University of Porto

Faculty of Nutrition Sciences and Faculty of Sciences

SALT INTAKE BY CHILDREN AND ADOLESCENTS
CONTRIBUTE FOR SALT REDUCTION STRATEGY

CONSUMO DE SAL EM CRIANÇAS E ADOLESCENTES
CONTRIBUTO PARA UMA ESTRATÉGIA DE REDUÇÃO DE CONSUMO DE SAL

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PALAVRAS-CHAVE: ADOLESCENTES; CRIANÇAS; SAL; ALIMENTAÇÃO; EXCREÇÃO URINÁRIA.

KEY WORDS: ADOLESCENTS; CHILDREN; SALT; FOOD; URINARY EXCRETION.

PERSONAL CONTRIBUTION IN RESEARCH WORK IN THIS THESIS

The candidate states that she was responsible for carrying out all work related data collection on the field, analysis and interpretation of the results and writing the papers resultants from study I to V.

Poema da malta das naus

Lancei ao mar um madeiro,
espetei-lhe um pau e um lençol.
Com palpite marinheiro
medi a altura do Sol.

Deu-me o vento de feição,
levou-me ao cabo do mundo.
pelote de vagabundo,
rebotalho de gibão.

Dormi no dorso das vagas,
pasmei na orla das prais
arreneguei, roguei pragas,
mordi peloiros e zagaias.

Chamusquei o pêlo hirsuto,
tive o corpo em chagas vivas,
estalaram-me a gengivas,
apodreci de escorbuto.

Com a mão esquerda benzi-me,
com a direita esganei.
Mil vezes no chão, bati-me,
outras mil me levantei.

Meu riso de dentes podres
ecoou nas sete partidas.
Fundei cidades e vidas,
rompi as arcas e os odres.

Tremi no escuro da selva,
alambique de suores.
Estendi na areia e na relva
mulheres de todas as cores.

Moldei as chaves do mundo
a que outros chamaram seu,
mas quem mergulhou no fundo
do sonho, esse, fui eu.

O meu sabor é diferente.
Provo-me e saibo-me a sal.
Não se nasce impunemente
nas praias de Portugal.

António Gedeão in "Teatro do Mundo", 1958

To my parents, my brothers and Ricardo,
without you nothing made sense.

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Abstract

Purpose: This thesis aims to describe dietary salt intake and to examine potential factors that could help to reduce salt intake. Thus aims to contribute to the development of salt reduction strategy. The specific objectives are to: i) evaluate salt consumption and dietary sources of sodium on a sample of Portuguese adolescents; ii) analyze the impact about salt consumption on hydration status; iii) assess the impact of soup added salt reduction on hedonic perception in children; iv) evaluate knowledge and practices related to added salt in food preparation by food handlers; and v) validate an new method able to quantify sodium in meals based on Ion Selective Electrode (ISE) methodology.

Methods: Data for objectives i) and ii) were collected from adolescents from LabMed Physical Activity Study, which was a 3-yr follow up study with Portuguese children and adolescents aged 12 to 18 years old. A convenience subsample of subjects (n=398) was invited to perform 24h urine collection. Urine collections were performed between 2012 and 2014. After a validation control of 24h urine collection, the final sample consisted of 200 adolescents with both valid urine collection and corresponding dietary recall. In order to answer to objective iii) a sensory analysis was performed and subjects were elderly (n=35) from two public nursing homes in Barcelos city and pre-school children (n=75) from Seia city. Baseline salt content of vegetable soups was previously analyzed by flame photometry and then sensory evaluation tests were performed with a less 30% salt content and a baseline salt content vegetable soup. Sensory description of the two soups was performed by elderly using a visual analogue scale for salt perception and hedonic perception and by children using a five-point facial scale for perceived liking. Data to objective iv) results from analysis of a questionnaire filled out by food handlers that work in a Portuguese catering company. Questionnaire had questions to assess knowledge about adequate intake of salt, major food groups contributing to salt intake, and the relationship between salt consumption and health; and to evaluate concerns about the use of salt, practices and difficulties. To validate the new methodology we execute a set of tests to determine specificity, linearity, precision and accuracy. Comparison of ISE with flame photometry (FP) was also performed using soups and meals from university canteen.

Results: The main results of this work are:

- Mean salt ingestion was above the World Health Organization recommendations. It was 9.5 g/d in boys and 7.8 g/d in girls. Major dietary sources for sodium intake

were cereal and cereal products (41%), meat products (16%) and milk and milk products (11%).

- Nearly one third of subjects were at risk of hypo-hydration. Higher sodium excretion was associated with a better hydration status.
- After 30% added salt reduction in vegetable soup, there were no significant differences in perceived liking by children (p=0.160).
- The majority of food handlers studied recognize the maximum advised level of salt intake and 70.6% agreed with reducing the added salt to meals. The major difficulty for reducing the salt content of the meals was the consumer's opinion (mentioned by 79.4%). Soups (36.4%) and salads (18.2%) were identified as components were could be more easy reduce salt.
- The ISE method is accurate, precise, linear, sensitive and selective, presenting an adequate linear working range to evaluate salt (sodium) content in meals and soups. Comparisons with internal reference method (FP) reveal a good correlation (R^2=0.902) without significant differences (p<0.387).

Conclusions: Adolescents had a high salt and low potassium diet, well far away from World Health Organization recommendations. Healthy eating promotion interventions are needed in order to decrease salt intake. In adolescents studied, vegetable soup, a rich potassium food typical on Mediterranean diet countries, contributed with 10% to total salt intake, and a 30% reduction of added salt during preparation is possible without affect perceived liking in children. Strategy to reduce salt content in foods must contemplate reduction of salt added by food handlers during cooking process and the implementation of a monitoring approach to strengthen regulatory or voluntary policies to reduce the supply of products with high salt content. Salt reduction strategy should consider consumer education to salt reduction and empower food handlers about how to reduce salt during food preparation. ISE method seems to be valid to evaluate salt (sodium) content in meals and soups and shows potential to be transformed into a rapid, portable and reliable device that allows control a large number of food samples.

KEYWORDS: ADOLESCENTS; CHILDREN; SALT; FOOD; URINE EXCRETION.

Resumo

Objetivo: Esta tese pretende avaliar a ingestão de sal e examinar os fatores potenciais que poderão ajudar a reduzir a ingestão de sal. Desta forma pretende contribuir para o desenvolvimento de uma estratégia de redução do consumo de sal. Os objetivos específicos são: i) avaliar o consumo de sal e as fontes alimentares de sódio numa amostra de adolescentes Portugueses; ii) analisar o impacto do consumo de sal no estado de hidratação; iii) avaliar o impacto de uma redução de sal adicionado em sopa na percepção hedónica de crianças; iv) avaliar o conhecimento e práticas dos manipuladores relacionadas com a adição de sal durante a confeção; e v) validar um novo método capaz de quantificar sódio em refeições baseado na metodologia de Eletrodo de Ião Seletivo (ISE).

Métodos: O dados para os objetivos i) e ii) foram recolhidos em adolescentes do estudo LabMed Physical Activity Study, que é um estudo longitudinal de 3 anos com crianças e adolescentes Portugueses entre os 12 e os 18 anos. Uma sub-amostra de conveniência (n=398) foi convidada para recolher a urina das 24 h. As recolhas de urina realizaram-se durante 2012 e 2014. Após uma validação das recolhas de urina das 24h, a amostra final consistiu em 200 adolescentes com recolha de urina válida e com recordatório alimentar correspondente ao dia da recolha. Para responder ao objectivo iii) foi realizada uma análise sensorial e os sujeitos foram idosos (n=35) de dois lares públicos da cidade de Barcelos e crianças do ensino pré-escolar (n=75) da cidade de Seia. O conteúdo habitual de sal das sopas foi previamente analizado através de fotometria de chama e depois os testes sensoriais foram realizados com sopa com menos 30% de sal e com sopa com o teor de sal habitual. A descrição sensorial das duas sopas foi realizada pelos idosos através de escala visual analógica para a percepção de sabor salgado e avaliação hedónica, e pelas crianças através de escala facial de cinco pontos para a percepção hedónica. Os dados para o objectivo iv) resultaram da análise de um questionário preenchido por manipuladores que trabalhavam numa empresa de restauração coletiva Portuguesa. O questionário tinha questões para avaliar o conhecimento acerca da ingestão de sal, maiores fontes alimentares do sal ingerido e a relação entre consumo de sal e saúde; e para avaliar as preocupações acerca do uso de sal, práticas e dificuldades. Para validar a nova metodologia realizamos uma série de testes para determinar a especificidade, linearidade, precisão e exactidão. A comparação do método ISE com a fotometria de chama (FP) também foi realizada usando amostras de sopas e refeições servidas em uma cantina universitária.

Resultados: Os maiores resultados deste trabalho foram:

- A ingestão média de sal estava acima das recomendações da Organização Mundial de Saúde. Esta ingestão foi de 9.5 g/d nos rapazes e 7.8 g/d nas raparigas. As maiores fontes alimentares da ingestão de sódio foram os cereais e produtos cerealiferos (41%), produtos de carne (16%) e leite e lacteos (11%).
- Cerca de um terço dos sujeitos estão em risco de hipo-hidratação. Uma maior ingestão de sódio estava associada a um melhor estado de hidratação.
- Depois de uma redução de 30% do sal adicionado à sopa, não existe uma diferença significativa na percepção hedónica em crianças (p=0.160).
- A maioria dos manipuladores estudados reconhecem o valor máximo de ingestão recomendado e 70.6% concordam com uma redução do sal adicionado às refeições. A maior dificuldade na redução do conteúdo de sal das refeições indicada foi a opinião do consumidor (mencionado por 79.4%). Sopas (36.4%) e saladas (18.2%) foram identificados como componentes mais fáceis a uma redução de sal.
- O método de ISE para o ião sódio é exacto, preciso, linear, sensível e seletivo, apresentando uma gama de trabalho linear adequada para a avaliação do conteúdo de sal (sódio) em refeições e sopas. Comparações com o método de referência interno (FP) revelou uma boa correlação (R^2=0.902) sem diferenças significativas (p<0.387).

Conclusões: Os adolescentes ingerem sal em excesso e défice de potássio, longe das recomendações da Organização Mundial de Saúde. Intervenções de promoção de alimentação saudável são necessárias de forma a reduzir a ingestão de sal. Nos adolescentes estudados, a sopa, um alimento típico dos países de dieta Mediterrânea, contribuiu com 10% da ingestão de sal, e uma redução de 30% do sal adicionado durante a confeção mostrou ser possível sem afectar a percepção hedónica das crianças. Uma estratégia para a redução do conteúdo de sal nos alimentos deve contemplar a redução do sal adicionado pelos manipuladores durante a confeção e a implementação de um plano de monitorização que fortaleça políticas regulatórias ou voluntárias de redução da oferta de produtos com elevado teor de sal. Esta estratégia deve considerar a educação do consumidor para a redução do sal e capacitar os manipuladores de como reduzir o sal durante a confeção. A metodologia de ISE demonstrou ser válida para avaliar o teor de sal (sódio) em refeições e sopas e demonstra potencial para ser transformada em um equipamento rápido, portátil e fiável para o controlo de uma grande quantidade de amostras alimentares.

PALAVRAS CHAVE: ADOLESCENTES; CRIANÇAS; SAL; ALIMENTAÇÃO; EXCREÇÃO URINÁRIA.

List of abbreviations

AI – Adequate intake level
EU – European Union
FP – Flame Photometry
FWR – Free Water Reserve
ISE – Ion Selective Electrode
IOM – Institute of Medicine
PA – Physical activity
PABA – Para-aminobenzoic acid
SB – Sedentary behavior
UL – Tolerable upper intake level
US – United States of America
UK – United Kingdom
WHO – World Health Organization

General Introduction

General introduction

The word salt in this manuscript follows the general usage that it means sodium chloride as used in cooking. Salt is made up of 40% sodium and 60% chloride.

For millions years our ancestors ate a diet to which no salt was added, however about 5000 years ago humans start to add salt to food and most populations became increasingly addicted.

The problem starts when salt consume became excessive to what is physiologically needed. The result was a general rise in blood pressure and other risks to health. A rise in blood pressure damages the arteries and it's the major cause of strokes and heart attacks, the leading cause of death thought the world. Recent trials and cohort studies shown that the effects of high salt intake in blood pressure starts early in infancy and its impacts remains in adulthood.

Information related to salt consumption on Portuguese adolescents is scarce and accurate data measured by "gold standard" method 24 hour urinary sodium excretion is inexistent. Furthermore the knowledge about the dietary sources of salt consumption in this age group is limited. The knowledge about these data is crucial to develop an intervention strategy that aims reduce the consumption of dietary salt.

The salt taste preference is an important factor that predicts high salt ingestion. Salt reduction without affect consumer preference has concerned food industry which is pushed to lowering salt content on their products. To know how far salt reduction can be made on staple foods as vegetable soup without affect hedonic preference for risk groups as children and elderly is very challenging.

About 80% of salt intake comes from added salt during food production and foods eaten away from home. For that reason is very difficult for consumers estimates their salt intake and reduce. Food handlers are an important piece in salt reduction during cooking process in catering industry and their knowledge and availability to embrace a salt reduction strategy in food preparation.

Salt content in foods served in catering are really hard to monitor due to complexity of the process. First meal items need to be collected in canteens or restaurants and then analyzed in laboratory. Sodium content in foods can be determined by diverse methods, including Flame Photometry, the intern reference method but are typically methods with several steps in sample preparation and no portable. The validation of a method that could be used in the development of a portable equipment to analyze salt content in foods immediately in canteens or restaurants could be very useful to support salt reduction policies.

On that way, the major aims of this thesis is to describe dietary salt intake, evaluate its impact on hydration status in adolescents and to examine potential factors that could help to reduce salt intake.

Included in this dissertation the following manuscripts published or submitted for publication:

1. Gonçalves C, Abreu S, Padrão P, Pinho O, Graça P, Breda J, Santos R, Moreira P. Sodium and potassium urinary excretion and dietary intake: a cross-sectional analysis in adolescents (submitted, under review).

2. Gonçalves C, Abreu S, Padrão P, Pinho O, Graça P, Breda J, et al. Association between sodium excretion and hydration status by Free Water Reserve: a cross-sectional analysis in adolescents. BMC Nutrition. 2015;1(1):17.

3. Gonçalves C, Monteiro S, Padrão P, Rocha A, Abreu S, Pinho O, Moreira P. Salt reduction in vegetable soup does not affect saltiness intensity and liking in the elderly and children. Food & Nutrition Research. 2014; 58

4. Gonçalves C, Pinho O, Padrão P, Santos C, Abreu S, Moreira P. Knowledge and practices related to added salt in meals by food handlers. Nutricias. 2014; 21:20-24.

5. Gonçalves C, Pena MJ, Moreira JL, Gabriel J, Moreira P, Alves A, Pinho O. Validation of a rapid and simple method to determine sodium content in food matrices based on Potentiometry of Sodium Selective Electrode. (Provisory patent waiting).

Part I – Background

1. Salt in health and disease – a delicate balance

The evolution of humanity was held for a long time in an environment where salt sources were very limited. Physiological adaptations were developed with salt specific taste receptors and highly effective hormonal and cellular transport systems for minimizing salt loss from the intestine, kidneys and skin. However, salt that used to be scarce has become ubiquitous, and high consumption induces a series of physiopathological responses such as changes in blood volume and hormonal and cellular changes [(1)]. These responses lead, in conjunction with other dietary and environmental factors, to hypertension, increased risk of stroke, coronary heart disease, and heart failure [(2-4)]. They also promote the development of osteoporosis [(5-8)], gastric cancer [(9-12)], and bronchial reactivity [(13-15)]. These non-communicable diseases are the major contributors to mortality and morbidity worldwide, especially in middle-income countries [(16)].

The effect of sodium excess on raise blood pressure is dependent on the associated counter anion. Studies involving salt-sensitive persons have shown that salt (sodium chloride), the traditional form of salt consumed, is related to hypertension [(17)], whereas other non-halide sodium salts, such as sodium citrate, sodium bicarbonate, or sodium phosphate, have none of these effects [(18)].

This relationship between salt intake and high blood pressure seems to happen early in life and at low levels [(19, 20)]. An interesting study performed by He F and MacGregor G based on the National Diet and Nutrition Survey for Young People program showed that an increase in salt intake of 1 g/day raised systolic pressure by 0.4 mmHg among children and adolescents from 4 to 18 years old [(21)]. Similar results have been published regarding US children and adolescents from 8 to 18 years old. In this study, sodium intake was positively associated with systolic blood pressure and is a risk for pre-high blood pressure and high blood pressure. The researchers also noted that this association was higher among those who are overweight or obese [(22)]. High blood pressure in childhood predisposes to hypertension in adulthood and increases the risk of developing cardiovascular disease, which can lead to premature death [(23)].

The impact of reduction in salt intake during infancy remains through adolescence and probably later in life. This hypothesis was tested in 1983 in a double-blind randomized trial in which 245 newborn infants were assigned to a normal-sodium diet and 231 to a low-sodium diet during the first six months of life. Systolic blood pressure was significantly lower in the low-sodium group than in the normal-sodium group at the end of the trial [20]. Fifteen years later, blood pressure was measured in 167 children from the original cohort. The adjusted systolic blood pressure was 3.6 mmHg lower and the diastolic pressure was 2.2 mmHg lower in the children who had been assigned to the low-sodium group compared to control group [24].

Children who are overweight, born preterm, small for gestational age, or are African American are at an increased risk of developing high blood pressure due to high salt intake because they are more likely to be salt sensitive [25]. Salt sensitive individuals who consume a diet high in salt seem to retain more sodium than sodium resistant individuals, indicating impaired renal sodium excretion. Those individuals in whom salt intake causes the least amount of change in arterial blood pressure are termed salt resistant, whereas those who experience larger changes are referred to as salt sensitive and are considered by some to be more likely to develop hypertension later in life. There are genetic mechanisms related to the salt sensitive phenotype [26, 27].

A study by Lava and Bianchetti reviews the effects of salt intake on blood pressure in children, and they conclude that there exists sufficient evidence to recommend restricted salt intake as a preventive measure, because high salt intake during childhood predicts elevated blood pressure during adulthood. Also, high salt intake increases the risk of hypertension in childhood and the development of taste preferences [25].

Indeed, adults today may still be affected by their childhood lifestyles, including diet. Diet in childhood may play an important role in developing cardiovascular diseases in adulthood [28]. For that reason a salt poor diet should start early in life, particularly in the first twenty years, so that the habits cultivated during childhood become sustained for the rest of life [29].

Some researchers argues that not so excessive (higher than 12.6 g salt) but also low salt intake (less than 6.7 g salt) are associated with increased mortality risk potentially because low sodium intake may be related to elevated renin-aldosterone activity, sympathetic nerve activation, and/or lipid abnormalities [30]. Overall, research has revealed mixed results regarding the impact of salt intake on various hormones. Sodium reduction has been associated with adverse health effects such as increased total cholesterol, increased low density lipoprotein cholesterol, and increased triglyceride and catecholamine levels [31, 32] mainly because lower sodium intake reduces blood volume without a concurrent reduction in blood lipids [33]. A enlightening systematic review concludes that a lower sodium intake has no adverse effect on blood lipids, catecholamine levels, or renal function and also corroborates the association between sodium intake and all stroke, fatal stroke, and fatal coronary heart disease events [34].

The role of potassium

Yin and Yang

In Chinese philosophy, yin and yang describes how apparently opposite forces are actually complementary and interdependent in nature, and how they interact to form a dynamic system in which the whole is greater than the assembled parts.

Potassium and sodium share a yin/yang relationship in the regulation of blood pressure [35]. INTERSALT was an important study in the field of sodium consumption and its impact on blood pressure. The study reported for the first time an inverse relationship between urinary potassium levels and blood pressure and a positive association of urinary sodium excretion and systolic blood pressure [36, 37].

A longitudinal study in the Netherlands had similar results. The sodium to potassium ratio showed an inverse relationship with blood pressure in childhood [38]. Another longitudinal study involving children aged 5 to 17 years old found that potassium and sodium to potassium ratio had showed an inverse relationship with blood pressure, and the effect of sodium intake on blood pressure may be more evident in the older groups of children [38].

Adrogué and colleagues synthesize the determinants and molecular pathways developed by sodium rich and potassium poor modern diets to establish hypertension. They conclude that sodium and potassium are intertwined in dietary intake, physiological roles, and vascular effects [39].

Salt intake increased in humans when they started adding salt to food in order to preserve it and make it more palatable. Years later, humans started to process their food, which lowered its potassium content due to losses during cooking and processing.

The chief environmental factor in the pathogenesis of primary hypertension and associated cardiovascular disease is not an isolated abundance of sodium or deficit of potassium on the body but the combination of the two events in the modern diet and the non-adapted kidneys, which are intrinsically formatted to conserve sodium and excrete potassium [39].

Dietary sodium excess and potassium deficiency play an important role in hypertension pathogenesis, independent cardiovascular risk factors, and measures of kidney function [40]. The Japan Cohort Study for Evaluation of Cancer Risks, followed 58730 subjects over two years and concluded that sodium intake was positively associated with mortality from total stroke, ischemic stroke, and total cardiovascular disease and that these conditions were similarly observed for nonoverweight and overweight persons. On other hand, potassium intake was inversely associated with mortality from coronary heart disease and total cardiovascular disease [41].

Potassium is a natural antidote for the pernicious effects of excessive sodium intake on blood pressure and cardiovascular risk. Clinical studies have shown that increased potassium intake significantly reduces systolic and diastolic blood pressure [42, 43], and epidemiological evidences suggest that potassium deficiency increases the impact of sodium excess on the pathogenesis of hypertension. Recently, the Prospective Urban Rural Epidemiology (PURE) study, conducted with 102,216 adults from 18 countries, found that a dominant inverse relationship existed between systolic blood pressure and urinary potassium excretion after adjustment for confounders [44].

These data are in concordance with findings from the Dietary Approach to Stop Hypertension (DASH)[45] trial, in which researchers studied the effects of different levels of dietary sodium simultaneously with a diet rich in vegetables, fruits, and low-fat dairy products. The results showed this potassium rich diet had a significantly effect in lowering blood pressure in all high, intermediate, and low dietary sodium intake subjects, and the effects were more pronounced for high than for the low sodium intake [46].

Dietary potassium exerts a dose-dependent inhibitory effect on sodium sensitivity in both normotensive and hypertensive subjects. The mechanism for these beneficial effects of high potassium diets may include vasodilation, enhanced urine flow, reduced renal rennin release, and negative sodium balance [39, 47].

Potassium is commonly found in a variety of unprocessed foods, especially fruits and vegetables. One of the best-studied diets for cardiovascular health is the Mediterranean diet, which promotes potassium rich foods by the consumption of fruits, vegetables, whole grains, and nuts [48]. The south of Portugal is very close to Mediterranean Sea and was once a country that followed the Mediterranean diet. However the adherence to a Mediterranean food pattern is decreasing [49]. Studies estimated that by adopting a Mediterranean lifestyle, the population could avoid 80 per cent of coronary artery disease, one third of acute myocardial infarctions and 70 per cent of strokes [50, 51].

Several authors argue that elevated sodium to potassium intake ratio has been more predictive of cardiovascular events and left ventricular mass than sodium or potassium alone [52-54]. These results reinforce the recommendation of a low-sodium/high-potassium diet as a critical strategy for the prevention and treatment of hypertension and cardiovascular disease [55].

2. Salt intake in Portugal and in Europe

Brief Note on Salt in Portuguese Culture

Ó mar salgado, quanto do teu sal
São lágrimas de Portugal!
Mensagem – Possessio Maris – Fernando Pessoa - 1934

Mark Kurlansky in his book A World History of Salt, explains that “salt is so common, so easy to obtain, and so inexpensive that we have forgotten that from the beginning of civilization until about 100 years ago, salt was one of the most sought-after commodities in human history” [56].

Salt was probably, since the beginning of time, the first mineral consciously used by man as a food supplement. Man’s growing need and concern to keep food in good condition led him to realize that the use of salt was one of the methods of food preservation. Many scholars believe that without salt, human society could not have made the leap from hunting-gathering to farming. According to legends, the discovery of saline springs resulted from the intense observation of nature as humans took note of places where animals went to lick salt [57].

The connection of man to salt has been documented since 2700 BC in China [58]. The Egyptians used salt in the mummification process in ancient times. Numerous tomb illustrations further suggest that salted mullet roe was popular in the day of the Pharaohs. In Greece, salt worked as a bargaining chip, and Roman soldiers were paid with salt rations known as "salarium Argentum". The importance of salt continued, and the "white gold", as it was known, has been a cause of wars and conflicts. In other empires and circumstances, the state exercised monopolies on the product. This occurred in the British and Portuguese Empires. England and Portugal formed an alliance, in which England traded naval protection for Portuguese sea salt. Portugal had salt production but needed protection for their boats, and England did not have salt but needed salt for its fish industry. The island “Sal” from the Cape Verde Islands owes its name to its important role in this alliance.

The “Salt March” is one of the most emblematic cases of the “power of salt”. Since the 18th century, the British dominated much of the territory now occupied by India, and all production and yield of salt were poured into the royal coffers. Indians were prohibited from collecting or selling salt, a staple food in the Indian diet. The Salt March, which took place from March to April 1930 in India, was an act of civil disobedience led by Mohandas Gandhi to protest British rule in India. During the march, thousands of Indians followed Gandhi from his religious retreat near the Arabian Sea coast, a distance of some 240 miles. The salt laws were in fact repealed one year later, and salt gathering became legal again. However, the damage had been done for England, and India finally was granted its independence in 1947 [59].

Up until the middle of the 19th century, the economic importance of salt was comparable to that of crude oil in our present-day economy [57]. Salt occurs naturally as rock-salt or in dissolved form as brine. Two entirely different strategies are applied as salt winning techniques: evaporation of brine and mining of rock-salt. Producing salt from brine involves several steps in which the sun provides evaporation and the separation of sodium chloride, which is the last to be precipitated, from other salts in sea water [57].

Portugal, by its natural, geographic, and climatic conditions, was early on an important producer of salt from brine by solar evaporation. This process requires the combination of a favorable geographic location, a coast with lagoons, sufficient amounts of sunlight and rainfall that is not so abundant that it will dilute the brine. One parameter that determines the efficiency of a saltworks is thus the ratio of water evaporation [60].

