Estimation of salt intake by 24 h urinary sodium excretion in a representative sample of Spanish adults

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Abstract
The present study reports the Na intake of a representative sample of Spanish young and middle-aged adults aged 18–60 years (n 418, 53·1 % women, selected from the capitals of fifteen provinces and the surrounding semi-urban/rural area), measured with a 24 h urinary Na excretion method. To validate the paper collection of 24 h urine, the correlation between fat-free mass determined by electrical bioimpedance (50·8 (SD 11·3) kg) and that determined via urinary creatinine excretion (51·5 (SD 18·8) kg) was calculated (r 0·633, P<0·001). Urinary Na excretion correlated with systolic and diastolic blood pressure data (r 0·243 and 0·153, respectively). Assuming that all urinary Na (168·0 (SD 78·6) mmol/d) comes from the diet, Na excretion would correspond with a dietary salt intake of 9·8 (SD 4·6) g/d, and it would mean that 88·2 % of the subjects had salt intakes above the recommended 5 g/d. Logistic regression analysis, adjusted for sex, age and BMI, showed male sex (OR 3·678, 95 % CI 2·336, 5·791) and increasing BMI (OR 1·069, 95 % CI 1·009, 1·132) (P<0·001) to be associated with excreting Na intake surveys.

Key words: Sodium: Intake: Urine: Urinary sodium excretion

A high level of salt intake is associated with high blood pressure and a greater risk of stroke and CVD(1–4). It is also directly associated with kidney disease, an increased risk of obesity and osteoporosis, the formation of kidney stones and stomach cancer (of which it is thought to be the main cause)(2,5).

Reducing population salt intake is one of the easiest, more efficient and cost-effective ways to reduce the burden of CVD and save health care costs, and this would result in a major improvement in public health(1,6).

The existing body of scientific evidence has pushed the Spanish Ministry of Health to develop a strategy to reduce population salt intake to the WHO/FAO(7) recommended level (5 g/d at the population level).

The measurement of 24 h urinary Na excretion is considered the ‘gold standard’ method for obtaining data of Na intake in population surveys because of the problems of underestimation of Na intakes based on dietary surveys in most studies(8).

The aim of the present study was to determine the baseline salt intake in the Spanish young and middle-aged adult population, as a first step in preparing public health measures and to enable later monitoring and evaluation of the governmental strategy.

Experimental methods
Study subjects
The study included 196 men and 222 women (total 418) aged 18–60 years (36·4 (SD 11·8) years), selected as a representative sample of the Spanish young and middle-aged adult population.
The sample size was planned, taking into account data provided by the Spanish Intersalt study\(^{29}\), to be representative for each sex, assuming a dropout rate of 25%. The initial sample size required was set at 406 participants. Sampling was performed in fifteen randomly selected provinces (selected with the proviso that the great majority of Spain’s autonomous regions be included), including the capital city of each province and a semi-urban/rural city (randomly chosen). The total number of sampling points was therefore 30. In each sampling point, participants were divided into six subgroups, taking into account their sex (male/female) and age (18–30, 31–44 and 45–60 years).

Individuals with a diagnosis of diabetes, hypertension or renal disease, or who had been prescribed diuretics, were excluded. All selected participants were healthy and lived in their own homes; neither hospitalised people nor those living in institutions were included in the present study.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Ethics Committee of the Faculty of Pharmacy (Universidad Complutense, Madrid, Spain). Written informed consent was obtained from all subjects.

The study populations and the number of participants by sex and age to be included in each place were selected in January and February 2009.

Participants were randomly selected among the residents of each population and were invited to take part in the study via telephone (or in person in some of the rural areas). When a participant was excluded at any site, or when participation was declined, another person of the same sex and age group was contacted. Of the 1835 people spoken to, 492 (26.8%) accepted the invitation to be included in the study. Of these, seventy-four were excluded. The final study sample therefore consisted of 418 participants (53.1% women; 22.8% of the originally contacted sample).

**Recording of health variables**

Information was collected from all participants on health problems, and on the consumption of medications (data required to determine whether the participants met the inclusion criteria), supplements and manufactured dietary foods. Blood pressure was measured in the right arm of seated participants following a 5 min rest period, using an Omron HEM-907XL automated sphygmomanometer (Omron Health Care, Inc., Vernon Hills, IL, USA), which is a valid method to use in the clinical setting\(^{11}\). All readings were taken by a trained technician\(^{11}\).

