <sup>[20]</sup>.

Although in the 60's there was a core of countries well distinct from the other European Union countries, with the proximity of a Mediterranean style eating pattern, as the years went by, these countries moved away from each other and the Mediterranean nature dimmed <sup>[21]</sup>.

One of the major problems of the Mediterranean diet (MD) is the difficulty of being set, as more than 15 countries are part of the Mediterranean owning cultures, religions and different eating habits <sup>[22,23]</sup>.

In 1992 the United States Department of Agriculture issued the first Pyramid associated to the Dietary Guidelines for Americans. In relation to it, in 1993, at the Boston Conference on MD, the first Mediterranean Pyramid was presented. It was then copyrighted in 1994 by Oldways, and has since been designed for the population of Greece, Spain and Italy, tailored for their different food habits <sup>[24]</sup>.

Considering the worldwide interest in the MD in the 2009, during the third International University Centre of Studies on Mediterranean Food Cultures Conference held in Parma, a consensus position on a new revised MD Pyramid was defined, moving from the current concept as just a model of healthy eating, to an updated well-being lifestyle concept <sup>[25]</sup>.

The new pyramid reflects the changing process that the MD is undergoing within the Mediterranean societies. This new graphic representation was conceived as a simplified main frame pyramid, to be adapted to the specific realities of different countries (e.g. portion sizes) and variations in the dietary pattern related to the various geographical, socio-economic and cultural contexts of the Mediterranean region <sup>[25-27]</sup>. The new pyramid provides key elements for the selection of foods, both quantitative and qualitative, indicating the relative proportions and consumption frequency of servings of the main food groups of this diet <sup>[25]</sup>.

The graphic representation follows the previous pattern: at the base, food items that should sustain the diet and provide the highest energy intake, and at the upper levels, foods to be eaten in moderate amounts and some of them left for special occasions <sup>[25]</sup>.

More specifically, at the base of the pyramid are the foods that should be consumed in every meal: vegetables and fruit (1-2 portions of vegetables and 1-2 portions of fruit for up to 3 portions per day); cereals (2-3 portions of bread, pasta, rice, couscous, spelt and the others). In the middle of the pyramid there are foods that should be consumed daily: milk and yogurt (2 portions); breakfast products such as cookies, biscuits, breakfast cereals, etc. (1 portion); extra virgin olive oil (2-3 portions); herbs and spices to flavor dishes. At the top of the pyramid, are the foods to be consumed on a weekly basis: food of animal origin such as meat, fish, eggs and cheese, which is recommended to be alternated with pulses (2-3 portions size), preferably in combination with cereals; potatoes (1-2 portions); nuts (3 portions). At the last step of the pyramid are the foods that should be eaten occasionally like sweets (1 portion per week). The wine, should be consumed with moderation and preferably during meals (1 glass per day for women and two glasses per day for men). Every day 1.5-2 liters of water should be guaranteed <sup>[26,27]</sup>.

A balanced composition of the main meals should include fruits, vegetables and cereals, complemented in a lower contribution to daily energy intake with other plant foods, dairy products and protein sources <sup>[25]</sup>.

In according to the Mediterranean model the pyramid takes into account physical activity, being recommended by WHO the practice of at least a 30 minutes per day <sup>[26,27]</sup>.

The traditional MD sought very early on the sustainable production of large quantities of food throughout the year and has always bet on food conservation <sup>[26,28]</sup>. It has a low environmental impact and is healthy for consumers, with a predominantly plant-based feeding, which incorporates a huge biodiversity of seasonal products, while the current Mediterranean dietary patterns are not sustainable <sup>[26]</sup>.

Is summary the MD is essentially characterized by the consumption of fruits, vegetables and legumes, nuts, whole grains, olive oil, fish, fat free dairy products, moderate alcohol consumption (red wine) and low consumption of red meats, refined cereals and sugars <sup>[12,15,17,22,23,29]</sup>.

Diet is believed to play an important role in hypertension, especially one high in sodium and low in potassium content [30].

The high consumption of foods rich in antioxidants (beta-carotene, polyphenols, anthocyanins, glutathione, vitamins C and E), various minerals (such as selenium), fibers, as well as the balanced ratio of essential fatty acids and low saturated fat intake, in combination with regular exercise, has been shown able to decrease the risk of hypertension, cardiovascular diseases and cancer [11,12,17,22,29,31,32].

There is a positive significant association between MD adherence and the mean consumption of potassium, due to the high content on fruits and vegetables, being that this consumption increases with higher MD adherence, in both men and women [33].

The strongest evidence for lowering blood pressure or preventing hypertension through dietary intervention includes adopting a dietary pattern such as the MD, getting plenty of potassium, limiting the amount of sodium in the diet and limiting alcohol consumption [34,35]. It is likely that other dietary factors, such as magnesium and fiber, may affect blood pressure, but the current evidence to support their recommendation is unsubstantiated [34].

In November 16th 2010, the MD was recognized as an Intangible Cultural Heritage of Humanity by UNESCO [25,36], who summarized it the following way: “The Mediterranean diet constitutes a set of skills, knowledge, practices and traditions ranging from the landscape to the table, including the crops, harvesting, fishing, conservation, processing, preparation and, particularly, consumption of food. The MD is characterized by a nutritional model that has remained constant over time and space, consisting mainly of olive oil, cereals, fresh or dried fruit and vegetables, a moderate amount of fish, dairy and meat, and many condiments and spices, all accompanied by wine or infusions, always respecting the beliefs of each community” [36].

