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Assessment of hydration status in a large population

Stephanie Baron[1]†, Marie Courbebaisse[1,2]†, Eve M. Lepicard[3] and Gerard Friedlander[1,2]*

[1]*Department of Physiology, European Hospital Georges-Pompidou, AP-HP, Paris Descartes University, Paris, France*
[2]*Inserm U845, Growth and Signalling Research Center, Paris Descartes University, 156 rue de Vaugirard, 75015 Paris, France*
[3]*Institute for European Expertise in Physiology, 21 Rue Leblanc, 75015 Paris, France*

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Abstract

Both acute and chronic dehydration can have important implications for human behaviour and health. Young children, non-autonomous individuals and the elderly are at a greater risk of dehydration. Mild hypertonic dehydration could be related to less efficient cognitive and physical performance and has been reported to be associated with frequently occurring pathological conditions, especially nephrolithiasis. The assessment of hydration status in a large sample appears to be of interest for conducting epidemiological and large clinical studies aimed at improving preventive and curative care. Especially in large-population studies, methods that are used have to be accurate, cheap, quick and require no technical expertise. Body weight change is widely used to determine acute hydration changes, but seems to be insufficiently accurate in longitudinal studies. Bioimpedance analysis methods enable the assessment of total body water content, but their use is still under debate. Because plasma osmolality directly reflects intracellular osmolality, it constitutes a good marker to assess acute hydration changes, but not chronic hydration status because it changes constantly. Moreover, venepuncture is considered to be invasive and is not suitable for a large-sample study, especially in children. Urinary markers appear to be good alternatives for assessing hydration status in large populations. Collection of urine samples is non-invasive and cheap. High technical expertise is not required to perform urinary marker measurements and these measurements can be carried out quickly. Thus, methods based on urinary markers are very well suited for field studies. Urine colour is probably the least sensitive marker despite its high specificity. Urine osmolality and especially urine specific gravity could be easily used for determining hydration status in large-sample studies.

Key words: **Hydration: Dehydration: Urinary indices: Osmolality: Urine specific gravity**

Both acute and chronic dehydration (body water deficit) can have important implications for human behaviour and health[1,2]. Mild dehydration is associated with altered cognitive performance and degraded mood[3,4] and also with impaired physical performance[5,6]. Mild dehydration could thus be associated with less efficient knowledge acquisition, especially during infancy and childhood, and also with less efficient professional activity.

Young children, non-autonomous individuals and the elderly are at a greater risk of dehydration, notably because they do not always have open and easy access to water and because their perception of thirst is neglected[7] or altered[8–10]. Although nephrolithiasis is the only disorder that has consistently been found to be associated with chronic low daily water intake[11], many other frequently occurring pathological conditions, such as constipation, asthma, CVD and chronic kidney diseases, could also be linked to insufficient fluid intake[12].

High medical costs, morbidity and mortality can thus result from dehydration, so this condition should be taken into account in the field of public health[13,14]. In the near future, epidemiological and interventional clinical trials will be needed to assess the impact of dehydration in a large sample. The lack of consistency in the evidence concerning hydration status and fluid intake requirements published to date is mainly due to the different methodologies used and also due to the complex and dynamic human fluid–electrolyte regulatory system that defies description as it changes constantly. That is why an attempt should be made to standardise methods for future studies. There is currently no consensus on a 'gold standard' for hydration status markers, particularly for mild dehydration. This indicates the need to define the

Abbreviations: AVP, arginine vasopressin; BIA, bioelectrical impedance analysis; BIS, bioelectrical spectroscopy; ECW, extracellular water; ICW, intracellular water; MF-BIA, multiple-frequency bioelectrical impedance analysis; SF-BIA, single-frequency bioelectrical impedance analysis; TBW, total body water.

* **Corresponding author:** Professor G. Friedlander, fax +331 43 06 04 43, email gerard.friedlander@inserm.fr

† These authors contributed equally to the present work.

best so-called field method to assess hydration status in a population of supposedly healthy people or patients.

To this end, the choice of accurate, easy-to-perform and non-expensive methods is fundamental. Although methods for assessing hydration status have been defined in previous reviews[15–17], the best methods suitable for assessing hydration status in a large sample have not been precisely discussed to date.

In this review, we focus on the need to study hydration status in a large population and on methods available to assess hypertonic dehydration status. We principally give an overview of methods applicable to a large sample.