**Anthropometric information**

Weight and height were determined using a digital electronic balance (Seca Alpha, GmbH & Company, Igeni, France; range 0.1–150 kg, precision 100 g) and a Harpenden digital stadiometer (Pfiffer, Carlstadt, NJ, USA; range 70–205 cm, precision 1 mm), respectively. For both measurements, participants were barefoot and wore only underwear. All data were collected following norms set out by the WHO\(^{12}\). BMI was calculated from the body weight (kg) and height (m\(^2\)) measurements.

Percentage body fat was determined by measuring electrical bioimpedance using an OMRON BF306 Body Fat Monitor (Shimogyo-ku, Kyoto, Japan), which is a valid method to use in the clinical setting\(^{13}\). Using the value for this variable and knowing the subject’s body weight, the fat mass and fat-free mass can be calculated.

\[
\text{Fat mass (kg)} = \text{body fat ()} \times \text{body weight (kg)} / 100, \\
\text{Fat-free mass (kg)} = \text{body weight (kg)} - \text{fat mass (kg)}.
\]

**Urine testing**

Volume, and Na, K and creatinine content of 24 h urine were determined. Urinary Na and K were quantified using an indirect potentiometer with selective solid membranes for each ion, connected to an Olympus AU 5400 autoanalyser (Mishima, Japan)\(^{14}\) (CV = 1.0% for Na and 1.1% for K). Quintiles (Q) of 24 h Na and K urine excretion were calculated:

\[
\text{Na (mmol): } Q_1 = 102.6, \quad Q_2 = 136, \quad Q_3 = 177.4, \quad Q_4 = 235.2, \quad Q_5 = 235.2; \\
\text{K (mmol): } Q_1 = 47, \quad Q_2 = 59, \quad Q_3 = 90, \quad Q_4 = 90.
\]

Creatinine was determined according to the modification of the Jaffé reaction using the same apparatus. The intensity of the colour developed was measured at a wavelength of 520 nm\(^{15}\) (CV = 2.8%). All reagents were supplied by Olympus.

To confirm the proper collection of 24 h urine, the correlation between urinary creatinine and the muscular mass of each subject was taken into account\(^{16}\). Fat-free mass was therefore calculated bearing in mind the creatinine excreted in the 24 h urine using the following equation:

\[
\text{Fat-free mass (kg)} = 0.02908 \times \text{creatinine (mg/dl)} + 7.38\quad^{17}
\]

The results were compared with the fat-free mass results obtained by the electrical bioimpedance method.

**Physical activity**

Participants completed a questionnaire on their usual physical activity\(^{18}\). This information was used to calculate estimated energy expenditure. Participants indicated the length of time spent sleeping, eating, playing sports, etc.
During working days and weekends. An activity coefficient was established for each participant by multiplying the time spent in each activity by established coefficients (19, 20) – 1 for sleeping and resting, 1·5 for very light activities (those that can be done sitting or standing up such as ironing, typing or painting), 2·5 for light activities (e.g. walking), 5 for moderate activities (e.g. playing tennis, skiing and dancing) and 7 for intensive activities (e.g. cutting down trees and playing basketball) – and then dividing them by 24 h.

These data provided two coefficients, one for weekdays and one for weekends. The weekday coefficient was multiplied by 6; the coefficient for Sunday was then added to this and the total was divided by 7. This provided a final activity coefficient for each participant, which was multiplied by the baseline expenditure (19, 20) to provide the theoretical energy expenditure for each participant.

According to the Institute of Medicine (21), four physical activity categories have been established:

- Sedentary: when the physical activity level was 1·0 to <1·4 (twenty-three persons).
- Low active: when the physical activity level was 1·4 to <1·6 (183 persons).
- Active: when the physical activity level was 1·6 to <1·9 (180 persons).
- Very active: when the physical activity level was 1·9 to <2·5 (twenty-nine persons).

**Socio-economic data**

Data were collected from all subjects on their educational level, job and number of people who live in the same house.

**Interviews**

Once the interviewer had contacted the volunteers, explained in detail the work to be undertaken and each participant had given their written consent to participate, three meetings were planned:

- **Day 1**: The following information was collected in this interview: personal data and health information (to confirm the inclusion criteria). Blood pressure was also recorded. The interviewer gave each participant the appropriate containers and instructions for collecting spot morning and 24 h urine samples.

- **Day 2**: The interviewer picked a spot morning urine sample up and reinforced the instructions for the proper collection of the 24 h urine sample (i.e. the collection of all urine during the day following the first urination of the day (discarded), but including the first urination of the day after). The spot urine sample was examined to determine its pH, density and the presence of abnormal substances using Combur Test® reactive strips (Roche Diagnostics, S.L., Barcelona, Spain); participants with abnormal findings were excluded. Anthropometric and activity data were also collected.