In December 4th 2013, Portugal, Cyprus and Croatia would join this group of accession countries and obtain this recognition. This way, the MD was definitely recognized, as a way of life in which physical activity, gathering around the table, the frugality and even way of producing and treating the food should be protected [36].

The first reports of the relationship between the MD and cardiovascular disease date back to the late 60's [22]. A large number of studies relates the MD to lower mortality, as well as a smaller incidence of cardiovascular disease (atherosclerosis, stroke), and certain

types of cancer and to the prevention of the development of hypertension and chronic diseases, such as type 2 diabetes mellitus and dyslipidemia [11-13,15,19,22,23,29,31,32]. Adherence to the MD has also been directly associated with physical activity, total energy intake, alcohol consumption, and educational level and inversely associated with both general obesity and abdominal obesity [37,38].

It was recently built in Portugal the Mediterranean Food Wheel, a graphical representation which shows the Mediterranean foods more related to the Portuguese eating pattern: oils and fats (olive oil/olives); vegetables (onion, garlic, kale, turnip greens, tomatoes, peppers, purslane); fruit (melon, fig, plum, citrus fruit, loquat, pomegranate); cereals and tubers (sweet potatoes, chestnut, pasta and wholegrain rice, oatmeal, rye bread, corn bread); meat, fish and eggs (sardine, horse mackerel, mackerel, tuna); dairy products (yogurt and cheese); legumes [39].

The MD in Portugal is characterized by 10 principles:

1. Frugality and simple cuisine that has in its base cooking that protects nutrients such as soups, casseroles and stews;
2. High consumption of plant products to the detriment of consumption of food products of animal origin, including vegetables, fruit, wholegrain bread and cereals, pulses dried and fresh, nuts and oleaginous fruits;
3. Consumption of plant products produced locally or close by, fresh and in season;
4. Consumption of olive oil as the main source of fat;
5. Moderate consumption of dairy products;
6. Use of herbs for seasoning instead of salt;
7. More frequent consumption of fish compared with low consumption and less frequent of red meats;
8. Low to moderate consumption of wine, and only on main meals;
9. Water as the main drink throughout the day;
10. User friendliness around the table [28,36,40].

Sodium can be hidden in well-known healthy foods (e.g. soups, cereals) [14] and dietary patterns such as M). Despite the recognized health benefits of this dietary pattern, there is a lack of knowledge regarding the association between dietary sodium and MD. Despite all the scientific evidence, the amount of sodium ingested by the Portuguese elderly population was never published.

## **Aims**

We aimed to quantify sodium excretion and to characterize the adhesion to the MD according to sociodemographic characteristics, and to evaluate the association between sodium excretion and the adhesion to the MD the in the Portuguese elderly population.

## Methods

### Study Population

A cross-sectional observational study was conducted in a random stratified cluster sample of 1500 older Portuguese,  $\geq 65$  years old, representative of the older national population in terms of age, sex, education and regional area, as described in a previously published protocol [41].

To achieve such a sample, a quota sampling approach was adopted using data from Census 2011, that shows that the number of Portuguese residents is equal to 10 562 178 and a total of 2 010 064 Portuguese older adults were identified, corresponding to 19% of the Portuguese population [2]. The recruited study sample corresponds to 0.075% of the Portuguese older population.

Inclusion criteria: subjects were eligible to participate in the study if they were aged  $\geq 65$  years; subjects were considered “Portuguese” if they had only Portuguese nationality and if their current tax residence was in Portugal.

In each regional area, three or more town councils with  $>250$  inhabitants were randomly selected.

The sample was composed of community-dwelling older adults and individuals institutionalized in retirement homes [2]. The participant was considered a community-dwelling individual if she/he slept in his own house or in the house of a family member or friend more than half the days of the preceding month.

The potential community-dwelling participants were contacted through home approach, telephone or via institutions, such as town councils and parish centers. Individuals institutionalized in retirement homes were contacted through the institution.

The potential participants were contacted by the interviewer who provided information about the study purposes and the methodology and invited them to participate. A document entitled “Information for the participant” was read by each potential participant or by a surrogate. In case of acceptance, all participants, or two representatives by participant, were asked to read and sign a duplicated “Informed consent” form.

Individuals presenting any condition that precludes the collection of venous blood samples and that precludes the collection of urine, such as dementia or urinary incontinence, were excluded from the study.

### Data Collection

Data was collected between December 2015 and May 2016, in an interview conducted by seven previously trained registered nutritionists.

Demographic data, cognitive performance, current and former professional occupation, lifestyle practices, health status and clinical history, nutritional status, cohabitation and household income, and adherence to a MD pattern were collected using a structured questionnaire.

### **Socio-demographic Data**

Demographic data included information on sex, date of birth, marital status and education, cohabitation and household income.

The applied age categories were 65-69; 70-74; 75-79; 80-84; 85-89;  $\geq 90$  years old [42].

Educational level was determined by the number of completed school years and the following categories were used: <4 years of schooling; first cycle (4 years of schooling); second cycle (6 years of schooling); third cycle (9 years of schooling); secondary (12 years of schooling); post-secondary (>12 years of schooling but no higher education); higher education (academic, vocational and advanced professional education) [42].

