

Agricultural University of Athens Department of Food Science and Human Nutrition Unit of Human Nutrition

Dissertation for the degree Master of Science in Human Nutrition, Public Health and Policy

A statistical exploitation of the Water Balance Questionnaire towards the formation of a hydration score

Georgios Sotiriou Karapanagos

ATHENS 2014

under the supervision of **Assoc. Prof. Maria Kapsokefalou**

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List of examiners:

- I. Professor Antonios Zampelas
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Declaration

Hereby I declare that I wrote this thesis myself with the help of no more than the mentioned literature and auxiliary means.

Up to now, this thesis was not published or presented to another examinations office in the same or similar shape.

Athens, 3rd February 2014

place and date

signature (Georgios Karapanagos)

Greek Synopsis

Στην παρούσα εργασία παρουσιάζεται η ανάπτυξη και προκαταρκτική αξιολόγηση σε υπάρχοντα επιδημιολογικά δεδομένα ενός δείκτη ενυδάτωσης, ενός βραχέος ερωτηματολογίου που θα υπολογίζει το ισοζύγιο ύδατος.

Η απουσία ενός τέτοιου εργαλείου αποτέλεσε το έναυσμα για την κατασκευή του από την ομάδα μας. Η ανάγκη προσδιορισμού του ισοζυγίου ύδατος γίνεται όλο και πιο έκδηλη από τη συσσώρευση επιδημιολογικών δεδομένων που συσχετίζουν την ήπια αφυδάτωση με αρκετές χρόνιες ασθένειες. Επιπλέον η ανακοίνωση από τον Ευρωπαϊκό Οργανισμό για την Ασφάλεια των Τροφίμων (EFSA) συστάσεων για την κατανάλωση νερού παρέχει τον οδικό χάρτη για την δημιουργία τέτοιου εργαλείου.

Το εργαστήριό μας στο παρελθόν κατασκεύασε ένα εκτενές ερευνητικό εργαλείο με τη μορφή ερωτηματολογίου συχνότητας κατανάλωσης τροφίμων για τον υπολογισμό του ισοζυγίου ύδατος. Έχοντας στη διάθεσή μας μια βάση δεδομένων για την πρόσληψη και αποβολή νερού από το σώμα μπορέσαμε να προτείνουμε και να αξιολογήσουμε ένα βραχύ ερωτηματολόγιο 12 πεδίων που βαθμολογεί ποιοτικά την ισορροπία ύδατος λαμβάνοντας υπ' όψιν τις κύριες πηγές πρόσληψης και αποβολής νερού από το σώμα.

Ο δείκτης αναφέρεται ως Δείκτης Ισορροπίας Ύδατος (στο κείμενο WBI ακρωνύμιο του Water Balance Index). Αποτελείται από τέσσερις δημογραφικές μεταβλητές και από 12 μεταβλητές συχνότητας κατανάλωσης (θετικής ή αρνητικής στη περίπτωση απώλειας). Το εύρος τιμών του δείκτη είναι 60 μονάδες. Η μέση τιμή του είναι 26 μονάδες με τυπική απόκλιση 3.23 μονάδες.

Ο προτεινόμενος δείκτης ενυδάτωσης μπορεί να αποτελέσει ένα εύχρηστο εργαλείο για τον προσδιορισμό του ισοζυγίου ύδατος. Ένα τέτοιο εργαλείο είναι χρήσιμο και για ατομικές καταγραφές άλλα και για πληθυσμιακές καταγραφές ώστε να παρακολουθείται ένας πληθυσμός και να ανιχνεύονται μεταβολές στην κατανάλωση ύδατος αλλά και στα επίπεδα ενυδάτωσης του πληθυσμού.

Synopsis

In this dissertation we present the development and exploratory evaluation, based on epidemiological data, of a hydration index, a short form of questionnaire that calculates water balance.

The absence of such an instrument triggered its development in our laboratory. The need to determine water balance figures becomes more and more pressing since epidemiological data suggesting a relation of between mild dehydration and a number of health issues accumulate. In addition the release by EFSA of recommendations on water consumption provides the blueprint for the construction of such an instrument.

Our lab has developed in the past a extensive Food Frequency Questionnaire, the Water Balance Questionnaire (WBQ), that thoroughly captures the water balance by gauging the water intake and loss from the body. Having on hand a database we were in position to investigate the significant water sources, positive and negative, to propose a short version of WBQ and eventually to evaluate it based on its psychometric properties.

The index is reffered to as Water Balance Index and comprises 4 preamble demografic fields as well as 12 consumption frequency variables. Its range is 60 units and its average value for the population of Athens, based on existing epidemiological data, is 26 units with 3.23 units of standard deviation.

The herein proposed water balance index may be useful in both atomic and community monitoring in order to detect water consumption and potential changes in hydration levels and consumption patterns.

Preface

The present work has been conducted in the Laboratory of Human Health of the Department of Food Science and Human Nutrition of the Agricultural University of Athens for the degree of Master of Science in Human Nutrition, Public Health and Policy.

The statistical software used was the PASW Statistics v.18 in Windows 8.1. The text and images have been created with the Oracle OpenOffice suite and two reference managers were used, the Mendeley Desktop, and JabRef.

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1 Introduction

The first three paragraphs make use of material coming from the following basic textbooks:

- Modern Nutrition in health and disease [1], 10th Edition (2006)
- Biochemie der Ernährung [2], 3. Aufgabe (2010)
- Physiologie des Menschen [3], 31. Aufgabe (2010)

1.1 Water is an essential element

Biomolecules of living cells exist and react almost exclusively in a aqueous milieu. Owing to water molecule's polarity crystal lattices can easily dissolve, and the arising ions become covered with water molecules (hydration shell). As a consequence ions can move relatively unrestricted and independent of one another in aqueous solutions, preserving at the same time their properties.

Water modifies also the properties of macromolecules, i.e. nucleic acids, proteins and carbohydrates, by forming low energy water bridges with the polar groups of these molecules. These modifications affect the conformation of macromolecules in many cases where spacious limitation have to be met in order for biochemical reaction to take place.

Hydrophobic interactions of non-polar molecules, like lipids, when in contact with water are a basic prerequisite for many biological functions. An elementary role play such hydrophobic interactions in the self-assembly and organization of biological structures e.g. polypeptide folding and tertiary protein structure.

Besides that, water appears as a reaction partner in numerous biochemical reactions, due to its polarity and high concentration. An example is the breaking of covalent bonds of biopolymers with the addition of water in what is known as hydrolysis reactions. The reverse of these reactions, the condensation reactions, produces water molecules. Another instance where water molecules are produced as byproduct is the oxidative catabolic reactions, and this water is called **oxidation water**.

The maintenance of consistency of the aqueous milieu of the cells -and the aqueous compartments of the body of any multicellular organism- is of absolute necessity and is ensured through strict homeostatic processes. Water was apparently the medium in which biomolecules first to living structures have evolved and no other medium seems to be able to substitute water for the earthly creatures in this respect. Thus water is considered an essential nutrient and the fulfillment of water requirements has priority over all other nutrients.

1.2 Water Balance

The water balance, i.e. the algebraic sum of water intake and water loss, must be always adjusted. Inevitable and facultative water loss must be within a short time compensated by the input of adequate amount of water.

Table 1 comes from the textbook "Biochemie der Ernährung"[2] and shows a **calculated** prototypical example of a daily water balance of an adult person. The figures of the water intake as well as those for the water loss of such a balance may considerably fluctuate. They are valid only to the extent where the conditions of the example are fulfilled. So the above figures have been calculated for a reference being of 1,72 m² surface assuming he/she stays still in a room of pleasant temperature. Under these conditions the unavoidable water losses through the skin and breath, collectively known as *perspiratio insensibilis*, amount to ca 840 mL. Elevated ambient temperature and physical activity, associated with increased breathing frequency and sweat secretion, can markedly this figure.

w a t	er loss		water intake				
(mL·day ⁻¹)			$(mL \cdot day^{-1})$				
obligatory facultative				obligatory	facultative		
perspiratio insensibilis	840		alimentary water	750			
urine	760	1000	oxidation water	320	1000		
		and more			and more		
faeces	100		drinking water	630			
obligatory minimum	1700		obligatory minimum	1700			

Table 1: An example of a daily water balance.

On the negative contribution site of the balance two more figures are to be credited: 100 mL water loss through the faeces and 760 mL contributed to urine. For the calculation of obligatory urine volume, the osmotically effective particles arising under the above mentioned conditions, and which should be through the renal pathway removed, are taken into account. Qualitatively significant for this calculation are the urea as an end product of the protein catabolism and the NaCl of the food-stuff consumed, the latter amounts to 156 mmol, given an adjusted electrolyte balance. Given the finite ability of the kidney to concentrate the osmotically active solutes, the minimum volume of diluting water can be calculated to 760 mL.

On the site of positive contribution, the volume of 750 mL was credited under alimentary water. This value is calculated under the assumption of a daily consumption of 1,25 Kg of food, with an averaged water content of 60%. The obligatory water gained through the oxidative catabolism of the energy-yielding nutrients (reference values: 1g of carbohydrates, triglycerides, protein - the latter to the step of urea formation - yields respectively 0.6g, 1.0 and 0.4g of H₂O) is estimated at 320 mL, based on a diet of 300g carbohydrates, 100g triglycerides and 100g of protein. And to level the water balance drinking water is calculated at 630mL.

It is obvious that both sides can be adjusted with facultative components. Under high ambient temperatures and heavy physical activity extreme losses of up to 20L through sweating can be observed and theses should be refunded with the analogous amount of fluids. In any case healthy individuals will adjust urine concentration to maintain the water balance.