In Portugal there were five important salines identified: Saline of Aveiro, Figueira da Foz, Setúbal, Alcácer do Sal, and Algarve [61]. In 2013, 91 thousand tones of sea salt were produced in Portugal. Algarve produces 95% of the national salt production due to best edaphoclimatic conditions [62].

The Portuguese proverb “Repartiu-se o mar e fez-se o sal” [63] literally means “divided up the sea and made the salt”. This proverb reflects the entire knowledge of the saltworker, which consists in evaporating the brine in the sun in such a way as to obtain successive separate crystallizations of the main dissolved salts. Salt workers walk on the dikes around the evaporation basins to harvest more pure salts.

Salt has a social importance, which is also could be a reason for people’s resistance to a reduction of consumption. Salt was almost a staple mineral in Portuguese culture, and is present in all household. A Portuguese ancient could say, “Não te has de fiar senão com quem comeres um moio de sal” [63]. This saying literally means “You should not trust except with whom you eat a lot of salt”. Thus it carries the meaning of a deep and enduring friendship, one nourished on the relations enjoyed by longtime table companions. Salt was one of the few, if not the only, spices that was almost always present at meals.

The use of salt is also linked to the gastronomic traditions of Portugal, characterized by foods like anchovies; salted hard cheeses; olives and brine-pickled; smoked food; pork meat and other meats preserved in salt and stuffed in natural casings from intestines; and the “faithful friend”, salt cod. Salt cod has a unique status in Portuguese cuisine and is a symbol of the Portuguese national identity (64). The Portuguese have a special hedonic taste for this salted food, which comes from a long time of eating salted fish, passed across a long time of socialization. Traditional salt cod consumed in Portugal, even after a day or more of soaking it in water, still has about 3.8 g of salt/100g (65).

For the Portuguese, the salt cod trade meant growth years for salt making. Setubal’s salt even earned a reputation throughout Europe for the dryness and whiteness of its large crystals, perfect for the curing of fish or cheese (56).

Salt consumption

The average 20th-century European populations consumed half as much food as the average 19th-century (56) populations, but the consumption of salt was still high. Moreover, salt was not necessary anymore to preserve foods.

Plausibly, the major factor that increased humans’ intake of salt was the discovery that meat and other foods could be preserved by putting them into a concentrated salt solution. Highly salted food suppresses the salt taste receptors in the mouth so that natural foods become insipid and unappetizing. It was probable the way how craving for salt was developed. Salt would then have been added to unsalted food to bring it up to the same concentrations as that of the preserved food. This salting of food was probably the way cravings for salt developed.

In the summer of 1982, 30 clinical epidemiologists met in Tuohilampi, Finland, for a two-week residential course. They were given the task of devising an epidemiological project that would overcome some of the objections raised against the salt and blood pressure hypothesis. To remedy the drawbacks of previous studies, it was proposed that researchers apply standardized methods across a variety of populations, simultaneously examining some major confounding variables. Thus, the INTERSALT study was born. This study was the largest international epidemiological comparative study in 52 centers worldwide representing both rich and poor countries, with 10079 adult men and women subjects (66). The main findings were published in 1989, but further analysis of the data has continued since. Salt intake (estimated by 24-hour sodium excretion) over a 24-hour period was analyzed. It varied from 0.05g per day in

Yanomamo Indians in Brazil to 16g per day in Tianjin in China [67]. Portugal was included in the group of countries in which mean salt consumption was higher than 12 g/day [67].

Subsequent to the INTERSALT study, a new epidemiological study that includes measurements of dietary sodium intake and urinary sodium excretion was designed and called INTERMAP [68]. INTERMAP included data from men and women, aged 40-59 years, from 17 population samples distributed in Japan, People's Republic of China, United Kingdom (UK), and the United States of America. As in the INTERSALT study, the highest mean urinary sodium excretion was found in China (mean salt intake estimated from urinary excretion was 17 g/day). In the UK, the only European country analyzed, had a mea salt intake of 9 g/day in men and 7g/day in women (data available from supplementary online appendix tables at *Journal of Human Hypertension* [69]).

Brawn et al. [70] in 2009 reviewed contemporaneous salt consumption data from around the world and concluded that salt consumption was well in excess of physiological needs. The European countries in this review are France, Finland, Italy, Netherlands, Spain, and UK.

In 2008, Member States of the European Union signed the EU Framework for National Salt Initiatives[71], and in 2012, the first monitoring report appeared [72].

Table 1 summarizes the most recent data on salt consumption in European countries. These countries are notorious for their populations' high intake of salt, which ranges between 5.4 to 17.2 g/day.

According to most recent data, Finland, France and UK have reported the lowest estimated salt intake, around 7 g/day. On the other hand, Hungary, Turkey, and Czech Republic have reported the highest estimated salt intake, around 17 g/day. In general, salt intakes are higher in men than in women. The most salient case of this finding is in Poland were men's salt intakes is reported to be almost twice that of women's salt intake.

No data about salt consumption before 2005 are available. In 2005, Polónia and colleagues [73] performed a study that estimates salt intake by urinary sodium excretion in a Portuguese adult sample. Although the sample selection was not representative of the population, this study showed that the Portuguese had high levels of salt consumption (12.3 ± 3.8 g/d).

Compared with Turkey or Hungary, Portugal had almost 7 g/day less salt intake; however, the consumption remains one of the highest according to the most recent results from PHYSA study [74]. Portugal has actually no nutrition monitoring system established and, therefore, salt intake reporting is particularly challenging.

The assessment of salt intake is hard work. Several methods can be used to estimate sodium intake: 24-hour urinary collection, duplicate diets, or dietary surveys. A 24-hour urine collection is considered the "gold standard" because 90% of the sodium ingested is excreted through the urine. The method also has the advantage of being unaffected by subjective dietary reporting. On the other hand, the greatest challenge in representative studies is the high participant burden, problems of completeness, and the accurate time of collection [(75)]. To avoid under and over urine collection, *Para*-aminobenzoic acid (PABA) ingestion during urine collection or 24-hour creatinine excretion based on age, sex, body weight, and protein intake are used as quality criteria to assess complete collection [(75)].

Some studies have used single or multiple spot urine samples to estimate 24-hour urinary sodium excretion. Although this methodology presents many potential advantages like easy collection and storage, the validity for estimating population sodium intake is highly controversial. The formulas to estimate 24-hour urinary sodium excretion from spot urine samples are not validated and show a more limited usefulness, due to greater intra-individual variability of sodium concentration, than that of 24-hour collections [(75)].

To estimate sodium excretion with the mean of consumption of ± 12 mmol/d and a 95% confidence interval, a single 24-hour urine collection from a sample population of 100-200 participants would be required; larger samples ensure greater precision [(76, 77)].

Table 1 – Most recent data about mean salt intake estimated by urinary sodium excretion or by dietary salt intake for adults from Europe.

Country (survey year)	References	Sampling	Age range, years	Measurement	Sample size	Mean salt intake ± SD, g/day[a]	Notes
France (2006-07)	EU Report [72]	Not defined	Adults	7 day dietary record (1g was added for salting at meals)	2646 men and women men women	 9.6 ± n/a 7.3 ± n/a	
Sweden (2005)	Hulthén et al. [78]	Random sample from GOOD Study in Gothenburg	18-20	Single 24 hour urine collection with PABA tablets	79 men	11.5 ± 4.0	
Finland (2002)	Laatikainen et al. [79]	Random sample stratified by age and sex from population lists of three areas	25-64	Single 24 hour urine collection	423 men Noth Karelia South-western Helsinki 486 women Noth Karelia South-western Helsinki	9.6 ± 3.8 9.9 ± 4.4 8.6 ± 5.4 7.5 ± 2.8 7.4 ± 2.9 7.0 ± 3.1	Also cited in Brown review.
Italy (2008/2012)	Donfrancesco et al. [80]	Random sample of adult population from 12 Italian regions	35-79	Single 24 hour urine collection	1114 men 1098 women	10.9 ± n/a 8.5 ± n/a	
Netherlands (2006/2010)	Hendriksen et al. [81]	Random sample from Doetinchem population and participants of the Doetinchem Cohort Study	19-70	Single 24 hour urine sample	317 men and women in 2006 342 men and women in 2010	8.7 ± n/a 8.5 ± n/a	Values are expressed as median salt.
Spain (2009)	Ortega et al. [82]	Random representative sample of Spanish adults	18-60	Spot morning and 24 h urine samples	196 men 222 women	11.5 ± 4.8 8.4 ± 3.9	Those with diabetes, hypertension, renal disease, taken diuretics or institutionalized are not included.
Belgium (2009)	Vandevijvere et al. [83]	Non random sample of Ghent and Liege	45-65	Two 24 hour urine collections in Ghent and one in Liege	114 men and women in Ghent 135 men and women in Liege	10.9 ± 3.8 10.0 ± 3.7	
Hungary (2009?)	Martos et al. [84]	Sample of Hungarian Diet and Nutritional Status Survey - joining to the European Health Interview Survey	adults	Unspecified method	Men Women	17.2 ± n/a 12.0 ± n/a	Article in Hungarian, some information's was not possible to obtain with abstract
UK (2005-06)	National Centre for Social Research [85]	Nationally representative sample	19-64	Single 24 hour urine collection with PABA tablets	294 men 398 women	9.7 ± 4.1 7.7 ± 2.9	Also cited in Brown review.
Slovene (2008?)	Ribicˇ et al. [86]	Random representative sample	25-65	Single 24 hour urine collection	61 men 82 women	13.0 ± 5.1 9.9 ± 4.3	
Turkey (2007)	Erdem et al. [87]	Random nationally representative sample	18 - >65	Single 24 hour urine collection	373 men 443 women	17.1 ± 6.6 16.1 ± 7.9	Pregnant, participants with cardiac/renal failure, chronic liver disease and diabetes mellitus were excluded.
Austria (2007)	EU Report[72]	Not defined	Adults	3 day dietary record, 24 hour dietary recall	3000 men and women men women	 9.0 ± n/a 8.0 ± n/a	
Bulgaria (2004)	EU Report[72]	Not defined	Adults	24 hour dietary recall	2282 men and women men women	 12.5-14.5 ± n/a 11.4 – 16.6 ± n/a	

[a] – salt intake was estimated from dietary and urinary data, considering that 1 mmol sodium = 23 mg, and 393 mg sodium=1 g salt. ? – date of data collection was not defined.

(Continued)

Table 1 – (continued) Most recent data about mean salt intake estimated by urinary sodium excretion or by dietary salt intake for adults from Europe.

Country (survey year)	References	Sampling	Age range, years	Measurement	Sample size	Mean salt intake ± SD, g/day[a]	Notes
Czech Republic (2003-04)	EU Report[(72)]	Not defined	Adults	Repeated 24 hour dietary recall	2282 men and women men women	16.6 ± n/a 10.5 ± n/a	
Czech Republic (2007-08)	Keyzer et al. [(88)]	Convenience subsample of EFCOVAL	45-65	Two 24 hour urine collections with PABA tablets	58 men 60 women	15.6 ± n/a 11.3 ± n/a	
Germany (2008-11)	Johner et al. [(89)]	Random sample from German Health Interview and Examination Survey for Adults	18-79	Spot urine samples	3340 men 3622 women	10.0 ± n/a 8.4 ± n/a	Values are expressed as median.
Norway (2007-08)	Keyzer et al. [(88)]	Convenience subsample of EFCOVAL study	45-65	Two 24 hour urine collections with PABA tablets	62 men 62 women	11.9 ± n/a 8.8 ± n/a	
Poland (2008)	EU Report[(72)]	Not defined	Adults	Household budget survey	4134 men and women men women	14.7 ± n/a 8.6 ± n/a	
Switzerland (2004)	EU Report[(72)]	Not defined	Adults	Unspecified dietary recall, 24 hour urine collection	13355 (urine samples from 100) men women	10.6 ± n/a 8.1 ± n/a	
Portugal (2005)	Polónia et al. [(73)]	Four convenience samples	20-71	Single 24 hour urine collection, some subjects had two collections in same month	426 men and women	12.3 ± 3.8	Subjects are: 245 hypertensive, 38 couples, 82 students, 61 workers.
Portugal (2011-12)	Polónia et al. [(74)]	Representative sampling population	18-90	Single 24 hour urine collection	3125 men and women	10.7 ± n/a	

[a] – salt intake was estimated from dietary and urinary data, considering that 1 mmol sodium = 23 mg, and 393 mg sodium=1 g salt. ? – date of data collection was not defined.

In children and adolescents, data about salt consumption are limited or absent in many countries.

In 1988, Knuiman et al. performed an observational study of diet and blood pressure among children from 19 European centers [90]. In **Table 2** we see the available data of salt consumption for children and adolescents of different ages from European countries. The table integrates the results of the Knuiman study and more recent studies.

Where data from boys and girls are available, boys had generally a higher or similar salt intake compared to girls. And salt intake increases with age, probably due to higher energy intake [70].

Notably, the use of a diet diary and food record as a method to assess salt intake was more prevalent in studies of children and adolescents than in those of adults. This method is less accurate than the 24-hour urinary sodium excretion due to an underestimation of salt added during the cooking process, a misdescription of food portions, or the omission of any intake during the day. However, the most recent studies show a predisposition to use the urinary method.

In the last 10 years, only Italy, Netherlands, UK, Poland, Iceland, and Portugal have had studies to estimate the dietary salt intake in children or adolescents. When we consider adolescence as a transitional stage of human development occurring between puberty and adulthood, which includes ages 12 to 18 [91], we find that results limited to Italy and UK only. These data highlight the lack of information on this population age group.

Diet during preadolescence and adolescence is known to have important consequences for health during adulthood; eating behaviors in adolescents could have future consequences like the development of cancer [92], obesity [93] and cardiovascular disorders [94].

More recently, three studies were published reporting Portuguese children's salt intake assessed by a 24-hour urinary collection. The first study was performed by Cotter et al. [95] and evaluates 24-hour urinary sodium excretion in children of 10-12 years at a school in the north of Portugal, and the mean salt intake was 7.8±2.5 g/ day. The other two studies were carried on 8 to 10 years old children and report salt intake from 6.1±1.9 g/day to 7.5±2.7 g/day [96, 97]. These findings represent a slightly higher value compared to children of the same ages in other European countries (Table 2).

Table 2 – Mean salt intake estimated by urinary sodium excretion or by dietary salt intake for children and adolescents from Europe.

Country (survey year)	References	Sampling	Age range, years	Measurement	Sample size	Mean salt intake ± SD, g/day[a]	Notes
Sweden (1986)	Knuiman et al. (90)	Random sample of boys from selected schools in Lund	8-9	Single 24 hour urine collection	40 boys	5.7 ± 1.9	
Sweden (1989-90)	Bergstrom et al. (98)	Non random sample of boys and girls from 4 schools in Umea	14-17	7-day diet diary	14 years 155 boys 189 girls 17 years 211 boys 176 girls	7.6 ± 2.1 5.7 ± 1.4 8.9 ± 2.3 5.7 ± 1.7	
Sweden (1993-94)	Samuelson et al. (99)	Random sample of boys and girls from Uppsala and Trollhattan	15	7-day diet diary	Uppsala 99 boys 104 girls Trollhattan 85 boys 110 girls	9.1 ± 2.5 6.9 ± 1.6 8.4 ± 2.0 5.8 ± 1.4	
Finland (1986)	Knuiman et al. (90)	Random sample of boys from selected schools in Turku	8-9	Single 24 hour urine collection	48 boys	5.5 ± 2.1	
Finland (1990-97)	Heino et al. (100)	Non random sample of boys and girls recruited from well-baby clinics in Turku	1-5	3-day weighed food record at 13 mo, 4-day weighed record at 3-5 yr	100 boys 13 mo 3 year 5 year 100 girls 13 mo 3 year 5 year	4.1 ± 1.4 4.9 ± 1.4 5.9 ± 1.4 4.0 ± 1.3 4.8 ± 1.2 5.5 ± 1.3	
Italy (1986)	Knuiman et al. (90)	Random sample of boys from selected schools in three areas	8-9	Single 24 hour urine collection	Catania: 45 Milan: 48 Rome: 45	7.7 ± 2.8 6.7 ± 2.4 6.7 ± 2.4	
Italy (1988)	Agostini et al. (101)	Sample of boys and girls from Corsico	11	Single 24-h dietary recall	55 boys 65 girls	8.1 ± 2.0 8.3 ± 2.7	
Italy (2013?)	Campanozzi et al. (102)	Non-random sample recruited by SIGENP operated by pediatricians among their patients	6-18	Single 24 hour urine collection	766 boys 658 girls	7.4 ± n/a 6.7 ± n/a	
Netherlands (1986)	Knuiman et al. (90)	Random sample of boys from selected schools in Turku	8-9	Single 24 hour urine collection	43 boys	5.3 ± 1.9	
Netherlands (1995)	Geleijnse et al. (24)	Non-random sample of children born to mother in Zoetermeer in 1980	15	Single overnight urine collection	96 boys and girls	5.0 ± 2.7	We consider subjects assigned to normal diet at birth for 6 mo
Netherlands (1993/ 2005)	Schreuder et al. (103)	Non-random sample of children attending a medical centre in Amsterdam	5-10	Single 24 hour urine collection	1993-95: 45 children 2003-05: 142 children	3.8 ± 2.3 5.9 ± 3.5	Mean weight was higher in the latter group
Netherlands (2002-03)	Huybrechts et al. (104)	Random representative sample of pre-school children	2.5-6.5	3-day food record	197 aged 2-3 years 465 aged 4-6 years	4.5 ± n/a 4.9 ± n/a	
Spain (1986)	Knuiman et al. (90)	Random sample of boys from selected schools in Madrid and Santiago	8-9	Single 24 hour urine collection	Madrid: 57 boys Santiago: 57 boys	7.1 ± 2.3 7.4 ± 2.3	
Spain (1993-94)	Maldonado et al. (105)	Random sample of children from public primary schools in the Almeria province	6-14	Single 24 hour urine collection	274 boys 279 girls	8.3 ± 4.1 7.4 ± 3.1	

[a] – salt intake was estimated from dietary and urinary data, considering that 1 mmol sodium = 23 mg, and 393 mg sodium=1 g salt. ? – date of data collection was not defined.

(Continued)

Table 2 – (continued) Mean salt intake estimated by urinary sodium excretion or by dietary salt intake for children and adolescents from Europe.

Country (survey year)	References	Sampling	Age range, years	Measurement	Sample size	Mean salt intake ± SD, g/day[a]	Notes
Belgium (1986)	Knuiman et al. (90)	Random sample of boys from selected schools in Ghent	8-9	Single 24 hour urine collection	38 boys	5.4 ± 2.2	
Belgium (2002-03)	Huybrechts et al. (104)	Random representative sample of pre-school children	2-6	3-day food record	Children 2-3 years Children 4-6 years	4.5 ± n/a 4.9 ± n/a	
Hungary (1986)	Knuiman et al. (90)	Two random sample of boys from selected schools in Budapest	8-9	Single 24 hour urine collection	46 boys 27 boys	8.5 ± 3.3 8.1 ± 2.3	
UK (1992-93)	Gregory et al. (106)	Nationally representative sample, NDNS	1.5-4.5	4-day weighed record	848 boys 827 girls	3.9 ± 1.2 3.8 ± 1.2	
UK (1992-93)	Brion et al. (107)	Random sub-sample of children enrolled in the ALSPAC cohort - Avon	4-8 mo	1-day diet diary 3-day diet diary	533 boys and girls 710 boys and girls	0.4 ± 0.1 1.4 ± 0.7	
UK (1997)	Gregory and Lowe et al. (108)	Nationally representative sample, NDNS	4-18	7-day weighed record	856 boys 845 girls	6.7 ± 2.1 5.5 ± 1.5	
UK (2007-10)	Marrero et al. (109)	Random selected sample of children and adolescents from London	5-17	Single 24 hour urine collection	126 children 5-6 years 111 children 8-9 years 103 children 13-17 years	3.8 ± 0.1 4.7 ± 0.2 7.6 ± 0.3	
Austria (1986)	Knuiman et al. (90)	Random sample of boys from selected schools in Vienna	8-9	Single 24 hour urine collection	43 boys	6.2 ± 2.4	
Bulgaria (1986)	Knuiman et al. (90)	Random sample of boys from selected schools in Sofia	8-9	Single 24 hour urine collection	58 boys	5.4 ± 1.9	
Germany (1986)	Knuiman et al. (90)	Random sample of boys from selected schools in three areas	8-9	Single 24 hour urine collection	Berlin: 44 boys; Freiburg: 46 boys; Heidelberg: 40 boys	6.0±3.7; 7.4±3.2; 6.2±2.1	
Germany (1998)	Deutsche Gesellschaft fur Ernahrung eV (110)	National sample	14-19	1-day weighed record and diet history	38924 boys and girls	8.1 ± n/a	
Germany (1996-03)	Remer et al. (111)	Non random sample of children participating in the DONALD study	6-12	Single 24 hour urine collection	178 boys 180 girls	8.0 ± 3.0 6.9 ± 2.7	
Germany (1996-03)	Shi et al. (112)	Non random sample of children participating in the DONALD study	12-18	Three 24 hour urine collections	212 boys 223 girls	7.7 ± n/a 6.3 ± n/a	Values are expressed as median and correspond to last assessment.
Poland (1986)	Knuiman et al. (90)	Random sample of boys from selected schools in Warsaw	8-9	Single 24 hour urine collection	60 boys	5.9 ± 2.3	
Poland (2013?)	Merkiel et al. (113)	Non random sample of children who attended last grade in pre-schools	6	3-day food record	56 boys 64 girls	8.1 ± 1.7 7.3 ± 1.4	
Iceland (2011?)	Kristbjornsdottir et al. (114)	Non random subsample of a national dietary survey from Reykjavik area	6	Single 24 hour urine collection with PABA tablets	58 boys and girls	4.2 ± 1.3	
Portugal (2012?)	Cotter et al. (95)	Non random sample of students recruited from North	10-12	Single 24 hour urine collection	139 boys and girls	7.8 ± 2.5	Considered values before intervention
Portugal (2014)	Oliveira et al. (97)	Non random sample of students	8-10	Single 24 hour urine collection	81 boys 82 girls	7.5 ± 2.7 6.1 ± 2.7	
Portugal (2013-14)	Correia-Costa et al.(96)	Random subsample of cohort study Generation XXI	8-9	Single 24 hour urine collection	159 boys 139 girls	6.8 ± 2.4 6.1 ± 1.9	

[a] – salt intake was estimated from dietary and urinary data, considering that 1 mmol sodium = 23 mg, and 393 mg sodium=1 g salt.; ? – date of data collection was not defined.

2.1. Salt taste preference

Hedonism and addiction are well known mates. Salt is an enhancer to taste of foods, however high levels of salt are unpleasant and repulsive. With exposure to consistent high levels of salt in foods high levels of salt become appetizing and people start to choose foods with high salt content. This is which we call “the fish that bites its own tail”, that is persons prefer salt rich foods because are habituated with high salt intake or had high levels of salt consumption because are exposed to foods rich in salt?

In young adults in their twenties sodium intake was positively associated with the preference for salty [(115)]. Other research suggests that preferred level of salt in food is dependent on the level of salt consumed and that this preferred level can be lowered after a reduction in sodium intake [(116)].

Cultural addiction to a high salt intake in modern humans is induced at premature ages. Newborn infants are either indifferent to or avoid moderate to high concentrations of salt [(117)], but by two or three years of age children commonly prefer salty foods over the same foods without salt [(118, 119)]. Infants demonstrate an increased preference for saline solutions over water at 4 months of age [(117)]. Some investigators have reported that a preference for a salty food in infants can be induced by exposure to the salted food, which means that salt preference is learned [(120, 121)]. A study of 10 to 12 years old school children showed that those who liked salty foods ingested more salt (measured by urinary excretion) [(122)].

Also, sodium depletion early in life seems to have profound effects on later salt preference [(123)], history of mineralofluid loss including maternal vomiting and infantile vomiting and diarrhea increase the avidity for salt later on adolescence [(124)].

Salt taste was innate salt-specific taste receptors activated by sodium ion [(125)]. When sodium activates epithelial sodium channels on taste receptors an afferent signal is sent to gustatory processing regions of the brain. Detection threshold, used as a measure of individual sensivity to sodium, was the level of sodium where and individual will be able to discriminate a sodium solution from water. The recognition threshold, occurs when sodium concentration is high enough to activate the taste receptors but also produce electrical impulses to the brain where are decoded and after which the taste quality can be identified. Sodium concentrations above the recognition threshold is termed suprathreshold [(126)].

Flavor was the combination of complex oral, nasal and texture sensations stimulated by food. Addition of ingredients with high impact on flavor as fresh herbs and spices, citrus, mustard impart distinctive flavors that could camouflage lower salt [(127)].

The perceived intensity of sodium chloride varies between individuals, a specific concentration required to induce a weak saltiness sensation in one individual may be strongly salty to another, and these differences appear to result from variation in taste sensation [(128)]. The preferred level of sodium in foods could be lowered over time if sodium levels consumed decreased, resulting in an increase in perceived salt intensity and decrease liking of high salt content foods [(129-131)].

Subsequent to infancy, exposure to salt is stimulated by commerce with snack foods, vending machines products, salty canned soups and greater frequency of meals taken outside home [(132)]. The saltiness preference matures with aging, and climaxing in a high intake in adolescence [(133)].

Exposures to foods that are typical of the surrounding culture are an important means of transferring flavor preferences within a culture and family habit [(133)]. Thus, exposures tend to merge the preferences of those individuals who share a food supply [(134)]. Indeed, the hedonic response to saltiness results from interplay of physiological, cultural and environmental factors that influence preference to high salt foods.

Salt was added to foods during industrial process due to its capacity to enhance taste and make products more enjoyable. Salt (sodium) masks unpleasant bitterness in products (like cheese or vegetables), increasing saltiness and sweetness and preserve food by lowering water activity to prevent microbial growth [(126, 135)]. Moreover, salt was incredibly cheaper which makes it a very attractive additive for food industry.

Reduce salt in foods reduce perceived saltiness, but also decrease perceived sweetness and increase bitterness, which may negatively impact the liking of foods [(126)]. We know that the acceptability of a product by consumer was highly correlated with the intent of purchase [(136)], for this reason was important to include the consumer in reformulation process to reduce salt content in foods. Understand the impact of salt reduction on food preferences and food intake is important especially among children and adolescents [(137)] to whom food choice is more related to taste.

The perception and acceptability of saltiness was product specific and the term “bliss point” refers to optimal salt concentration to consumer. This optimal salt concentration seems to varies with consumer characteristics like age [(127)] (**Figure 1**). This inverted “U” function of added salt can be used in formulating foods, by testing the acceptance of different salt concentrations with many consumers.