The regional areas used are defined in the Nomenclature of Territorial Units for Statistical purposes: Alentejo, Algarve, Azores, Lisbon Metropolitan Area, Center, Madeira and North [43].

## **Clinical Data**

Cognitive performance was assessed by the Portuguese version of the Mini Mental State Examination® (MMSE) [44]. This test examines the functions of orientation, registration, attention and calculation, recall, language and ability to follow simple commands. It consists of 30 questions each scored one point if correct. The cut off scores for cognitive impairment are as follows: individuals with no education,  $\leq 15$  points; 1 to 11 years of years of school completed,  $\leq 22$  points; and  $> 11$  years of school completed,  $\leq 27$  points.

For the individuals identified as presenting cognitive impairment, the “Informed consent” was asked to be signed by two representatives and all data was provided by a person close to the participant, such as a family member or caregiver.

Data on subjective general health was collected using questions withdrawn from the Portuguese National Health Survey 2005-2006. They concern self-reported diagnosis of chronic diseases in the past 12 months, namely the presence of hypertension and also pharmacological treatment and use of nutritional supplements, name and number of daily doses. Current and former tobacco use and consumption of alcoholic beverages were also examined.

## **Anthropometric Assessment**

Detailed information about each participant’s nutritional status encompasses the assessment of the anthropometric measurements that were collected following standard procedures [45].

Mid upper arm, waist and calf circumferences were measured with a metal tape measure from Lufkin with 0.1cm resolution.

Triceps skinfold thickness was obtained using a Holtain Tanner/Whitehouse skinfold calliper with 0.2mm resolution.

Standing height was obtained with a calibrated stadiometer (Seca 213) with 0.1cm resolution. For participants with visible kyphosis or when it was impossible to measure standing height, height was obtained indirectly from non-dominant hand

length (in centimetres) <sup>[46]</sup>, measured with a calibrated paquimeter from Fervi Equipment with 0.1 cm resolution.

Body weight (in kilograms) was measured with a calibrated portable electronic scale (Seca 803) with 0.1kg resolution, with the participants wearing light clothes. When it was not possible to weigh a patient, body weight was estimated from mid-upper arm and calf circumferences <sup>[47]</sup>.

The Portuguese version of the Mini-Nutritional Assessment® (MNA) - Short Form was also applied <sup>[48,49]</sup>, consisting on six questions targeting food intake, weight loss, physical and mental status and anthropometry through body mass index (BMI) assessment. BMI was calculated using the standard formula (weight [in kilograms]/height [in meters]<sup>2</sup>). A participant scoring  $\leq 7$  out of 14 points was classified as undernourished, one that scores between 8 and 11 is at risk of undernutrition and one scoring between 12 and 14 points was considered well-nourished <sup>[48]</sup>.

### **Physical Activity Assessment**

Physical activity was assessed by the short form of the International Physical Activity Questionnaire <sup>[50]</sup>. It gathers information regarding the previous seven days, namely on how many days and how much time the participant spent: walking or hiking (at home or at work, moving from place to place, for recreation or sport); sitting (at a desk, visiting friends, reading, studying or watching television); moderate activities (carrying light objects, hunting, carpentry, gardening, cycling at a normal pace or tennis with two pairs); vigorous activities (lifting heavy objects, agriculture, digging, aerobics, swimming, playing football, cycling at a fast pace).

A standardized algorithm was used to calculate kcals expended per week, which allowed to categorize physical activity level in low or high. The lowest quintile of physical activity was identified for each gender, being that physical activity was considered low for men when kcals per week were  $<383$ , and  $<270$  for women <sup>[51]</sup>.

## **Mediterranean Diet Pattern Assessment**

The adherence to the MD was evaluated with the Portuguese version of PRevenção com dieta Mediterránea (PREDIMED) [52]. This tool tests the effectiveness of the MD on the primary prevention of cardiovascular diseases and consists of 14 questions, each scored with zero or one point. The criteria for assigning one point are established in the questionnaire itself, and a final score  $\geq 10$  indicates a good adherence to the MD.

The PREDIMED study was a multicenter (11 recruiting centers), randomized, controlled, single-blinded 4-year clinical trial that aimed to assess the effects of a Mediterranean-type diet on the risk of major cardiovascular events, conducted in Spain [37,38,53]. It was the first large trial to randomize high-risk individuals to follow either one of two MD (with virgin olive oil or with mixed nuts) or a control diet for primary cardiovascular prevention, in order to support a causal association between adherence to Mediterranean-type diets and cardiovascular risk [53]. Despite being an interventional study, the PREDIMED study provided a unique opportunity for conducting the long-term follow-up of a large observational cohort of high cardiovascular risk subjects in a Mediterranean setting. The pilot study of the PREDIMED trial suggested that a Mediterranean-type diet was a safe strategy to reduce the levels of major cardiovascular risk factors after a 3-month follow-up [38]. This is a valid tool for rapid assessment of adherence to the MD, useful in clinical practice [54].