1.3 Physiology of water

The water content of an adult of normal weight is about 60 percent, and to put things in context to a person of 60 to 70 Kg corresponds a water amount of 36 to 42Kg. A water loss that corresponds to 10% of body weight is enough to cause severe metabolic impair. A loss of 20% of body weight due to dehydration is not compatible with life. Infants and children are even more sensitive to a distort-ed water balance.

Different cell types contain different amounts of water. A hepatocyte (a liver cell) for example contains ca 70% water while an adipocyte (fat cell) contains on average only 20% water. Even greater is the discrepancy of water content of the various tissues of the body, given that in addition to the different cell types they contain markedly different materials that fill in the space between them. Suffice to indicate the example of the osseous (bone) tissue where calcium fraction is extremely high in the extracellular space. The scale is almost complete from 0.2% water content for enamel to 99% for the vitreous humour of the eye. The water content of the major (in terms of mass) tissues of the body is given in the following table.

tissue	Water content
skeleton	20-25%
adipose	30%
muscle	73-76%
connective	80%

Table 2: Water content of various tissues.

The difference in relative water content of the adipose tissue and the rest main tissues, results in a significant difference in the percentage and absolute water content of a person depending on the distribution of these tissues. So two persons with the same weight, the one tall and thin and the other with a higher BMI, will have notably different water in their bodies. Similarly, the well established differences in tissue distribution between sexes affects inevitably the percentage water content of the body.

Finally, the decrease of the intracellular volume (see next paragraph), as a result of muscle tissue shrinkage, in older ages corresponds to a reduced water portion in the total body mass of a person.

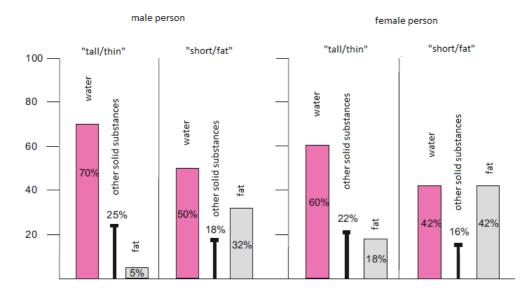


Figure 1: Water, fat and other dry matter (proteins, carbohydrates, nucleic acids ect) expressed as percentage of total body weight in relation to gender and body type.

So to summarize the mean water content of a person of normal weight is taken 75% if it is an infant, 60% if he is a man, 55% if he/she is elderly and 50% if she is a woman (Figure 1). **Total body water** (TBW) is to be found in two separated compartments: the **intracellular volume** - the water content of the cytoplasm and the subcellular organelles- and the **extracellular volume** – that would be the rest. The extracellular space is further divided into the **interstitial volume** – the fluid immediately in contact with the cells, their local environment-, the **plasma volume** – the aqueous fluid in the circulatory system- and the **transcellular** one, comprising the fluids of the CNS (*liquor cerebrospinalis*), fluids of the orbits (eye cavities) and the fluids of the derivative cavities of the coelom (pleural, pericardial and peritoneal cavities). Counting luminal fluids of the GI track or the urinary track is technically wrong since both are extracorporeal spaces. As it can be seen in the figure in older ages while the intracellular volume and plasma remain constant the extracellular volume is shrunken.

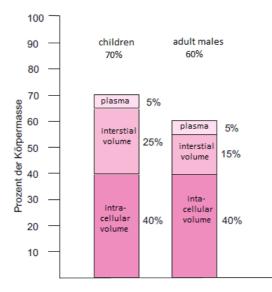


Figure 2: Water distribution in the different body fluid compartments in children and adults as a percentage of body weight. The water content of the body was assumed 70% for kids and 60% for adults.

Between these compartments there is constant bidirectional water exchange, in particular between intracellular and interstitial volumes as well as between interstitial and plasma volumes. From the skin, the kidneys and the lungs water current is efferent.

1.4 Recommendations for water consumption

Individual water needs vary considerably depending on diet, activity, environmental temperature, and humidity and thus a general water requirement for free living individuals is difficult to establish. In the past it was customary to express recommendations in proportion to the amount of energy expended under average environmental conditions. According to the "Referenzwerte für die Nährstoffzufuhr" [4] :

"The guidelines for the amount of total water intake is for an adult person ca $250 \text{mL} \cdot \text{MJ}^{-1}$ ($\approx 1 \text{mL} \cdot \text{Kcal}^{-1}$), for an older person more than $250 \text{mL} \cdot \text{MJ}^{-1}$ ($> 1 \text{mL} \cdot \text{Kcal}^{-1}$), and for breast-feeding infants ca $360 \text{mL} \cdot \text{MJ}^{-1}$ ($\approx 1.5 \text{mL} \cdot \text{Kcal}^{-1}$)."

These recommendations are in line with the Adequate Intake (AI) for *total water* set by the DRI Committee. It is intresting to point out at this point the recurring theme of the underestimated importance of water as a nutrient. Water came into frame only in the years 1997-2004, while the nutritional recommendations on their current form date from the year 1941. The table with the AI for total water of the DRI and the matching one of the DGE/ÖGE/SGE/SVE[‡] are to be found in the Appendix X.

Dietary Guidelines of the USDA

Before moving to the EFSA's position a few points related to water from the last Dietary Guidelines of the USDA [5], these of the year 2010, are worthy to mentioned. It is stated explicitly, in a separate highlighted box, the unanimity of the scientific community on the matter:

"A special note about Water

Total water intake includes water from fluids (drinking water and other beverages) and the water that is contained in foods. Healthy individuals, in general, have an adequate total water intake to meet their needs when they have regular access to drinking water and other beverages. The combination of thirst and typical behaviors, such as drinking beverages with meals, provides sufficient total water intake.

Individual water intake needs vary widely, based in part on level of physical

[‡] DGE: Deutsche Gesellschaft für Ernährung, ÖGE: Österreichische Gesellschaft für Ernährung, SGE: Schweizerische Gesellschaft für Ernährung, SVE: Schweizerische Vereinigung für Ernährung

activity and exposure to heat stress. Heat waves have the potential to result in an increased risk of dehydration, especially in older adults.

Although the IOM set an Adequate Intake (AI) for total water, it was based on median total water intake estimated from U.S. dietary surveys. Therefore, the AI should not be considered as a specific requirement level."

A second point stressed throughout the document is the promotion of the idea that water content of foods is something to be taken into account when making our choices regarding caloric load and hydration in order to determine the overall eating pattern. In several locations in the document there is a effort to clarify the last-mentioned argument:

"Foods high in water and/or dietary fiber typically have fewer calories per gram and are lower in calorie density, while foods higher in fat are generally higher in calorie density."

and

"Research has investigated additional principles that may promote calorie balance and weight management. However, the evidence for these behaviors is not as strong. Some evidence indicates that beverages are less filling than solid foods, such that the calories from beverages may not be offset by reduced intake of solid foods, which can lead to higher total calorie intake. In contrast, soup, particularly broth or water-based soups, may lead to decreased calorie intake and body weight over time."

Finally, as it is also apparent in the above quote, the energy content of beverages is also accented. On the following paragraph a detailed presentation of the state of affairs in the USA is given.

"Remember that beverages count

Beverages contribute substantially to overall dietary and calorie intake for most Americans. Although they provide needed water, many beverages add calories to the diet without providing essential nutrients. Their consumption should be planned in the context of total calorie intake and how they can fit into the eating pattern of each individual. Currently, American adults ages 19 years and older consume an average of about 400 calories per day as beverages. The major types of beverages consumed by adults, in descending order by average calorie intake, are: regular soda, energy, and sports drinks; alcoholic beverages; milk (including whole, 2%, 1%, and fat-free); 100% fruit juice; and fruit drinks. Children ages 2 to 18 years also consume an average of 400 calories per day as beverages. The major beverages for children are somewhat different and, in order by average calorie intake, are: milk (including whole, 2%, 1%, and fat-free); regular soda, energy, and sports drinks; fruit drinks; and 100% fruit juice. Among children and adolescents, milk and 100% fruit juice intake is higher for younger children, and soda intake is higher for adolescents."

EFSA's opinion

EFSA, the European Food Safety Authority, has issued two crucial documents in 2010 [6] and 2011 [7] expressing within them its opinion on the Dietary Reference Values for water and on the substantiation of the health claims related to water as a food/food component. The documents summarize the research findings and knowledge we have around water consumption and health, and serve the important role of the reference material with which heath professional and public heath officers can support their work.

With regard to the Dietary Reference Values figures are given in a detailed tabular form (Appendix) as well as in verbal form. In particular:

"The Panel concludes that available data for adults permit the definition of adequate intakes and that these adequate intakes should be based both on observed intakes and on considerations of achievable or desirable urine osmolarity. Adequate total water intakes for females would have to be 2.0 L/day (P 95 3.1 L) and for males 2.5 L/day (P95 4.0 L). The Panel defines the same adequate intakes for the elderly as for adults. Despite a lower energy requirement, the water requirement in the elderly per unit of dietary energy becomes higher because of a decrease in renal concentrating capacity."

Additional and detailed guidelines are given for every age group and for pregnancy. EFSA also emphasizes the importance of plurality in the sources of water.

"The Panel has decided that the reference values for total water intake should

include water from drinking water, beverages of all kind, and from food moisture."