Definition and regulation of hydration status

The balance between water outputs and water inputs defines hydration status. Excess loss of water or insufficient intake of water induces a state of dehydration. This dehydration is hypertonic when water loss exceeds electrolyte loss, leading to a higher blood electrolyte concentration and thus to an increased plasma osmolality. To equilibrate osmolality between the intracellular and extracellular compartments, an obligatory increase in plasma osmolality inducing a shift in water from the intracellular to the extracellular compartment occurs. An increase in plasma osmolality thus implies intracellular dehydration[18–20].

Water is mainly lost via kidney excretion and sweating. Other routes of loss are the respiratory tract and the faeces. Water excretion via the kidney removes solutes from the blood, and a minimum obligatory urine volume is required to remove the solute load. Obligatory urine volume is defined as the water volume necessary to excrete 24 h urine solutes at the age-related lower limit of maximum urine osmolality. The lower limit of maximum urine osmolality in individuals living in industrialised countries has been estimated to be 830 mOsm/kg minus 3·4 mOsm/kg per year starting from the age of 20 years[16]. In an 18–25°C environment, a healthy sedentary adult will have moderate water losses ranging from 1·8 to 3·0 litres/d[21]. These water outputs must be counterbalanced by water intake to maintain a neutral hydration balance. Most of the water intake in humans is from pure water (about 61 % of the total daily water intake), while the water that they consume in the form of beverages or water present in foods represents less than 40 % of the total daily water intake[22].

Only a small amount of water (250 ml/d) is produced by metabolism in humans. Because water balance is highly dependent on dietary intakes and nutrient availability, body water balance is highly regulated. A loss of 1 % of body water is usually compensated within 24 h. Both water intake and renal water losses are controlled to achieve water balance, while sweating and expiration of regulatory vapours are not regulated. Minute changes in plasma osmolality are the main factors that stimulate the two main homeostatic mechanisms: release of the antidiuretic hormone arginine vasopressin (AVP) and thirst. AVP is synthesised in the supraoptic and paraventricular nuclei of the hypothalamus and is released from the posterior pituitary[23] and controls renal water reabsorption. An increase in plasma osmolality immediately triggers the release of AVP, which in turn activates the reabsorption of water from urine by the kidney, the main effective regulator of water loss[24]. The discriminatory power of renal excretion measures for assessing dehydration status is thus always secondary to that of plasma osmolality changes[25]. Following renal water reabsorption, urine osmolality increases, reflecting the concentration capacity of the kidney. Together with the release of AVP, an increase of 1 or 2 % in plasma osmolality elicits thirst and thus water intake.

Dehydration consequences and related disorders

Water is vital to life: when fluid deficit exceeds 8 %, death may occur[21]. Before this extreme state occurs, dehydration is manifested as various signs and symptoms[12]. Altered cognitive performance[26–28], degraded mood and headache symptoms[4] have been reported to be associated with dehydration in adults and children[29–31]. A recent review has concluded that being dehydrated by just 2 % impairs performance in tasks that require attention, psychomotor and immediate memory skills, as well as assessment of the subjective state, whereas performance in long-term and working memory tasks and executive functions is better preserved[3]. However, as emphasised by Secher & Ritz[32], these data have been derived from a small number of children and are not generalisable to older adults (mean age about 60 years) to support a relationship between mild dehydration and cognitive function. Lastly, data are currently lacking in frail elderly and demented individuals.

The role of hydration in physical activity, particularly in athletes, is of considerable interest and is well described in the scientific literature[5,6]. Although environmental temperature and heat tolerance of individuals should be taken into account, physical performance is also affected by dehydration. The performance of adults in strength and power exercises is generally less affected when compared with endurance or repeated intense performance[33]. Rehydration can reverse deficits due to dehydration such as reduced endurance, increased fatigue, altered thermoregulatory capability, reduced motivation and increased perceived effort and can also reduce oxidative stress induced by exercise and dehydration[34]. During exercise, children may be at a greater risk of involuntary dehydration than adults. Children may not recognise the need to replace lost fluids, and both children and coaches need to be given specific guidelines regarding fluid intake[7].

Popkin *et al.*[12] suggested that the replacement of water with sugar-sweetened beverages, juice and milk is associated with a reduced energy intake. The literature concerning the effect of water intake on energy intake in children is very limited, but a German school intervention study with water has suggested that the effects of water on the overall energy intake of children are comparable to those in adults[35].