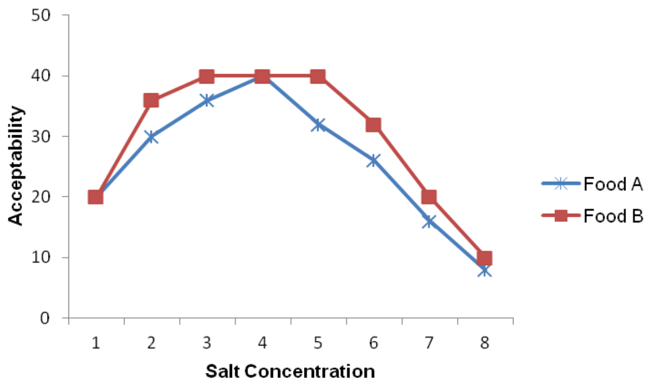

FIGURE 1- Hypothetical analysis of optimal salt levels in two foods, A and B;
For food A, with a sharp optimum, it may be difficult to reduce salt levels quickly if it is now manufactured or served at concentration level 4. For food B, if it is currently manufactured or sold at level 4, it may be relatively easy to reduce it to level 3, since this is equally acceptable.

In a study with school-aged children was showed that it may be relevant to adapt salt reduction to the specific type of food and salt content could be easily reduced below the current content in palatable foods (such pasta), whereas moderate salt content is sufficient but necessary in less palatable foods, such as vegetables [(137)].

2.2. Salt content in foods

Besides taste, salt modifies flavor, controls microbial growth, and alters nutrient availability and the texture/ consistency of food. Salt also has specific processing functions in different food categories as aid extraction methods, food formulation and the malting and fermenting of foods [(138, 139)]. Salt or sodium-containing ingredients (like sodium citrate, sodium nitrate and others) are added during food processing or cooking process due to it many functional attributes.

The range of salt content for foods varies widely, inclusively the same food could have different salt content based on different brands or processing. However salt is naturally present in few foods, for example milk, the contribution to total salt intake was almost than 12%, the large majority of salt was added to food during processing or cooking [(140)]. In **Table 3** can be noted the effect of processing and/or food cooking as major factors influencing salt content in foods. Variations in salt content of soups, cooked pasta, rice or even bread could be quite large.

The magnitude of the problem to estimate salt content in foods becomes more evident due to enormous quantity of new food products that daily appearing on the market and the difficulty to estimate salt losses with some food preparation practices for example soaking or rinsing of foods prior to preparation or discarding salted cooking water.

Table 3 – Salt content in various foods according to Portuguese Table Foods [65].

Food Item	Description	Sodium (mg/100g)	Salt (g/100g)
Cheese	Emmental	394	1.0
	Cheese from Irelands	991	2.5
	"Évora" cheese	3255	8.3
	"Camembert"	576	1.5
	"Serra"	792	2.0
	"Quark"	37	0.1
Buillon cubes, powdered broths	Chicken buillon cube	16300	41.5
	Cow buillon cube	15000	38.2
Smoked meats	Salami "*salpicão*"	4336	11.0
	Pork sausage "*paio*"	3507	8.9
	Sausage "*linguiça*"	2909	7.4
	Meat sausage "*chouriço de carne*"	2634	6.7
	Smoked ham "*presunto*"	2570	6.5
	Ham "fiambre"	1875	4.8
Vegetables	Olives	2100	5.3
	Olives in brine	2712	6.9
	Raw Carrots	58	0.1
	Pickles	1376	3.5
	Raw potatoes	9	0.02
	Masheed potatoes	128	0.3
Fish	Boiled cod	1228	3.1
	Grilled cod	1245	3.2
	Boiled shrimp	1600	4.1
	Boiled hake	190	0.5
Cereals and cereal products	Bread	550	1.4
	Corn Flakes	702	1.8
	Maria cookies	418	1.1
	Boiled pasta	238	0.6
	Raw pasta	5	0.01

It makes really difficult capture salt content of every products and makes challenging to accurately assess dietary sodium intake of individuals. As can be seen in **Figure 2**, home-made and ready-meal versions of two dishes, highlight the differences in salt content between varieties for common foods.

Food composition tables or databases provided detailed profiles of the nutritional composition of foods, typically for a particular country. They are essential to determining the sodium content in foods consumed as identified in food consumption surveys. Depending on which product a person chooses to eat the sodium ingested could vary greatly.

For this reason is necessary researchers had an ongoing commitment to kept up-to-date food databases using nutrition labeling or chemical analyses.

Sodium content in foods can be determined by diverse methods. Most modern analytical procedures utilize methods which are related to the atomic structure of the sodium ion, including Flame Photometry (FP), the Mohr or Volhard titration procedures or ion-selective electrode (ISE). All these methods are faster and less expensive than analysis by atomic absorption spectroscopy or inductively coupled plasma-atomic emission spectroscopy.

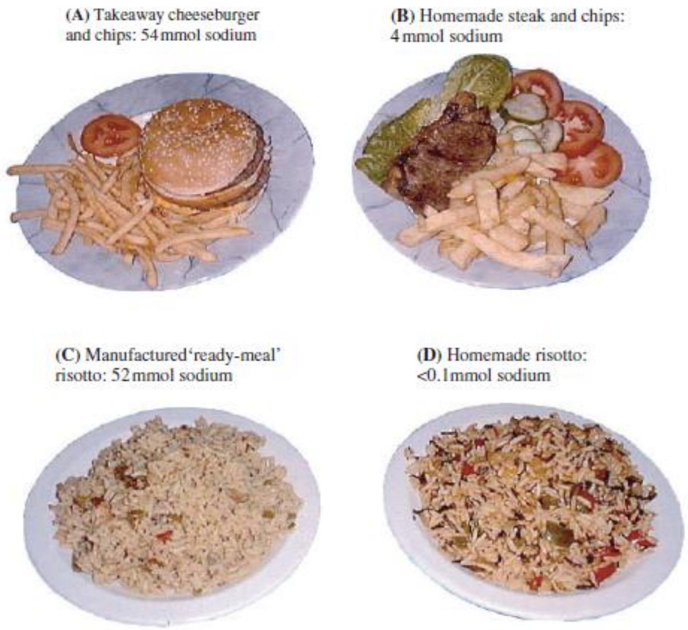

FIGURE 2 – The sodium content of manufactured (A, C) and home-made foods (B, D) meals
Sodium: 1 mmol = 23 mg; Salt: 1 g = 393 mg sodium. From: Salt intake around the world: implications for public health [70].

Some methods are official methods of analysis for numerous specific products, as FP [141] for bread analysis, considered the internal reference method for food analysis. However this methodology presents some limitations such as not being portable and having too much sample preparation steps. The demand for a portable, quick and user friendly instrument to analyse sodium content of foods in catering and industry could be part of the solution to monitoring salt content in foods by food security and industry agents.

Some portable instruments in market are based on conductivity methodology, which is not specific for sodium. It measures the total concentration of ions in solution and cannot distinguish on electrolyte or ion from another. These equipments although their portability, are only indicated to analyse high sodium solutions as brine, ketchup, soy sauce, etc. because on these solutions was not expected other ions on higher concentration than sodium.

The development and implementation of tools to both monitor and accurately evaluate sodium content in foods must be an important aim to researchers [142].

2.3. Sources of salt in diet

To reach the recommendations of daily salt consumption, the first step was to assess which foods contributed to the consumption of the population.

In European countries the large proportion of salt ingested is added in food production and foods eaten away from the home, is estimated that 75-85% of salt intake comes from these sources and 10-12% occurs naturally on foods, similarly proportion to be from added salt at home or at table [70, 143]. On other hand, Asian countries, like China, added salt during cooking and sauces is the major source of salt intake (around 70%) and salt from processed foods is low (almost 7%) [53].

In the United Kingdom, the main contributors to total salt intake is cereal and cereal products (35%), with white bread alone providing 14%, meat and meat products (26%), and milk and milk products (8%) [144].

An interesting study developed by Saunders et al. [145] in an urban municipality from England clearly showed that population of this area lives at a short walk from at least one takeaway restaurant, and single meal on there could containing over 173% of guideline daily amount. This situation becomes more serious given the evidence that young people commonly eat takeaway foods several times a week [146]. The consumption of snacks between meals, as pizzas, chips and sandwiches has become a popular habit among adolescents and can contribute with 1.4 g for daily sodium intake [147].

Our group published in 2012 results from a study where was analyzed sodium content present in vegetable soups served in several canteens as kindergartens, schools and nursing homes. Average amount of sodium could reach 269.06 mg/100g of soup, i.e. in a 300 g portion was 2049 mg of salt [148]. The salt content of available foods in the marketplace and from restaurants make difficult for consumers to meet dietary guidelines.

The custom of eating out in restaurants or canteens has become routine and takes place from early ages. In Finland, the majority of children and adolescents generally start from 2 or 3 years old consuming at least one daily meal outside home [28].

In a study with 638 Caucasian Portuguese adult hypertensive patients, the subgroup that ingest more salt, ate significantly higher amounts of vegetables, sauces, bread (account for 20-27% daily salt intake), cheese, fries and sausages/cold meat [149].

The major sources of sodium intake in Portuguese adolescents assessed by food frequency questionnaire were starchy foods, meat, dairy, seafood and fast-food [150]. No data are available related to food sources of sodium in children.

Similar sources of salt intake was found in England children and adolescents, being major contributors cereal and cereal products (36%), meat products (18%) and milk and milk products (11%). In this population bread alone contributes to 15% of total salt intake [109].

Flemish pre-school children had too high salt intake and bread (22%) and soup (13%) are important contributors to total salt intake [104].
In Spanish children added salt to meals during cooking or at the table seems to be the principal source of sodium (21.2%), followed by chips (12.1%), white bread (11.3%) and ham (6.3%) [151].
White bread was identified as a most important contributor to total salt intake in every country and at all ages, because it is consumed in consistent quantities by all segments of populations.

2.4. Recommendations

Historically there have been indirect health benefits from salt through the prevention of diseases caused by the spoiling and wastage of food; however with vulgarization of technological methods in food preparation, preservation, and distribution, the need for salting food for safety proposes has nearly been abrogated [19].
Salt is sodium chloride, the conjugation of the cation sodium and the anion chloride. About 98% of ingested salt is absorbed mainly in the small intestine, and remains in extracellular compartments. In people with minimal sweat loss, the amount of sodium excreted in the urine is roughly equal to the amount consumed [140].
Sodium is the most abundant extracellular cation and is pivotal for fluid balance and cellular homeostasis. Normal plasma sodium concentration is maintained in narrow limits, deviations of less than 1% of trigger corrective measures [152]; thus, a raised plasma sodium concentration stimulates both thirst and renal water conservation. For this reason, excess salt intake does not raise plasma sodium concentration (hypernatremia) if corrective measures like fluid ingestion are available. Then the resulting increase in extracellular fluid volume due to fluid ingestion stimulates increased sodium excretion through urine.
This sodium balance is influenced by various systems and hormones, including the renin-angiotensin-aldosterone axis, the sympathetic nervous system, atrial natriuretic peptide, the kallikrein-kinin system, various intrarenal mechanisms, and other factors that regulate renal and medullary blood flow [153]. Sodium depletion by reducing plasma volume and renal perfusion stimulates the production of renin from the kidneys, which generates the vasoconstrictor hormone angiotensin. Angiotensin stimulates sodium retention in the kidneys and stimulates adrenal secretion of aldosterone, which reduces sodium concentration in the feces, saliva, and sweat. Conversely, excess of sodium suppresses of salt retention mechanisms but also activates of natriuretic mechanisms. Two hormones are involved: the atrial natriuretic peptide (produced by the cardiac

atria) and active sodium transport inhibitors (probably produced within the brain). They increase sodium excretion and lower arterial pressure.

Sodium is also involved in the conduction of nerve impulses and in muscle contraction control [(154)].

Human population has demonstrated the capacity to live with both extremes of sodium intake, from less 18.4 mg/ day found in Yanomamo Indians from Brazil to 5152 mg/ day in Japanese men [(70)].

The minimal amount of sodium required to replace losses is estimated to be no more than 180 mg/ day (8 mmol/d) [(153)]. Compared with the average intake of most populations, the amount of sodium to maintain homeostasis is very low [(155)].

Table 4 summarizes the recommendations for sodium intake in each age group issued by national and international organizations.

According to Institute of Medicine (IOM) [(153)], sodium requirements are based on adequate intakes (AI) established to ensure that the overall diet provides an adequate intake of other nutrients and covers sodium sweat losses in individuals who are exposed to high temperatures or who become physically active. IOM also determines a tolerable upper intake level (UL) representing the highest level of daily nutrient intake that is not likely to pose risk effects for most people. In the specific case of sodium, UL is based on the impact of sodium on blood pressure. It also represents total intake from foods, water, and supplements.

Table 4 – Dietary recommendations for sodium (mg of sodium per day).

<table>
<tr><th rowspan="2">Life Stage Group</th><th colspan="2">IOM[1]</th><th rowspan="2">WHO[2]</th><th rowspan="2">DGA 2010[3]</th><th rowspan="2">DGS[4]</th></tr>
<tr><th>AI[5]</th><th>UL[6]</th></tr>
<tr><td>0-6 months</td><td>120</td><td>ND[6]</td><td></td><td></td><td></td></tr>
<tr><td>6-12 months</td><td>370</td><td>ND[6]</td><td></td><td></td><td></td></tr>
<tr><td>1-3 years</td><td>1000</td><td>1500</td><td rowspan="4"><2000 adjusted downward based on energy requirements</td><td rowspan="4"><1500</td><td rowspan="4"><2000 adjusted downward based on energy requirements</td></tr>
<tr><td>4-8 years</td><td>1200</td><td>1900</td></tr>
<tr><td>9-13 years</td><td>1500</td><td>2200</td></tr>
<tr><td>14-18 years</td><td>1500</td><td>2300</td></tr>
<tr><td>19-50 years</td><td>1500</td><td>2300</td><td rowspan="3"><2000</td><td><2300</td><td rowspan="3"><2000</td></tr>
<tr><td>51-70 years</td><td>1300</td><td>2300</td><td><1500</td></tr>
<tr><td>> 70 years</td><td>1200</td><td>2300</td><td><1500</td></tr>
<tr><td>Persons with diabetes, hypertension or chronic kidney disease.</td><td>-</td><td>-</td><td>-</td><td><1500</td><td>-</td></tr>
</table>

[1] IOM – Institute of Medicine (www.nap.edu). ; [2] OMS – World Health Organization; [3] DGA 2010 – Dietary Guidelines for Americans 2010; [4] DGS – Direção Geral de Saúde; [5] AI – Adequate intake, may be used as a goal for individual intake; [6] UL – The maximum level of daily nutrient intake that is likely to pose no risk of adverse effects; [6] ND – not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts.

For adolescents, IOM recommends an ingestion of sodium below 2300 mg/day, which corresponds to 6 g/day of salt.

More recently, the US Department of Agriculture and US Department of Health and Human Services provided a document to help Americans choose a healthy eating pattern, the Dietary Guidelines for Americans [156]. This document advised that children and adolescents sodium intakes in should be less than 1500 mg/day (4 g/day of salt).
In 2012, the World Health Organization (WHO) [157] issued a guideline that provides recommendations on the consumption of sodium to reduce the number of subjects with non-communicable diseases. WHO recommends that sodium intake be less than 2000 mg/day (5 g/day of salt) and adjusts this recommendation downward for children based on their energy requirements.
The Portuguese government (Direção-Geral da Saúde) and other members of the European Union follow the recommendations of WHO [158].

3. Salt reduction initiatives

In 2006, WHO organized a technical meeting to develop recommendations for Member states on interventions to reduce salt intake and they conclude that interventions to reduce population salt intake have been shown to be highly cost-effective and highlighted the urgency to national specific programs to reduce salt intake [(76)]. In 2013, all members states of WHO signed up to the target to reduce salt intake in population by 30% by 2025 with a target 5 g per day [(159)].

European Union (EU) developed in 2008 a Framework for National Salt Initiatives to describe a common vision for a general European approach towards salt reduction [(71)], and Portugal reported that the Framework help to define and support the strategy and to empower actions against salt [(72)]. In 2013 was reported the situation and actions taken in the EU and its Member States since 2008 [(160)].

The United Nations General Assembly recognize the important role of salt in prevention and control of non-communicable diseases and reaffirm the political commitment in response to the challenge of reduce salt intake [(161)].

Reduction in salt consumption is one top priority in political commitments because was one of the most cost effective strategy to reduce non communicable diseases[(162, 163)], it was estimated that the reduction of population-wide salt consumption by only 15% would avert up to 8.5 million deaths in 23 high-burden countries over 10 years [(163, 164)]. It is expected that reducing daily salt intake of population to less than 5 grams a substantially reduction on the burden of cardiovascular disease and mortality in several European countries occur [(165)].

Several European countries have already carried out salt reduction strategies, United Kingdom (UK) and Finland are the most successfully examples. However other low-income and middle-income European countries have not even developed a salt reduction strategy [(1, 166)].

The UK salt reduction strategy has led to a 15% reduction in the average salt intake of the population that was 9.5 g/d in 2001 to 8.1 g/d in 2009 [(167)]. This strategy has worked on a voluntary basis with industry, most of processed foods available in supermarkets have now less 20 to 30% salt content. The targets to salt reduction were stipulated for 80 food categories, and these targets are reviewed each 2 years. At the same time a major public health campaign was promoted to sensitize consumer to salt reduction [(168)].

Finland was one of the first countries to starts a systematic approach to decrease salt intake in the population. The Finnish approach to diminished salt result in a reduction of salt intake by 12 g/day in 1979 to less than 9 g/day in 2002 as measured by 24-h urinary sodium [169]. The Finnish program beyond mass media campaigns, co-operation with the food industry and implementing salt labelling legislation, had disseminated the replacement of conventional table salt by a sodium-reduced, potassium-and magnesium-enriched mineral salt known as Pansalt. In the early of 1990s Government set rigorous salt labeling legislation applied for all the food categories where foods that are high in salt are required to carry a 'high salt content' warning [170].

The most popular approach to reduce national salt intake in Portugal was the adoption of national legislation in relation to salt content in bread. In 2009, salt content in bread was limited to 1.4g per 100g [171]. In 2013, Ministry of Health reports the strategy to reduce salt consumption in Portugal [158]. **Figure 3** shows the four strategic objectives in Portuguese program: (1) provide labeling able to help consumer decision making, (2) modify the availability creating conditions for foods with more salt become more inaccessible, (3) raise awareness and empower citizens to a lower salt intake and (4) implement evaluation systems of intake and monitor the supply of salt in food.

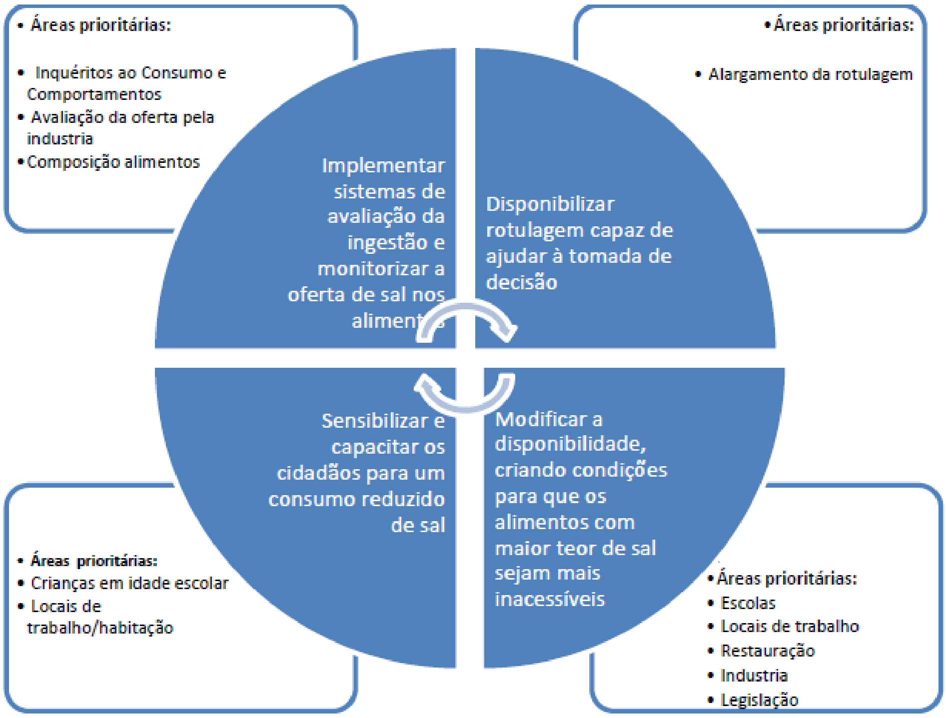

FIGURE 3 - Strategic objectives and priority areas to reduce salt intake in Portugal. From: Estratégia para a redução do consumo de sal na alimentação em Portugal [158].

In 2015, the Portuguese Government assumed that salt reduction was imperative for public health, and promoted the creation of the interministerial Working Group [(172)] with the mission to set measures quantifiable and monitored over time to reduce population salt intake, particularly by reducing the supply of food products high in salt, namely in catering.

The Working Group presented 14 proposals that summarize the priorities and strategies adopted by consensus among food industry, government elements and researchers [(173)]. The consensus document highlights the necessity to set quantifiable reduction targets and monitor in terms of consumption (population / consumer) and supply (food and meals on sale).

In United States children and adolescents are included on core messages about decrease salt consumption based on concerns about the development of preferences for salt taste at young ages and the increasingly earlier development of high blood pressure [(127)]. Adults today may still impacted by their childhood diet [(28)]. Consequently the influence of childhood habits is important when planning educational strategies for the primary prevention of diseases.

4. Aims

The main aim of this thesis was to assess dietary salt intake in adolescents, its dietary sources and examine potential factors that could help to develop strategies to reduce salt intake.
This thesis is organized by studies that aims answer the following specific objectives:

1. To describe dietary salt intake and its impact on hydration status in adolescents:
   (i) describe dietary sodium, potassium and sodium-to-potassium ratio excretions (Study 1);
   (ii) evaluate the compliance of sodium and potassium intake guidelines (Study 1);
   (iii) investigate the main food sources to total dietary sodium and potassium intake in Portuguese adolescents (Study 1);
   (iv) assess the association between urinary sodium excretion and hydration status by FWR (Study 2).

2. To examine potential factors that could help to reduce salt intake:
   (i) assess the perceived saltiness intensity and liking of a vegetable soup after 30% reduction of the usual sodium content, in a sample of institutionalized elderly and community preschool children (Study 3);
   (ii) establish a baseline on food handler's knowledge, practices and behavior with respect to salt handling (Study 4);
   (iii) describe and validate a new electrochemical method that allows an easy and accurate determination of salt content in foods (Study 5).

Part II - Methods

Methods

Data for **study 1 and 2** were derived from Longitudinal Analysis of Biomarkers and Environmental Determinants of Physical Activity Study (LabMed Physical Activity Study), which was a 3-yr follow up study with Portuguese children and adolescents aged 12 to 18 years old. This school-based prospective cohort study was carried out in 4 Portuguese cities from the North Region (Barcelos, Vila Nova de Gaia, Ílhavo and Braga). LabMed Physical Activity Study aimed to evaluate the independent and combined associations of dietary intake, physical activity (PA) and sedentary behaviour (SB) on fitness levels and on cardiovascular and inflammatory markers; the early determinants of cardiovascular and inflammatory markers; the independent and combined associations of dietary intake, motor coordination, PA and SB on academic achievement; the associations of perceived environmental features and PA and SB levels; and the psychosocial correlates of PA and SB levels.

All participants in this study were informed of its goals and the parent or guardian of each subject provided a written informed consent for his/her child to participate. The study was conducted in accordance with the World Medical Association's Helsinki Declaration for Human Studies. The Portuguese Data Protection Authority (process number 1112434/2011), the Portuguese Ministry of Science and Education (approval number 0246200001/2011) and Faculty of Sport, University of Porto, approved the study.

Baseline data was collected in the fall of 2011,for 1,229 adolescents aged 12 to-14 years (7th grade) and 15 to-18 years (10th grade).

A convenience subsample of subjects (n=398) was invited to perform 24h urine collection and 250 subjects (63%) voluntary agreed to perform 24 h urine collections. Urine collections were performed between 2012 and 2014. After a validation control of 24h urine collection 50 urine collections were rejected (20%). Thus, the final sample consisted of 200 adolescents (82 boys) with valid urine collection and corresponding dietary recall. Urinary collection was also approved by Ethical Commission of University of Porto (process number: 16/2012).

The **study 3** was a sensory analysis and subjects were elderly (n=35) from two public nursing homes in Barcelos city and pre-school children (n=75) from Seia city. Due to exclusion criteria applied to elderly subjects, that were have renal diseases or hyponatremia, the final sample was 29. Cognitive status of all elderly subjects was evaluated. The final sample of pre-school children consisted in 49 subjects, due to

parent's assignment of consent form. All provided written informed consent were obtained from participants.
Baseline salt content of vegetable soups was previous analyzed by flame photometry and then sensory evaluation tests were performed with a less 30% salt content and a baseline salt content vegetable soup. The sensory evaluation took place in usual lunchrooms at the institutions at typical lunch period.
Sensory description of the two soups was performed by elderly using a visual analogue scale with 10 cm line for salt perception and hedonic perception and by children using a five-point facial scale for perceived liking.

Data to **study 4** result from analysis of a questionnaire filled out by food handlers that work in a Portuguese catering company. The questionnaire was developed and reviewed after a pretest (on 10 food handlers) by our researchers' team. Questionnaire has three main parts: 1) assessment of knowledge about adequate intake of salt, major food groups contributing to salt intake, and the relationship between salt consumption and health; 2) evaluation of the concerns about the use of salt, practices and difficulties; 3) characteristics of the business unit and sociodemographic characteristics of the subjects.

Study 5 was performed with a sodium ion-selective electrode from Sentek (United Kingdom) combined to a Crison potentiometer (Spain). The steps of sample preparation and calibration were described detailed in corresponding paper. Validation consisted in the assessment of a set of parameters as specificity, linearity, precision and accuracy in several food categories. Food samples used to compare ISE with FP were collected between February and May 2015 in 7 canteens from a public university in Porto. The collection was performed in random days, without the notification to the institutions; in each visit one sample were weighted (SECA scale, Germany) and transported in polyethylene bags. The final set of samples consisted of 2 soups and 6 meals (n=2 meat, n=2 fish, n=2 vegetarian).