## **Biological Samples**

Food frequency questionnaires or a 24h recall approach tend to underestimate sodium intake, and since 90% or more of ingested sodium is excreted by the kidneys, the gold standard for assessing sodium intake in populations is 24h urinary sodium excretion [55,56]. This method objectively measures dietary sodium intake from food and from salt added during cooking and at the table [55], and involves administering para-aminobenzoic acid three times during the urine collection which is almost completely excreted in the urine during a 24h urine collection [56].

A sample of blood and the 24h urine was collected for each participant. A certified laboratory, Labco Portugal, was responsible for blood and urine samples collection and analyses.

For the urine collection the interviewers gave the participants oral and written instructions on how to proceed for the collection and storage of the volume of 24h urine. A 24h urine container was also provided.

The following urinary markers were quantified: urine volume (ml); urinary creatinine (mg/day), measured by the Jaffe method (reaction with alkaline picrate) [57]; urine osmolality (mOsm/kg); urinary sodium (originally expressed in mEq/day, it was converted to mg/day by using the molecular weight of sodium (23mg Na = 1mmol Na or 1mEq Na), for comparative purposes); urine density for 24 hours.

Sodium excretion in the urine of 24 hours is recognized as the most rigorous method in determining indirect consumption of salt [19], being validated by the determination of urinary creatinine in order to obtain the best estimate possible of average salt consumption (the sample was considered inadequate if the creatinine level was below 0.4g/24h for women and 0.6g/24h for men) [19,55,58]. The subjects whose urine sample didn't respect the creatinine parameters (<0.4mg/day for females; <0.6mg/day for males) or minimum necessary volume ( $\leq 500$ ml) were excluded from the analysis, therefore we were left with 1321 subjects, verifying a female majority with 766 subjects, and only 555 male subjects.

The hydration status was evaluated by Free Water Reserve (FWR) (ml/24h) [59-64], calculated by subtracting 24h urine volume to obligatory urine volume necessary to excrete the actual 24h urine solutes at the mean 2 standard deviation value of maximum urine osmolality (Uosm) [65]  $[(\text{Solute in urine 24h (mOsm / day)} / (830 - 3.4 \times (\text{age} - 20))]$  allowing the classification of the 24h hydration status (euhydrated vs. hypohydrated subjects or at risk of hypohydration) [60,66].

Positive values of FWR indicate euhydration (between the mean - 2 SD of maximum Uosm and the mean + 2 SD of minimum Uosm), negative values indicate risk of hypohydration (Uosm  $\geq$  mean - 2 SD of maximum Uosm) [60,63,65].

## Bioethics

This research was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association [67]. The study protocol was submitted for approval by the Portuguese National Commission of Data Protection (n.º 9427/2015), and has been approved by an Ethics Committee for health from the Social Sciences and Health department from Faculdade de Medicina da Universidade do Porto (FMUP) (n.º PCEDCSS – FMUP 15/2015). Written informed consent was required to all study participants or their legal guardians.

## Statistical Analysis

A total of 1321 subjects for whom information on sodium excretion and adherence to MD was available were considered for data analysis.

All data were analyzed using Statistical Package for the Social Sciences for Windows, version 24.0.0.0.

The cardinal variables were checked for normal distribution, and since the sample is larger than 30 subjects we used de Z of Kolmogorov-Smirnov test. The data significantly deviate from a normal distribution, therefore this variables are presented as mean (maximum and minimum) and categorical variables as frequencies.

Comparisons of the non-normal distributed variables between groups of study were performed using a nonparametric test for 2 independent samples, in this case the U of Mann-Whitney test (for 2 groups).

Associations between categorical variables were tested using the Pearson's chi squared test.

A multivariable binary logistic regression model was conducted to evaluate the association between urinary sodium excretion and the adherence to the MD. Odds Ratios (OR) and respective 95% Confidence Intervals (CI) were calculated.

A  $p$ -value  $<0.05$  was considered to indicate statistical significance.

## Results

### The Portuguese Elderly, Sodium Excretion and Mediterranean Diet Adhesion

Excessive sodium excretion was observed in 80.0% of men and 91.5% of women whereas a good adhesion to the MD was reported by 42.2% of women and 46.4% of men.

Tables 1 and 2 present the proportion of elderly with adequate/excessive sodium excretion and with good/bad adhesion to MD, according to socio-demographic, anthropometric, lifestyle and health characteristics.

Elderly with excessive sodium excretion were younger than those with adequate sodium excretion (women: 75 vs. 79 years,  $p<0.001$ ; men: 74 vs. 77,  $p=0.003$ ). Compared to very elderly ( $\geq 80$  years), the prevalence of excessive sodium excretion among subjects with 65-79 years was higher both in women (76.7% vs 58.7%,  $p<0.001$ ) and men (88.1% vs 79.4%,  $p=0.013$ ).

Excessive sodium excretion was more common among elderly who lived at home compared to institutionalized subjects, although the difference was only significant among women (73.3% vs. 41.7%,  $p<0.001$ ).

Married participants showed a higher prevalence of excessive sodium excretion when compared to unmarried (women: 81.8% vs. 65.6%,  $p<0.001$ ; men: 89.7% vs. 80.7%,  $p=0.003$ ).

A lower proportion of excessive sodium excretion was observed among participants with lower per capita household income although this association was statistically significant only among women.

High physical activity has been associated with higher sodium excretion in both women and men (women: 73.3% vs. 62.5%,  $p=0.010$ ; men: 87.7% vs. 77.8%,  $p=0.012$ ).