The EFSA's opinion on dietary values of water was followed a year later by a second document titled "Scientific opinion on the substantiation of health claims related to water and maintenance of normal physical and cognitive functions (ID 1102, 1209, 1294, 1331), maintenance of normal thermoregulation (ID 1208) and "basic requirement of all living things"(ID 1207) pursuant to article 13(1) of Regulation (EC) No 1924/2006". The latter document corroborates the scientific findings relating dehydration and health. With regard to the health claims two out of three claims were found ultimately valid under Article 13 of the Regulation (EC) No 1924/2006, the third one was judged general and not sufficiently defined, not referring to any specific health claim.. The scientific panel considers that a cause and effect relationship has been established between dietary intake of water and maintenance of normal physical and cognitive functions as well as between the dietary intake of water and maintenance of normal thermoregulation. For both claims it is considered that, in order to obtain the claimed effect, at least 2.0L of water should be consumed per day. Such amounts can be easily consumed as part of a balanced diet. The target population is the general population. As for the last claim is indeed very general and non-specific.

1.5 Mild dehydration and health

In the past water metabolism related morbidity was examined mainly towards the extremes of the of severe dehydration and water intoxication and drowning. There is increasing evidence, however, that mild dehydration may also account for many disease states [8]. Several reviews have appeared focusing on this problem. Manz and Wentz searched and classified the published results within the period of 4 years (2001-4) relating to three hydration status markers: a) intake (water or fluid), b) urine output (volume OR osmolality) and c) hydration. They conclude that:

"Good hydration has been shown to reduce the risk of urolithiasis (category Ib evidence¹), constipation, exercise asthma, hypertonic dehydration in the infant, and hyperglycemia in diabetic ketonoacidosis (all category IIb evidence²) and it is associated with reduction in UTIs³, hypertension, fatal coronary heart disease, venous thromboembolism, and cerebral infarct (all category III evidence⁴)."

¹ Evidence from at least one randomized, controlled trial. (categories used by the writers of the review)

² Evidence from at least one other type quasi-experimental study.

³ Urinary track infections.

⁴ Evidence from descriptive studies such as comparative studies, correlation studies, and case-control studies.

Popkin et al offer a seemingly more complete review on water metabolism and health. Water's importance for prevention of nutrition-related noncommunicable diseases is explored. In addition to the above results they explore the relation of hydration to delirium, headache, skin and cognitive performance. Their interest stems from the conclusion that populations have moved to the consumption of larger portions of fluids coming from caloric beverages. The problem in both reviews is the exploration of hydration as a function of liquid intake only. This used to be the way people considered hydration if it is examined at all, anyway.

1.6 Translational research and Public Health

The landscape of biomedical research is undergoing a vast reestablishment apparently as a result of momentous changes in all its constitutional parts. Mainly the new globalized economy and health theory, the novel structure of information exchange (through the internet) and the worldwide enlargement of the academic institution affected in an unprecedented way the form of the establishment.

Since year 2000 a term has dominated the discussion around medical research and most importantly its funding. The translational medicine, a term first appeared in mid nineties, came into frame to bridge the gap between the basic research and the clinical practice, the "bench-to-bedside" gap, as most people initially understood it. In 2005, Elias Zerhouni, from his post as the director of the National Institute of Health (NIH), declares that:

"It is the responsibility of those of us involved in today's biomedical research enterprise to **translate** the remarkable scientific innovations we are witnessing into health gains for the nation. In order to address this imperative, we at the NIH asked ourselves: What novel approaches can be developed that have the potential to be truly transforming for human health?" [9]

What needed to be clarified was the exact meaning and the boundaries of this new translational research as most people initially confined it to faster and more effective connection between animal and laboratory research and clinical practice [10]. From the part of health services researchers and public health investigators the above goal is only the starting point. For the latter community the aim is to close the "bench-to-bedside" gap by:

improving access, reorganizing and coordinating systems of care, helping clinicians and patients to change behaviours and make more informed choices, providing reminders and point-of-care decision tools, and strengthening the patient-clinician relationship [Woolf 2008].

So Woolf goes on and presents in his article the outcome of the NIH's Clinical Research Roundtable which identified two definitions of translational research abbreviated T1 and T2. These represent the two "translational blocks" in the clinical research enterprise. Our research is eventually embraced by the T2, whose "laboratory is the community and the ambulatory care settings, where population-based interventions and practice-based research networks bring the results of T1 research in public."

I quote the following passage from Wool's article because it seems to capture the very essence of the challenges of the research we are undertaking:

T2 requires different research skills: mastering of the "implementation science" of fielding and evaluating interventions in real world settings and the disciplines that inform the design of those interventions, such as clinical epidemiology and evidence synthesis, communication theory, behavioral sciences, public policy, financing, organizational theory, system redesign, informatics and mixed methods/qualitative research. ... T2 struggles more with human behaviour and organizational inertia, infrastructure and resource constrains, and the messiness of proving the effectiveness of moving targets under conditions that investigators cannot fully control. [11]

1.7 Indices as quantifying instruments

An index is a scalar variable trying to encapsulate complex information. In clinical medicine there are scores to assist in making diagnoses or prognoses, scores to assists therapeutic decision making and to evaluate therapeutic results and scores to help physicians when informing and advising patients. Indicative examples of such scores are the Nutritional Risk Screening (NCR2002) designed to *identify hospital patients in danger of malnutrition*, the CURB-65 score which predicts *30-day mortality in patients with community acquired pneumonia* or the IAS-AGLA score of the Working Group on Lipids and Atherosclerosis of the Swiss Society of Cardiology which calculates *a 10-year risk of myocardial infarction for people living in Switzerland* [12]. Apart from this more traditional view scores seem to accomplish the very essence of a new medical landscape, what has come to be known as Translational Medicine, and in particular the T2 (see previous paragraph 1.6Translational research and Public Health). According to the National Institute of Health (USA)

T2 is the "translation of results from clinical studies into everyday clinical practice and health decision making" and the wide dissemination of research results and readiness to use in everyday health care decision making" [11]. Particularly, in the area of nutrition the current concern of public health has moved from problems of nutritional deficiency to problems of excesses and imbalances. Improving dietary patterns and, in turn, improving nutritional status, is viewed as a key way to improve public health [13]. Main concern of the emerging indexing tools, as opposed to former efforts, is to be able to assess dietary patterns instead of concentrating on single nutrients or even distinct foodstuffs. As it is pointed out by Panagiotakos et al. "People do not eat isolated nutrients or food items, but they consume meals consisting of a variety of foods with complex combinations of nutrients" [14]. The really advantageous aspect of this approach is its affinity to real life conditions. Dietary components don't function in isolation and their true benefit might only be effected synergistically to presence of other dietary components of the same diet.

Numerous such translational scores in nutrition have appeared the last decades. Each such score is attached to a set of dietary recommendations or to a particular dietary pattern. This holistic approach is more relevant to the way scientists comprehend public health nowadays and it has become possible due to the advancement in statistical and computational methods. Notable examples in each of the two types of indices are the Healthy Eating Index and its various offsprings encompassing the US Dietary Guidelines for Americans and the MedDietScore assessing adherence to the Mediterranean diet. Tools like these are very useful both in monitoring the dietary habits of the population in time in order to intervene if necessary and as research tools in epidemiological surveys.

The general approach is to select a limited number of non-overlapping elements that can adequately mirror the targeted dietary system. These elements are then scaled under the rationale of the dietary pattern and the final score is simply the summation of the weighted, if necessary, individual scores.

2 Objectives

The main objective of the current project is to explore the possibility of developing a Water Balance Index, in the form of a short questionnaire, that would be capable of discriminating between dehydrated, euhydrated and hyperhydrated subjects and that could be used as hydration gauging instrument on large surveys acknowledging the perplexity of accurate evaluation of hydration state.

To this end we need to perform the following steps:

- 1. choose the elements of the Water Balance Index
- 2. describe the exploratory WBI variables, based on mock replies from an existing database
- 3. Check its validity and reliability

In the following three chapters will describe the methods and results for every one of the three steps mentioned above.

In short, to achieve the first objective we identified the potentially relevant variables that qualitatively can contribute to hydration by scientific argument and discussion and by reviewing the literature. The guiding map will inevitably be EFSA's recommendations on water consumption.

Having composed this short questionnaire, which literally tries to emulate its ancestor, the Water Balance Questionnaire, we use the large (n=840) data base formed by the latter instrument to monk replies to the WBI questions. This procedure will provide us with a pseudo-distribution of the WBI score for the general population of Athens and the characteristics of the index's variables. The deliverable of this step is score's frequency distribution based on n=840 subjects

The final step will be the statistical exploitation of this distribution and of the index components distributions in order to measure validity, dimensionality and internal consistency (do items measure an underlying/latent construct), in this case the Crombach's α .

3 Methods

In this chapter, we present the methodological details of all the steps undertaken in order to develop and evaluate, in an exploratory level, the proposed water balance index (WBI). In particular the *WBI development and scoring rationale*, the procedure of *WBI scores' calculation* for epidemiological data conducive to its evaluation and finally the *evaluation plan* are described. The logic and design of the analysis plan follows closely the methodology applied for the development and evaluation of two very successful scores already in use, as these are described in the "Dietary patterns: A Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk" [14] and to a technical report on the development of the second version of the Healthy Eating Index (HEI-2005) [15].

3.1 Score Development

The general aims of the Water Balance Index (WBI), which will inevitably shape it, are in accordance with these of the aforementioned scores (HEI and MedDiet). In particular, its primary function should be the evaluation of the hydration rank of its respondent. The Index score is designed to reflect the hydration status of an individual by identifying and recording both the intake and the losses of water. In that it resembles the goals of the Water Balance Questionnaire, its "parental" instrument, which calculates a balance score between water intake by the individual and water loss from the body.

The WBI aspires to become an easy-to-use tool by the general population as well as by the research and policy making communities, so it is crucial that it be short and accessible.