The characteristics of each study which made this dissertation are shown in **Table 5**. The full description concerning sample size, variables assessment and statistical analyses are presented in the corresponding paper at the methods section.

Table 5 – Summary of the characteristics of the studies integrated in the dissertation.

Study	Type	Main variables	Sample size	Age (years)	Major Statistics
Study 1 Sodium and potassium urinary excretion and dietary intake: cross-sectional analysis in adolescents.	Cross sectional	Single 24 hour urine collection Dietary record	118 girls 82 boys	14.9 ± 1.4 14.7 ± 1.3	Independent Sample T-test Mann-Whitney U test Chi-square test Spearman's correlation coefficient
Study 2 The relationship between high sodium excretion and hydration status by Free Water Reserve: cross-sectional analysis in adolescents.	Cross sectional	Single 24 hour urine collection	118 girls 82 boys	14.9 ± 1.4 14.7 ± 1.3	Linear regression model
Study 3 Salt reduction in vegetable soup does not affect saltiness intensity and liking in the elderly and children.	Sensorial analysis	Visual analogue scale Five point facial scale	29 elderly 49 children	79.7 ± 8.9 4.5 ± 1.3	Wilcoxon signed rank test Spearman's correlation coefficient
Study 4 Knowledge and practices related to added salt in meals by food handlers.	Cross sectional	Questionnaire	68 subjects	Between 26-44	Pearson's chi-square test
Study 5 Validation of a rapid and simple method to determine sodium content in food matrices based on Ion Selective Electrode methodology	Laboratorial	Sample foods collected from university canteens	8 samples: 2 soups 2 meat dishes 2 fish dishes 2 vegetarian dishes	Not applicable	Linear regression model Paired Student t test Spearman's correlation coefficient

Part III – Results

Study 1 - Sodium and potassium urinary excretion and dietary intake: a cross-sectional analysis in adolescents

(Submitted and under review)

[Submitted – under review]

Sodium and potassium urinary excretion and dietary intake: a cross-sectional analysis in adolescents

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Short Title: Sodium and Potassium intake in adolescents

ABSTRACT

BACKGROUND: Hypertension is the leading cause for heart disease and stroke, the leading causes for mortality and morbidity worldwide and a high sodium-to-potassium intake ratio is considered a stronger risk factor for hypertension than sodium alone.

OBJECTIVE: This study aims to evaluate sodium and potassium urinary excretion, and to assess the food sources of these nutrients in a sample of Portuguese adolescents.

DESIGN: Cross-sectional study with a sample of 398 Portuguese adolescents. Sodium and potassium excretion were measured by one 24h urinary collection, and coefficient of creatinine was used to validate completeness of urine collections. Dietary sources of sodium and potassium were assessed using a 24h dietary recall.

RESULTS: Valid urine collections were provided by 200 adolescents (118 girls) with median age 14.0 in both sex (p=0.295). Regarding sodium, the mean urinary excretion was 3725 mg/d in boys and 3062 mg/d in girls (p<0.001), and 9.8% of boys and 22% girls meets the World Health Organization (WHO) recommendations for sodium intake. Concerning potassium, the mean urinary excretion was 2237 mg/d in boys and 1904 mg/d in girls (p<0.001) and 6.1% of boys and 1.7% of girls meets the WHO recommendations for potassium intake. Major dietary sources for sodium intake were cereal and cereal products (41%), meat products (16%) and milk and milk products (11%); and for potassium intake, main sources were milk and milk products (21%), meat products (17%) and vegetables (15%).

CONCLUSIONS: Adolescents had a high sodium and low potassium diet, well far away from WHO recommendations. Health promotion interventions are needed in order to decrease sodium and increase potassium intake.

KEY WORDS: urinary sodium, urinary potassium, intake, adolescents, salt

INTRODUCTION

Evidence demonstrates that high sodium intake increases blood pressure and has an impact in endothelial dysfunction, cardiovascular function and structure, kidney disease, and cardiovascular morbidity and mortality (1, 2). Hypertension is recognized as a primary risk factor of heart disease and stroke, both leading causes of death worldwide (3). Recent data regarding sodium intake show that populations around the world are consuming far more sodium than is physiologically necessary (4). In response, the World Health Organization (WHO) considers the reduction of sodium intake to be a priority concern (5), and WHO member states have agreed to work toward a global 30% reduction until 2025 (6).

At the same time, interest in potassium intake has grown, namely because potassium attenuates sodium's negative effects, by reducing stroke rates and cardiovascular risk (7) and increasing urinary sodium excretion (8). Furthermore, low dietary intakes of potassium potentiate the sodium sensitivity of blood pressure (9) and the risk of hypertension, while the relationship between sodium and blood pressure strengthens if the urinary sodium-to-potassium ratio (Na+/K+) is considered instead of only sodium excretion rate (10). High Na+/K+ intake is considered to be a stronger risk factor of hypertension and cardiovascular disease than each of these nutrients alone (11, 12), and the benefits of higher intake of potassium are particularly important when sodium intake is high (13).

Data from around the world suggest that the average potassium consumption in the populations of many countries is less than the 2730-3120 mg/d, which was the reference recommended by the 2002 Joint WHO/ Food and Agriculture Organization expert consultation (14).

The WHO recommends a maximum sodium intake of 2000 mg/d (15) for children and a potassium intake of at least 3510 mg/d, which should be adjusted downward for children based on their energy requirements (16).

In Portugal, cerebrovascular and cardiovascular diseases are the major causes of death (17), and nearly 42% of the population has hypertension (18). In the adult population, the latest data indicate an average consumption of 4200 mg sodium/d and 2900 mg potassium/d (18), whereas data purporting the youngest population remain scarce. To the best of our knowledge, no study has yet characterized sodium and potassium intake in Portuguese adolescents using the gold standard method of 24 hour urine collection (19). This method is considered the "gold standard" for assessing the distribution and average intake of sodium in a representative population (20, 21).

Accurate estimates of sodium and potassium intake are essential for monitoring the effectiveness of current actions to reduce sodium intake and to improve efforts to increase potassium consumption.

In this study, we thus aimed to (i) describe dietary intakes of sodium and potassium and Na+/K+; (ii) assess their compliance with sodium and potassium intake guidelines and (iii) investigate the main food sources of total dietary sodium and potassium intake among Portuguese adolescents.

METHODS

Study Design

Data for the present cross-sectional study came from a 3-yr follow up study with Portuguese children and adolescents – Longitudinal Analysis of Biomarkers and Environmental Determinants of Physical activity (LabMed Physical Activity Study). The LabMed Physical Activity Study aimed to evaluate independent and combined associations of dietary intake, physical activity and sedentary behavior on fitness levels and other factors in 12 to 18 years old children and adolescents.

From the participating schools all students enrolled in the 7th and 10th grade classes in 2011 scholar year were invited to participate in the study (n=1678). The sample for this analysis consisted of a convenience subgroup of LabMed Physical Activity Study aged 13 to 18 years old (n=398) that was invited to perform 24 h urine collection and 250 subjects (63%) voluntary agreed to perform 24 h urine collections. Urine collections were performed between 2012 and 2014. After a validation control of 24h urine collection (described below) 50 urine collections were rejected (20%). Thus, the final sample consisted of 200 adolescents (82 boys) with both valid urine collection and corresponding dietary recall.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Ethical Commission of University of Porto. Written informed consent was obtained from all subjects and caregivers.

24h Urine Collection

Participants were asked to complete a 24-hour urine collection. Participants and caregivers received oral and written instructions on how to collect complete 24-h urine samples. They were instructed to discard the first morning void and to collect all urine over the following 24-hour including the first void on the next morning, and the time of the start and finish collection was recorded in a questionnaire. Considering the participant's comfort and for feasibility purposes, urine collections were held on Sundays and during the collection period subjects were asked to store the collected urine in a cool place. Urine samples were sent to a certified laboratory to be analyzed for urinary creatinine (mg/day) (Jaffé reaction, Siemens Advia 1650), urinary sodium (mEq/day) and urinary potassium (mEq/day) (indirect ion-selective electrodes methodology, Siemens Advia 1800). Sodium and potassium excretion was reported in mEq/day, however, for comparative purposes, it was converted to mg/day by using their molecular weight. Na^+/K^+ was also calculated as sodium (mg/day) divided by potassium (mg/day).

For 24-hour urine collection validation, quality control was used calculating 24-hour urinary creatinine excretion in relation to body weight according to age group (22). If the urine collection was incomplete (n=50) or if the subjects had felt ill (n=0) or took medication on the day of collection (n=2) the urine specimen was rejected and not considered for analysis. The subjects that refer having felt ill during the day of collection agree to collect 24-hour urine again.

Dietary Record

A 24 hour dietary recall referring to the day of urine collection was collected by trained interviewers using a photographic book and household measures to quantify portion sizes (23). Energy and nutritional intake were estimated using an adapted Portuguese version of the nutritional analysis software Food Processor Plus (ESHA Research Inc., Salem, OR, USA). The nutrient content of basic food was taken from standard nutrient tables (24), whereas the content of commercial food (e.g. pizza and ready-to-eat-food) was derived from labeled ingredients and nutrients.

The food codes used were categorized into 13 major food groups (25): (1) cereal and cereal products (bread; breakfast cereals; biscuits, cakes, puddings, scones, doughnuts; pasta, rice, and other cereal-based products); (2) meat products (chicken and turkey dishes; sausages; bacon and ham; red meats); (3) milk and milk products (milks, yogurts and cheeses); (4) vegetable and potato products (potato products); (5) soups and sauces (vegetable soups and sauces); (6) fruits; (7) fish and seafood dishes (cod fish); (8) oils and fats; (9) egg and egg dishes; (10) sugars, preserves, and confectionery; (11) fast food (pizza; sandwiches, burgers, filled wraps; salted snacks and fried snacks; fried potatoes); (12) beverages (soft drinks; water; hot beverages); (13) other foods (beans, pulses, canned fruit and pickles). The contribution of these 13 food groups to sodium and potassium intake was calculated. An additional analysis was performed to evaluate the contribution of each food group to total sodium and potassium intake only in participants who ingested foods included in that food group

For validation of records and to check for underreporting, the ratio of reported energy intake (EI) and estimated basal metabolic rate (BMR) according to Schofield (26) was used as proposed by Goldberg et al. (27) taking into account age, sex, body weight, and height. Using the formulas proposed by Goldberg et al. (27) we recalculated individual specific cut offs with the following modifications: the new estimates of the Physical Activity Level assuming light physical activity given for the different age/sex groups adolescents (28), using coefficients of variation for EI (23%) (29) and the use 1 recorded day.

Therefore, records with EI:BMR ratios below the cut offs values depending on the subject's age and sex was considered as a not plausible measurement of the actual 1d energy intake in our further analysis and were excluded (n=22, 11%).

Anthropometric measures

For weight and height measurements we used a digital scale (Tanita Inner Scan BC 532, Tokyo, Japan) and a portable stadiometer (Seca 213, Hamburg, Germany) respectively. All measurements were performed with participants in light clothing, without shoes, and according to standard procedures (30).

According to BMI-for-age x-scores from the WHO, participants were classified in thinness (<-2SD), normal weight, overweight (> +1 SD), or obese (> +2 SD) (31). The classification was performed using WHO AntroPlus Software (32).

Socioeconomic status

As an indicator of the socioeconomic status of the household, the Family Affluence Scale (FAS) was used (33). The FAS is a four-item questionnaire that helps students report their family income objectively: it evaluates the sum of scores regarding whether the family owns a car, whether the student has his/her own bedroom, the number of family vacations during the past 12 months, and the number of computers the family owns. The final score ranges from 0 to 9 points, the highest score mean a higher socioeconomic status.

Statistical analysis

All statistical analyses were performed using SPSS 21.0 Inc.. A p-value <0.05 was considered to indicate statistical significance. The Kolmogorov-Smirnov test was used to assess the assumption of normality. Independent Sample T-test or Mann–Whitney U test were performed to compare continuous variables, and Chi-Square test was used for categorical variables. Dietary sources of sodium and potassium were reported through the thirteen food and beverages categories. A proportion to total food sodium or potassium was used to report results as mean percentage contribution of each category. Nutrients and food intake were also energy-adjusted according to residual method (34), and adjusted values were used to evaluate the differences between sexes. Spearman's correlation coefficient was used to identify and test the strength of a relationship between urinary sodium with dietary sodium and urinary potassium with dietary potassium.

RESULTS

Descriptive characteristics of the sample are presented in Table I. Adolescents were on average 14.0 years old and most exhibited a BMI in the normal range.

Table I - Sample characteristics of the study sample (13-18 years) (n=200).

	Boys	**Girls**	***p***
Age (y) [a,b]	14.7±1.3	14.9±1.4	0.295
Weight (kg) [a,b]	60.2±12.2	55.1±9.3	0.001
Height (m) [a,b]	1.7±0.1	1.6±0.1	<0.001
Weight status (%)[c]			
Thinness	1.2	0.8	
Normal weight	73.2	74.6	<0.001
Overweight	17.1	17.8	
Obese	8.5	6.8	
FAS [a,b]	6.5±1.8	6.7±1.6	0.704

[a] Values are mean ± standard deviation
[b] Between-sex analysis by Mann-Whitney U test.
[c] Analysis by χ2 for categorical variables.
FAS - Family Affluence Scale

Results from urine collection are shown in Table II. Boys showed a greater median sodium and potassium excretion than girls (p<0.001). Of all participants, 83% exhibited sodium intake above upper-limit recommendations (2000 mg/day), while 96.1% showed potassium intake below recommendations (3510 mg/day). The Na+/K+ were similar in both sexes (1.7 in boys and 1.6 in girls, p=0.109).

Table II - Urinary data on sodium and potassium excretion by sex (13-18 years)[a]

	Boys n=82	**Girls n=118**	***p***
Sodium (mg/day)[a,b]	3725±1445	3062±1379	<0.001
% of compliance[c]	9.8	22	
Potassium (mg/day)[a,b]	2237±704	1904±593	<0.001
% of compliance[d]	6.1	1.7	
Na/K ratio[a,b]	1.7±0.6	1.6±0.6	0.109

[a] Values are mean± standard deviation.
[b] Analysis by Mann-Whitney U test.
[c] Percentage of participants with sodium excretion below 2000 mg/d.
[d] Percentage of participants with potassium excretion above 3510 mg/d.
Na/K ratio – sodium to potassium ratio

In this study, sodium and potassium intakes determined by dietary records correlated with 24 hour sodium and potassium excretion values (r=0.152. p=0.031 for sodium and r=0.229, p=0.001 for potassium).

As shown in Table III boys reported higher energy intake than girls (p<0.001). From dietary records, mean sodium intake was 2649 mg/d for boys and 2106 mg/d for girls (p<0.001), which corresponds to a mean estimated salt intake of 7 g/day for boys and 5 g/day for girls. Mean potassium intake was 2998mg/d for boys and 2471 mg/d for girls (p=0.009).

Table III - Dietary intake of the sample by sex (13-18 years) crude and adjusted to total energy intake (n=178).

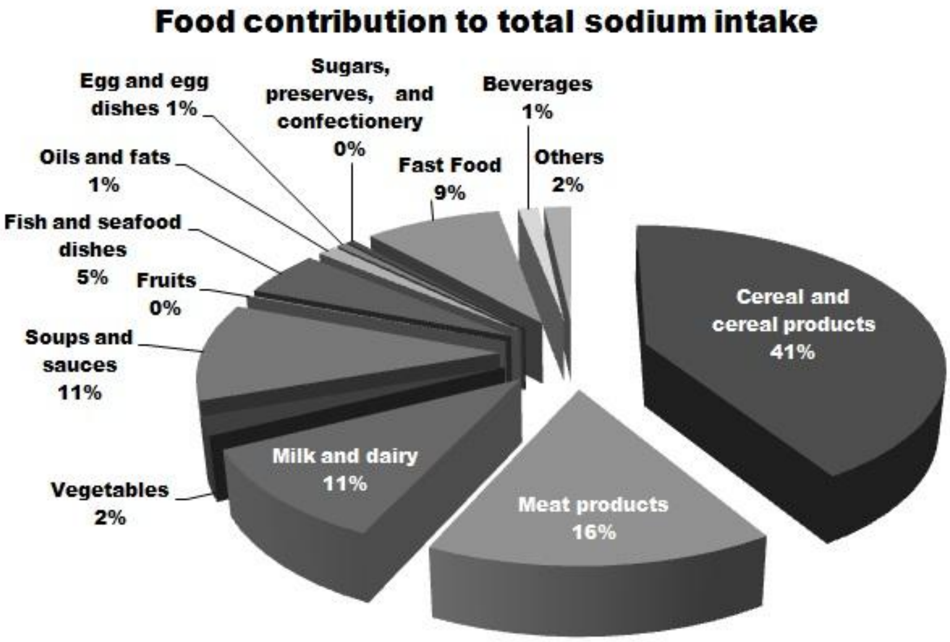
	Boys	Girls	*p*	*p**
Nutritional intake				
Total energy (kcal/d)[a,b]	2449±929	1981±665	<0.001	<0.001
Carbohydrates (g/d)[a,b]	285±113	242±83	0.002	0.305
Carbohydrates (%TEI)[a,b]	47±7	49±8	0.081	0.196
Sugars (g/d)[a,b]	131±64	115±57	0.020	0.407
Protein (g/d)[a,b]	103±53	79±32	<0.001	0.614
Protein (%TEI)[a,b]	17±4	16±4	0.528	0.375
Fat (g/d)[a,b]	93±42	73±34	<0.001	0.907
Fat (% TEI)[a,d]	34 ± 7	33 ± 7	0.205	0.810
Dietary fiber (g/1000kcal)[a,b]	6±3	7±3	0.073	0.089
Total food (g/d)[a,b]	2540±916	2219±735	0.017	0.889
Total water (g/d)[a,b,e]	1781±713	1632±609	0.221	0.456
Potassium (mg/d)[a,b]	2998±1368	2471±1068	0.009	0.524
Sodium (mg/d)[a,b,f]	2649±1586	2106±1204	<0.001	0.287
Salt (g/d)[a,b,f]	7±4	5±3	<0.001	0.287
Na/K ratio[a,b]	1.7±0.6	1.6±0.6	0.192	0.350
Dietary intake				
Cereal and cereal products (g/d)[c,b]	286 (227, 357)	225 (162, 309)	0.001	0.219
Breads (g/d) [c,b]	93 (45, 143)	80 (45, 120)	0.261	0.673
Breakfast cereals (g/d) [c,b]	30 (30, 40)	30 (30, 40)	0.124	0.520
Biscuits, cakes, puddings, scones, doughnuts (g/d) [c,b]	60 (29, 124)	75 (40, 117)	0.516	0.045
Pasta, rice, and other cereal-based products (g/d) [c,b]	151 (97, 224)	97 (43, 180)	<0.001	0.012
Meat products (g/d) [c,b]	160 (120, 252)	132 (80, 212)	0.007	0.799
Chicken and turkey dishes (g/d)[c,b]	118 (93, 220)	105 (99, 173)	0.444	0.871
Sausages (g/d) [c,b]	40 (20, 60)	25 (14, 45)	0.122	0.166
Bacon and ham (g/d) [c,b]	30 (23, 60)	30 (23, 45)	0.428	0.826
Red meats (g/d) [c,b]	119 (90, 195)	112 (56, 160)	0.090	0.328
Milk and milk products (g/d) [c,b]	545 (292, 841)	471 (304, 659)	0.066	0.172
Milk (g/d) [c,b]	516 (283, 841)	400 (280, 545)	0.013	0.012
Cheeses (g/d) [c,b]	30 (28, 60)	30 (28, 56)	0.860	0.709
Vegetable and potato products (g/d)[c,b]	123 (73, 175)	121 (63, 224)	0.844	0.182
Potato products (g/d) [c,b]	155 (93, 350)	155 (116, 231)	0.772	0.860
Soups and sauces (g/d) [c,b]	363 (117, 541)	363 (174, 368)	0.499	0.706
Vegetable soups (g/d) [c,b]	363 (363, 541)	363.0 (261.5, 458.8)	0.102	0.179
Sauces (g/d) [c,b]	15 (11, 68)	10 (8, 20)	0.184	0.315
Fruits (g/d) [c,b]	301 (174, 487)	174 (132, 328)	0.100	0.191
Fish and seafood dishes (g/d)[c,b]	184 (108, 272)	120 (75, 227)	0.139	0.235
Cod fish (g/d) [c,b]	236 (71.0, -)	114 (68, 233)	0.496	0.545
Oils and fats (g/d) [c,b]	10 (4, 27)	10 (5, 21)	0.528	0.183
Egg and egg dishes (g/d) [c,b]	100 (58, 100)	46 (22, 50)	0.030	0.026
Sugars, preserves, and confectionery (g/d) [c,b]	16 (10, 37)	12 (8, 25)	0.073	0.923
Fast food (g/d) [c,b]	150 (100, 333)	150 (82, 202)	0.850	0.372
Pizza (g/d) [c,b]	220 (191, 445)	301 (200, 403)	0.941	0.289
Sandwiches, burgers, filled wraps (g/d) [c,b]	219 (219, 219)	219 (80, 219)	0.396	0.749
Salted snacks and fried snacks (g/d) [c,b]	104 (52, 229)	144 (88, 147)	0.767	0.462
Fried potatoes (g/d) [c,b]	100 (100, 150)	100 (50, 123)	0.213	0.258
Beverages (g/d) [c,b]	720 (476, 1140)	825 (540, 1047)	0.576	0.036
Soft drinks (g/d) [c,b]	435 (200, 615)	495 (220, 707)	0.373	0.111
Water (g/d) [c,b]	410 (90, 720)	440 (140, 846)	0.537	0.729
Hot beverages (g/d) [c,b]	30 (4, 122)	102 (10, 295)	0.202	0.336
Other foods (g/d) [c,b]	69 (22, 185)	81 (21, 127)	0.826	0.930

[a] Values are mean ± standard deviation.
[b] Analysis by Mann-Whitney U test.
[c] Values are median (P25, P75).[d] Analysis by Student t test for continuous variables.
[e] Water from foods, beverages and metabolic water.
[f] Estimated from dietary records without considering household salt, 393 mg sodium=1 g salt.
* Adjusted for energy intake.
TEI – Total Energy Intake.

The major food sources of sodium in participants' diet (Figure 1) were cereal and cereal products (mean 41%, including bread (mean 16%);pasta, rice and other cereal-based products (mean12%) and breakfast cereals (mean 5%)), meat products (mean 16%, including bacon and ham (mean 7%), sausages (mean 4%), red meats (mean 3%) and chicken and turkey dishes (mean 2%)) and milk and milk products (mean 11%, including milk (mean 4%) and cheese (mean 4%)). Fast food contributed mean of 9% to total sodium ingestion, including the contribution of pizza (mean 3%), salted and fried snacks (mean 1%), fried potatoes (mean 3%) and sandwiches, burgers and filled wraps (mean 2%).

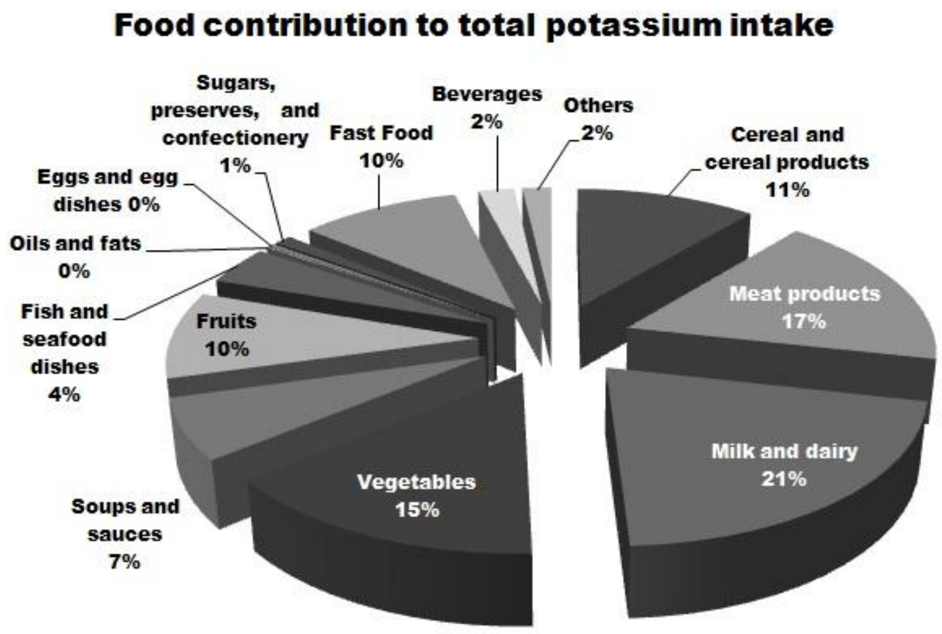
By contrast, milk and milk products (mean 21%, including milk (mean 14%) and cheese (mean 1%)); meat products (mean 17%, including red meats (mean 9%) and chicken, turkey dishes (mean 5%), bacon and ham (mean 2%) and sausages (mean 1%)) and vegetables (mean 15%) were major sources of total potassium intake (Figure 2). Fast food contributed 10% to total potassium ingestion, including the large contribution of fried potatoes (mean 8%).

Figure 1 – Contribution (%) of dietary sources for total sodium intake to total sample of subjects (n=178).



The contribution of each food category is expressed in mean percentage.

Figure 2 – Contribution (%) of dietary sources for total potassium intake to total sample of subjects (n=178).



The contribution of each food category is expressed in mean percentage.

The contribution of food groups to total sodium and potassium intake by sex, considering only the subjects that ingest foods included in groups is shown in Table IV.

Table IV – Contribution (%) of dietary sources for total sodium and potassium intake by sex considering subjects that consume those food groups (n=178).