A lower proportion of excessive sodium excretion was found among participants with bad health status perception compared to those with reasonable and good health status perception, although this association was significant only among women.

Finally, males with hypertension had a more excessive excretion of sodium (87.7%) vs those without hypertension (77.4%,  $p=0.009$ ), the same as with hypohydrated or at risk subjects vs euhydrated (92.9% vs 80.5%,  $p<0.001$ ).

No other statistically significant associations were found between sodium excretion and socio-demographic variables.

Regarding the adherence to MD, 44.9% of men and 41.8% of women reported a good adherence.

The adherence to the MD was associated with a lower age among women and higher education in both sexes.

A good adherence to the MD was more frequent among married participants, although the association was statistically significant only among women (49.3% vs. 37.5%,  $p=0.002$ ).

Participants with good health status perception showed a higher proportion of adherence to the MD, compared to those with bad and reasonable health status perception, although the differences were statistically significant only in women.

No other statistically significant associations were found between the adherence to MD and socio-demographic variables.

**Table 1 Socio-demographic, anthropometric and lifestyle characteristics of female participants, by sodium excretion and Mediterranean Diet adherence**

Variable	Sodium Excretion		<i>p</i> <sup>c</sup>	MD Adhesion		<i>p</i> <sup>c</sup>
	Adequate (<2000mg/day) <sup>a</sup>	Excessive (≥2000mg/day)		Bad (<10 points) <sup>b</sup>	Good (≥10 points)	
<b>N</b>	220	546		446	320	
<b>Age (years)</b>						
Mean (min-max)	79 (65-100)	75 (65-93)	<0.001*	76 (65-100)	75 (65-95)	0.002*
Elderly (65-79 years old), n (%)	125 (23.2%)	411 (76.7%)	<0.001**	292 (54.5%)	244 (45.5%)	0.001**
Very Elderly (≥80 years old), n (%)	95 (41.3%)	135 (58.7%)		154 (67.0%)	76 (33.0%)	
<b>Educational level</b>						
No studies, n (%)	49 (36.8%)	84 (63.2%)	0.095**	90 (67.7%)	43 (32.3%)	0.003**
<4 years of schooling, n (%)	55 (31.8%)	118 (68.2%)		109 (63.0%)	64 (37.0%)	
4 years of schooling, n (%)	90 (25.5%)	263 (74.5%)		194 (55.0%)	159 (45.0%)	
5 to 12 years of schooling, n (%)	19 (24.7%)	58 (75.3%)		43 (55.8%)	34 (44.2%)	
>12 years of schooling, n (%)	7 (23.3%)	23 (76.7%)		10 (33.3%)	20 (66.7%)	
<b>Residence</b>						
Home, n (%)	192 (26.7%)	526 (73.3%)	<0.001**	417 (58.1%)	301 (41.9%)	0.750**
Institution, n (%)	28 (58.3%)	20 (41.7%)		29 (60.4%)	19 (39.6%)	
<b>Marital Status</b>						
Married, n (%)	169 (34.4%)	322 (65.6%)	<0.001**	307 (62.5%)	184 (37.5%)	0.002**
Not Married, n (%)	50 (18.2%)	224 (81.8%)		139 (50.7%)	135 (49.3%)	
<b>Professional Occupation</b>						
Non Active, n (%)	217 (28.9%)	535 (71.1%)	0.543**	440 (58.5%)	312 (41.5%)	0.239**
Active, n (%)	3 (21.4%)	11 (78.6%)		6 (42.9%)	8 (57.1%)	
<b>Household Income</b>						
Low (100-500€/month), n (%)	55 (27.9%)	142 (72.1%)	0.019**	118 (59.9%)	79 (40.1%)	0.059**
Medium (>500-1000€/month), n (%)	25 (15.8%)	133 (84.2%)		75 (47.5%)	83 (52.5%)	
High (>1000€/month), n (%)	9 (18.4%)	40 (81.6%)		25 (51.0%)	24 (49.0%)	
<b>Physical Activity</b>						