The WBI is designed in light of EFSA's publication in 2010 on Dietary Reference Values for water. The main point of the EFSA's report we took into consideration is the following:

> "Water is consumed from different sources, which include drinking water (tap and bottled water), beverages, moisture

content of foods, and water produced by oxidative processes in the body. Water intake from beverages and foods is defined as total water intake, while the sum of total water intake and oxidation water constitutes total available water." [6]

This dictated the inclusion of all three major dietary sources of water - namely water itself, water from beverages and moisture of foods – as items in the index. As regards the beverages WBI items tried to cover the whole spectrum of options. The final choices regarding solid food items were eventually determined by water content and consumption patterns. The water content data were retrieved from the USDA National Nutrient Database [16].

3.2 Evaluation of the WBI

We evaluated the performance of the WBI, by assessing its psychometric properties, including several types of validity and a measure of reliability, all listed in Table 4. In order to accomplish the latter we converted a monthly food frequency questionnaire into the WBI items and calculated the WBI score for every participant of a cross-sectional study.

Data Sources

The evaluation was conducted with the calculated scores of 892 persons from the general population of Athens, Greece. The data came from a study conducted by our group in Agricultural University of Athens which compared water balance, intake and loss in summer and winter [17]. In this study the Water Balance Questionnaire (WBQ) [18] developed by Malissova also in our lab, was administered to 480 subjects during summer (July and August 2010) and to 412 during winter (December 2010 and January/February 2011) stratified by age and gender (plus to a 100 subjects for energy normalization). The sample is presented in Table 3.

Participants completed the semi-quantitative food frequency questionnaire, WBQ [18] in reference to the previous month's dietary consumption. The WBQ (presented in Appendix B) consists of 58 fields of groups of food items selected according to their water content (USDA National Nutrient Database). Alien aliments (e.g. exotic fruits) or aliments rarely consumed from the general population in Greece were not included. The reference portion was stated explicitly for every field next to the food items, and the frequency of consumption was recorded in seven non-overlapping response categories, namely 'never', 'once a month', '1-3 times per month', '1-2 times per week', '3-6 times per week', 'once per day' and 'twice or more per day'. Habits on drinking beverages, except water, were monitored with 19 questionnaire items, again reference portions were stated for each group next to the corresponding beverages and the responses' spectrum consisted of the following

six non-overlapping categories: 'never/seldom', '1-2 times per week', '3-6 times per week', '1-2 times per day', '3-4 times per day' and '5 or more times per day'. Water consumption survey was more exhaustive inquiring on two 10 point scales the glasses and 0.51 bottles of water consumed (each point being either a glass or half a bottle).

		female n (%)	male n (%)	total n (%)
	age (year)			
	<19	38 (8)	25 (6)	63 (13)
÷	20-39	83 (17)	82 (17)	165 (35)
summer	40-64	91 (19)	83 (17)	174 (36)
S	>65	39 (8)	39 (8)	78 (16)
	total	251 (52)	229 (48)	480 (100)
	age (year)			
	<19	37 (9)	37 (9)	74 (18)
winter	20-39	58 (14)	63 (15)	121 (29)
	40-64	73 (18)	70 (17)	143 (35)
WIL	>65	37 (9)	37 (9)	74 (18)
	total	205 (50)	207 (50)	412 (100)

Table 3: Age and sex distribution of participants of the WBQ evaluation study.

Urination and defecation were recorded on the basis of frequency. This questionnaire surveys additionally the physical activity level by means of the International Physical Activity Questionnaire (IPAQ) [19] and sweating are recorded as well, twice on a 10-point scale, once under activity and once under sedentary conditions.

Calculation of mock replies

The syntax for the score calculations is presented in Appendix C. Besides the obvious computations converting food frequencies recorded in WBQ to WBI scores a few points need to be clarified. As for the beverages that are typically consumed in volumes different from those of the rest of the group they belong, they were scaled, in all cases down, with appropriate factors. In particular greek coffee/espresso portions were scale down by a factor of 4, that is a portion of esspresso corresponds

to 1/4 of a normal coffee, the cappuccino group by a factor of 1.6 (reference coffee volume the classic mug) and both wine and alcohol by a factor of 2, with reference to an average beer glass. The sweat item of the questionnaire was calculated in the following way. IPAQ variables are of two sorts. The one gives duration of exercise in minutes per day whenever someone is involved in some sort of exercise (three levels are included) and the other gives the number of days in a week someone actually undertakes some sort of physical activity. So to calculate the average duration of exercise in minutes per day one should multiply these two variables and divide by 7, the number of days in a week. The amount of sweat lost by a person during a day is the summation of the sweat lost in each of these time intervals, calculated by multiplication by a personal scaling factor derived by the WBQ variable on sweat quantity produced under different conditions (described in the previous section). The final step in order to get a score is to rank each person into a category from one to five. Two separate computations were performed one assuming maximum value of sweat volume the litre, since 95% of the sample does not overcome total volumes of 975mL, and one taking the whole sample into account. The maximum volume in either case receives 0 score and the minimum receives five, the scoring here descending monotonically exactly as the urine frequency item scoring.

3.3 Analysis Plan

Table 4 comprises the analysis we undertook to verify to what extend can we trust the proposed Index and to justify further action in order to confirm its validity.

Content validity

This is a qualitative scrutiny of the Index to reveal if the various features that make up the investigated mode, in this case water balance, are included. To this end we have checked the WBI components against EFSA's recommendations.

Construct validity

First the distributional properties of the index plus its components were calculated to check their sensibility. In addition the underlying structure of the index was sought through principal component analysis (PCA) on the WBI items. This kind of analysis reveals the number of independent factors comprising the index.

The underlying structure of the index was examined through factor analysis. The method applied was the principal axis factoring on the 12 components of the score. The syntax and the Scree plot is

presented.

```
FACTOR
 /VARIABLES score anapsiktika score ximoi score gala score kafes score alcohol
score wasser score idrotas score oura score vegetable score fruit score soup
score giaourti
  /MISSING LISTWISE
 /ANALYSIS score anapsiktika score ximoi score gala score kafes score alcohol
score wasser score idrotas score oura score vegetable score fruit score soup
score giaourti
  /PRINT INITIAL CORRELATION DET KMO EXTRACTION ROTATION
 /FORMAT BLANK(0.3)
  /PLOT EIGEN
  /CRITERIA MINEIGEN(1) ITERATE(25)
  /EXTRACTION PAF
  /CRITERIA ITERATE(25)
  /ROTATION VARIMAX
  /METHOD=CORRELATION.
```

Reliability

To check the reliability of the WBI we examined its internal consistency, the degree to which multiple components within an index measure the same underlying, unidimensional, latent construct, by calculating the Cronbach's coefficient alpha. This "statistic" is mathematically equivalent to the average of the correlations among all possible split-half combinations of the index components. To understand further the relationships among components, we examined the inter-component correlations.

	Psychometric property	Evaluation question	Analysis strategy
	Content validity	Does the index capture the key points of EFSA's recommendations	Checked WBI components against EFSA's scientific opinion
Validity		Does the Index measure what it is supposed to be measuring compared with a hydration index?	Estimated Spearman's ρ (rho) between WBI and urine colour
	Construct validity	What is the underlying structure of the index components, i.e., does it have more than one dimension?	Examined structure by using a principals components analysis
		Are the total and component scores sufficiently sensitive to detect meaningful differences?	Examined population distributions of total component scores
Reliability	Internal consistency	How reliable is the total index score if water balance is found to have one dimension	Determined Cronbach's coefficient α (alpha)
		What are the relations among the index components?	Estimated Spearman's p (rho)
		Which components have the most influence on the total score?	Estimated correlations between each component and sum of al others?

Table 4: Psychometric properties of the Water Balance Index evaluated

4 Results

4.1 The WBI questionnaire

The WBI questionnaire (see Appendix A) is divided in two sections, a preamble (Table 5) with four fields inquiring general information and the main body (Table 6) of the questionnaire with the water balance gauging items.

The preamble (Table 5) consists of the two standard confounding variables, those of sex and age, which are meant to be filled in by the respondent and two weather related questions which depending the actual form of the questionnaire -web application or hard copy- will be filled in either automatically by the software or manually by the experimenter.

 Table 5: The preamble fields of the Water Balance Index

confounding	weather conditions
gender	ambient temperature
age	humidity

The main body of the questionnaire (Table 6) comprises 12 items which are divided in three conceptually distinct sections. The first captures intake from water and beverages, the second the water intake from solid foodstuffs and the final the loss of water from the body.

The first section captures virtually all major liquid sources of water. The second section, that of solid sources includes four items which have been chosen on the basis of water content and consumption frequency(Figure 3). Fruits and vegetables have both high water intake and should by consumed on a daily basis in amounts that can contribute meaningful to water intake figure. Soups and yogurt/ice cream are equally high in water content and thus can contribute significantly to water intake albeit the fact their consumption is largely seasonal. The water losses from the body are quantified through two questions on urination and on perspiration. The losses from defecation and breathing are compensated with water generated through oxidative

catabolic reactions of metabolism. It is also speculated that the relation between water available from increased consumption of proteins and carbohydrates is proportional to the losses of water through increased respiration in order to balance the energy equilibrium.

Water (tap and bottled)		
water based beverages (coffee, tea etc)		
milk based beverages (milk, milkshake, etc)		
fruit based beverages (fruit juices)		
soft drinks (regular and light)		
Beer and wine		
fruits		
vegetables		
soups		
yogurt and ice-cream		
urination frequency		
perspiration intensity		

Table 6: The Water Balance Index component items.