It has been shown that cardiovascular function is impaired under dehydration conditions. Heart rate increases and blood pressure decreases more rapidly during dehydration. Rehydration improves cardiac function under 2 % body weight dehydration conditions[36,37].

Bar-David *et al.*[29] reported a higher rate of kidney stone formation in a hot environment. Moreover, there is strong

evidence that urine dilution in stone formers contributes to the reduction of the average recurrence interval and also the recurrence rate[38]. A high intake of fluids, especially water, is still the most powerful and certainly the most economical means of preventing nephrolithiasis, and it is often not used to advantage by stone formers[39].

Dehydration may also affect kidney function as high fluid intake is associated with a lower risk of chronic kidney diseases[40] or with a slower decline in kidney function[41]. Interestingly, in the last study, this association was found to persist even after adjustment for age, sex, baseline estimated glomerular filtration rate, use of medications for hypertension (including diuretics), proteinuria, diabetes and CVD.

Less strong evidence links good hydration status to a reduced incidence of constipation, exercise asthma and hyperglycaemia in individuals with diabetic ketoacidosis. Good hydration status is associated with a reduction in the risk of urinary tract infections, hypertension, fatal CHD and venous thromboembolism, but this needs to be confirmed by clinical trials. For other conditions such as bladder and colon cancers, evidence for a preventive effect of maintaining good hydration status is not consistent[12]. It has recently been reported that fluid intake of more than 2000 ml/d might be a protective factor in secondary stroke prevention[42].

In a recent review, Armstrong[11] has reported that urolithiasis is the only disorder that has consistently been associated with chronic low daily water intake, whereas evidence suggests that in conditions such as obesity and type 2 diabetes, increased water intake may reduce energy intake in some individuals.

The elderly (aged >65 years) are particularly at an increased risk of dehydration because they exhibit a decrease in thirst sensation and at the same time have an impaired kidney capacity to concentrate urine[8–10]. However, in a recent German population-based observational study, the median total water intake was found to decrease with an increase in age in only males[43]. Obligatory urine volume was found to increase in both sexes due to a decreased concentration capacity of the kidney. The latter was balanced by a decrease in non-renal water losses, leaving the free water reserve and therefore hydration status almost unchanged, showing that total water intake requirements do not change with age, although ageing affects several parameters of water metabolism. Reduced sweat loss with increasing age appears to be primarily responsible for this observation[43]. We must keep in mind that this study was conducted under conditions of free water access in the elderly, which is not the case in those who are institutionalised. Children are dependent on adults for access to water, and a larger surface area:volume ratio makes them more susceptible to changes in skin temperatures, linked to ambient temperature shifts[44,45].

Determination of daily fluid intake requirements

The scientific and medical communities have made recommendations regarding daily water intake to fulfil water requirements in infants, children and adults of both sexes. These recommendations are not based on minimal intake as a lot of factors can lead to an increased water output and a negative water balance. Indeed, environmental temperature, altitude, humidity level, physical activity and diet can affect water requirements[46,47]. Calculations of the recommended water intake made by the European Food Safety Authority (EFSA) are based on the ideal urine osmolality of 500 mOsm/kg to provide a safe margin of a 'free water reserve'[46]. The majority of experts recommend a daily fluid intake of more than 2 litres/d in stone formers to maintain a diuretic state of at least 2 litres/d to optimise urine dilution[48].

With regard to the impact of seasonal variations on hydration status, it has been reported that dehydration secondary to a heat wave is potentially very harmful, particularly in some susceptible subpopulations, such as the elderly[49,50], and in fragile patients, i.e. patients on antipsychotics[51] and patients suffering from cystic fibrosis[52]. However, it has been shown that hydration status assessed by central venous pressure in patients with heat stroke could be normal, indicating that rapid intravenous rehydration should be avoided to prevent overload problems[53]. Moreover, it is now well known that athletes who train in hot weather are hypohydrated while drinking *ad libitum* during practice because water intake is not sufficient to replace their sweat loss[54,55]. This is also the case in manual workers working under extremely hot conditions[56,57]. A single study has been conducted in 547 children living in the Mediterranean region to evaluate the effects of seasonal changes in the climate on urine specific gravity and blood pressure. Surprisingly, seasonal changes in Mediterranean climate did not lead to changes in the hydration status of the children, suggesting that the decrease in blood pressure observed during summer should not be attributed to the hydration status[58].