Low (<270), n (%)	54 (37.5%)	90 (62.5%)	<b>0.010**</b>	89 (61.8%)	55 (38.2%)	0.334**
High (≥270), n (%)	166 (26.7%)	456 (73.3%)		357 (57.4%)	265 (42.6%)	
<b>Health Status Perception</b>						
Bad, n (%)	68 (39.5%)	104 (60.5%)	<b>0.002**</b>	114 (66.3%)	58 (33.7%)	<b>&lt;0.001**</b>
Reasonable, n (%)	96 (25.1%)	287 (74.9%)		242 (63.2%)	141 (36.8%)	
Good, n (%)	55 (26.6%)	152 (73.4%)		86 (41.5%)	121 (58.5%)	
<b>Cognitive Performance</b>						
MMSE Normal, n (%)	139 (26.4%)	388 (73.6%)	0.524**	324 (61.5%)	203 (38.5%)	0.569**
MMSE with Cognitive Impairment, n (%)	8 (21.6%)	29 (78.4%)		21 (56.8%)	16 (43.2%)	
<b>Chronic Diseases</b>						
Hypertensive, n (%)	182 (28.9%)	447 (71.1%)	0.368**	376 (59.8%)	253 (40.2%)	0.120**
Non Hypertensive, n (%)	32 (25.0%)	96 (75.0%)		67 (52.3%)	61 (47.7%)	
<b>Nutritional Status</b>						
MNA Well-nourished (≥12 points), n (%)	156 (25.2%)	464 (74.8%)	<b>&lt;0.001**</b>	349 (56.3%)	271 (43.7%)	<b>0.025**</b>
MNA at Risk and Undernourished (<12 points), n (%)	64 (43.8%)	82 (56.2%)		97 (66.4%)	49 (33.6%)	
<b>BMI</b>						
Normal (<25Kg/m <sup>2</sup> ), n (%)	27 (26.2%)	76 (73.8%)	0.505**	59 (57.3%)	44 (42.7%)	0.326**
Overweight (25-29.9Kg/m <sup>2</sup> ), n (%)	98 (27.7%)	256 (72.3%)		216 (61.0%)	138 (39.0%)	
Obesity (≥30Kg/m <sup>2</sup> ), n (%)	92 (31.2%)	203 (68.8%)		163 (55.3%)	132 (44.7%)	
<b>Hydration Status</b>						
Euhydrated, n (%)	164 (30.7%)	370 (69.3%)	0.065**	305 (57.1%)	229 (42.9%)	0.345**
Hypohydrated or at Risk, n (%)	56 (24.1%)	176 (75.9%)		141 (60.8%)	91 (39.2%)	

\* – U of Mann-Whitney

\*\* – Pearson's X<sup>2</sup>

<sup>a</sup> Sodium excretion was classified as adequate and excessive (i.e. <2000, ≥2000mg/day) by following WHO recommendations.

<sup>b</sup> MD adhesion was classified to good and bad (i.e. ≥10, <10 points on PREDIMED) according to what's specified in the literature.

<sup>c</sup> *p* values were derived through U of Mann-Whitney test for non-normal distributed variables, and Pearson's X<sup>2</sup> for categorical data. *p*<0.05 was considered significant.

**Table 2 Socio-demographic, anthropometric and lifestyle characteristics of male participants, by sodium excretion and Mediterranean Diet adhesion**

Variable	Sodium Excretion		<i>p</i> <sup>c</sup>	MD Adhesion		<i>p</i> <sup>c</sup>
	Adequate (<2000mg/day) <sup>a</sup>	Excessive (≥2000mg/day)		Bad (<10 points) <sup>b</sup>	Good (≥10 points)	
<b>N</b>	77	478		306	249	
<b>Age (years)</b>						
Mean (min-max)	77 (65-92)	74 (65-96)	<b>0.003*</b>	74 (65-96)	76 (65-91)	0.415*
Elderly (65-79 years old), n (%)	51 (11.9%)	378 (88.1%)	<b>0.013**</b>	237 (55.2%)	192 (44.8%)	0.924**
Very Elderly (≥80 years old), n (%)	26 (20.6%)	100 (79.4%)		69 (54.8%)	57 (45.2%)	
<b>Educational level</b>						
No studies, n (%)	8 (15.1%)	45 (84.9%)	0.843**	37 (69.8%)	16 (30.2%)	<b>0.013**</b>
<4 years of schooling, n (%)	12 (16.2%)	62 (83.8%)		42 (56.8%)	32 (43.2%)	
4 years of schooling, n (%)	43 (14.2%)	259 (85.8%)		171 (56.6%)	131 (43.4%)	
5 to 12 years of schooling, n (%)	10 (10.4%)	86 (89.6%)		46 (47.9%)	50 (52.1%)	
>12 years of schooling, n (%)	4 (13.3%)	26 (86.7%)		10 (33.3%)	20 (66.7%)	
<b>Residence</b>						
Home, n (%)	72 (13.4%)	466 (86.6%)	0.060**	299 (55.6%)	239 (44.4%)	0.240**
Institution, n (%)	5 (29.4%)	12 (70.6%)		7 (41.2%)	10 (58.8%)	
<b>Marital Status</b>						
Married, n (%)	43 (19.3%)	180 (80.7%)	<b>0.003**</b>	134 (60.1%)	89 (39.9%)	0.059**
Not Married, n (%)	34 (10.3%)	297 (89.7%)		172 (52.0%)	159 (48.0%)	
<b>Professional Occupation</b>						
Non Active, n (%)	75 (13.8%)	469 (86.2%)	0.676**	302 (55.5%)	242 (44.5%)	0.206**
Active, n (%)	2 (18.2%)	9 (81.8%)		4 (36.4%)	7 (63.6%)	
<b>Household Income</b>						
Low (100-500€/month), n (%)	9 (13.2%)	59 (86.8%)	0.187**	41 (60.3%)	27 (39.7%)	0.101**
Medium (>500-1000€/month), n (%)	13 (10.6%)	110 (89.4%)		62 (50.4%)	61 (49.6%)	
High (>1000€/month), n (%)	3 (4.3%)	66 (95.7%)		29 (42.0%)	40 (58.0%)	
<b>Physical Activity</b>						