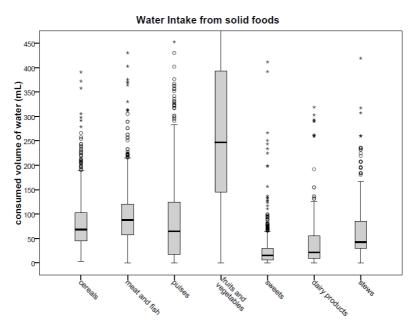


Figure 3: Box Plots of water intake from the different food groups as recorded in the WBQ evaluation study.

Levels of measurement

Water intake items are all probed in a quantitative frequency basis. Portion size is specified for each item and the frequency of consumption is sought. Water loss is more difficult to quantify, since both urine volume and sweat volume are hard to estimate. So for the urination a daily frequency question is posed and the perspiration is inquired through a visual-analog scale quantifying the amount of sweat lost by the individual in a day. Scale ranges from "I did sweat at all today" to "I have sweated too much today".

4.2 Content Validity

The key recommendations of EFSA's report, linked to related components of the WBI, are listed in Table 7.

EFSA's recommendation	WBI component	Comment
Sources of water are drinking water, beverages, food moisture and water from substrate oxidation	Items 1 through 10 record the water sources except oxidation water (see below)	
Losses of water occur via the skin, lung, urine and faeces.	Items 11 and 12 record these water losses except the skin and lung losses (see below)	
Oxidation water varies from about 350 mL·day ⁻¹ to 600mL·day ⁻¹		
Transepidermal water diffusion amounts to 450mL·day ⁻¹ , while 250 to 350mL·day ⁻¹ are exhaled with respiration	No item for these two elements was included in the Index	These two elements cancel each other out

Table 7: WBI components mapped to EFSA's recommendations.

From the validation study of the WBQ and from a currently running study in our lab we could compare the WBQ score to an hydration index, namely urine colour of 24 hours samples for a 100 individuals. The latter index serves as a marker of hydration, so a positive correlation with our mock index is indicative of its validity. The Spearman's rank order correlation resulted in a ρ value of 0.311 p(2-tailed)=0.002.

4.3 Construct Validity

Table 8 presents the various descriptive characteristics of the Water Balance Index as derived from its evaluation on the subjects (n=828) of the WBQ evaluation study [17]. The expected value of the index is about 26 and is the same for both sexes.

	Sex of subjects				
	Male	Female	Total		
Mean	25.96	25.88	25.92		
Standard Deviation	3.26	3.20	3.23		
1 st Quartile	24	24	24		
Median	26	26	26		
3 rd Quartile	28	28	28		
Minimum	17	19	17		
Maximum	36	40	40		
Kurtosis	0.122	1.341	0.641		
SE of kurtosis	0.231	0.249	0.170		
Skewness	0.166	0.611	0.366		
SE of skewness	0.116	0.125	0.085		

Table 8: Descriptive characteristics of the Water Balance Index.

Normality is assessed visually by superposition of normal curves on the frequency histograms of the WBI scores (see Figure 4 and Figure 5) and by creating the Q-Q plot of the Index.

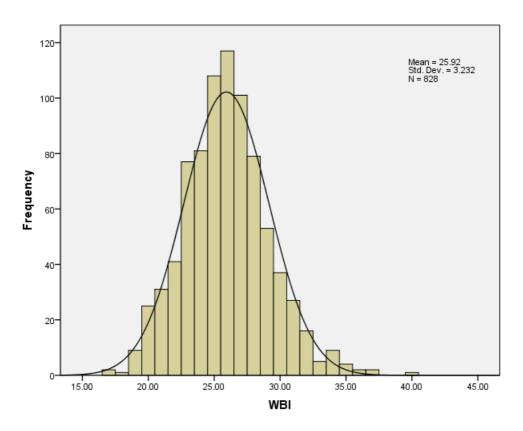


Figure 4: Water Balance Index score frequency histogram for the whole sample.

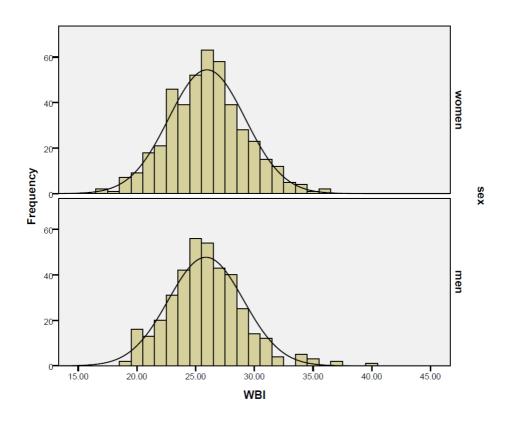


Figure 5: WBI frequency distribution broken down by sex.

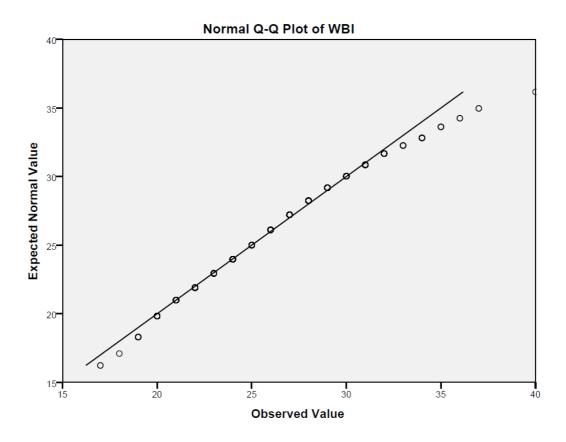


Figure 6: The WBI Q-Q plot.

The range of the WBI score associated with its ability to capture a wider window of cases and discriminate them is demonstrated in Table 9. This table shows the percentile description of the WBI variable. Below are given the SPSS steps and syntax for its calculation.

(Analyze \rightarrow Descriptive Statistics \rightarrow Explore. Dependent List: the WBI score, Statistics: Percentiles)

EXAMINE VARIABLES=WBI /PLOT NONE /PERCENTILES(5,10,25,50,75,90,95) HAVERAGE /STATISTICS NONE /MISSING LISTWISE /NOTOTAL

			Ρ	ercentiles					
sex		Percentiles							
			5	10	25	50	75	90	95
women	Weighted Average	WBI	21	22	24	26	28	30	32
	Tukey's Hinges	WBI			24	26	28		
men	Weighted Average	WBI	21	22	24	26	28	30	31
	Tukey's Hinges	WBI			24	26	28		

A more detailed presentation of the percentile score of each WBI point is given on the following graph (Figure 7). To get this plot, I have sorted the WBI variable and ranked it into percentiles and finally computed the score of each point. Immediately follows the syntax and right after it the plot.

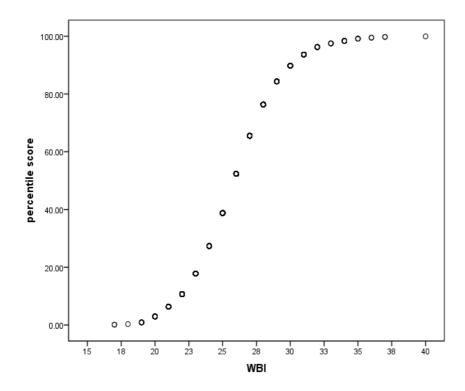


Figure 7: Percentile scores of the WBI points.

The syntax for the calculation of the Figure 7 variable is given below:

```
SORT CASES BY WBI (A).

RANK VARIABLES=WBI (A)

/RANK

/PRINT=NO

/TIES=MEAN.

COMPUTE percent_scores=(((RWBI-0.5)/828)*100).

EXECUTE.

GRAPH

/SCATTERPLOT(BIVAR)=WBI WITH percent_scores

/MISSING=LISTWISE
```

The following table (Table 10) contains mean score values and percentile distributions of the components of the Index.

As we expected from the original data, consumption of soups and yogurt/ice cream are very limited by the study population, and thus they don't seem to affect water intake significantly. They would still be part though of the WBI because of their high water content which can make a notable difference in cases where consumption of these products is higher. Beverages consumption is broken down into the five variables comprising the 1st section the score. Water per se and sweat and urination frequency variables are at variance.

The underlying structure of the index was examined through factor analysis. The method applied was the principal axis factoring on the 12 components of the score. The syntax and the Scree plot is presented.

```
FACTOR

/VARIABLES ...

/MISSING LISTWISE

/ANALYSIS ...

/PRINT INITIAL CORRELATION DET KMO EXTRACTION ROTATION

/FORMAT BLANK(0.3) /PLOT EIGEN

/CRITERIA MINEIGEN(1) ITERATE(25)

/EXTRACTION PAF

/CRITERIA ITERATE(25)

/ROTATION VARIMAX

/METHOD=CORRELATION.
```

	Percentile											
Component	Mean	10 th	20 th	30 th	40 th	50 th	60 th	70 th	80 th	90th		
water	3.95	2	3	3	4	4	5	5	5	5		
Fruit-based beverages	1.29	1	1	1	1	1	1	1	2	2		
Milk-based beverages	1.49	1	1	1	1	1	2	2	2	2		
Coffee and related beverages	1.64	1	1	1	2	2	2	2	2	2		
Sodas/refreshments	1.27	1	1	1	1	1	1	1	2	2		
Alcoholic beverages	1.31	1	1	1	1	1	1	1	2	2		
fruits	2.35	1	1	2	2	2	2	3	5	5		
vegetables	2.40	1	1	2	2	2	2	3	3	4		
soups	1.06	1	1	1	1	1	1	1	1	1		
Yogurt and ice cream	1.40	1	1	1	1	1	1	2	2	2		
urination	3.51	3	3	3	3	4	4	4	4	4		
perspiration	4.23	3	4	4	4	5	5	5	5	5		

Table 10: Means and distributions of the Water Balance Index components.