- **Day 3**: Participants provided their 24 h urine sample to the interviewers. After measuring the volume, it was divided into three aliquots and stored. Some information on socio-economic data was collected.

In those cases in which the collection of either the spot morning or 24 h urine sample had to be repeated, further meetings were planned.

The same questionnaires were used in all thirty areas and were completed following the same order. All study was performed between January and September 2009.

**Statistical analysis**

Means and standard deviations, and ranges as well as median and interquartile range (p50 (p25–p75)) were calculated for all variables and the normality of the data was checked.

The association between sex and the studied variables was assessed; for quantitative variables, Student's t test was used (or the Mann–Whitney test if the distribution of results was not homogeneous), and for qualitative variables, a χ² test was used.

When the quantitative variables are statistically different between sexes, the significance appears in the mean column if the variable is homogeneous and in the median column if the variable is non-homogeneous.

In order to assess that 24 h urine collection was properly performed, Pearson’s linear correlation coefficients between fat-free mass determined by electrical bioimpedance and that determined by the creatinine content of the 24 h urine were calculated. Likewise, a linear correlation between systolic blood pressure and diastolic blood pressure, and 24 h urinary Na excretion was assessed. Relationships between variables were examined by multiple linear regression, controlling for potential founders (sex, age and BMI). Logistic regression analysis was used to identify risk or protection factors, expressing the OR and the 95% CI.

BMI was analysed according to 24 h urinary excretion of Na and K quintiles.

ANOVA was used to analyse the trends in Na and K excretion by age, BMI and physical activity categories.

All calculations were made using RSIGMA BABEL Software (Horus Hardward, Madrid, Spain). The significance was set at P<0·05.

**Results**

Personal, anthropometric and blood pressure data of the participants are shown in Table 1. Creatinine, Na and K excretion and the Na/K ratio were significantly higher in men than in women. Nevertheless, Na:creatinine and K:creatinine ratios were higher in women than in men (Table 2).
### Table 1. Personal, anthropometric characteristics of the study population by sex and age

(Means values, standard deviations, medians and interquartile ranges or percentages)

<table>
<thead>
<tr>
<th></th>
<th>Total (n 418)</th>
<th>Men (n 196)</th>
<th>Women (n 222)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>sd</td>
<td>Median</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36·4</td>
<td>11·8</td>
<td>36</td>
</tr>
<tr>
<td>Present smokers (%)</td>
<td>22·5</td>
<td>19·9</td>
<td>24·8</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>71·83</td>
<td>14·81</td>
<td>70·6</td>
</tr>
<tr>
<td>Ht (cm)</td>
<td>168·0</td>
<td>9·93</td>
<td>168</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25·33</td>
<td>4·14</td>
<td>24·9</td>
</tr>
<tr>
<td>Overweight (%)</td>
<td>34·2</td>
<td>13·6</td>
<td>34·2</td>
</tr>
<tr>
<td>Obese (%)</td>
<td>29·0</td>
<td>8·1</td>
<td>29·0</td>
</tr>
<tr>
<td>BF (%)</td>
<td>21·0</td>
<td>7·9</td>
<td>21·0</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>50·8</td>
<td>11·3</td>
<td>50·8</td>
</tr>
<tr>
<td>FFM-EBI (kg)</td>
<td>51·5</td>
<td>18·8</td>
<td>51·5</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>116·6</td>
<td>16·4</td>
<td>116</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>73·9</td>
<td>11·2</td>
<td>73·9</td>
</tr>
</tbody>
</table>
| BF, body fat; FM, fat mass; FFM-EBI, fat-free mass obtained by electrical bioimpedance; FFM-C, FFM estimated from 24 h urine creatinine excretion. Values were significantly different with respect to sex: ***$P<0·001$.† (p50 (p25–p75)).