Low (<270), n (%)	20 (22.2%)	70 (77.8%)	<b>0.012**</b>	52 (57.8%)	38 (42.2%)	0.582**
High (≥270), n (%)	57 (12.3%)	408 (87.7%)		254 (54.6%)	211 (45.4%)	
<b>Health Status Perception</b>						
Bad, n (%)	15 (20.3%)	59 (79.7%)	0.059**	44 (59.5%)	30 (40.5%)	0.357**
Reasonable, n (%)	27 (10.5%)	231 (89.5%)		147 (57.0%)	111 (43.0%)	
Good, n (%)	35 (15.7%)	188 (84.3%)		115 (51.6%)	108 (48.4%)	
<b>Cognitive Performance</b>						
MMSE Normal, n (%)	46 (12.9%)	310 (87.1%)	0.104**	201 (56.5%)	155 (43.5%)	0.222**
MMSE with Cognitive Impairment, n (%)	8 (22.9%)	27 (77.1%)		16 (45.7%)	19 (54.3%)	
<b>Chronic Diseases</b>						
Hypertensive, n (%)	55 (12.3%)	393 (87.7%)	<b>0.009**</b>	252 (56.3%)	196 (43.8%)	0.413**
Non Hypertensive, n (%)	21 (22.6%)	72 (77.4%)		48 (51.6%)	45 (48.4%)	
<b>Nutritional Status</b>						
MNA Well-nourished (≥12 points), n (%)	65 (13.4%)	421 (86.6%)	0.366**	265 (54.5%)	221 (45.5%)	0.444**
MNA at Risk and Undernourished (<12 points), n (%)	12 (17.4%)	57 (82.6%)		41 (59.4%)	28 (40.6%)	
<b>BMI</b>						
Normal (<25Kg/m <sup>2</sup> ), n (%)	9 (10.2%)	79 (89.8%)	0.488**	55 (62.5%)	33 (37.5%)	0.332**
Overweight (25-29.9Kg/m <sup>2</sup> ), n (%)	35 (15.4%)	193 (84.6%)		122 (53.5%)	106 (46.5%)	
Obesity (≥30Kg/m <sup>2</sup> ), n (%)	33 (14.7%)	191 (85.3%)		122 (54.5%)	102 (45.5%)	
<b>Hydration Status</b>						
Euhydrated, n (%)	59 (19.5%)	243 (80.5%)	<b>&lt;0.001**</b>	162 (53.6%)	140 (46.4%)	0.440**
Hypohydrated or at Risk, n (%)	19 (7.1%)	235 (92.9%)		144 (56.9%)	109 (43.1%)	

\* – U of Mann-Whitney

\*\* – Pearson's X<sup>2</sup>

<sup>a</sup> Sodium excretion was classified as adequate and excessive (i.e. <2000, ≥2000mg/day) by following WHO recommendations.

<sup>b</sup> MD adhesion was classified to good and bad (i.e. ≥10, <10 points on PREDIMED) according to what's specified in the literature.

<sup>c</sup> *p* values were derived through U of Mann-Whitney test for non-normal distributed variables, and Pearson's X<sup>2</sup> for categorical data. *p*<0.05 was considered significant.

Association between the Adherence to the Mediterranean Diet and Sodium Excretion

Table 3 presents the odds ratios for the association between the adherence to the Mediterranean Diet and adequacy of sodium excretion in the studied sample.

After adjusting for potential confounders (age, region, educational level, nutritional status, marital status and PREDIMED) excessive sodium excretion was associated with a good adherence to the MD in men (OR=1.94, 95% CI: 1.03-3.65).

**Table 3 Odds ratios for the association between adherence to the Mediterranean Diet and adequacy of sodium excretion in Portuguese elderly**

	Females		Males	
	Adequate Sodium Excretion	Excessive Sodium Excretion	Adequate Sodium Excretion	Excessive Sodium Excretion
	Reference	OR (95% CI)	Reference	OR (95% CI)
<b>MD (Good Adhesion vs. Bad Adhesion)</b>	1	0.91 (0.62 – 1.34)	1	1.94 (1.03 – 3.65)

OR, odds ratio; 95% CI, 95% confidence interval.

## Discussion

In the present study, in males it was shown that a higher sodium excretion was associated with a higher likelihood of exhibiting good MD adherence, proving our hypothesis that this diet can be source of high sodium content. This high sodium content must most likely come from meal preparation rather than food choices.

A study conducted in Spain also showed that adherence to MD does not seem to be associated with a clinically relevant improvement in health-related quality of life [68].

After considering sodium intake according to age, the older In addition to the results on the association between the adherence to MD and sodium excretion, our study showed a very high prevalence of excessive sodium excretion affecting more than eight out of every ten subjects. This result is in concordance with global data. In 2010, estimated mean intakes of sodium in 181 of 187 countries, exceeded the WHO recommendation of 2 g/day [8,69].

Older subjects presented lower sodium excretion. This could be explained by a bigger concern with health over the years, adding to a higher tendency to pathologies the older a person becomes. Furthermore, increasing age may be associated with higher compliance with sodium intake recommendations, due to an overall decrease in food intake in the later life [70], although age-related-change in taste buds may also drive some elderly to consume more seasonings and less salt [71].

This results may go against what was expected since there is both a decline in glomerular filtration rate and an increased incidence of renal disease with advancing age [72], which sometimes contributes to increased urinary Na losses in the elderly [71,73].

Institutionalized female subjects had more adequate intake of sodium than those who lived at home, which may be explained by the type of meals provided in these institutions, with more careful and nutritionally adapted meals prepared to them. On the opposite, the other group of elderly may have a higher number of meals eaten outside their homes, or have a different type of dietary patterns, with higher intake of processed foods and added salt.