The plot shows the amount of variance contributed by each of the principal components or factors. The optimal number of factors is determined by looking for the location in the graph where the curve formed by connecting the dots starts to form a flat, parallel to the abscissa line. In Figure 8 we observe that the line appears to plateau between the 7th and 9th factor. The PCA provides evidence that no one single linear combination of the components of the WBI could account for a significant proportion of the covariation in the water consumption pattern observed in the WBQ seasonality evaluation data.

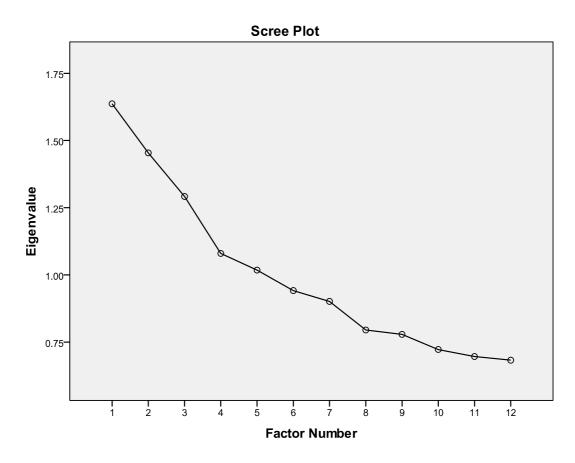


Figure 8: Scree plot from principal component analysis of the Water Balance Index showing the amount of variance accounted for by each successively extracted factor.

4.4 Reliability

Table 12 shows the relationships among the WBI components. For the most part, correlations are low. Alcohol due to its limited consumption failed to give significant correlations.

To measure internal consistency of the index we calculated the Cronbach's α measure. In PASW this

analysis is to be found under Analyze \rightarrow Scale \rightarrow Reliability Analysis. Selecting the components of the index and performing the task for the complete set of data as well as in the broken down by the sex variable we ended up with the following Table 11:

	Cronbach's a	Cronbach's α based on standardized items	Number of items
Men	0.252	0.279	12
women	0.265	0.291	12
Total	0.247	0.279	12

Table 11: The Cronbach's alpha value assessing reliability of the WBI.

	sodas	juices	milk	coffee	alcohol	water	sweat	urine	vegetables	fruits	soups	yogurt
sodas	1											
juices	.195	1										
milk	_	.121	1									
coffee	_	_	125	1								
alcohol	.182	.079	095	_	1							
water	_	.125	.132	_	_	1						
sweat	_	_	088	_	_	246	1					
urine	_	070	_	_	_	_	.083	1				
vegetables	149	_	.094	.150	_	.073	_	_	1			
fruits	080	.097	.092	.097	_	.090	086	096	.263	1		
soups	_	_	_	_	_	099	.111	_	.126	007	1	
yogurt	.088	.117	.093	_	-	.116	_	_	.134	.146	_	1

Table 12: The Spearman rho correlations of	of the WBI components.	Only significant values at r	o<.05 are included.
	<i>j</i>		

5 Discussion

5.1 Content Validity

The juxtaposition of the WBI components and EFSA's recommendations on water consumption though simple, guarantees the content and face validity sought for our index. That is, the WBI contains the water intake and loss variables dictated by EFSA's recommendations, the latter embodying the current scientific knowledge. Another important consequence of the chosen construction is the association of any given score other than the very low or high with a great number of possible water consumption patterns.

5.2 Construct validity

The distribution of the WBI total scores is wide and lacks skewness, we don't observe in our data significant clusters of individuals in either end of the scale. This is a particularly good feature since the WBI scores were constructed from 1 month data by rounding off portions, a trick that could cause floor or ceiling effects, that is scores bunching on the edges of the scale.

The PCA analysis confirms the multidimensional nature of the water consumption.

5.3 Reliability

A basic form of reliability, namely the test-retest repeatability, which detects whether an index can be expected to yield the same score, time after time, in identical situations, could not be performed since we only had one set of WBQ epidemiological data.

Internal consistency was found low, Cronbach's α =0.247, a less than moderate value, something desired anyway. Given we know that water consumption is multidimensional, as demonstrated by the principal component analysis and the intercorrelations of the WBI components, we don't expect internal consistency. The poor value of the coefficient alpha is due to the fact that components measure different, independent aspects of the water consumption behaviour. This feature of the WBI score allows component scores to be useful along with the total score.

5.4 Weaknesses and further research

The major failure of the WBI Score as developed thus far is our inability to correlate it with biochemical indices. The problem lies in the fact that WBI in its current form is preoccupied with water consumption and loss and not water balance per se. This indicates that scoring should be modified by scaling factors that would proportionate portions into water quantity. Models to this direction are already in the way and show encouraging results.

The ultimate step of course to be undertaken is an confirmatory validation study. We need to check the WBI on the field, with real answers and data collected for that purpose. A parallel study with focus groups will refine WBI components and and give it its final form.

5.5 Conclusion

We have developed a Water Balance Index. A short questionnaire with 12+4 variables that gauges water balance.

Various validity tests were performed by applying the WBI to a Water Balance database. These tests confirmed its plausibility and pointed out amendments and refinements to be employed.

A confirmatory validation is the eventual step to finalize the instrument.

Appendix A

item	Question	quantitative frequency						
1	How many glasses of water do you consume daily?	0-1	2-3	4-5	6-7	8+		
2	How many glasses of fruit juices do you consume daily?	0	1	2	3-4	5+		
3	How many cups of coffee/tea do you consume daily?	0	1	2	3-4	5+		
4	How many glasses of sodas/refreshments do you consume daily?	0	1	2	3-4	5+		
5	How many glasses of milk-based beverages do you consume daily?	0	1	2	3-4	5+		
6	How many glasses of alcohol do you consume daily?	0	1	2	3-4	5+		
7	How many portions of vegetables do you consume daily?	0	1	2	3	4+		
8	How many portions of fruits do you consume daily?	0	1	2	3	4+		
9	How many portions of soups do you consume daily?	0	1	2	3	4+		
10	How many portions of yogurt/ice cream do you consume daily?	0	1	2	3	4+		
11	How many times do you urinate in a day?	0-1	2	3	4	5+		
12	How much did you sweat in a day?			visual analo	og scale			

Appendix B

THE WATER BALANCE QUESTIONNAIRE

A. Demographics – Socioeconomic										
Sex: 1. male 0. female		Year of birth:	Year of birth:							
Address (optional): Phone (optional):										
Height(cm):	Total years of study (from 1st Primary school):									
Profession: 1. unemployed	2. se	elf-employed person	Marital status:	1.	Single, never married	2. Married				
3. employee	4.¦ci	ivil servant		3.1	Widower/Widow	4. Divorced				
5. university student 6. pensioner			Number of chi	ildrei	n:					
	7.	Other								

B. Lifestyle Features								
Do you receive medication? 1_{\perp}^{\dagger} Laxatives 2. Diuretics 3.	Do you receive medication? 1 Laxatives 2. Diuretics 3. Other							
Do you receive diet supplements? 1. Yes 0. No If yes, please specify								
Have you been diagnosed with: Diabetes 1. Yes 0. No Are you pregnant? 1. Yes 0. No								
Urinary tract infection $1.$ Yes $0.$ No	If yes, which month							
Renal dysfunction 1. Yes 0. No	Are you visiting a dietician? 1. Yes 0. No							
Do you feel/have: 1. Chills 2. Constipation 3. Diarrhea 4. Lack of concentration 5. Lack of energy								

C. Physical Activity

We would like to investigate the type and level of intensity of physical activity that you do as part of your everyday life. The questions below refer to the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself as a physically active person. Please think about all activities you do at **work** or at home, to **get from place to place**, and during your **spare time** for recreation, **exercise or sport**.

Think about all the vigorous activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that require hard physical effort and make you breathe much harder than normal. Think **only** about those physical activities that you did for **at least 10 minutes at a time.**

1. During the **last 7 days**, on how many days did you do vigorous physical activities such as heavy lifting, digging, aerobics, or fast bicycling?

.....days per week

2. How much time did you usually spend doing vigorous physical activity on one of those days?

...... hours per dayminutes per day don't know/not sure

Think about all the **moderate** activities that you did in the **last 7 days**. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for **at least 10 minutes at a time.**

3. During the last 7 days, on how many days, on how many activities you do a moderate physical activity such as carrying light loads, bicycling at a regular pace or double tennis? Please, do **not** include walking.

.....days per week

4. How much time did you usually spend doing moderate physical activities on one of those days?

......hours per dayminutes per day don't know/not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to move from place to place, and any other walking that you might do for recreation, sport, exercise or leirure

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

.....days per week

6. How much time did you usually spend walking on one of those days?

...... hours per dayminutes per day don't know/not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting at dinner or lying down to watch television. Do not include sleeping.