### Table 2. 24 h urine data

(Means values, standard deviations, medians and interquartile ranges)

<table>
<thead>
<tr>
<th></th>
<th>Total (n 418)</th>
<th>Men (n 196)</th>
<th>Women (n 222)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>sd</td>
<td>Median</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>1617·3</td>
<td>694·0</td>
<td>1500</td>
</tr>
<tr>
<td>Creatinine (mmol)</td>
<td>13·42</td>
<td>5·72</td>
<td>12·2</td>
</tr>
<tr>
<td>Na (mmol)</td>
<td>188·0</td>
<td>78·6</td>
<td>154·0</td>
</tr>
<tr>
<td>K (mmol)</td>
<td>77·1</td>
<td>32·3</td>
<td>75·0</td>
</tr>
<tr>
<td>Na:K</td>
<td>2·57</td>
<td>1·29</td>
<td>2·28</td>
</tr>
<tr>
<td>Na:creatinine</td>
<td>13·44</td>
<td>6·60</td>
<td>12·5</td>
</tr>
<tr>
<td>K:creatinine</td>
<td>5·78</td>
<td>2·56</td>
<td>5·25</td>
</tr>
</tbody>
</table>

Values were significantly different with respect to sex: **$P<0·001$, ***$P<0·01$.† (p50 (p25–p75)).
A positive, significant correlation (r 0·633; P<0·001) was found between the fat-free mass determined by electrical bioimpedance (50·8 (sd 11·3) kg) and that determined by the creatinine content of the 24 h urine (51·5 (sd 18·8) kg). No significant differences were observed between these results (Table 1). This shows that the 24 h urine was adequately collected.

Assuming that the Na eliminated in the urine comes from the diet (168·0 (sd 78·6) mmol/d) (Table 2), this excretion would correspond with a dietary salt intake of 9·8 (sd 4·6) g/d in the whole population and of 11·5 (sd 4·8) and 8·4 (sd 3·9) g of salt/d in men and women, respectively. It would mean that 88·2 % of the subjects (92·8 % of men and 84·1 % of women) had intakes of over 5 g of salt (NaCl)/d (85 mmol of Na/d) (maximum recommended)(7).

Systolic blood pressure (r 0·243) and diastolic blood pressure (r 0·153) correlated positively and significantly with 24 h urinary Na excretion. Linear regression analysis showed that systolic blood pressure increased with 24 h urinary Na (β = 0·24 ± 0·06; P<0·001), as did the diastolic blood pressure (β = 0·022 ± 0·007; P<0·01).

Taking into account the influence of sex, age and BMI on the likelihood of excreting >200 mmol Na/d in the urine, logistic regression analysis showed this to be more likely among men (OR 3·678, 95 % CI 2·336, 5·791) and normal-weight and overweight people had significantly lower 24 h urinary Na excretion than obese people (Table 3 and Fig. 1).

K excretion increased with age, and physical activity had no influence on Na or K excretion (Table 3). Na (Table 3 and Fig. 1) and K (Fig. 1) excretion increased with BMI, and normal-weight and overweight people had significantly lower 24 h urinary Na excretion than obese people (Table 3 and Fig. 1).

**Discussion**

There have been very few studies on salt intake in Spain and none has been performed in a sample representative of the Spanish population as a whole. Capita & Alonso-Calleja(22) reported an intake of 8·01 g/d in a population of 20–40-year-olds in León, a result similar to that obtained in the present study. However, other authors report much smaller intakes. For example, Campillo et al.(23) recorded an intake of only 4·93 g/d in 122 elderly subjects from Extremadura, while Schröder et al.(24) reported 5·48 g/d to be the mean intake of 986 people (25–74-year-olds) in Gerona. The subjects in these two studies can be said to have had low Na intakes (perhaps because both populations involved elderly persons); however, it might also be possible that there was notable underestimation of this intake in the dietetic recording these studies have entailed.

A number of problems hinder the accurate estimation of Na intake, especially the fact that subjects can underestimate their intake of Na-containing foods and the amount of extra salt added to their food. Certainly, it is very difficult to know how much salt has been left on the plate, and it can be very hard to determine the salt content in food and drinking-water over time(1). Thus, in agreement with numerous authors(1,8,25), the best way to determine salt intake is probably via the amount excreted in 24 h urine.

If all the salt in the urine comes from the diet, then 88·2 % of the subjects in the present study had intakes over 5 g of salt/d (the maximum recommended intake)(7). Thus, in this study, salt intake was probably via the amount excreted in 24 h urine.

K excretion is probably lower than that of Na. Indeed, in the present study, weak correlations were found between Na excretion and BMI (r 0·633; P<0·001), age (r 0·243; P<0·01), and BMI (r 0·243; P<0·01). The relationship between urinary Na excretion and BMI was stronger in men (r 0·633; P<0·001) than among those of normal weight (r 0·243; P<0·01) and it was stronger among men (r 0·633; P<0·001) than among those with problems of overweight/obesity (r 0·243; P<0·01). It would mean that 88·2 % of the subjects (92·8 % of men and 84·1 % of women) had intakes of over 5 g of salt (NaCl)/d (85 mmol of Na/d) (maximum recommended)(7).