Unmarried participants showed a lower sodium excretion when compared to those who were married. This may happen because either they cook for themselves and are more careful or don't know how to do it, so resume to simpler foods.

Low physical activity was connected to a more adequate sodium excretion than those with a high activity. Physical inactivity is a risk factor for coronary heart disease in the elderly, predisposing to the development of hypertension, adverse lipid and haemostatic profile (exercise training seems to induce renal haemodynamic alterations and stimulates electrolyte excretion), and left ventricular dysfunction <sup>[74,75]</sup>. In addition, diet is related to physical activity, since exercise will result in higher requirements <sup>[69]</sup>.

Some studies have found that urinary elimination of sodium was higher among those with problems of overweight/obesity than among those of normal weight, and normal-weight and overweight people had significantly lower 24h urinary sodium excretion than obese people <sup>[74]</sup>.

Females with lower per capita household income had lower sodium excretion, and those with medium and high household incomes had the higher excretion, which can be explained by an easier access to different food products, some of which may be high in sodium. Radhika et al also found that higher income levels associated with higher salt or sodium intake among south Indian adults <sup>[76]</sup>.

Women with a bad health status perception were the ones who showed a more adequate sodium excretion, which is logical since those who already think their health is damaged will probably be more careful, as opposed to those who feel good about their health and will probably be more carefree.

Studies of dietary habits of lower social economic status groups have emphasized lack of nutrition knowledge <sup>[77]</sup>, lack of cooking skills, lack of motivation <sup>[78,79]</sup>. A lack of nutrition knowledge <sup>[80]</sup>, apathy toward nutrition prevention messages <sup>[81]</sup>, and an erroneous perception of body weight <sup>[82,83]</sup> have all been cited as potential explanations for unhealthy dietary habits among disadvantaged groups <sup>[84]</sup>.

Some studies found that a higher education translates into a higher socioeconomic status, and predicted better dietary adherence, but those findings did not hold for long-term adherence <sup>[85,86]</sup>.

In these study, we observed that female subjects at risk or undernourished showed a more adequate sodium excretion than those well-nourished. This can be explained by institutionalization of undernourished subjects which is usual, guaranteeing that they receive an adapted care, which can also happen at home by the caregivers part.

Finally, men with hypertension showed an excessive sodium excretion, which is in accordance with hat is expected since excessive sodium intake has been repeatedly associated with hypertension <sup>[10-17]</sup>.

When analyzing MD adhesion, more male subjects showed a good MD adhesion than female subjects, which was found by most previous studies <sup>[86-88]</sup>. The exception was a family intervention study <sup>[89]</sup> in which, since mothers generally plan the family meals, were more inclined to set positive eating habits. These findings can lead us to think that spouses and children influence eating habits and meal preparations, leading to this disparate findings <sup>[86,90]</sup>. By the same logic, we verified that married women showed a higher adhesion do MD when compared to unmarried.

Regarding educational level we observed that the subjects without studies were the ones with the largest bad adhesion percentage, and those with the higher level of studies, had the largest good adhesion. This meets previous studies results that a higher educational level, translates in greater nutritional knowledge and greater income, which predicts better dietary adherence, however this results don't appear to be long term <sup>[85,86]</sup>.

The MOLI-SANI study, conducted in an Italian population showed that education, among other socioeconomic factors became largely associated with greater adherence to a MD <sup>[91]</sup>.

Women with a bad health status perception were the ones who showed a lower adhesion, and those with a good perception had the higher adhesion. Women at risk or undernourished showed a lower MD adhesion than those well-nourished, since they probably have worse eating habits, especially nutritional wise. Baseline health status may indicate how much a person values his or her health, and how it can influence peoples eating habits <sup>[86]</sup>, and practicing a nutritional pattern classified as healthy can lead to having a better health perception.

Regardless of this findings it has still been associated with lower mortality among the elderly and should still be promoted in this age group due to the benefits it presents on other health variables <sup>[68]</sup>.

## **Conclusions**

Excessive sodium excretion was observed in 80.0% of men and 91.5% of women whereas a good adherence to the MD was reported by 42.2% of women and 46.4% of men.

After adjusting for potential confounders, excessive sodium excretion was associated with a good adherence to the MD in men (OR=1.94, 95% CI: 1.03-3.65). No such a significant association was found in women.

These results highlight the need for implementing strategies to reduce sodium intake even when following a MD pattern. Nevertheless, MD is still a healthy eating pattern, with several other health benefits, and should therefore still be encouraged. However, special orientation should be given regarding the meal preparation in order to avoid high sodium content particularly to women.

The success of a dietary intervention to induce changes in the overall food pattern should revolve around several strategies, among them training of dietitians, negotiation, goal setting, self-monitoring, length and intensity of intervention and patient motivation.

## **Limitations**

The main limitation of this study was the use of the PREDIMED questionnaire for the evaluation of adherence to the MD, given that it is not validated for the Portuguese population.

Another limitation was the fact that apart from a questionnaire to assess the adherence to the MD, there wasn't any dietary and eating behavior assessment to determine eating habits in general, making the sodium intake evaluation dependent only on the excretion evaluation. This evaluation was performed having in account only a single sample, which may not be representative of usual sodium intake.

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