7. During the last 7 days, how much time did you spend sitting on a week day?

...... hours per dayminutes per day don't know/not sure

Is the past 7 days representative of your regular physical activity: 1. Yes 0. No

C. Mark HOW OFTEN you consumed the following foods during the last month:											
Caution, answer considering as portion the quantity that is entered in the parenthesis.											
(t = times, g = gram, pcs = pieces, c = cup = 240 ml)											
	Never/	1-3 t/	1-2 t/	3-6 t/	1 t/	$\geq 2 t/$					
	Rarely	month	week	week	day	day					
White bread (1 slice 30gr)											
White toasted bread (2 pcs)											
Whole grain bread (1 slice 30gr)											
Whole toasted grain bread (2 pcs)											
Thessaloniki sesame bread ring, pita bread, bread for burger (1 pcs)											
Breadsticks or crackers (2 pcs), rusks (1 pcs), cookies (2 pcs)											
Cereals ($\frac{1}{2}$ c), cereals bar (1 pcs)											
Beef (steak) (1p~150 grams)											
Hamburgers (2 pcs), meatballs (4 pcs), minced meat (1c)											
Chicken/turkey (all types) (150 gr)											
Pork (steak, piece skewer) (150 grams)											
Lamb, goat, deer, rabbit, lamb chops (150 grams)											
Ham, meat products (1 slice),											
Sausages (1 medium), bacon (2 slices)											
Fish (150 g)											
Seafood (octopus, squid, shrimp) (150 grams)											
Lentils, beans, chickpeas (1 c) (1 dish = 2 cups)											
Giant beans $(1 \text{ dish} = 2 \text{ cups})$											
Fish soup (1 portion=250ml)											
Meat soup, chicken soup (1 portion=250ml)											
Vegetable soup, mushroom soup, (1 portion=250ml)											
Soup with pasta (eg frumenty, noodles) (1 portion=250ml)											
Spinach with rice/cabbage with rice(1 p=250ml), stuffed tomatoes (2 pcs)											
Pastitsio, moussaka, eggplant with meat (1 portion = 150 grams)											
Peas, fresh beans, okra, artichokes (1 portion=250ml)											
Rice, spaghetti, pasta, noodles (1 cup)											
Boiled potatoes, mashed potatoes (1 medium/ $\frac{1}{2}$ c)											
French fries (½ portion)											
Tomato, cucumber, carrot, pepper (1 c. raw)											
Lettuce, cabbage, spinach, rocket(1 c. raw)											
Broccoli, cauliflower, courgette, $(\frac{1}{2} c. boiled)$											
Herb, leek, spinach, celery ($\frac{1}{2}$ c. boiled)											
Apple, pear (1 medium), orange (1 medium), tangerine (2 medium)											
Water melon ($\frac{1}{2}$ slice), melon (1 slice)											
Pineapple, avocado (2 slices), banana (1 medium)						1					
Grape, cherries (15 psc), strawberries (1 c)	1		1	1	1	1					
Peaches (1 medium), apricots (3-4 medium), nectarines(1 medium)	1		1	1		1					
Dried fruits (¹ / ₄ c.)											
Dried nuts, nuts (1 cup)											
Yoghurt complete or light (1 tub)											
Anthotyro, manouri or cream cheese (30 gr)						+					
			+	+		+					
Feta, white cheese, hard cheese (30 gr)	+		1	+		+					
Cheese non-fat or low fat (light, cottage) (30gr)				-							
Egg (boiled, fried, omelet) (1 pcs)											
Pies (ex. Cheese pie, spinach pie) (1 portion)											
Sweets, pastries, pie (1 pcs)											
Jams (1 serving)											
Stewed fruit(1 pcs)	 										
Jelly (1 pcs)											
Ice cream, milk shake, pudding, rice pudding (1 pcs)											

C. Mark HOW OFTEN you consumed the following foods during the last month:

Г

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	Never/ Rarely	1-3 t/ month	1-2 t/ week	3-6 t/ week	1 t/ day	$\geq 2 t/$ day
Croissant (1), wafer (1 pcs), cake(1 slice), biscuits (3-4)						
Chocolate (all types) (1 medium = 60 gr)						
Chips, corn curl, pop corn (1 bag =70 gr)						
Honey, jam (1 teaspoon)						
Olives (10 small /5 large)						
Oil (any) (1 tsp.)						
Sauce (eg mayonnaise, ketchup, mustard) (1 spoon.)						
Your soups (vegetable soup, pulses) are usually:	1. Dilute (t	hin)	2. J	elly (thick	()	

D. Mark the quantity of water	Fluid Cons you consur		during the la	ast month		
A. Do you use glass to drink water? $1.$ Yes 0.	No					
If yes, mark how many glasses of water you consumed per $1 \begin{vmatrix} 2 & 3 & 4 \\ 2 & 3 & 4 & 5 \\ 1 & 2 & 3 & 4 & 5 \\ 1 & 5 & 6 & 7 & 8 & 9 \\ 1 & 0 & 1 & 1 \\ 1 & 0 & 1 \\ $		sumed more,	how many			
B. Do you use bottle to drink water? 1. Yes 0						
If yes, mark how many bottles of 500 ml you consumed $\frac{1}{2} \begin{vmatrix} 1 \\ 1 \end{vmatrix} \begin{vmatrix} 1 \\ 2 \end{vmatrix} \begin{vmatrix} 1 \\ 2 \end{vmatrix} \begin{vmatrix} 2 \\ 2 \\ 2 \\ 2 \end{vmatrix} \begin{vmatrix} 2 \\ 2 \\ 2 \\ 2 \\ 2 \end{vmatrix} \begin{vmatrix} 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2$	per day: if you con		, how many			
(small size water bottle: 500ml, middle size water bottle						
Mark HOW OFTEN you cons Caution, answer considering as					3-4 t/ day	>5 t/ day
Fruit juice (1 glass or ³ / ₄ of small juice pack)	Karely	WEEK	week		uay	uay
Soft drinks, light soft drinks, carbonated water /soda (1						
glass or ³ / ₄ of small can)						
Milk, chocolate milk, chocolate (hot drink) (1 glass)						
Coffee in a small cup (e.g. Greek, Espresso) Decaffeinated Coffee in large cup, Coffee in large cup						
(e.g. cappuccino, french, freddo) (1 cup)						
Milk shake, sorbet (1 glass)		_		_		
Tea, other herbal teas (e.g. chamomile, peppermint) (1 cup)						
Isotonic/energy drinks (1 glass)						
Alcoholic drinks (wine, beer, whisky, vodka, raki, ouzo) (1 glass)						

F. Elimination of body fluids										
The quantity of your body sweat expulsion during exercise from 1 (minimum) until the 10 (maximum) corresponds to:										
The quantity of your body sweat expulsion in regular conditions from 1 (minimum) until the 10 (maximum) corresponds to:										
The expulsion of urine from your body corresponds to: $1t/day$ 2-4t/day 5-7t/day 8-10t/day more often										
The expulsion of faeces from your body corresponds to: $\geq 1t/day 5-6t/week 3-4t/week 1-2t/week 1t/10days$										

H. Trends in fluid consumption								
Do you usually carry water with you when you are out of home:	Yes	No						
Do you consume water from the bottle when you are at home:	Yes	No						
Do you consume bottled water:	Yes	No						
Do you consume water during the exercise:	Yes	No						
If you do please mark the quantity								
Do you consume isotonic/ energy drinks during the exercise:	Yes	No						
If you do please mark the quantity								
Do you consume liquids before you feel thirsty:	Yes	No						
Do you consume water for taste/pleasure:	Yes	No						
When you are thirsty, do you prefer to consume other fluids instead of water:	Yes	No						
Does consumption of fluids cause you the feeling of saturation:	Yes	No						
Do you how much water should a man consume per day:	If Yes, mark t	he quantity						
Do you how much water should a woman consume per day:								

Appendix C

 \ast convert ffq answers for liquid foods to glass per day. needs to be multiplied by numbers of glasses (last field).

RECODE

freskos_xymos_froutvn sysk_xymos_froutvn_100 nectar anapsyktika anapsyktika_light mineral_water gala sokolatouxo_gala sokolata_rofhma tsai other_afepsima kafes_ellhnikos kafes_decaf kafes_cappuchino milkshake grannita isotoniko_poto krasi mpyra other_alcohol (0=0) (SYSMIS=0) (1=0.066) (2=0.214) (3=0.643) (4=1) .EXECUTE .

* final step: multiplication of above frequency with the last column. COMPUTE f freskos xymos froutvn = freskos xymos froutvn* q freskos xymos fr .EXECUTE . COMPUTE f sysk xymos froutvn 100 = sysk xymos froutvn 100 * q sysk xym fr 100 .EXECUTE . COMPUTE f nectar= nectar* q nectar .EXECUTE . COMPUTE f anapsyktika= anapsyktika* q anapsyktika .EXECUTE . COMPUTE f anapsyktika light = anapsyktika light * q anapsyktika light .EXECUTE . COMPUTE f mineral water = mineral water * q mineral water .EXECUTE . COMPUTE f_gala= gala* q_gala .EXECUTE . COMPUTE f_sokolatouxo_gala= sokolatouxo_gala* q_sok_gala .EXECUTE . COMPUTE f sokolata rofhma = sokolata rofhma * q sok rof .EXECUTE . COMPUTE f_tsai= tsai* q_tsai .EXECUTE . COMPUTE f_other_afepsima = other_afepsima * q_other_afepsima .EXECUTE . *dierw me 4 gia na parw portion se koupa galikou. COMPUTE f kafes ellhnikos = (kafes ellhnikos * q kaf ellhn)/4 .EXECUTE . COMPUTE f kafes decaf = kafes decaf * q kaf dec .EXECUTE . *dierw me 1.6 gia na parw portion se koupa galikou. COMPUTE f kafes cappuchino = (kafes cappuchino * q kaf capp)/1.6 .EXECUTE . COMPUTE f milkshake= milkshake* q milkshake .EXECUTE . COMPUTE f_grannita= grannita* q_grannita .EXECUTE . COMPUTE f_isotoniko_poto = isotoniko_poto * q_isotoniko_poto .EXECUTE . *dierw me 2 gia na parw portion se potiri mpiras. COMPUTE f krasi= (krasi* q krasi)/2 .EXECUTE . COMPUTE f_mpyra= mpyra* q_mpyra .EXECUTE . COMPUTE f other alcohol = (other alcohol * q other alcohol)/2 .EXECUTE . $^{\ast} \text{compute}$ score variables for glasses and cups, excluding water. COMPUTE portions anapsiktika=RND(SUM(f anapsyktika,f anapsyktika light,f grannita,f isotoniko poto)). EXECUTE. COMPUTE portions ximoi =RND(SUM(f freskos xymos froutvn,f sysk xymos froutvn 100,f nectar)). EXECUTE. COMPUTE portions kafes=RND(SUM(f tsai,f other afepsima,f kafes ellhnikos,f kafes decaf,f kafes cappuchino)). EXECUTE. COMPUTE portions gala=RND(SUM(f gala,f sokolatouxo gala, f sokolata rofhma,f milkshake)). EXECUTE.