	% CONTRIBUTION TO SODIUM INTAKE						**% CONTRIBUTION TO POTASSIUM INTAKE**					
	n	Boys	n	Girls	p	p*	n	Boys	n	Girls	p	p*
Cereal and cereal products	73	39.5 ± 16.2	105	42.5 ± 18.0	0.369	0.543	73	10.9 ± 7.7	105	11.3 ± 8.8	0.921	0.588
Breads	62	18.1 ± 10.7	85	19.4 ± 11.6	0.627	0.759	62	4.6 ± 3.1	85	5.1 ± 3.9	0.678	0.631
Breakfast cereals	36	9.0 ± 5.9	47	10.6 ± 7.6	0.262	0.869	36	3.4 ± 3.6	47	4.1 ± 3.0	0.033	0.674
Biscuits, cakes, puddings, scones, doughnuts	45	10.0 ± 7.6	75	16.4 ± 10.4	0.002	0.004	45	4.6 ± 4.6	75	4.6 ± 4.6	0.725	0.675
Pasta, rice, and other cereal-based products	60	16.5 ± 11.7	83	14.1 ± 10.8	0.125	0.112	60	3.3 ± 3.7	83	3.4 ± 5.5	0.071	0.231
Meat products	71	18.3 ± 14.1	95	15.7 ± 13.5	0.162	0.694	71	19.3 ± 11.8	95	18.2 ± 12.8	0.250	0.575
Chicken and turkey dishes	25	4.4 ± 2.7	42	5.3 ± 4.0	0.364	0.646	25	12.3 ± 9.1	42	12.6 ± 8.7	0.631	0.174
Sausages	22	17.9 ± 12.7	14	16.3 ± 12.4	0.626	0.817	22	3.6 ± 2.6	14	3.0 ± 1.9	0.770	0.729
Bacon and ham	31	15.1 ± 9.9	42	18.2 ± 8.2	0.059	0.119	31	5.2 ± 4.0	42	5.4 ± 4.6	0.746	0.788
Red meats	48	6.9 ± 8.4	54	4.8 ± 6.9	0.086	0.295	48	16.9 ± 11.3	54	15.9 ± 11.5	0.634	0.279
Milk and milk products	71	10.4 ± 7.8	97	12.0 ± 8.9	0.313	0.378	71	21.6 ± 13.0	97	22.7 ± 13.4	0.848	0.632
Milk	61	5.2 ± 5.0	85	5.2 ± 4.2	0.365	0.731	61	17.6 ± 10.7	85	16.9 ± 11.7	0.467	0.154
Cheeses	26	10.5 ± 6.5	35	12.4 ± 7.4	0.226	0.345	26	3.1 ± 2.3	35	3.2 ± 2.8	0.919	0.780
Vegetable and potato products	45	5.3 ± 11.6	77	1.7 ± 3.9	0.930	0.626	45	16.1 ± 16.8	77	23.3 ± 19.2	0.156	0.126
Potato products	24	8.8 ± 15.2	47	2.0 ± 4.6	0.114	0.328	24	27.3 ± 16.8	47	31.5 ± 17.8	0.362	0.982
Soups and sauces	34	23.0 ± 15.2	42	28.6 ± 15.1	0.146	0.958	34	16.3 ± 11.9	42	15.2 ± 10.8	0.892	0.519
Vegetable Soups	24	29.6 ± 12.0	32	31.4 ± 12.9	0.703	0.290	24	21.3 ± 9.6	32	19.2 ± 9.1	0.389	0.098
Sauces	9	4.8 ± 5.3	7	2.3 ± 1.1	0.958	0.874	9	2.9 ± 3.6	7	1.3 ± 2.2	0.791	0.634
Fruits	36	0.2 ± 0.4	61	0.1 ± 0.1	0.308	0.018	36	17.9 ± 11.7	61	17.7 ± 15.3	0.638	0.123
Fish and seafood dishes	18	17.0 ± 17.6	36	17.2 ± 15.3	0.741	0.752	18	17.1 ± 14.5	36	12.9 ± 11.8	0.359	0.121
Cod Fish	3	22.9 ± 13.9	13	24.3 ± 19.1	0.840	0.840	3	1.6 ± 1.1	13	2.4 ± 2.5	0.638	0.737
Oils and fats	34	2.2 ± 2.9	61	2.2 ± 3.8	0.907	0.609	34	0.1 ± 0.1	61	0.1 ± 0.2	0.480	0.826
Egg and egg dishes	5	6.8 ± 5.4	17	4.0 ± 3.3	0.290	0.784	5	3.3 ± 1.4	17	3.1 ± 2.3	0.724	0.290
Sugars, preserves, and confectionery	42	1.3 ± 2.1	59	0.8 ± 1.0	0.566	0.431	42	2.6 ± 6.2	59	1.4 ± 2.0	0.730	0.935
Fast Food	38	18.5 ± 17.3	35	24.0 ± 20.1	0.279	0.112	38	25.9 ± 15.3	35	26.7 ± 14.9	0.829	0.922
Pizza	5	36.6 ± 19.8	8	41.3 ± 13.0	0.558	0.724	5	23.7 ± 19.3	8	19.9 ± 8.9	0.590	0.814
Sandwiches, burgers, filled wraps	7	27.0 ± 16.6	7	22.2 ± 19.7	0.749	0.406	7	15.9 ± 13.0	7	13.6 ± 6.0	0.655	0.565
Salted snacks and fried snacks	4	21.2 ± 11.2	4	27.1 ± 19.4	0.564	0.999	4	10.5 ± 3.0	4	7.7 ± 6.5	0.508	0.999
Fried potatoes	32	7.7 ± 4.7	26	9.4 ± 8.1	0.731	0.783	32	23.0 ± 10.1	26	24.8 ± 13.2	0.900	0.553
Beverages	71	1.2 ± 1.1	105	1.4 ± 1.2	0.087	0.075	71	2.6 ± 7.4	105	2.4 ± 5.3	0.021	<0.01
Soft drinks	45	0.9 ± 1.2	53	1.2 ± 1.5	0.196	0.196	45	0.3 ± 0.3	53	1.4 ± 3.9	0.841	0.492
Water	55	0.7 ± 0.6	85	0.9 ± 0.7	0.194	0.407	55	0.6 ± 1.6	85	0.5 ± 1.0	0.356	0.052
Hot beverages	9	0.6 ± 0.8	26	0.2 ± 0.2	0.763	0.489	9	14.9 ± 15.7	26	5.2 ± 7.4	0.234	0.378
Other foods	16	6.7 ± 8.2	28	7.2 ± 9.3	0.626	0.526	16	8.3 ± 11.0	28	7.0 ± 10.1	0.479	0.759

The contribution of each food category is expressed in mean percentage ± standard deviation.

* Adjusted for energy intake.

DISCUSSION

To the best of our knowledge, this study has marked the first in Portugal to estimate sodium and potassium intake in a large group of adolescents aged 13 to 18 years using 24-hour urine excretion. Our results have shown that 83% of participants exceed the recommended sodium intake and that 96.5% did not meet recommendations for potassium intake.

Portuguese data related to sodium excretion are scarce. A study of Portuguese children aged 10-12 years showed that sodium excretion was 3072±985 mg/d (35), while a more recent study with children aged 8-10 years reported that median sodium excretion was 2737 mg/d in boys and 2104 mg/d in girls (36). Comparing our results to those representing other European adolescents, Portuguese boys seem to have a mean sodium excretion greater than that of Italian (2967 mg/d) (37), Spanish (3270.6 mg/d) (38), UK (i.e., London) (3401.47 mg/d) (25) and German (3013 mg/d) (39). Similar results were found between Portuguese and other European girls, except Spanish ones (2888.8 mg/d) (38). Across the board sodium excretion was greater in boys than in girls (25, 36-39), probably due to their higher food intake.

Conversely, other studies have evaluated sodium intake in children and adolescents via dietary recall. In Australian adolescents (aged 14-16 years) mean dietary sodium intake was 3190 mg/d, a value that increased with age (40). In French adolescents median sodium intake was 2245 mg/d (41) and in Korean adolescents mean sodium ingestion was 4100 mg/d (42). Results from our study related to sodium intake via dietary recall are more similar to French adolescents.

Although the amount of potassium excretion in our study was well below the minimum value recommended by the WHO, our data are similar to those reported by Geleijnse et al. (43) regarding 233 children aged 5-17 years, for whom mean potassium excretion was 1704.3 mg/d and was higher in boys. Among Portuguese children (8-10 years old) mean potassium excretion was 1701±594 mg/d in boys and 1682 ±541 mg/d in girls.

Na+/K+ intake is a critical environmental risk factor of hypertension and cardiovascular outcomes than either electrolyte alone (44). In our study, mean urinary Na+/K+ was 1.7 in boys and 1.6 in girls, these values are greater than Na+/K+<1 mmol/mmol (<0.59 mg/mg) according to WHO recommendations (45). Higher Na+/K+ is associated with increased risk of cardiovascular diseases and all-cause mortality (11, 46), as well as relates to Western diets heavily reliant on processed foods high in added sodium and low in potassium (38).

Na+/K+ excretion among our study's participants was smaller than the median ratio found in Portuguese children (2.73 in boys and 2.33 in girls) (36) and in Dutch adolescents (ratio was 3.3) (43), as well as in Italian children and adolescents (ratio was 3.79±1.68) (47). However, the ratio in our participants is still three times greater than 0.59 mg/mg, which raises concerns about poor dietary behavior early in childhood.

Participants in our study did not meet the recommended intake for sodium or potassium, and major dietary contributors of sodium and potassium were identified in order to improve food-based intervention programs.

Correlations between dietary and urinary data found in our study were comparable with those reported by other authors (41, 48). The possible explanation of this result could be due to the difficulty of food record to estimate accurately food quantities and food ingredients especially if the subjects ate out of home.

Taking into account the overall intake reported for our sample of adolescents, the food groups that contribute most to total sodium intake are cereals and cereal products (mean 41%) and meat products (mean 16%). Meaning that in addition to bread (mean 16%), sodium added during the cooking process is the chief source of sodium intake as in pasta, rice, and other cereal-based products (mean 12%), meat products (mean 16%) and fish and seafood products (mean 5%) and vegetable soup (mean 10%). Our data show that in individuals who consumed vegetable soup (approximately 15% of participants), soup contributed roughly a third to their total sodium intake (33% for boys and 31% for girls; Table IV). Conversely, in individuals who consumed processed foods such as fast food (about 41% of participants), such foods became an important contributor to their total sodium intake.

Since the largest proportion of sodium is added during food manufacture or preparation, including for vegetable soup and bread, changes in recipes to reduce sodium content could be possible without disturbing other nutrient values (49) and consumer preferences. In a study of children and the elderly, 30% salt reduction in vegetable soup was achieved without compromising perceived saltiness (50). This result is especially important because in Portugal the amount of sodium in a 300 g portion of vegetable soup cooked outside home may be as great as 1316 mg (51).

To diminish salt added during cooking processes, it is necessary to devise nutrition education strategies that increase population awareness of salt reduction and develop technology to control amounts of added salt.

At the same time, industry and food politics need to engage efforts to make processed foods with low sodium content available on the market. Portugal's salt reduction plan (52) includes as a strategic objective the modification of the availability of products with low salt content, via the participation of industry in reformulating food with higher salt content. Therefore, since 2009 in Portugal, sodium content in bread was legally limited to 550 mg/100g (53), though this limit was exceptionally permissive compared to that of other countries' legislation (54, 55). Bread is probably the most important staple food of modern diets (56) as well as of the traditional Mediterranean diet of the early 1960s (57). Thought salt (i.e., sodium chloride) has a technical and functional role in the manufacture of bread (58), the production of bread reduced in salt is feasible (58), and incremental reductions of 30% to 50% have proven to be acceptable to consumers (59-61) .

Surprisingly, the top sources of potassium among all sample of participants were milk and milk products (24%) and meat products (20%), as explained by the low intake of fruit and vegetables (2% and 9%, respectively; data not shown). These data may reflect the low rate of adolescents that follow the recommendation to eat at least 400g of fruit and vegetables daily (62). The intake of fruits and vegetables as a natural source of potassium is inversely related to the risk of stroke (63) and associated with more varied and higher quality diet (64).

Since food processing reduces the naturally amount of potassium in many food products (65), diets high in processed foods and low in fresh fruits and vegetables and coupled with high sodium intake found in our study, suggest that Portuguese adolescents have moved away from a typical Mediterranean diet toward dietary patterns common in industrialized countries (41). Developing strategies to improve fruit and vegetable consumption would thus increase potassium intake, as well as offer other beneficial health effects (66).

In the context of our study, vegetable soup deserves particular attention, for it is a staple food of the Mediterranean diet and of Portuguese dietary habits, consumed daily by 67% of population (67) and promoted by healthy eating education programs (68). Vegetable soup is a traditional Portuguese food rich in vegetables, low in energy-density, and high in dietary fiber that was associated with a lower risk of obesity (69). In our study, vegetable soup was an important contributor to total potassium intake, however, its contribution to total sodium was exceptionally high and therefore detrimental. This typical food seems to show the potential for improving potassium intake if its consumption could be stimulated in adolescents and if added salt were diminished.

A strategy that emphasizes reducing sodium added to foods, promote potassium rich foods and promote reformulation of processed and package foods could achieve greater public health benefits than restricting sodium alone (46).

Our study is strengthened by the 24-hour urine collection, the clinical gold standard method, and by our sample size. The 24-hour urine collection objectively measured dietary sodium intake and captured sodium intake from food and salt added during the preparation of meals and at the table. Bias stemming from under or over collection was decreased by 24-hour urine creatinine excretion quality control and self-report. The fact that we performed only one urine collection per participant could be a limitation; however, urine collections were measured in a large quantity of participants and showed a response rate of 41.6% similar to that of other studies (19).

Another potential limitation of our study is the possible underestimation of sodium and potassium intake in 24-hour dietary recall, as has been previously reported (48, 70). Nevertheless, 24-hour recalls were administered by trained researchers using a photographic book and household measures to quantify portion sizes, which minimized this potential limitation. It should also be noted that food composition data in software used for calculating nutrient intakes might also introduce bias into dietary data. Therefore, we careful check the sodium and potassium composition in the foods consumed by participants. We should additionally note that the collection of urine and the respective diet recall refers to Sunday. Although variation in day-to-day dietary intake exists, at their age our participants were more likely concerned about what their peers think about them, and the thought of completing urine collection during week days was roundly rejected by adolescents and their parents at the outset of the study. For this reason, we performed urine data collection on Sunday only.

CONCLUSIONS

In conclusion, in this study we found that Portuguese adolescents have high sodium intake and low potassium intake compared to WHO recommendations. The low rate of achieving potassium intake recommendations raises concerns about the potential detachment of Portuguese adolescents' dietary habits from a Mediterranean diet. Sodium reduction in staple foods such as bread and vegetable soup could be effective for decreasing sodium intake, while, the promotion of milk, fruit and vegetable intake could similarly increase potassium intake.

DISCLAIMER

João Breda is a staff member of the World Health Organization. The authors are responsible for the content and writing of this paper, which does not necessarily represent the decisions, stated policy or views of the World Health Organization.

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CONFLICT OF INTEREST

None of the authors declare conflict of interest.

AUTHORS' CONTRIBUTIONS

CG, SA, RS and PM were responsible for the conception and design of the study; CG, SA and RS were responsible for the collection of data; CG, SA, PP, OP, PG, JB, RS and PM were responsible for data interpretation; CG draft the manuscript and all the authors reviewed and approved the final version of the manuscript.

ETHICAL STANDARDS DISCLOSURE

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Ethical Commission of University of Porto. Written informed consent was obtained from all subjects and caregivers.

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Study 2 – Association between sodium excretion and hydration status by Free Water Reserve: a cross-sectional analysis in adolescents

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Association between sodium excretion and hydration status by Free Water Reserve: a cross-sectional analysis in adolescents

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Abstract

Background: Excessive sodium intake is excreted through urine and could affect hydration status. This study aims to describe hydration status in adolescents and to assess the association between hydration status by free water reserve (FWR) and urinary sodium excretion.

Methods: Two hundred participants (118 girls), aged 13–18 years completed the study. Median urinary sodium excretion was measured in one 24-hour collection and was used as a proxy for sodium intake. FWR (measured urine volume minus the obligatory urine volume) was used for characterization of hydration status, and linear regression models were used to evaluate the association between urinary sodium excretion and FWR, adjusted for water and energy intake in boys and carbohydrate, fiber, and water intake in girls.

Results: The participants median urinary sodium excretion was 3645.5 mg/d for boys and 2702.5 mg/d for girls ($p < 0.001$). Median FWR was positive in both sex groups; however, 40.2 % of boys and 31.4 % of girls ($p = 0.195$) were at risk of hypo-hydration status. Linear regression models showed that urinary sodium excretion was a significant predictor of FWR for both sexes (Crude Model $\beta = 0.114$, $p = 0.003$ for boys and $\beta = 0.160$, $p < 0.001$ for girls; Adjusted Model $\beta = 0.120$, $p = 0.002$ for boys and $\beta = 0.142$, $p < 0.001$ for girls).

Conclusions: Nearly one third of subjects were at risk of hypo-hydration. Higher sodium excretion was associated with a better hydration status obtained by FWR. However, as the majority of subjects consume sodium above recommendations, preventive measures to promote better hydration status should focus in increasing the level of total water intake.

Background

The preservation of an adequate hydration status (HS) in adolescents has been recognized as important and related to the ability to regulate body temperature and cognitive performance [1]. Although population-representative data on urine osmolality are scarce, existing data suggest that cell dehydration may be prevalent in healthy, free-living children at school [1, 2]. They could even be in a state of chronic voluntary dehydration [3]. Water intake could be suboptimal in some segments of the population such as adolescent boys and elderly [4].

HS can be accessed through subjective observations and by objective non-invasive measurements [5]. The subjective observations, such as skin turgor or thirst sensation, have been described as less reliable than objective laboratorial measurements due to the lack of consistency of measurements among measurers [5]. Current evidence suggest that urine indices are more sensitive than other methods; particularly osmolality as been recognized as one of the most accurate means to assess an individual's hydration status [5, 6]. However, osmolality is a measure of concentration and a new and suitable quantitative measure of individual 24-hour euhydration status was developed, using the concept of free water reserve (FWR), which corresponds to the difference between the measured urine volume and the ideal urine volume necessary to excrete the actual 24-hour urine solutes at the mean 2

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standard deviation value of maximum urine osmolality (Uosm) [6].

The identification of the determinants of HS is crucial. Unfortunately, data on this topic is scarce, particularly regarding the impact of other nutrients besides water intake on hydration physiology [7]. The HS is mainly influenced by water [6], which may be obtained by drinking water and other beverages as well as from moisture content in food [8, 9]. To prevent dehydration, humans have a sensitive network of physiological controls to maintain body water and fluid intake by thirst [10], and this homeostatic mechanism is triggered by the ingestion of dietary salt [11, 12].

Salt intake (sodium chloride) is a major factor in controlling urinary volume, which is significantly related to urinary sodium excretion [13]. Where salt intake increases there is an increase in both plasma sodium concentration and plasma osmolality [14, 15]. Sodium accounts for more than 90 % of all osmotically-active extracellular fluid solutes and is the major determinant of plasma volume. Plasma volume variation stimulates extra-renal and intra-renal sensors that segregate antidiuretic hormone. This hormone regulates plasma volume by adjusting sodium and water excretion in urine in order to maintain normal concentrations of sodium [14].

Plasma osmolality variations also stimulate thirst, a subjective perception that provides the stimulus to drink fluids due to a neural mechanisms activated by cellular dehydration [11, 16]. The expressions of thirst have been characterized as a combination of sensations that increase with dehydration and decrease with rehydration (that is, restoration of fluid balance) [17]. Salt intake is a major factor in controlling urinary volume and therefore fluid intake [13].

High salt intake can result in renal excretion of water [18, 19], since the excretion of excess sodium requires excretion of water through urine [8] and could affect the hydration status of children and adolescents.

In fact, children have less surface area-to-mass ratio for evaporative cooling, are less inclined to replace fluids, and therefore are especially susceptible to dehydration. Few studies have evaluated the impact of sodium intake (assessed by urinary sodium excretion) on hydration status. Ute et al. aimed to answer this question with German adolescents and reported that the children's hydration status was not affected by salt intake perhaps due to a compensatory increase in beverage consumption [8]. Our study was performed with adolescents from Portugal, a southern European country with Mediterranean climate, characterized by a temperate climate with a dry season in summer. Temperature was an important differentiating factor that could produce different results due to the warmer climate than was experienced by German adolescents. The Mediterranean climate could lead to water loss by sweat and increased need for fluid intake to replenish hydration status.

Therefore, the aim of this study was (a) to describe the hydration status in a sample of healthy Portuguese adolescents and (b) to assess the association between urinary sodium excretion and hydration status by FWR in this sample.

Methods

Study design and sampling

Data from this cross-sectional study resulted from LabMed Physical Activity Study (Longitudinal Analysis of Bio-Markers and Environmental Determinants of Physical Activity).

For the present study we assessed a sub-sample of 250 adolescents who were willing to participate, aged between 13 and 18 years, from schools in Braga district, with urinary excretion data collected across two time blocks, September 2012–April 2013 and September 2013–April 2014, excluding the warmer months of the year.

Quality control was used by calculating 24-hour urinary creatinine excretion in relation to body weight according to age group [20] and incomplete urine collections were repeated; subjects that had felt ill, had reported renal problems or took drugs in the day of collection were also not included for the present analysis (rejected $n = 50$, 20 %). Therefore, the final sample comprised 200 adolescents (82 boys) with valid urine collection and corresponding dietary recall.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the Ethical Commission of University of Porto. Written informed consent was obtained from all participants and caregivers.

Urine sampling and hydration markers

Participants and caregivers received oral and written instructions on how to collect complete 24-hour urine samples. All participants were instructed to discard the first morning void and to collect all urine over the following 24-hour including the first void on the following morning, the time of the start and finish collection was asked to be recorded in a questionnaire. During the collection period, subjects were asked to store collected urine in a cool place. All samples were sent to a certified laboratory to be analyzed sodium and potassium by indirect ion-selective electrodes methodology (Siemens Advia 1800), creatinine by Jaffé reaction (Siemens Advia 1650) and osmolality by sum of solute particles (exocinase method and Siemens Advia 1650/1800 equipment). Urine samples were analyzed for urinary creatinine (mg/day), and urinary sodium (mEq/day); sodium excretion was reported in mEq/day, however, for comparative purposes, it was converted to mg/day by using the molecular weight of sodium. Estimated salt intake was calculated from analyzed (24 h urine) sodium excretion (1 g salt = 393 mg sodium).

Hydration status was assessed using urinary markers, namely 24-hour urinary volume (mL), 24-hour Uosm (mOsm/kg), and FWR (ml/24 h) as determined and described previously [21]. Since concentration ability decreases not until age of 20 [6, 22], 830 mosm/1000 g is the mean maximum urine osmolality used to establish FWR in adolescents [8]. Positive values of FWR indicate euhydration, negative values the risk of hypo-hydration [21]. Risk of hypo-hydration correspond to the Uosm mean - 2 SD of maximum Uosm, and euhydration to Uosm between the mean −2 SD of maximum Uosm and the mean + 2 SD of minimum Uosm [21].

Dietary survey

A 24 h dietary recall referring to the day of the urine collection was collected by trained interviewers using photo book and household measures to quantify portion sizes [23]. Energy and nutritional intake were estimated using an adapted Portuguese version of the nutritional analysis software Food Processor Plus (ESHA Research Inc., Salem, OR, USA). The nutrient content of basic food was taken from standard nutrient tables, whereas the content of commercial food, e.g. pizza, ready-to-eat-food was derived from labelled ingredients and nutrients. Water from solid and fluid foods (total water in g per day), recorded from the 24 h dietary recall, was calculated using data from the Food Processor Plus (ESHA Research Inc., Salem, OR, USA).

Anthropometric measures

Height was measured to the nearest millimeter in bare or stocking feet with the adolescents standing upright against a stadiometer (Crymych, Pembrokeshire, UK). Weight was measured to the nearest 0.10 kg, with adolescents lightly dressed using portable electronic weight scale (Tanita Inner Scan BC 532, Tokyo, Japan). Body mass index (BMI) was calculated as weight (kg) divided by square height (m^2), and participants were classified according to World Health Organization (WHO) BMI reference values [24], in normal weight, overweight, and obesity. Underweight subject ($n = 1$) was combined with subjects in the normal weight category, due to the fact that represented a very small proportion of the sample.

Physical activity

Physical activity and participation in sports were measured by means of a short self-report questionnaire that was administered individually [25]. The answers were coded from 1 to 3, 1 representing inactivity or very low activity, 2 moderately intensive or frequent activity, and 3 frequent or vigorous activity. The physical activity questionnaire consisted of questions concerning frequency of physical activity, intensity of physical activity, frequency of vigorous physical activity, hours spent on vigorous physical activity, average duration of a physical activity session, and participation in organized physical activity. After coding, a sum index of physical activity was calculated.

Socioeconomic status

As an indicator of the socioeconomic status of the household, the Family Affluence Scale (FAS) was used (which ranged from 0 to 9 points, being higher socioeconomic status corresponding to highest score) [26]. The FAS is a four-item questionnaire that helps students report their family income objectively: It evaluates the sum of scores regarding whether the family owns a car, whether the student has his/her own bedroom, the number of family vacations during the past 12 months, and the number of computers the family owns. FAS was used as a continuous variable as well due by other authors in a number of analyses focusing on health gradients [26–28].

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the assumption of normality. Independent samples T-test or Mann–Whitney U test were performed to compare continuous variables and the $\chi 2$ test was used for categorical variables to assess differences between sample characteristics, dietary and nutritional data and urinary data stratified by sex.

Receiver operating characteristic (ROC) curves were used to analyse the potential diagnostic accuracy of sodium excretion to identify adolescents with low hydration status and to find the best trade-off between sensitivity and specificity. The area under the ROC curve (AUC) represents the ability of the test to correctly classify the participants with euhydrated status and risk of hypo-hydration. AUC values range between 1 (a perfect test) and 0.5 (a inadequate test).

Spearman's rank correlation coefficient was performed to assess the relationship between sodium excretion (mg/d) and urinary volume (ml/d), Uosm (mosm/kg), and FWR (ml/d). Kruskal–Wallis one-way analysis of variance and Mann–Whitney U test were used to identify differences for sodium excretion grouped by quartiles and by below or at above the upper limit recommendation (2000 mg/d), respectively.

Linear regression was used to estimate the association between the 24-hour urinary sodium excretion and the FWR. There were no interactions for sex x FWR (p-value for interaction = 0.420), however data from girls and boys were analyzed separately based on the existing sex differences in Uosm [29]. The following variables were considered as potential covariates of FWR: BMI, energy intake (kcal/d), carbohydrate intake (% energy), fat intake (% energy), protein intake (% energy) and total water intake (g/d resulted from beverages and solid foods ingested), socio-economic status and physical activity. All variables were initially tested simultaneously, and after only those variables that significantly predicted the FWR ($p < 0.05$) and substantially modified the coefficient of sodium excretion (mg/d) by 10 % were included in the models. The crude model – Model 1, included FWR as dependent continuous variable and sodium excretion as the independent variable. For boys the adjusted model – Model 2, included FWR as dependent variable and sodium excretion, energy, total water intake. For girls the adjusted model – Model 2 included FWR as dependent variable and sodium excretion, energy, fiber (g/1000 kcal), carbohydrate intake % energy and total water intake as independent variables.