The mean 24 h urinary Na excretion recorded in the present study is similar to the 165·4 mmol/d (9·7 g of salt/d)
recorded in Manresa and to the 175·2 mmol/d (10·3 g of salt/d) recorded in Torrejón, the two Spanish populations included in the Intersalt study(9). It also resembles the 155·0 (sd 62·4) mmol/d (9·12 g of salt/d) reported by Stamler et al.(27) in a study of 10 020 subjects (from fifty-two populations worldwide) aged 20–59 years (also part of the Intersalt Study). In addition, it is similar to values reported from Australia, Finland, the USA and the UK and in a study on white South Africans(26–32). However, these figures are higher than those reported from populations in Cameroon, Ghana and Nigeria, and from the Caribbean nations of Barbados, St Lucia and Jamaica(33,34). They are also higher than those recorded in studies undertaken in Venezuela, Holland and France(3,35). However, adults in China and Japan have urinary Na excretions much higher than those reported in the present study. This is not surprising since salt intake in these countries tends to be higher, mainly owed to the high consumption of soya sauce and the custom of adding a lot of salt to food (both during cooking and at the table)(33,34,36).

The 24 h urinary elimination of K and creatinine observed in the present study was similar to that recorded by other authors(3,34,37).

In the present study, K excretion was lower in the youngest group of the population (Table 3). There is both a decline in glomerular filtration rate and an increased incidence of renal disease with advancing age(38), which may contribute to increased urinary Na and K losses in this population(39). However, the maximum age of the population studied in the present study was 60 years, not being high enough to produce this type of kidney impairment. Thus, lower urinary K excretion observed in the youngest age group (Table 3) could be due to lower consumption of certain foods rich in K, such as pulses, fruits and vegetables. It is a common situation and has been observed in different studies on dietetic habits in young and adolescents(40,41).

As reported in most of the studies, the intake and excretion of Na was higher among men (Table 2), perhaps because of their overall higher food intakes and differences in food habits(3,29,32,42).

In the present study, significantly higher 24 h urinary Na excretion was observed in obese than in normal-weight and overweight people. The higher Na intake and urinary elimination seen in subjects with the higher BMI has also been described by other authors(5,31). Particularly, this finding is in agreement with the results shown in the recent Olivetti Heart Study population(43), where 940 Italian men in the age range of 25–75 years were examined. Previous studies from this group suggested that abnormalities in renal tubular Na handling may occur in overweight and obese individuals(44,45); in particular, in these individuals, enhanced fractional proximal Na reabsorption was detected, possibly leading to extracellular fluid volume expansion and high blood pressure. The Olivetti Heart Study showed that in normal-weight individuals, the physiological regulation of Na reabsorption at the proximal tubule was maintained; that is, the higher the Na intake, the lower its reabsorption at the proximal tubule. In contrast, in overweight/obese individuals, the control of tubular Na handling seemed to be altered, being independent of the total amount of Na ingested(45). In addition, the highest 24 h urinary Na excretion observed in obese people could be due to the poorer food habits of these persons, as well as the use of salt to make food more palatable, favouring a higher food intake. Different studies have demonstrated that, if many foods taste better when flavour enhancers such as monoglutamate sodium are added to them, then there could be a risk of inducing over-consumption of these foods and perhaps over-intake of energy leading to weight gain(46). These findings support the concept that reduction of dietary Na should be particularly effective in overweight/obese individuals as they combine the attitude to consume more salt with a reduced ability to excrete the excess dietary Na.

With respect to physical activity, although exercise training seems to induce renal haemodynamic alterations and stimulates electrolyte excretion(47), in the present study, urine Na and K excretion by very active people was similar to that in the rest of the groups (Table 3). This is due to the fact that none of the participants had significant exercise training and the maximum level of physical activity reported in the present study was not enough to induce renal haemodynamic alterations and stimulate electrolyte excretion.

Given the health problems, such as hypertension(48) and CVD(49), associated with excessive salt intake, it would appear that public health initiatives are needed to reduce its consumption(1,2). These might include engaging with the food industry to reduce the large amount of salt commonly included in processed foods(50); initiatives to limit salt consumption can only be effective if such industry involvement is secured(1,51). Indeed, such a campaign is underway in Spain, promoted by the Spanish Agency for Food and Nutritional Safety; the results of the present study may be useful in its endeavours.

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