COMPUTE portions_alcohol=RND(SUM(f_krasi, f_mpyra, f_other_alcohol)). EXECUTE.

*SCORE for WATER.

*calculate water from water, syntax copy-paste from kalokairino.sps (olgas' code).
RECODE

glasses water vrysi ml water vrysi glass water emfial ml water emfial (SYSMIS=0) .EXECUTE . COMPUTE w glasses water vrysi = glasses water vrysi*240 .EXECUTE . COMPUTE w glass water emfial = glass water emfial*240 .EXECUTE . COMPUTE w mineral water = mineral water * q mineral water * 240 * 0.955 .EXECUTE . COMPUTE water from water = w glasses water vrysi + w glass water emfial + ml water vrysi+ ml water emfial.EXECUTE . COMPUTE freq_wasser_a=water_from_water/250. EXECUTE. * alternative rechnung. COMPUTE f ml vrysi=ml water vrysi/250.EXECUTE. COMPUTE f ml emfial=ml water emfial/250.EXECUTE. COMPUTE freq_wasser_b=RND(SUM(glasses_water_vrysi,glass_water_emfial,f_mineral_water,f_ml_vrysi,f_ml_emfial)).EXECUTE. *score for water a and b versions. COMPUTE portion wasser a=RND(freq wasser a). EXECUTE. COMPUTE portion wasser b=RND(freq wasser b). EXECUTE. ***** SCORES ******* RECODE portions_anapsiktika (0=1) (1 thru 2=2) (3 thru 4=3) (5 thru 6=4) (7 thru Highest=5) INTO score anapsiktika. EXECUTE. RECODE portions ximoi (0=1) (1 thru 2=2) (3 thru 4=3) (5 thru 6=4) (7 thru Highest=5) INTO score ximoi.EXECUTE. RECODE portions gala (0=1) (1 thru 2=2) (3 thru 4=3) (5 thru 6=4) (7 thru Highest=5) INTO score gala. EXECUTE. RECODE portions kafes (0=1) (1 thru 2=2) (3 thru 4=3) (5 thru 6=4) (7 thru Highest=5) INTO score kafes. EXECUTE. RECODE portions alcohol (0=1) (1 thru 2=2) (3 thru 4=3) (5 thru 6=4) (7 thru Highest=5) INTO score alcohol. EXECUTE. RECODE portion wasser a (0 thru 1=1) (2 thru 3=2) (4 thru 5=3) (6 thru 7=4) (8 thru Highest=5) INTO score_wasser. EXECUTE. * IPAQ score [min / day]: calculate minutes of exersice per day. Multiplying week-frequency (daysq G 1,q G 3 or q G 5) by duration (min- q G 2,q G 4 or q G 6). RECODE $q_G_1~q_G_2~q_G_3~q_G_4~q_G_5~q_G_6~(SYSMIS=0)$. EXECUTE . COMPUTE min entoni askhsh = q G 1 * q G 2/7 . EXECUTE . COMPUTE min metria askhsh = q G 3 * q G 4/7 . EXECUTE . COMPUTE min_aplh_askhsh = $q_G_5 * q_G_6/7$. EXECUTE . * i am calculating sweat quantity by adding up IPAQ score (min/day) for vigorous and moderate/walking activity multiplied by the 10-scale for vigorous vs normal sweat loss. COMPUTE posotita_idrota=(min_entoni_askhsh * idrvtas_askhsh) + ((min_metria_askhsh+min_aplh_askhsh)*idrvtas_kanonika). EXECUTE. * i am scaling to 5, considering max the product value (posotita idrota) 1000 (95% of population is below 972.36), thru division by 100

* AND rounding to the nearest integer. COMPUTE scaled_idrotas=RND((posotita_idrota*4)/1000). VARIABLE LABELS scaled idrotas 'scaled idrotas'. EXECUTE.

RECODE scaled_idrotas (0=5) (1=4) (2=3) (3=2) (4 thru Highest=1) INTO score_idrotas. EXECUTE. RECODE oura freq (1=5) (2=4) (3=3) (4=2) (5 =1) INTO score oura. EXECUTE. \ast convert ffq answers for solid food to portions.

RECODE

psarosoupa kreatosoupa xortosoupa soupa_zymarikvn arakas domata marouli mprokollo xorta karpouzi mhlo staffylli ananas rodakino giaourti pagvto (0=0) (SYSMIS=0) (1=0.066) (2=0.214) (3=0.643) (4=1) (5=3) . EXECUTE .

* portions of vegetables, fruits and soups, rounded to the nearest integer. COMPUTE portions_vegetable=RND(SUM(arakas,domata,marouli,mprokollo,xorta)). EXECUTE. COMPUTE portions_fruit=RND(SUM(karpouzi,mhlo,staffylli,ananas,rodakino)). EXECUTE. COMPUTE portions_soup=RND(SUM(psarosoupa,kreatosoupa,xortosoupa,soupa_zymarikvn)). EXECUTE. COMPUTE portions_giaourti=RND(SUM(giaourti,pagvto)).EXECUTE.

*Scores for vegetables, fruits and soups, r stands for rounded values.
RECODE portions_vegetable (Lowest thru 0=1) (1=2) (2=3) (3=4) (4 thru Highest=5) INTO
score_vegetable. EXECUTE.
RECODE portions_fruit (Lowest thru 0=1) (1=2) (2=3) (3=4) (4 thru Highest=5) INTO score_fruit.
EXECUTE.
RECODE portions_soup (Lowest thru 0=1) (1=2) (2=3) (3=4) (4 thru Highest=5) INTO score_soup.
EXECUTE.
RECODE portions giaourti (Lowest thru 0=1) (1=2) (2=3) (3=4) (4 thru Highest=5) INTO score giaourti.

EXECUTE.

References

[1] J., Shils et al. (Ed.), 2006. Modern Nutrition in Health and Disease. Lippincott Williams & Wilkins,

[2] Daniel, G. R. H., 2010. Biochemie der Ernährung. Spektrum akademischer Verlag, .

[3] Heckmann, R. (Ed.), 2010. Physiologie des Menschen. Springer Verlag, .

[4] D-A-CH, D.Jenine, B. (Ed.), 2008. Referenzwerte für die Nährstoffzufuhr. UMSCHAU, .

[5] Services, H. (2010). Dietary Guidelines for Americans

[6] **EFSA Panel** (2010). *Scientific Opinion on Dietary Reference Values for water*, EFSA Journal Vol 8 : 2-3.

[7] **EFSA Panel (2011)**. Scientific Opinion on the substantiation of health claims related to water and maintenance of normal physical and cognitive functions (ID 1102, 1209, 1294, 1331), maintenance of normal thermoregulation (ID 1208) and the basic requirement of all livin, EFSA Journal Vol 9 : 1-2.

[8] Manz, F. & Wentz, A. Hydration status in the United States and Germany. *Nutr. Rev.* 63, S55–62 (2005).

[9] Zerhouni, E. A. Translational and clinical science--time for a new vision. *N. Engl. J. Med.* **353**, 1621–3 (2005).

[10] DeMaria, A. N. Translational research? J. Am. Coll. Cardiol. 62, 2342-3 (2013).

[11] Woolf, S. H. The meaning of translational research and why it matters. *JAMA* 299, 211–3 (2008).

[12] Huber, U., Rösli, A., Ballmer, P. E. & Rippin, S. J. Scores in der Allgemeinen Inneren Medizin. *Ther. Umsch.* 70, 559–66 (2013).

[13] Kennedy, E. T., Ohls, J., Carlson, S. & Fleming, K. The Healthy Eating Index: design and applications. *J. Am. Diet. Assoc.* **95**, 1103–8 (1995).

[14] **Panagiotakos, D. B., Milias, G. a, Pitsavos, C. & Stefanadis, C**. MedDietScore: a computer program that evaluates the adherence to the Mediterranean dietary pattern and its relation to cardiovascular disease risk. *Comput. Methods Programs Biomed.* **83**, 73–7 (2006).

[15] Guenther, P. M., Reedy, J. & Krebs-Smith, S. M. Development of the Healthy Eating Index-2005. J. Am. Diet. Assoc. 108, 1896–901 (2008). [16] U.S. Department of Agriculture, Agricultural Research Service. 2013. USDA National Nutrient Database for Standard Reference, Release 26. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata

[17] Malisova, O., Bountziouka, V., Panagiotakos, D. B., Zampelas, a & Kapsokefalou, M. Evaluation of seasonality on total water intake, water loss and water balance in the general population in Greece. *J. Hum. Nutr. Diet.* **26 Suppl 1**, 90–6 (2013).

[18] Malisova, O., Bountziouka, V., Panagiotakos, D. B., Zampelas, A. & Kapsokefalou, M. The water balance questionnaire: design, reliability and validity of a questionnaire to evaluate water balance in the general population. *Int. J. Food Sci. Nutr.* **63**, 138–44 (2012).

[19] Hallal, P. C. & Victora, C. G. Reliability and validity of the International Physical Activity Questionnaire (IPAQ). *Med. Sci. Sports Exerc.* **36**, 556 (2004).