Data were analysed using IBM Statistics for Windows, Version 21.0 (Armonk, NY: IBM Corp) and Med Calc software v.10.4.5 (MedCalc Software, Mariakerke, Belgium). A *p*-value <0.05 was considered to indicate statistical significance. In this report, descriptive analysis is presented in terms of median and interquartile range, unless otherwise stated.

Sample size was calculated a priori for linear regression model considering 4 predictors. For a power sample ≥ 80 %, a medium effect size ($f^2 = 0.15$) and $\alpha = 0.05$ we had to enrol 85 subjects. Additionally since in boys the sample was lower than 85, we performed a post-hoc test to assess the power sample. According to the linear regression model results and $\alpha = 0.05$ power sample was higher than 80 %.

Results

Descriptive and nutritional characteristics of the participants based on dietary records are present in Table 1.

Table 2 shows data from urinary collection. Urinary volume and Uosm does not differ significantly between boys and girls. Median urinary sodium excretion was 3645.5 mg/d for boys and 2702.5 mg/d for girls ($p < 0.001$).

Table 1 Descriptive and nutritional characteristics of the participants by sex

	Number	Boys	Girls	*p*
Age (y)[a,b]	200	14.0 (14.0; 16.0)	14.0 (14.0;16.0)	0.295
Weight (kg)[a,b]	200	60.0 (51.7; 66.3)	54.4 (47.5; 59.0)	0.001
Height (m)[a,b]	200	1.69 (1.64; 1.73)	1.61 (1.57; 1.65)	<0.001
Weight status (%)[c]	200			
Normal weight		72.0	73.7	<0.001
Overweight		20.7	13.6	
Obese		7.3	12.7	
FAS[a,b]	200	7.0 (5.0; 8.0)	7.0 (6.0; 8.0)	0.704
Physical Activity[a,b]	190	14.0 (12.0, 18.0)	17.0 (14.3, 20.0)	<0.001
Total energy (kcal/d)[a,b]	200	2210.1 (1800.1; 2948.5)	1862.7 (1598.4; 2239.7)	<0.001
Carbohydrates (g/d)[a,b]	200	264.0 (206.9; 345.1)	238.3 (188.2; 288.3)	0.005
Carbohydrates (%TEI)[d,e]	200	47.0 ± 7.4	49.4 ± 8.3	0.192
Protein (g/d)[a,b]	200	90.9 (69.7; 118.1)	72.9 (57.8; 91.1)	<0.001
Protein (%TEI)[a,b]	200	16.2 (13.9; 18.7)	15.6 (13.5; 18.4)	0.344
Fat (g/d)[a,b]	200	84.8 (58.2; 117.8)	67.8 (50.5; 81.1)	<0.001
Fat (% TEI)[d,e]	200	33.8 ± 7.0	32.6 ± 6.7	0.292
Dietary fiber (g/1000 kcal)[a,b]	200	5.3 (4.3; 7.1)	6.1 (4.8; 8.2)	0.020
Total food (g/d)[a,b]	200	2475.7 (1873.4; 3070.7)	2207.5 (1710.5; 2609.8)	0.014
Total water (g/d)[a,b,f]	200	1652.5 (1249.7; 2189.1)	1599.1 (1220.0; 1944.8)	0.216
Caffeine (mg/d)[a,b]	200	14.8 (1.2; 57.3)	10.4 (0.6; 49.2)	0.574
Sodium (mg/d)[a,b,g]	200	2845.2 (1866.3; 3449.8)	2145.3 (1527.2; 2779.7)	<0.001
Salt (g/d)[a,b,f]	200	7.2 (4.7; 8.8)	5.5 (3.9; 7.1)	<0.001

TEI Total Energy Intake, *FAS* Family Affluence Scale
[a]Values are medians (P25; P75)
[b]Between-sex analysis by Mann–Whitney U test
[c]Analysis by χ2 for categorical variables
[d]Values are means ± SD
[e]Analysis by Student t test for continuous variables
[f]Water from foods, beverages and metabolic water
[g]Estimated from dietary records without considering household salt, 393 mg sodium = 1 g salt

Table 2 Urinary data on sodium excretion and hydration status (13–18 years)[a]

	Boys	Girls	*p*
Creatinine (mg/d)[a,b]	1440.0 (1164.5; 1717.0)	1094.0 (978.0; 1238.5)	<0.001
Volume (ml/d)[a,b]	1100.0 (837.5; 1300.0)	1025.0 (700.0; 1412.5)	0.923
Osmolality (mosm/kg)[c,d]	715.7 ± 172.3	597.42 ± 193.1	0.247
FWR (ml/d)[a,b]	173.2 (−137.5; 509.2)	373.2 (−105.7; 832.1)	0.059
Euhydration status (%)[e]	59.8	68.6	0.195
Risk of Hypohydration status (%)[e]	40.2	31.4	
Sodium (mEq/d)[a,b]	158.5 (114.5; 197.8)	117.5 (95.5; 159.5)	<0.001
Sodium (mg/d)[a,b,f]	3645.5 (2633.5; 4548.3)	2702.5 (2196.5; 3668.5)	<0.001
Salt intake estimation (g/d)[a,b,f]	9.3 (6.7; 11.6)	6.9 (5.6; 9.3)	<0.001

[a]Values are medians (quartile 1 and quartile 3)
[b]Analysis by Mann–Whitney U test
[c]Values are means ± SD
[d]Analysis by Student t test for continuous variables
[e]Analysis by χ2 for categorical variables
[f]Estimated salt intake from urinary sodium excretion (393 mg sodium = 1 g salt)

Median FWR was positive in both sex groups however, 40.2 % of boys and 31.4 % of girls was at risk of hypo-hydration status.

For the whole sample, ROC analysis showed that sodium excretion has a predictive ability to discriminate subjects at risk of hypo-hydration from euhydrated subjects (AUC = 0.65, 95 % CI: 0.582–0.718, $p = 0.005$; sensitivity = 50.6 % and specificity = 76.5 %) Corresponding values for girls and boys were: boys AUC = 0.73, 95 % CI: 0.617–0.819, $p = 0.020$ (sensitivity = 67.6 % and specificity = 75.0 %), and girls AUC = 0.65, 95 % CI = 0.554–0.733, $p = 0.021$ (sensitivity = 54.4 % and specificity = 76.9 %).

In Table 3 it can be seen that urinary volume and FWR were positively correlated with urinary sodium excretion in both boys and girls respectively. Boys and girls in the category of sodium excretion above upper limit for sodium intake recommendations (78.0 % of girls and 90.2 % of boys) had higher mean values for urinary volume and FWR than the category with less 2000 mg/d of sodium excretion.

Two linear regression analysis models (crude and adjusted) were used to describe the relationship between urinary sodium excretion and FWR for boys and girls (Table 4). Both models clearly showed a significant and positive association between urinary sodium excretion and FWR in boys (crude model $\beta = 0.114$, $p = 0.003$ and adjusted model $\beta = 0.118$, $p = 0.002$) and girls (crude model $\beta = 0.160$, $p < 0.001$ and adjusted model $\beta = 0.129$, $p = 0.001$).

Discussion

To our knowledge, this is the first study examining cross-sectional associations between urinary sodium excretion and FWR in adolescents from a southern European country. Our results show that about 40 % of boys and one third of girls were at risk of hypo-hydration status. And, urinary sodium excretion was a significant independent predictor of FWR for both sexes. Suggesting that a high-sodium diet was associated with a better hydration status in adolescents, assuming that all sodium excreted through urine came from diet.

Data on 24-hour urinary sodium excretion in children and adolescents, the best marker for sodium intake, is scarce. Our results show that in this sample of adolescents, salt intake is high; median sodium intake was 3645.5 mg/d in boys and 2702.5 mg/d in girls ($p < 0.001$), thereby exceeding maximum daily intake recommendations of 2300 mg/d [30] and WHO recommendations of 2000 mg/d [31]. Our findings show that sample salt ingestion (median salt intake 9.3 g/d in boys and 6.9 g/d in girls) of these adolescent's was slightly higher than the values found in Portuguese children aged 10–12 years old, whose mean salt intake was 7.8 ± 2.5 g/d [32].

In our study, no significant sex differences were observed in urine Uosm ($p = 0.247$) and elevated Uosm defined as over 800 mmol/kg [33] was verified in 16.1 % of girls and 37.8 % of boys. In industrialized countries, a sex difference in Uosm is common, however, it is not a universal finding. Males have been commonly shown to have higher Uosm than females [6]. In the United States, adolescents have been found to display Uosm values similar to ours (649 mosm/kg for boys and 540 mosm/kg for girls) but with significant sex differences [34]. A German study found higher Uosm in boys than girls, leading the authors to suggest that sex difference in Uosm could be caused by a higher water density of ingested food (ml/kcal) and a lower insensible water loss (ml/kcal) in girls than boys [29].

We use FWR to categorize the hydration status of participants because it was been defined as a suitable quantitative measure of individual 24-hour euhydration [6]. Our results indicate that 40.2 % of boys and 31.4 % of girls

Table 3 Hydration status indicators and its relation to sodium excretion divided in quartiles and according to upper limit recommendation (2000 mg/d) and by sex[a]

	Sodium excretion (mg/d)						Sodium excretion		
	Q1	Q2	Q3	Q4	p^b	ρ $(p)^c$	<2000 mg/d	> = 2000 mg/d	p^d
Boys (n)	20	21	21	20			8	74	
Volume (ml/d)[a,b]	775.0 (600.0; 987.5)	1000.0 (725.0; 1250.0)	1100.0 (900.0; 1175.0)	1325.0 (1150.0; 1600.0)	<0.001	0.585 (<0.001)	725.0 (450.0; 900.0)	1100.0 (900.0; 1300.0)	0.001
Osmolality (mosm/kg)[a,b]	677.5 (527.3; 791.3)	703.0 (547.5; 922.0)	776.0 (699.5; 847.0)	761.0 (553.3; 832.8)	0.337	0.150 (0.178)	704.5 (620.8; 828.8)	725.0 (565.3; 824.0)	0.702
FWR (ml/d)[a,b]	−78.0 (−293.2; 349.8)	29.5 (−341.6; 528.3)	181.3 (−97.9; 338.3)	459.9 (79.7; 961.0)	0.015	0.358 (0.001)	−122.9 (−624.8; 189.6)	226.4 (−108.6; 535.7)	0.036
Risk Hypo-hydration status (%)[c]	65.0	47.6	33.3	15.0	0.011		75.0	36.5	0.035
Girls (n)	29	30	30	29			26	92	
Volume (ml/d)[a,b]	775.0 (550.0; 1075.0)	975.0 (737.5; 1270.0)	975.0 (767.5; 1312.5)	1450.0 (1250.0; 1700.0)	<0.001	0.512 (<0.001)	737.5 (537.5; 1012.5)	1200.0 (800.0; 1487.5)	<0.001
Osmolality (mosm/kg)[a,b]	589.0 (415.5; 750.5)	544.0 (397.3; 686.3)	689.0 (455.5; 793.3)	572.0 (497.5; 656.0)	0.201	0.113 (0.222)	564.5 (420.3; 738.8)	528.0 (458.3; 743.8)	0.610
FWR (ml/d)[a,b]	44.6 (−304.2; 517.5)	304.2 (−134.5; 813.7)	165.4 (−215.2; 758.0)	748.2 (509.0; 964.5)	0.004	0.323 (<0.001)	52.6 (−293.4; 464.2)	512.0 (−43.4; 867.3)	0.022
Risk Hypo-hydration status (%)[c]	44.8	30.0	36.7	13.8	0.073		46.2	27.2	0.065

[a]Values are median (percentile 25; percentile 75)
[b]Analysis by Kruskal Wallis
[c]Spearman correlation test
[d]Analysis by Mann–Whitney U test

Table 4 Multivariate regression models predicting FWR (ml/day) by daily sodium excretion (mg/d) stratified by sex

	Model 1[a]		Model 2[b]	
	FWR		FWR	
	β (CI 95 %)	*p*	β (CI 95 %)	*p*
Boys ($n = 82$)	0.114 (0.040, 0.189)	0.003	0.118 (0.046, 0.189)	0.002
Girls ($n = 118$)	0.160 (0.080, 0.240)	<0.001	0.136 (0.060, 0.212)	0.001

[a]Model 1 – unadjusted model
[b]Model 2 – model 1 additionally adjusted for total water intake and energy intake (for boys) and carbohydrate intake (% energy), fiber (g/100 g energy) and total water intake (for girls)

were at risk of hypo-hydration status and the median was positive in both sexes. Similar results were found in the DONALD Study, which found that FWR increased significantly with age in both boys and girls [8].

Our study indicates that 24-hour urinary sodium excretion positively affects hydration status measured by FWR in both sexes, suggesting that adolescents probably compensate the high sodium intake with greater fluid ingestion. Alexy et al. [8], in a sample of healthy adolescents, showed a positive association between sodium excretion and FWR in girls, although hydration status was not significantly affected by salt intake in boys. Our results suggest that total body water was replaced faster than it was lost by adolescents. Ingestion of more salt seems to particularly stimulate regulatory mechanisms that maintain plasma volume, provided that exogenous fluid intake increases proportionally to water lost.

The association between salt intake and hydration status is not observed across all age groups. In a study of Portuguese community-dwelling elderly people, higher sodium intake was associated with a poorer hydration status. This difference to our results may be explained by the lower ability of elderly people to compensate their higher sodium intake with increasing fluid intake [35].

In our study, the male euhydrated participants consumed more water ($p = 0.009$) and female euhydrated subjects consumed more hot beverages ($p = 0.023$) (data not shown). According to our knowledge of adolescents' beverage intake across Europe, water is the largest contributor to fluid consumption followed by sugar-sweetened beverages [36]. He et al. [37] demonstrated that during childhood, salt is a major determinant of fluid consumption including sugar-sweetened soft drinks; however, we did not found an association between salt intake and sugar-sweetened soft drinks consumption (data not shown).

According to Table 3, urinary sodium excretion is associated with the FWR and the subjects of the upper quartiles of sodium excretion have higher value of FWR. Such that, subjects that ingest salt above maximum daily intake recommendations had a better median hydration state, however, 25 % of subjects in this category have a negative value of FWR which means that they are at risk of hypo-hydration. Thus, it seems that the total fluid intake was not sufficient to compensate water losses in these individuals. Therefore, total water intake should be promoted as a strategy to improve the hydration status concomitantly with reductions in sodium intake to minimize its negative impact on other health outcomes, such as hypertension [31].

Our findings should be interpreted taking into account the study's limitations and strengths. A major strength of this study was the sample size and high quality of dietary record and urine collections. Indeed data on 24-hour urinary excretion in adolescents are scarce. Our rejection of 50 incomplete urinary collections (20 %) was similar to other studies [8, 38] and indicates good compliance.

One limitation of this study was that subjects were not randomly selected from the general population but were recruited from LabMed Study participants. Also, we collected one urine specimen per subject and therefore long-term extrapolation on hydration status cannot be draw. However, other studies have evaluated hydration by this method since water balance is regulated over 24-hour periods [30].

Conclusions

Over one-third of adolescents were at risk of hypo-hydration. Higher sodium excretion was associated with a better hydration status assessed by FWR in this sample of adolescents. Nevertheless, the majority of subjects consume sodium above recommendations, thus preventive measures to promote better hydration status should focus in increasing the level of total water intake.

Abbreviations

HS: Hydration status; FWR: Free Water Reserve; FAS: Family Affluence Scale; BMI: Body mass index; WHO: World Health Organization; Uosm: Urinary osmolality.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CG, SA, RS and PM were responsible for the conception and design of the study; CG, SA and RS were responsible for the collection of data; CG, SA, PP, OP, PG, JB, RS and PM were responsible for data interpretation; CG draft the manuscript and all the authors reviewed and approved the final version of the manuscript.

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Study 3 – Salt reduction in vegetable soup does not affect saltiness intensity and liking in the elderly and children

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Salt reduction in vegetable soup does not affect saltiness intensity and liking in the elderly and children

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Abstract

Study background: Reduction of added salt levels in soups is recommended. We evaluated the impact of a 30% reduction of usual added salt in vegetable soups on elderly and children's saltiness and liking evaluation.
Methods: Subjects were elderly and recruited from two public nursing homes (29 older adults, 79.7 ± 8.9 years), and preschool children recruited from a public preschool (49 children, 4.5 ± 1.3 years). This study took place in institutional lunchrooms. Through randomization and crossover, the subjects participated in two sensory evaluation sessions, on consecutive days, to assess perceived saltiness intensity (elderly sample) and liking (elderly and children samples) of a vegetable soup with baseline salt content and with a 30% salt reduction. Elderly rated perceived liking through a 10 cm visual analogue scale ['like extremely' (1) to 'dislike extremely' (10)] and children through a five-point facial scale ['dislike very much' (1) to 'like very much' (5)].
Results: After 30% added salt reduction in vegetable soup, there were no significant differences in saltiness noted by the elderly ($p = 0.150$), and in perceived liking by children ($p = 0.160$) and elderly ($p = 0.860$).
Conclusions: A 30% salt reduction in vegetable soup may be achieved without compromising perceived saltiness and liking in children and the elderly.

Keywords: *elderly*; *preschool children*; *salt*; *hedonic evaluation*; *soup*

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High sodium intake has been associated with the etiology and pathogenesis of hypertension (1) and cardiovascular diseases (CVD), which is the leading cause of mortality and morbidity worldwide (2).

The World Health Organization (WHO) recommends the consumption of less than 2,000 mg sodium per day for adults and the adjustment of 2,000 mg sodium downward based on the energy requirements of children (2). In this global scenario, the reduction of population sodium intake is one of the most urgent strategies to put into practice (3). However, in Portugal, the intake levels are well above these recommendations, reaching an average of 4,800 mg per day in adults (4), and children and the elderly are two important population subgroups to target for lower sodium intake (5).

Since most sodium is consumed in the form of sodium chloride, which is table salt (approximately 40% sodium), the European Salt Framework established a benchmark of a minimum of 16% salt reduction over 4 years for all food products, also encompassing salt consumed in restaurants and catering, including vegetable soup (6). In Portugal, the amount of salt in a 100 g portion of vegetable soup may reach 1,073 mg in nursery homes, and 1,098 mg in kindergarten, and one of the key approaches to decrease sodium intake is to target added salt in soup preparation (7). In order to achieve this goal, a reduction benchmark of 16% against the individual baseline food levels was established by the European Commission (8), but strong controversy exists regarding the choice of cutback levels in each food group. In France (3), United Kingdom (3), United States (9), and Czech Republic (6), the targets for sodium reduction in soups are 7, 15, 8, and 50%, respectively. Therefore, the establishment of a sodium reduction benchmark for vegetable soup should be worked on a country level with highest priority in order to accomplish a significant reduction of sodium intake without compromising sensorial characteristics, such as liking and saltiness perception, which may compromise food

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acceptance (10). It has been reported that the reduction of added sodium to foods ranging between 10 and 48% of baseline levels may not be detected by taste receptors (11–15); however, the large majority of these studies involves trained panelists, and commercial soups or soups with salt substitutes. On the other hand, the new directions to help consumers to eat healthier focus on training in food preparation practice, including in the catering sector, to reduce salt usage in their kitchens. In this scenario, different benchmarks of salt reduction should be tested across the life cycle, in order to significantly reduce sodium intake without affecting consumer acceptability (10, 11, 13, 15, 16).

Thus, the objective of this study was to assess the perceived saltiness intensity and liking of a vegetable soup after 30% reduction of the usual sodium content, in a sample of institutionalized elderly and community preschool children.

Materials and methods

Sampling

Elderly

Elderly subjects ($n = 35$) who attended from two public nursing homes (NH1 and NH2) were invited to participate in the study, and interviewed through a structured questionnaire about general health conditions. Subjects with renal diseases or hyponatremia were excluded ($n = 6$), resulting in 29 elderly subjects remaining. Cognitive status was evaluated using the mini-mental state examination (MMSE) (17), and all had MMSE ranked >18. The elderly provided written informed consent.

Children

All children from a public preschool were also invited to participate ($n = 75$). Letters were distributed to all parents outlining the aims of the study along with a consent form. Forty-nine parents signed and returned the filled-out form, and all of these children gave verbal consent, therefore the sample consisted of 49 children.

Study design

Each set of participants – elderly and children – was randomly divided into one of two groups to perform the saltiness and liking evaluation of vegetable soups in two sessions on separated days.

Each group tasted and evaluated the soups following a crossover design, in which the subjects were randomly assigned to two different arms of the study, one consuming a vegetable soup with baseline sodium content and the other having the soup with 30% salt reduction, switching the soups with the two different levels of sodium content afterwards. Other ingredients in the soups besides salt were the same in both groups. The study was also single blinded such that the subjects were unaware of the salt content of the soup they were assessing.

The sensory evaluation took place in the usual lunchrooms at the institutions, in order to minimize the effects due to changes in the physical environment, and at the typical lunch period – between 12:00 and 13:00 am – which is also the time when participants are most alert to perform sensory testing. Furthermore, the researchers visited the institutions several times before the test so that participants became familiar with them.

Salt composition of vegetable soups

According to our previous experiences (7), no specific amount of added salt is used for different kinds of vegetable soups, and the added quantities widely vary even for a soup with the same recipe. Hence, for the estimation of the baseline sodium content of soup, we computed the mean sodium content of the consecutive days prior to the trial (7 consecutive days in nursing home and 5 days in kindergarten), and estimated the added salt reduction of 30% in order to obtain a soup in the trial.

To estimate baseline sodium content, four samples of soup were collected, two samples before adding salt in confection by a food handler and two samples after added salt, in each day. Baseline added salt was calculated subtracting average sodium before adding salt (sodium from vegetables) to average sodium after added salt, and the average sodium content of the 7 days/5 days was considered as the baseline.

Sodium content was determined using flame photometry method, the intern reference method to analyze sodium in food matrices (18). Samples preparation procedure was adapted to soups from one validated method proposed to quantify sodium content in bread (19). All soup samples were stored in plastic containers at 4°C until analysis. After homogenization of each soup (Robot 300 IX, Taurus, Oliana, Spain), 2 g was sampled and 2 ml of nitric acid was added. The mixture was shaken during 90 min to allow the food matrix's to complete hydrolysis. Then, 20 ml of water was added, and the mixture was again homogenized using an electric homogenizer (Ultra Turrax blender T25, Sotel, Staufen, Germany). Volume was completed up to 40 ml and shaken for 30 min, followed by centrifugation (4,000 rpm, 15 min; Labofuge 6000® Haerus model, Burladingen, Germany). Finally, 1 ml of aqueous supernatant was diluted up to 40 ml of deionized water before reading in the flame photometer (Model PFP7, JenWay, Staffordshire, England).

For sensory evaluation, the vegetable soups were presented in a white ceramic plate at 68–72°C. Soups were served with nectar consistency and stored in the first day for the second refrigerated at 4°C, without added salt. Before the test soups were heated, soups were divided in two groups: in one group, the amount of added salt corresponded to the usual amount in baseline soups, and

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in the other group of soups, the amount of added salt was reduced by 30% compared to baseline. For the vegetable soups prepared for the elderly, a traditional recipe was selected, consisting of 1.5 kg of potatoes, 2.0 kg of white cabbage, 1.5 kg of Portuguese cabbage, 0.5 kg of onions, and 1.0 kg of carrots. For preschool children, the usual recipe for the soup was prepared with 7.0 kg of potatoes, 1.3 kg of onions, 2.3 kg of carrots, 2.5 kg of leek, and 30 g of garlic.

Sensory evaluation

A sensory description of the two soups with the two different sodium contents was performed; elderly evaluated saltiness and liking using a visual analogue scale (VAS) with 10 cm line scale [from 'extremely' (1) to 'not at all' (10) for salt perception and 'like extremely' (1) to 'dislike extremely' (10) for hedonic perception], and children evaluated liking through perceived liking ranking using a five-point facial scale (FS), by means of smiles icons that express feelings from 'dislike very much' (1) to 'like very much' (5). Given the low educational attainment in the elderly, a prior explanation of how to fulfill the VAS was given by a trained researcher.

Sociodemographic and anthropometric data

A questionnaire was applied to elderly and to the children's parents in order to collect data on age, and the highest education qualification completed. Weight and height measurements were performed in the elderly using standardized procedures (20); in the case of children, these anthropometric measures were reported by parents. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Elderly were classified as overweight if their BMI exceeded 25 kg/m^2 and obese if their BMI exceeded 30 kg/m^2, and children were classified as at risk of overweight if BMI at or above the 85th percentile and lower than the 95th percentile and obese if BMI at or above the 95th percentile for children of the same age and sex according to United States Centers for Disease Control and Prevention criteria (21).

Statistical analysis

Mean and standard deviations (SD) were used to describe continuous variables with normal distribution; otherwise, medians, minimum and maximum were presented.

Data analysis was performed with SPSS (version 17, Chicago, IL). The Shapiro–Wilk test was used to assess the assumption of normality, as VAS and FS variables did not reach normality, its median scores were compared using a non-parametric test for paired data, the Wilcoxon signed rank test. Spearman correlation coefficient was used to measure the degree of the association between pairs of variables. A p value of <0.05 was regarded as significant.

The impact of reduced added salt level on sensory evaluation is described separately for children and elderly.

Results

Elderly subjects ($n = 29$, 20 females) were 79.7 ± 8.9 years old with BMI of 25.7 ± 3.9 kg/m^2 (41.4% overweight and 13.8% obese); 62.1% had basic education (1–4 years) and 34.5% could not read or write. Children ($n = 49$, 26 girls) were 4.5 ± 1.3 years old with a BMI of 16.0 ± 1.5 kg/m^2 (16.3% overweight and 12.2% obese). Almost half (49%) of the children's parents reported to have attained a high education level; however, 18.3% had nine or less schooling years.

The baseline sodium concentrations in soups from each institution are shown in Table 1. The values of added sodium to soups (mg of sodium/100 g of soup) were 300.7 ± 6.6 mg in NH1, 206.7 ± 7.3 mg in NH2, and 147.0 ± 8.5 mg in preschool.

The sensory evaluation of the two soups (one with baseline salt content and another with 30% added salt reduction) is presented in Table 2. There were no significant differences in saltiness and liking between the two soups. Furthermore, no significant correlation was found between saltiness and liking in the elderly ($\rho = -0.714$, $p = 0.475$).

Discussion

The overweight and obesity prevalence values found in the elderly and children samples were similar to values reported in the same age groups in Portugal (22, 23).

A 30% added salt reduction did not change the saltiness and liking perception of a vegetable soup in both preschool children and elderly subjects. These results encourage for achieving higher sodium reductions than those typically advised, namely with decreases between 4 and 10% per year (9), without affecting hedonic response, particularly among children and the elderly. Nevertheless, these results should be considered with caution, and this reduction ought to be tested in soups with other vegetables, since optimum added sodium levels to increase saltiness or sweetness, or decrease bitterness, (24) may vary according to vegetable ingredients. Previous studies (10, 14, 25, 26) addressed sodium reductions and the combinations of extra aroma and salt replacers to substitute sodium (11) in adults although more research is needed to understand and control the human liking for salt (27), particularly among children and the elderly. Malherbe et al. found similar results in adults and conclude that it is possible to reduce the sodium content in soup by about 30% without significantly changing acceptability and pointed to the masking of other ingredients, partial adaptation, as well as the decreased absolute sensitivity and increased differential sensitivity as possible reasons (13).

Table 1. Sodium content in vegetable soups (mg sodium/100 g soup)[a]

		Before adding salt				After adding salt				Salt added during the cooking process			
		Mean ± SD	Med	Min	Max	Mean ± SD	Med	Min	Max	Mean ± SD	Med	Min	Max
NH1	Soup day 1	4.5 ± 0.1				212.4 ± 4.6				207.9 ± 4.5			
	Soup day 2	5.5 ± 1.6				315.2 ± 23.7				309.7 ± 22.1			
	Soup day 3	1.3 ± 1.2				290.8 ± 12.2				289.5 ± 11			
	Soup day 4	5.5 ± 1.5				308.3 ± 11.4				302.8 ± 9.9			
	Soup day 5	17.2 ± 2.6				334.4 ± 1.9				317.2 ± 0.7			
	Soup day 6	7.4 ± 4.2				267.8 ± 12.3				260.4 ± 8.1			
	Soup day 7	2.7 ± 0.8				420.0 ± 12.3				417.3 ± 11.5			
	Mean of the 7 days soups	6.3 ± 4.6	4.6	1.3	17.2	307.0 ± 11.2	308.3	212.4	420.0	300.7 ± 6.6[b]	302.8	207.9	417.3
NH2	Soup day 1	2.4 ± 0.6				358.1 ± 10.2				355.7 ± 9.6			
	Soup day 2	13.5 ± 2.9				185.4 ± 4.1				171.9 ± 1.2			
	Soup day 3	33.9 ± 2.0				166.4 ± 10.2				132.5 ± 8.2			
	Soup day 4	18.9 ± 0.2				175.3 ± 14.2				156.4 ± 14.0			
	Soup day 5	21.4 ± 1.3				204.9 ± 5.0				183.5 ± 3.7			
	Soup day 6	130.1 ± 1.0				300.9 ± 6.5				170.9 ± 5.5			
	Soup day 7	0.6 ± 0.4				226.9 ± 9.1				226.3 ± 8.7			
	Mean of the 7 days soup	31.5 ± 6.2	15.7	0.6	130.1	231.1 ± 13.5	212.5	166.4	300.9	206.7 ± 7.3[b]	196.8	132.5	355.7
PS	Soup day 1	3.0 ± 1.1				164.6 ± 3.5				161.6 ± 2.4			
	Soup day 2	3.0 ± 0.5				107.6 ± 2.9				104.6 ± 2.5			
	Soup day 3	3.0 ± 0.5				110.2 ± 8.4				107.2 ± 8.0			
	Soup day 4	18.5 ± 0.9				67.2 ± 5.9				48.7 ± 5.0			
	Soup day 5	18.5 ± 0.9				318.0 ± 19.5				299.5 ± 18.6			
	Mean of the 5 days soup	9.2 ± 0.7	6.1	3.0	18.5	153.5 ± 8.0	131.9	67.2	318.0	144.3 ± 7.3[b]	125.8	48.7	299.5

[a]Sodium obtained using flame photometry methodology; vegetable soups included four vegetables and potatoes.
[b]Values used to perform a 30% salt reduction.
SD = standard deviation; Med = median; Min = minimum; Max = maximum; NH1 = Nursing Home 1; NH2 = Nursing Home 2; and PS = preschool.

Strong concerns about the consumption of salt in vegetable soup exist in Portugal. The soups analyzed to establish the baseline (7 days in institutionalized elderly and 5 days for preschool children) take part in the usual menu cycle in both institutions, and regardless of the recipe, they are usually composed of four vegetables plus potatoes. Thus, the soup used in the trial symbolizes a general traditional vegetable composition of any of the soups analyzed in the baseline, with no expected significant variations in the nutritional composition, other than added sodium. The latter may vary according to the food handlers/cookers since no standardized amount of added sodium is established for each recipe.

Accordingly, in our study, salt added in soups during the cooking process (Table 1) varies widely between different collection days and between institutions, probably because the sodium added to soups varied according to intrapersonal and interpersonal food preparation prac-

Table 2. Evaluation tests according to the salt content in the soup (hedonic and perceived salt ratings)

		Vegetable soups with usual added salt		Vegetable soups with 30% reduction of added salt		
	Hedonic perception	Mean ± SD	Median (Min; Max)	Mean ± SD	Median (Min; Max)	*p*
Elderly (*n* = 29)	Saltiness	5.7 ± 3.2	5.0 (3.4; 9.1)	6.9 ± 2.5	7.8 (4.9; 9.3)	0.150
	Liking	2.1 ± 2.8	0.8 (0.2; 2.7)	2.2 ± 2.9	0.7 (0.3; 4.0)	0.860
Preschool children (*n* = 49)	Liking	4.4 ± 0.5	4.0 (4.0; 5.0)	4.41 ± 0.54	4.0 (4.0; 5.0)	0.160

SD = standard deviation; Min = minimum; Max = maximum.

tices without standardization (7). Moreover, the levels of salt found in the set of days used as 'baseline' are similar, or even lower than others reported in the literature (7, 28). For that reason, it is not expected that soup used in the trial was 'over-salted'.

According to a study that evaluated the sodium content of Portuguese vegetable soups in NH, elementary schools and kindergartens, subjects eating two portions of soup per day may reach 67% of the upper limit of sodium intake, when considering the mean values of sodium in vegetable soups (7) and the values found in this study were similar to ours. Given the low content of sodium in non-processed vegetables, nutrition education approaches to target sodium reduction should focus on training in cooking practice to significantly decrease added salt (according to the present study, about one third reduction in relation to usual levels may be acceptable) in vegetable soups produced both at home and in catering industries. A major challenge is to ensure that salt reductions do not exceed consumer expectations from a hedonic perspective which is a difficult task considering that bliss point depends on the individual and on his hedonic relation with a particular food (29), with some products largely punished on their hedonic evaluation even with small sodium reductions (9). However, sensitivity, perception of the intensity, preference and hedonic response to salt are independent measures; thus even individuals who are able to detect salt reduction may still maintain high ranking hedonic responses (30). Our results suggested that in vegetable soup, it is possible to moderately decrease the added salt without affecting both the consumer salty perception and the hedonic value attributed.

Salt is used in the food industry and during the cooking process due to its capacity to improve sensory properties of food (31), and each food preparation process or technology of processing has its own specific challenges as salt has multiple functions. In the case of soup preparation, adding salt may be a very cheap way to positively influence the taste of soup. For individuals who are used to tasting high levels of salt, its sudden reduction may cause the rejection of food (9, 32). For this reason, a small reduction of added salt, which is not detected by salty taste receptors, might be a valuable strategy to reduce sodium intake, and this study adds 30% as a possible benchmark for reduction.

Few studies have assessed the impact of a reduction of the sodium content of vegetable soup on hedonic perception of consumers in different age groups. Drewnowski et al. (33) showed that older adults prefer less salty soups than young adults; these results are also supported by Kremer et al. (34). However, it is largely assumed that people aged over 70 may have suffered changes in the function of ion channels and receptors of taste buds, with a consequent decrease of the threshold for detection and identification of flavor (35). Accordingly, Murphy and Withee have shown that the elderly have difficulty in detecting sweet and salty tastes (36), leading to an increased preference for salt (37), which could result in a higher sodium intake with the associated adverse health consequences. However, our findings showed that the elderly and children did not differently evaluate the soups with different sodium contents, which may be a good indicator for the process of reduction of added salt in vegetable soups in these age groups, leaving soup as a perfect vehicle of micronutrients. Vegetable soup is part of the food culture in southern Europe (38), being a culinary preparation with a suitable consistency for anyone with weak dentition (common in the elderly and children populations), with easy digestibility, high content of vitamins, minerals and fiber, that may also contribute to lower risk of overweight and obesity (39, 40). The nutritional and metabolic advantages of vegetable soup intake may further include hydration and anti-inflammatory properties (41), being strongly advised to consume it twice a day, in order to counteract the low intake of vegetables in Portuguese adults (42) and children (43) and prevent non-communicable diseases (44).

The results of the present study should be interpreted while taking into account its strengths and limitations. The study was carried out in the habitual familiar context for participants and during the period that they usually take their meals, leaving these variables constant over the intervention. Another strength of the study was the use of a single-blinded crossover model. On the other hand, participants were institutionalized; that is, the cooking process was the responsibility of the institution. Thus, the results should not be speculated to other population groups, neither the food used to taste stimuli; the perception of sodium reduction in vegetable soup should not be generalized to other foods because the effect of salt appears to be food specific. Another limitation of this study is that salt reduction was carried out a particular kind of vegetable soup recipe; however, we can achieve other values of reduction of salt content in other types of vegetable soup with other ingredients without affecting saltiness and hedonic perception.

For that reason, in the future, it will be important to consider other vegetable ingredients and recipes, and other values for the reduction of added salt in order to formulate targeted measures to control sodium intake, and set realistic and culturally relevant goals in food policy.

In conclusion, a 30% salt reduction in soups can be achieved without affecting perceived saltiness intensity and liking among the elderly and children, considering the reported mean baseline content of sodium.

Conflict of interest and funding

The authors declare no conflict of interest.

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Study 4 - Knowledge and practices related to added salt in meals by food handlers

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Knowledge and Practices Related to Added Salt in Meals by Food Handlers

Conhecimento e Práticas dos Manipuladores Relacionados com a Adição de Sal nas Refeições

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ABSTRACT

Objectives: To assess the knowledge and concerns about salt intake and added salt in meals, and to identify difficulties and proneness for a reduction of added salt in foods.

Methodology: The present study was based on a questionnaire sent by mail to a randomly selected sample of 100 business units from a catering industry, so that food handlers filled out and returned the questionnaires through the mail. The return rate was 70%, and 68 subjects were considered after the rejection of 2 questionnaires.

Results: The majority of the subjects (80.3%) recognized the maximum advised level of salt intake and 70.6% agreed with reducing the added salt to meals. The major difficulty for reducing the salt content of the meals was the consumer's opinion (mentioned by 79.4%). Soups and salads were identified as major candidates to salt reduction, by 36.4% and 18.2% of the participants, respectively.

Conclusions: Most of food handlers were aware about the recommended salt intake values, and are open to salt reduction strategies in food preparation.

KEYWORDS: Catering, Food handlers, Knowledge, Salt

RESUMO

Objectivos: Avaliar o conhecimento e preocupações relacionadas com a ingestão de sal e com a prática da adição de sal nas refeições, e identificar dificuldades e predisposição para uma possível redução do sal adicionado nos alimentos.

Metodologia: Este estudo baseou-se nos resultados de um questionário enviado por correio para uma amostra randomizada de 100 unidades de uma empresa de restauração colectiva, para que os manipuladores da unidade preenchessem e devolvessem os questionários através de correio. A taxa de resposta foi de 70% e 68 sujeitos foram considerados após uma rejeição de 2 questionários.

Resultados: A maioria dos sujeitos (80,3%) reconhecem o nível máximo de ingestão de sal preconizado e 70,6% concordam com uma redução no teor de sal adicionado às refeições. A maior dificuldade para a redução do teor de sal nas refeições foi a opinião do consumidor (mencionado por 79,4%). Sopas e saladas foram identificadas como melhores candidatas a uma redução do teor de sal, por 36,4% e 18,2% dos sujeitos, respectivamente.

Conclusões: A maioria dos manipuladores tem conhecimento dos valores de ingestão de sal recomendados, e estão receptivos a uma estratégia de redução do teor de sal na confecção de refeições.

PALAVRAS-CHAVE: Catering, Conhecimento, Manipuladores, Sal

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INTRODUCTION

Clinical and epidemiological studies have shown strong evidence of the link between excessive salt consumption and several chronic diseases (1). The World Health Organization recommends consuming less than 5 g/day (2) to prevent chronic diseases. However, in Portugal salt consumption is estimated to be 12.3 g/day (3).

Interventions to reduce population-wide salt intake have been shown to be highly cost-effective, hence the urgency to implement strategies tackling the reduction of salt intake, such as those involving the catering industry level. Moreover, at the present, eating in canteens or outside the home may be associated with high levels of salt intake (4-6).

The interaction between health professionals and the catering industry should encourage harmonizing the salt content of served meals according to the lowest threshold possible to simultaneously promote good health and avoid dissatisfaction among consumers. Thus, it is important to involve catering industry in exploratory studies of behaviors like this to formulate an intervention with potential to reduce salt consumption (7).

The objectives of the study were to assess: the knowledge, perceptions, and concerns about salt intake and added salt in meals; food handlers practices associated with the use of salt; the predisposition for a reduction of added salt in meals; and the difficulties and target foods for a reduction of added salt.

MATERIALS AND METHODS

Subjects and Procedures

This research was conducted in Uniself, a Portuguese catering company, and the subjects included in this study were workers that produce meals and manage the canteens for kindergartens, schools, nursing homes and prisons. Questionnaires were distributed to a randomly selected sample of 100 company's business units from North and Center of Portugal by mail without preference or intentional choice of any particular business unit, so that food handlers (one element that was responsible for cooking in each business unit) filled out and returned the questionnaires through the mail. All participants were informed that the questionnaire was anonymous and individual. The return rate was 70%, however 2 questionnaires were rejected because they were incomplete (68 subjects were considered).

The questionnaire was developed and reviewed after a pretest (on 10 food handlers) by a team of nutritionists, and consisted of three main parts: 1) assessment of knowledge about adequate intake of salt, major food groups contributing to salt intake, and the relationship between salt consumption and health; 2) evaluation of the concerns about the use of salt, practices and difficulties; 3) characteristics of the business unit and sociodemographic characteristics of the subjects.

Statistical Analysis

All analyses were performed using Statistical Package for Social Sciences v.20 (SPSS, Chicago, Illinois, US). Pearson's chi-square tests were performed to examine if there were any relationship between demographics (gender, age and school level), knowledge (recommended sodium intake) and practices (taste before add salt and taste after add salt). A p-value of <0.05 was regarded as significant.

RESULTS

The target consumers of these business units were children (50%), adults (22.1%), elderly (1.5%), and two or more age groups (26.5%). The subjects were 92.5% female, 60% were between 26-44 years old, and 50.7% of the handlers had 9 or less schooling years. Regarding the subject's professional status, 20.6% were 2nd level cooker, 20.6% were catering supervisors, and 35.4% had "Other" status (described as cookers 3rd level in 98% of cases).

Major results related to the assessment of knowledge about adequate intake of salt, and the evaluation of the concerns about the use of salt, practices and difficulties are described in Table 1.

Large majority of the respondents (91.2%, n = 61) said that has heard or read something about an adequate intake of salt and about 80.3% think that this value is less than 5 grams per day. Half of the respondents classified salt intake in Portugal as high (39.7%) and very high (10.3%). Food produced in catering industry was identified as major contributor to salt intake by 4.5% of respondents, being the major contributor's sausages and smoked sausages (69.7%) and fast food (12.1%). Adding salt to foods is associated with few benefits to human health (31.8%) and 95.5% of respondents has identified excessive consumption of salt with the development of hypertension.

Vast majority (94.1%) has concerned about quantity of salt added to meals and 75.0% believe that the amount of salt present in the food produced in their business unit does not affect the health of the consumer.

One usual quantity measured previously (63.2%), taste of food handler responsible for cooking process (33.8%) and the consumer acceptance of food (26.5%) was factors that determine quantity of salt added to foods.

About a quarter of respondents (26.5%) always tastes foods before adding salt however 55.8% tastes less then frequently foods before adding salt. After salt addition 70.6% always taste foods, however some respondents rarely (1.5%) or never (2.9%) taste foods.

About the possibility of reducing the amount of added salt in prepared meals, 70.6% agree and over half declare that meals would become similar if a reduction of usually salt used was performed (51.5%).

Opinion/knowledge of consumers (80.6%) and opinion/knowledge of food handlers (25.3%) was

TABLE 1: Survey answers about knowledge, concerns, practices and difficulties

Questions	Answer	%
Have you heard or read any information about an adequate intake of salt?	a) yes	91.2
	b) no	8.8
Which do you think is the value of the recommended daily salt intake?	a) < 5g/day	80.3
	b) 6-7.9 g/day	13.6
	c) 8-10.9 g/day	6.1
	d) 11-13.9 g/day	0
How much do you think is the consumption of salt in Portugal?	a) very low	2.9
	b) low	10.3
	c) moderate	36.8
	d) high	39.7
	e) very high	10.3
In your opinion, which factor contributes more to total salt intake?	a) pizzas	4.5
	b) sausages and smoked sausages	69.7
	c) cheese	0
	d) food produced in catering	4.5
	e) homemade food	4.5
	f) fast food	12.1
	g) bakery and pastry	4.5
Monitoring added salt to foods has health benefits?	a) many benefits	1.5
	b) some benefits	21.2
	c) no benefits	16.7
	d) few benefits	31.8
	e) very few benefits	28.8
Is the excessive intake of salt associated with any effects on health: (multiple answers)	a) enhance health	20.6
	b) mood changes	8.8
	c) hypertension	95.6
	d) osteoporosis	10.3
	e) stomach cancer	27.9
	f) any influence	0

TABLE 1 (continuation): Survey answers about knowledge, concerns, practices and difficulties

Questions	Answer	%
Do you have some concern about the quantity of added salt in foods produced in your business unit?	a) yes	94.1
	b) no	5.9
The quantity of salt present in foods produced in your business unit...	a) is beneficial to health of consumer	20.6
	b) don't affect health of consumer	75.0
	c) is harmful to health of consumer	2.9
	d) I don't have notion about the quantity	1.5
What are the most important factors that determine the quantity of salt added to foods/meals produced in your business unit?	a) the consumer acceptance	26.5
	b) the price	1.5
	c) the taste of food handler responsible	33.8
	d) one usual quantity, measured previously	63.2
	e) other	7.4
Do you usually taste foods before adding salt?	a) never	17.6
	b) rarely	19.1
	c) sometimes	19.1
	d) frequently	17.6
	e) always	26.5
Do you usually taste foods after adding salt?	a) never	2.9
	b) rarely	1.5
	c) sometimes	5.9
	d) frequently	19.1
	e) always	70.6
What do you think about trying to reduce added salt to foods in your business unit?	a) disagree totally	5.9
	b) disagree partially	17.6
	c) indifferent	2.9
	d) agree partially	30.9
	e) agree totally	39.7
If you reduce the quantity of salt usually added to foods, the meals produced would become:	a) much worse	1.5
	b) worse	36.8
	c) similar	51.5
	d) better	5.9
	e) much better	2.9
In your opinion, what difficulties you may have in salt reduction in your business unit?	a) opinion/knowledge of consumer	79.4
	b) time spend in reduction	1.5
	c) opinion/knowledge of food handlers	25.0
	d) the costs associated	5.9
	e) never try	1.5
	f) other difficulties	4.4

identified as major difficulties in a possible salt reduction.

The food groups more frequently named as targets to reduce their salt content were: soups (36.4%); salads (18.2%); rice/pasta/potato/pulses (16.7%); and bread (15.2%). Meat (7.6%) and fish (6.1%) products were less mentioned.

Knowledge and practices were not significantly associated with the sociodemographic characteristics of the respondents (Table 2).

TABLE 2: Relationship between sociodemographic characteristics and knowledge/practices

	Gender	School Level	Age
Knowledge[a]	0.435	0.516	0.516
Taste before add salt[a]	0.923	0.981	0.548
Taste after add salt[a]	0.239	0.276	0.696

[a] Analysis by χ^2 for categorical variables

DISCUSSION

The results showed that food handlers, the main players in the production of meals, are concerned, and had good knowledge about the recommended salt intake values and health problems associated with high salt intake.

The foods groups identified as major contributors of salt intake was in accordance to reported sodium composition of these foods (8). These results are encouraging, however, given that many courses of culinary have no specific nutrition content is no good reason to suppose that chefs know any more about nutrition than the general public (9).

Food produced in catering was considered by 4.5% respondents as major contributor to salt intake, however the contribution by catering to total salt intake may be underestimated by respondents. Recent data from U.S. population shows that foods consumed in restaurants could contribute by 24.8% to 27% from total salt intake (10) and other food sources contribute with 4.1% of salt from sausages, 6.3% from pizzas, 3.5% from cheese and 7.3% from bakery products (8).

The contribution of salt in meals was considered by almost all the respondents as beneficial or not affecting consumer health. Nevertheless, mass catering may be an excellent mean for decreasing salt intake (11), and some studies showed that a single meal or a single component like soup provided in canteens may contribute to exceed the adequate daily intake of salt (12, 13).

A further aspect in the present study was the absence, or the occasional practice, to taste the foods in the meal before adding salt by about one third of the subjects, while the vast majority tastes the foods only after adding salt. Similar results was found by Johns and colleagues and suggest that salt addition depends on the chefs' palates, which tend to be less sensitive due to regular exposure to salt (9, 14) and this could have impact on quantity of salt added. Using standardized measures and procedures to add salt in

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meal preparation could be a strategy to monitor the food handler's activities related to achieve the desired final salt composition of served meals. The amount of added salt to foods was based, in most cases, in the usual quantity that was used, and about one third of the subjects rely on the taste of food handler responsible for preparation.

The European Union framework for national salt initiatives established a benchmark of a salt reduction for all food products, also encompassing salt consumed in catering (15). Most food handlers agreed with a possible reduction in salt added to foods, and the majority reported to believe that the meals produced would be similar or better. These responses may be due to social desirability or to the belief that the amount of salt being used is modest and a further reduction would not affect the taste or the consumer health.

Although this study shows a predisposition of food handlers toward the possibility of changing their practices, such as reducing the added salt to foods, factors such as consumer behavior may affect this change (16). If a genuine effort to introduce meals with a lower salt composition is made, the consumer should also be willing to choose these meals, in order to maintain the sales in the subsequent time.

Food handler's knowledge of how to reduce salt in food preparation without affecting consumer acceptance should be supported. The United Kingdom's salt reduction program successfully reduced the average salt intake of the population developing a specific work in association with the catering sector including reviewing kitchen practices and menu planning (17).

In order to perform effective actions, it will be necessary to concentrate efforts among catering associations, consumers and food handlers. Companies should be encouraged to create sustainable programs to maintain adequate salt content of the meals served, and if necessary, to reformulate some recipes, implement consumer information and awareness campaigns (18).

CONCLUSIONS

Most of food handlers were aware about the recommended salt intake values and health problems associated with excessive salt intake, and are open to salt reduction strategies in food preparation.

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Study 5 - Validation of a rapid and simple method to determine sodium content in food matrices based on Ion Selective Electrode methodology

(Provisory patent waiting)

[Provisory patent waiting]

Validation of a rapid and simple method to determine sodium content in food matrices based on Potentiometry of Sodium Selective Electrode

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ABSTRACT

High salt (sodium chloride) intake is related with the development of non-communicable diseases. Worldwide, governments are developing strategies to reduce salt intake on populations by industry's salt reduction in products. Accurate, cheap, portable and reproducible determination of sodium in meals is challenging because strategies to reduce sodium consumption contemplate a monitoring approach to empower fiscal and regulatory policies.

The aim of this study was to develop and validate an analytical method for quantitative determination of sodium in meals using direct potentiometry with sodium ion-selective electrode (ISE).

The method is accurate, precise, sensitive and selective, presenting an adequate linear working range. Comparison with an internal reference method (by flame photometry) reveals a good correlation (R^2=0.902) without significant differences ($p<0.387$). The average relative error between the two methods was 15%. Methodological issues and applications of the method are discussed.

KEYWORDS: ion selective electrode, sodium, meals, flame photometry, salt, food analysis

1. INTRODUCTION

Salt is responsible for around ninety nine percent of sodium intake and the salt consumption in the majority of the European countries is ranged between 8 and 12 grams per day (1). The World Health Organization recommends maximum intake of 5 grams per day (2). In Portugal the most recent data shows that in adult population mean salt intake is 10.7 g/d (3). Salt, besides endogenous sources, is added during cooking process and during food processing by the food industry in order to promote flavor and increase shelf life.

Based on robust evidence that related high salt (sodium chloride) intake with the development of non-communicable diseases including cardiovascular and renal diseases (4-6), and the conclusive scientific evidence that reduction of salt consumption reduces those risks (7, 8), the Member States of European Union created a common European Union Framework on voluntary national salt initiatives (1).

Each country is developing strategies to reduce salt intake on populations by industry's voluntary salt reduction in products (9), increasing awareness about salt consumption on population and/or legislative approaches (1, 10). The implementation of these strategies should contemplate a monitoring approach to empower fiscal and regulatory policies. Thus, rapid, portable and reliable methods are needed to control in loco a large number of food samples.

The official method for determination of salinity in foods is Mohr's titration, where the concentration of chloride ion titrated with silver nitrate solution is used to calculate sodium content of the sample (11). Some disadvantages are associated with this method like the de-coloration pre-treatment required for foods with deep color, the adjustment to nearly neutral condition before titration, insensitive for foods with insoluble fibrous material, and possible side reactions of silver ion with other anions such as carbonate or phosphate (12), resulting in poor method accuracy.

Another method that is validated for some foods like bread (13) and is commonly used for the direct detection of sodium concentration in foods is flame photometry (FP) (11, 14, 15). It has been often referred as the internal reference method and its advantages are the simplicity and low cost, however, sample pretreatment is time-consuming and it requires the use of a flame photometer, a non-portable equipment. In this technique the sample solution is sucked into a nebulizer and thus into the fuel stream of a fuel gas-compressed air flame. The flame provides energy to the elements leading to the excitement of their valence electrons. The light emitted is then detected by a detector after passing through a wave-length selector, being the intensity of light emitted directly proportional to the atoms in the solution (16). Although being precise and accurate, this method has not yet been applied, nor even validated, to a wide set of food products, specially cooked meals.

Besides the titrimetric and photometric methods, direct potentiometry through ion-selective electrode (ISE) for sodium (17) or for chloride (18, 19) have also been applied for the determination of food salinity. This methodology is very simple, quick and low cost, and with the additional advantage to become portable. ISE methodology is based on the measurement of the electrical potential difference between a selective electrode and a reference electrode using Nernst equation.

In 80's some researchers (17, 20, 21) developed works where sodium determination in foods was performed by ISE. However, meal analyses were not contemplated in their studies. Recently, Brewster and colleagues (22) applied ISE methodology for salt content analyses of the meals, however method validation on meals are not referred.

The aim of the present study was to validate a faster procedure based on ISE method for sodium that can be applied to quantification of salt in meals (soup and dish).

2. MATERIALS AND METHODS

2.1 Sample collection and pretreatment

Samples were collected between February and May 2015 in 7 canteens from a public university in Porto, Portugal. The collection was performed in random days, without the notification to the institutions; in each visit one sample were weighted (SECA scale, Germany) and transported in polyethylene bags. The final set of samples consisted of 2 soups and 6 meals (n=2 meat, n=2 fish, n=2 vegetarian). In the laboratory, soups were homogenized using a hand blender (Eletric Co 450 W, Portugal) and meals containing fish or meat with bones were deboned and weighted again, than all components of each meal were homogenized using a blender (Moulinex 700 W, France). The homogenized mass obtained from samples was distributed on PTFE 60 ml containers and stored in a freezer (-18ºC) until it was used.

2.2 Chemicals and Reagents

All reagents were of analytical grade and solutions were prepared with deionized water, obtained from Seralpur PRO 90 CN and Seradest LFM 20 Water Purification System.

For sodium analysis by ISE, NaCl (99.5%, Sigma, USA) was used, and the ion strength adjustment buffer (ISAB) was prepared from ammonium chloride solution (purity 99.5%, Scharlau, Spain) and ammonia (Pronalab, Mexico) at a concentration of 4 M. For the analysis of sodium by FP, sodium standard solution (1,000 ppm) was supplied

by JenWay (England) and nitric acid 70% (HNO3) was purchased from Sigma (USA). Solutions used for calibration curves were stored in the refrigerator.
To test the interference of potassium, calcium and magnesium in ISE method, we used KCl (99.5%, Pronalab, Portugal), CaCl2 (95%, Panreac, Spain) and Mg(OH)2 (99%, Fluka, Japan).
All PTFE materials were cleaned bathed in 10% HNO_3 overnight, and rinsed twice with double distilled water.

2.3 Analysis of sodium by Flame Photometry

FP was conducted according to Vieira et al. (13). A flame photometer (PFP7, JenWay England) was used. Propane gas and air were supplied as the source of flame. The flow rate of the fuel was adjusted to obtain a maximum sensitivity. Standard curve with sodium concentrations 9.10^{-6}, 2.10-5, 4.10^{-5}, 1.10^{-4}, 2.10^{-4} M was established daily and the signal of 2.10^{-4} M standard was checked occasionally during the analysis.
A portion of 2g (scale Kern ALS 120-4, Ziegelei, Germany) from each homogenized sample was taken and 2 ml of HNO3 was added and manually shaken periodically until 90 min. Then, the mixture was completed with 20 ml of deionized water and mixed using a Ultra Turrax blender (Ultra Turrax blender T25, Sotel, Germany). The volume was completed up to 40 ml and after 30 min was centrifuged during 15 min at 4,000 rpm (Labofuge 6000† Haerusmodel, Germany). Finally, 1 ml of supernatant was diluted up to 40 ml and a reading was taken.

2.4 Analysis of sodium by Ion Selective Electrode

Measurements were performed with a combined sodium ion-selective electrode from Sentek (United Kingdom, Part Number 315-77) combined to a Crison potentiometer (Spain).
Standard calibration curve with sodium concentrations 5.10^{-4}, 10^{-3}, 5.10^{-3}, 10^{-2}, 5.10^{-2}, 10^{-1} M was established. The electrode was calibrated daily, using two standard solutions of NaCl 10^{-1}M and 10^{-2} M bracketing the expected concentration of the samples to be measured. Periodically the response of the 10^{-2} and 10^{-1} M standards were checked during the samples analyses. The temperature of the standard solutions and sample solutions was measured by a digital thermometer (Lacor, Spain) within ±0.1 ºC.
In a 120 ml beaker containing a stirring bar, 100 ml standard solution was added. Beaker was placed on a magnetic stirrer (Crison, Spain) at moderate speed. ISAB 4M

(2 ml) was added to standard solution, the electrode was immersed and a reading was taken.
A portion of 10 g (scale Kern ALS 120-4, Ziegelei, Germany) from each homogenized sample was taken and completed to 100 ml with deionized water. This mixture was placed in a 120 ml beaker containing a stirring bar and 2 ml of ISAB 4M was added. Samples were placed on a magnetic stirrer (Crison, Spain) at moderate speed. The electrode was immersed in the sample solution and a reading was taken.

2.5 Method implementation

Based on Ehling (23) experimental methodology of sodium analysis by ISE on labeled low-sodium foods, the present method was adapted to meals served in canteens and in order to make the method quicker in order to allow its adaptation to a portable device. Based on these objectives the same dilution of 10 g of sample completed up to 100 ml of deionized water was kept for all samples.

2.6 Method validation

For the evaluation of specificity the interference of potassium (K^{+}), calcium ($Ca^{2+)}$ and magnesium (Mg^{2+}) was studied because these ions were the most frequent on these food matrices, beyond sodium, according to Portuguese Food Composition Table (24). The procedure consisted of three tests with different concentrations of each ion in addition to 10.0g of vegetable soup. The added mass of K+ was 71.0 mg in Test 1, 143.0 mg in Test 2, and 283.4 mg in Test 3. The added mass of Ca2+ was 20.6 mg in Test 1, 41.2 mg in Test 2, and 82.3 mg in Test 3. The added mass of Mg2+ was 6.4 mg in Test 1, 12.5 mg in Test 2, and 29.5 mg in Test 3. The mass added to the Test 2 was the average usual concentrations of these ions in soup (24).
The sodium standards (5.10^{-4}, 10^{-3}, 5.10^{-3}, 10^{-2}, 5.10^{-2}, 10^{-1} M) were used to establish the calibration curve.
To evaluate the precision by coefficient of variation 6 independent measurements were performed in the same day (same analyst same calibration) on a sample of soup (concentration 151 mg/100g i.e. $6.6.10^{-2}$ M) and on a sample of lasagna (labeled 394 mg/100g i.e. $1.7.10^{-1}$ M). The intermediate precision was also evaluated on separate days (different measurements, the same analyst and different calibrations) from two different samples: one soup (labeled 369 mg/100g i.e. $1.6.10^{-1}$ M) and one sample of lasagna (labeled 394 mg/100g i.e. $1.7. 10^{-1}$ M).
The accuracy of ISE method was evaluated through two tests: 1) standard additions method with calculation of the percentage of recovery (%R) and 2) comparison with FP method. In the first test, samples (cooked rice and soup) were analyzed by FP and ISE

before and after the addition of 401 mg/100g and 572 mg/100g. In the second test all samples were analyzed in duplicate in both methods (FP and ISE) and the difference of results was evaluated.

2.7 Statistical Analysis

Linear regression was performed to measure linearity of method. Median values, 25th and 75th percentiles, maximum and minimum values were calculated for each food group. For each food group, the sodium concentration calculated from FP and ISE were statistically compared. The paired Student t test and correlation coefficient between FP and ISE were calculated. The difference in the Na+ values estimated by the two methods was analyzed by calculation of average relative error. Statistical tests were performed with IBM SPSS Statistics for Windows, version 20.0 (IBM Corp. Armonk, NY).

3. RESULTS AND DISCUSSION

The validation of an analytical method is crucial, since it allows obtaining reliable and suitable data. During the validation process, several parameters should be assessed, such as, specificity (ability of a method to measure sodium in the presence of all expected components of the sample), linearity (ability of a method to obtain results directly proportional to the sodium concentration in a certain range) and the correspondent limit of detection, precision (concordance grade of repeated measurements under specific conditions) and accuracy (the closeness of the measurements to the true value).

Validation consisted in the assessment of a set of parameters as specificity, linearity, precision and accuracy in several food categories.

The parameters resulting from validation of ISE method for sodium analysis in meals and soups are summarized in Table 1. The tests with K^+, Ca^{2+} and Mg^{2+} showed that these ions do not significantly influence the results using the ISE method (interference less than 1% in all cases).

Table 1 - Summary of parameters resulted from Ion Selective Electrode validation in samples of soups and meals

	Linearity, M	Detection limit, M	Quantification limit, M	Precision (CV %)
ISE	$5.10^{-4} – 1.10^{-1}$	4.10^{-6}	5.10^{-4}	1.6-10.0%

The observed linearity corresponds to the working range from 11 to 2299 mg/100g in the samples. This range is consistent with our objective because foods processed in canteens had salt added during cooking process and the results are expected, or have been previously described, between these values (25-27). On the other hand, the ISE working range is broader than the FP, which is 22 to 563 mg/100g (28). The regression equation between the measured difference of potential and the decimal logarithm of the sodium concentrations in standard solutions at 30ºC (Fig. 1) shows a slope of 57.2 mV and a coefficient of correlation 0.999.

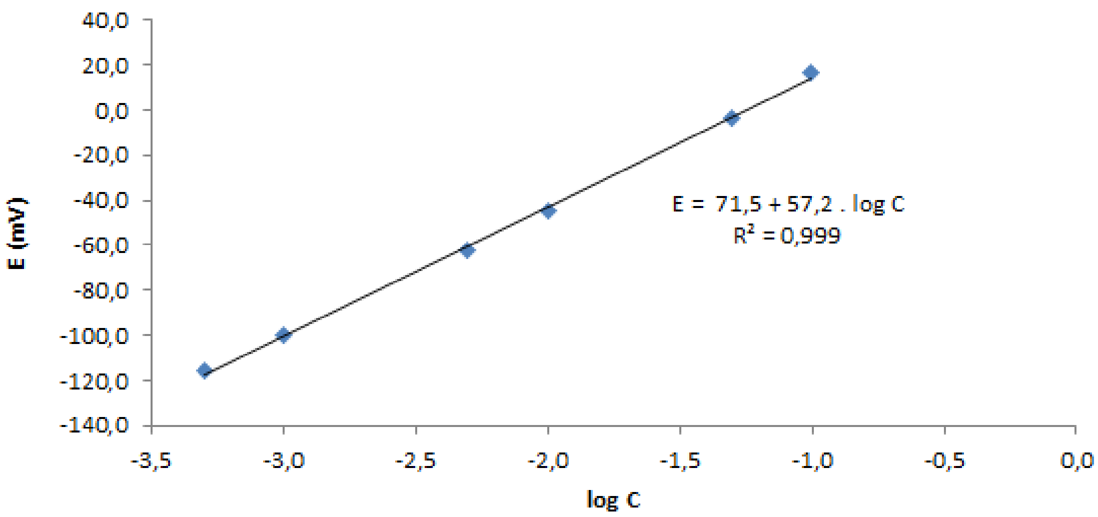


Fig 1. Calibration curve for sodium ISE obtained for sodium standard solutions in range of concentration from 5.10-4 to 10-1 M, at 30ºC.

From the calibration curve it was possible to obtain the parameters: (i) lower limit linear response (LLLR = 12 mg/100g i.e. 5.10-4 M) which is the minimum value that can be used in quantitative analysis and (ii) practical detection limit was 0.1 mg/100g, and is the minimum concentration value from which it is possible to detect the presence of sodium ion (29). The LLLR present in our method was lower than that obtained by Ehling (23) (25 mg/100g) for the determination of sodium in low-sodium foods, defined by U.S. Food and Drug Administration as foods with 140 milligrams or less sodium per reference amount (30).

A new calibration curve was constructed daily and linearity was verified by compatibility with the slope of calibration curve and the expected slope by Nernst equation, accordingly to:

$$E = E^0 + 2{,}3.\frac{R.T}{n.F}.\log C \qquad \text{(equation 1),}$$

where E is the difference of potential between the sensor and the reference electrodes (in mV), Eo is the constant for the given eletrochemical cell, R is the ideal gas constant, T the temperature in Kelvin, n the ionic charge and F the Faraday constant. Accordingly, the factor 2.3RT/nF has a theoretical value of 59.17 mV at 25ºC.

The control of temperature during the measurements was important since the slope can be affected by samples temperature. At 30ºC (Fig.1) the slope has a deviation of 4.8% and at 10ºC the slope presents a deviation of 6.4% compared to theoretical slope.

The intra-assay coefficient of variation obtained by 6 independent measurements was 1.6% in soup and 10.0% in lasagna. The inter-assay coefficient of variation was 7.1% in soup and 10.0% in lasagna. The coefficient of variation of different extractions analyzed on the same day is low but slightly increases in case of intermediate precision and in case of solid foods. However, this variation does not affect the purpose of the method; it is sufficiently precise to assess sodium in food samples.

The recovery tests by standard additions were performed to determine the accuracy of the method. Samples were analyzed before and after the addition of known amounts of sodium, expecting a recovery of 100% for an accurate method. Table 2 shows that recoveries for ISE were between 89 ± 2% and 115 ± 1%. Recoveries between 80% and 110% are accepted for method validation for food analysis (31).

Table 2 Accuracy of method assessed by 2 food samples analyzed by ISE (sodium ion-selective electrode) and FP (flame photometry).

Sample	ISE		FP	
	Spike, mg/g	% Mean ± SD	Spike, mg/g	% Mean ± SD
Soup	401	98 ± 6	401	101 ± 9
	572	115 ± 1	572	97 ± 1
Cooked rice	149	101 ± 0	149	93 ± 1
	276	111 ± 8	276	103 ± 10

By the comparison of results of samples analyzed both by FP and ISE (Table 3) it can be seen that both methods had a linear relationship (y=ax+b) established by the regression equation y= 1.32x – 33.47 with a good correlation (R^2 = 0.902).

Table 3. The statistics of sodium determination by ISE – sodium ion-selective method and FP – flame photometry method (n=8 samples).

Paired *t* test sample	Linear regression data			Probability of *t* test
	Slope [b]	Interception [b]	r^2	
ISE vs FP	1.32 (0.18)	-33.47 (37.67)	0.902	0.387

b Standard deviations of slope and intercept were included in parentheses.

The comparison between both methods should be done in light of the characteristics and limitations of each of the methods. FP is widely used in routine determination of sodium in foods. However this method was not suitable to become portable, which is our focus on validation of ISE method.

FP is prone to a number of interferences, as wider bandpass of the wavelength selector (proneness to interference from other analytes), the uniformity of aspiration and nebulization and maintenance of consistent flame (32). When the analyte is present in a complex matrix such as food sample the method is more susceptible to interferences, for example the presence of magnesium and phosphates depress sodium radiations (33). To reduce interferences, an approach involves spiking samples with an element that is known to be absent and to use this as an internal standard by which fluctuations may be determined and thus corrected for. Lithium is widely used in this context as an internal standard for the clinical determination of sodium and potassium in blood or serum (34).

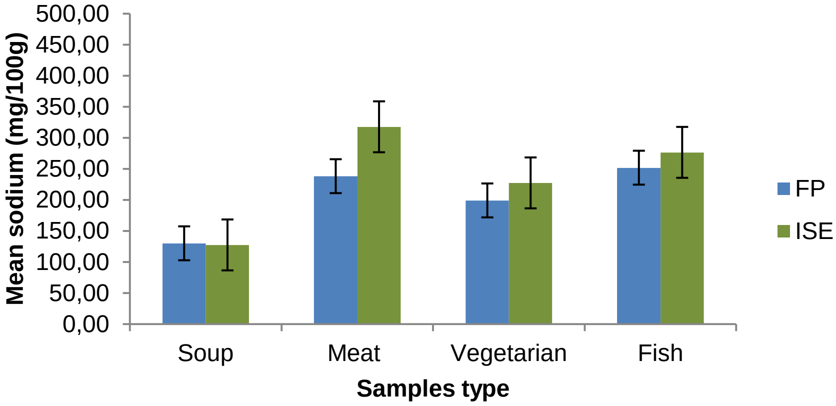
In this work the sample was prepared as described by Vieira and colleagues (13) in bread, without adding an internal standard element because using the addition of reference material they proved that addition of internal standard element was not necessary on food samples. In biological samples the addition of standard element was more common. A comparison between both methods was performed by Albert et al (35) in serum and urine samples. They used lithium as an internal standard reference in FP in order to obtain a greater accuracy and enable a precise compensation of changes in the flame. They found good degree of agreement on comparing the two methods for measuring sodium.

The average relative error between the methods was 15%, and in most of cases ISE method measured sodium concentration higher than the FP method.

The average levels of sodium in samples (mg/100g) are presented in Fig. 2, as well, as the relative standard deviations. A general tendency was observed for ISE which elevated results compared to FP, however, the simplicity and low cost of the ISE method are obvious advantages. The tendency for higher values accessed by ISE compared with FP was also obtained by other authors (35, 36) on serum and urine analyses.

The higher mean sodium content were found in fish and in meat dishes and the lowest was found in soup samples. Fish and soup samples presented the highest standard deviation of values on both methods. The high dispersion of values indicates greater variability in sodium content among products.

Plácido and colleagues (18) concluded on their study that ISE should be used in an initial screening phase and only samples which values near to legal limit should be forward to be analyzed by reference method. On the other hand is prudent that the authorities supply information about the analytical procedure which has to be followed for legal purposes in order to compare sodium ion selective electrode evaluation with the reference method.



	Food Type	n	ISE Mean ± SD	ISE Min	ISE Max	FP Mean ± SD	FP Min	FP Max
	Meat	2	317 ± 47	284	350	238 ± 28	218	258
	Fish	2	276 ± 56	237	316	252 ± 58	211	293
	Vegetarian	2	227 ± 4	224	230	199 ± 3	196	201
	Soup	2	127 ± 56	88	167	130 ± 56	91	169

Fig 2. Sodium content in samples using ISE – sodium ion-selective method and FP – flame photometry method according type of sample (n=8 samples). Standard error bars.

4. CONCLUSION

The ion selective electrode methodology for determination of sodium in meals provides a faster, less tedious and inexpensive alternative compared with FP for determination of salinity in meals (soup and dish). The validated analytical method proved to be reliable, quick and simple since it only needs sample collection, crushing, dilution and direct reading after addition of the ISAB.

The European Union Framework on voluntary national salt initiatives contemplates monitoring salt content of foods as a key element and the method validated had potential to be adapted to a portable device and allows the monitor of salt content in meals on kitchens and canteens.

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Part IV - General discussion and conclusions

Discussion

Findings from this thesis contribute to the understanding of salt consumption, particularly in Portuguese adolescents, and provide important information to contribute to the development of salt reduction strategies.

Study 1 provides, for the first time, salt intake calculated by 24-h sodium excretion and the prevalence of accomplishment of WHO recommendations in a reasonable sample of Portuguese adolescents. Our sample of adolescents with a valid 24 h urine collection was 200 subjects (118 girls). A single 24 hour urine collection from a sample population of 200 people was adequate to estimate salt intake with 95% confidence interval about the mean of consumption of +/- 12 mmol/d [174].

The adolescents studied ingested excessive amount of salt, being 9.5 g/d (3725 mg sodium/d) in boys and 7.8 g/d (3062 mg sodium/d) in girls. This clearly excessive value, where only 9.8% of boys and 22% of girls meets the recommendation of ingestion under 5 g/d [157], is alarming because the impact in health of this elevated ingestion remains to older ages [24]. Furthermore to worsen this scenario the ingestion of potassium is visibly in deficit, the sodium to potassium ratio was 1.7 in boys and 1.6 in girls.

Although this level of salt ingestion in adolescents is disturbing it is not surprising, because similar ingestion patterns are present in other European countries [90, 102, 105, 109] and in US [156], but highlights the necessity of interventions for salt reduction. Adolescence is a period characterized by increased autonomy and independent decision making and dietary intake is one way for adolescents express their independence [175]. Their health-related behaviours established can often continue into adulthood and evidence recommends that public health practitioners and policy makers consider new strategies to improve food literacy in adolescence [176].

In Study 2 we analyze the relation between salt intake and hydration status, considering the importance of an adequate hydration balance on physical and mental parameters of adolescent's health [177-179], and the interrelation between water and sodium intake to maintain fluid homeostasis [180].

Our data suggests that higher sodium ingestion may impact hydration parameters being associated with a higher FWR. Thereby adolescents probably compensate high salt intake with greater fluid ingestion. However, this capacity to compensate high salt intake with fluid ingestion does not seem to be present across all levels of sodium intake, since 25% of adolescents with sodium ingestion above 2000 mg/d (5 g salt/d) had at risk of hypo-hydration status. Also, a previous study in elderly showed that higher sodium intake was associated with a poorer hydration status assessed by urine osmolality [181]. This could corroborate the hypothesis that not all persons with different sodium intakes may be able to compensate in the short-medium term high salt intake with fluid ingestion.

In order to develop strategies to reduce salt intake in adolescents it is important to understand which food sources contribute most to salt intake. According to Study 1, the major dietary sources of salt in adolescents were cereal and cereal products (41%), meat products (16%) and milk and milk products (11%). Cereal and cereal products group includes bread (16%), which salt content was ruled by legislation to 1.4 g/100g [171], however, other countries where salt content are legislated present lower salt allowed values than Portugal [182, 183].

The largest proportion of salt is added during food manufacture or cooking process, which emphasizes the importance and magnitude of interventions in the food industry, catering and home cooking practices.

Despite vegetable soup being consumed by only 15% of adolescents, its consumption contributes to around one third of salt ingestion. Previous studies had also showed that one portion of soup (300g) could provide 54% of the daily adequate salt intake [148]. Given the relevance of vegetable soup in the Portuguese traditional dietary patterns, its importance on Mediterranean diet, its high nutritional value and negative association with obesity [184], salt reduction in vegetable soup deserves our attention.

The findings from Study 3 showed that a reduction of 30% of added salt in a vegetable soup can be performed without compromise hedonic perception by children. It is important study the acceptability of a reduced salt product by consumer because if reformulation affects pleasant perception the intent of purchase could decrease. Small reductions of salt in the food should naturally be followed by reductions in salt preference over time, so it is important starts early during childhood modulating taste preferences.

Catering industry has an important role in consumers salt ingestion through meals served in canteens, restaurants and take away services for example. Food handlers, as culinary chefs and other food service professionals, are central players in meal production. Nutritionists and public health agents should encourage and support salt reduction on catering industry through communication and communication with food service professionals.

On our Study 4, we analyze food handlers' knowledge and practices related to salt that could may affect the provision of these foods in catering units [185]. The target consumers of the meals produced on business units of food handlers studied were children in 50% of the cases. We find that 70.6% agree with the possibility of reduce salt during cooking process, however opinion/ knowledge of consumers (80.6%) and opinion/ knowledge of food handlers responsible to cook were identified as most important difficulties.

According to our results, strategies to reduce salt in catering should contemplate technical support and training for food handlers to execute best practices as taste foods before adding salt, reduce salt using herbs and other spices, to choose ingredients with lower salt content and measuring the salt content of foods. Companies should also be encouraged to create sustainable programs to reduce salt content of the meals served by reformulating recipes and implementing food education programs to consumers in order to raise awareness about salt reduction.

The difficulties could be higher in smaller restaurants than in big catering companies due to smaller buying power and fewer resources. Reduce salt could imply investments in ingredients and staffing and due to tight budget of some companies, reduce salt could be uncertain [(185)]. The food companies associations and public health agents have the responsibility to be able to motivate and develop appropriate strategies to this smaller business.

The great difficult in implement a salt reduction strategy in catering could be the necessity to monitor salt and track progress in salt reduction over time. Findings from our Study 5 suggest that ISE methodology is accurate and precise to evaluate salt content in foods like soups and meals. This methodology is easier than FP and has potential to be adapted to a portable device. With this evolution we could empower all stakeholders of the salt reduction strategy to be able to assess the current salt content and decrease it. We can imagine, the case of meals served to a school or hospital, this device allows that salt content was previously evaluated and then corrected if it is excessive. Thus, efforts to not provide meals with high salt content could be more effective.

Conclusions

In conclusion, salt intake in this sample of Portuguese adolescents was clearly excessive and exceeds WHO recommendations in the vast majority of subjects. To lower consumer salt intake a comprehensive strategy should be designed and should take into account that the major sources of sodium identified in this study were cereal and cereal products, meat products and milk and milk products: i) reductions of salt content on cereal and cereal products group and meat products group should have greater impact on reduction of adolescents daily salt intake; ii) specific product salt reductions should be studied to achieve reductions that are not perceptible to the consumer, for example, a 30% salt content reduction in vegetable soup seems reasonable; iii) consumer awareness campaigns should be contemplated to sensitize about salt reduction on foods and don't compromise salt reductions carry on by catering and food industry; iv) food handlers are an important key on salt reduction on catering industry and specific measures to empower they should be considered; and v) it is important implement an effective monitoring system that can be supported by the use of a device that may be easy to use and provide reliable results about sodium composition of foods.

Future steps in investigation

Future studies should consider the evaluation of high salt intake across other groups in the life cycle and also the impact of excessive sodium intake in health and disease, particularly during adolescence.

It is important to maintain a regular surveillance among Portuguese adolescents to monitor their behavior and salt consume patterns. Adolescents are a particular segment of population that are no longer children but not yet adults. This period of life has long been recognized as a period of heightened risk-taking and, accordingly, a stage that requires special attention from researchers (186). Research with adolescents must guide current and future efforts to promote healthy behavior and also to prevent risky behaviors that are prevalent during this stage of development.

In order to enhance the monitor of salt content of meals served by catering or by the food industry by food security, industry agents or consumers becomes important to develop and disseminate a fast, portable and easy friendly devices such as ISE methodology. Also, it is relevant test the validity of ISE method on other food matrix.

The research about salt consumption must continue in cooperation with the food industry, in order to help solve technical and safety problems resulting from salt reduction in food products.

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