Studies carried out in diverse populations have shown that daily water intake requirements are not met in children[59,60]. Stookey[61] showed that adults are also sensitive to dehydration. In this study, based on plasma tonicity measured, 60 % of the 14 855 American community-dwelling adults (aged 20–90 years) giving blood for the Third National Health and Nutrition Examination Survey were found to have hypertonic plasma. Besides problems related to disease and the fact that elderly people have a reduced thirst sensation that affects water balance regulation, this study demonstrated that the recommended daily water intake is not met in adults, just as in children.

In a study carried out in eighty-four subjects aged 81–86 years, the mean fluid intake from drinks was found to be 950 ml for women and 1330 ml for men[62]. Only 45 % of the women and 35 % of the men drank at least 1000 ml of drinks a day, with 1000 ml of daily fluid intake from only drinks being the French recommended dietary intake for the general population. Actually, the scientific basis for water intake recommendations for the elderly is scarce, and the recommendations made by different nutrition societies are not consistent.

The high recurrence rate in stone formers strongly suggests that their daily fluid intake is insufficient: up to 85 % of all stone patients could be at a lower risk of stone recurrence with elementary reorientation of their lifestyle and dietary habits, the most important being higher fluid intake[63].

Patients' compliance with this very simple preventive measure could be improved by autoevaluation of their hydration status.

Need to define the best method to assess hydration status in a large population

Dehydration may have a potential economical and sociological impact in terms of cognitive and physical performance. Mild dehydration could thus be related to less efficient knowledge acquisition, especially during infancy and childhood, altered professional activity and more frequent work stoppages. Dehydration could also have a high impact in the field of public health as it appears to be a risk factor for highly prevalent pathological conditions, such as nephrolithiasis, which is a disease affecting about 5–10 % of the population in industrialised countries worldwide with high clinical and economical costs[64]. Lastly, ageing[65] and obesity epidemic in developed countries[66] highlight the need to study the potential impact of hydration status and of water intake on morbidity and mortality in the elderly and on energy intake.

Due to all these reasons, assessing hydration status in a large sample appears to be of great interest for conducting epidemiological and large clinical studies aimed at improving preventive medicine and also medical supervision in patients, especially stone formers, and in elderly institutionalised people. This may have some important implications for those responsible for forward planning in health care facilities.

'Field' methods for assessing hydration status: advantages and disadvantages

A 'field method' should be able to be performed in a large sample in everyday-life conditions, while remaining reliable enough to give access to scientifically useful data. It should consequently be ideally non-invasive, acceptable for the majority of people, cheap, easy to perform (not time consuming, not requiring high technical expertise and with a very few pre-analytical requirements), reproducible, sensitive enough and without a large inter-individual variability within a given population. In the following sections, we focus on the methods available for a large-sample study. A summary of the methods available is given in Table 1.

Assessment of body water

Body weight change. Determination of body weight change is probably the simplest method for assessing water loss during physical exercise for a short period of time. Total body water (TBW) content corresponds to about 60 % of body weight[20]; thereby, acute changes in body water content can be assessed by determining body weight change. Moreover, this method can be performed easily as it is quick and does not require technical expertise. It is commonly assumed that during physical exercise, body weight loss essentially equals the water loss occurring due to sweating. No other body component is lost at such a rate[17]. Harvey *et al.*[67] have recently found that during a match the body weight of nine football players varies in correlation with other indices

Table 1. Advantages and disadvantages of methods available for assessing hydration status

Techniques	Fluids used	Practicability	Accuracy	Risk for subjects	Intra-individual variability* (%)	Inter-individual variability* (%)
Body weight change (acute context only)	All (extracellular and intracellular fluids)	Not expensive, quick, low technical expertise required	Moderate	Non-invasive	1·1	26·6
Bioelectrical impedance	Uncertain	Moderate cost, time consuming, technical expertise required	Moderate	Non-invasive	–	–
Plasma osmolality	Extracellular fluid	Not expensive, quick, medium technical expertise required	Moderate	Invasive (venous puncture)	1·3	1·5
Urine osmolality	Excreted urine	Not expensive, quick, medium technical expertise required	Moderate	Non-invasive	28·3	57·9
Urine specific gravity	Excreted urine	Not expensive, quick, low technical expertise required	Moderate	Non-invasive	0·4	1·0
Urine colour	Excreted urine	Low cost, quick, low technical expertise required	Moderate	Non-invasive	30·9	47·4
Saliva osmolality	Saliva	Not expensive, quick, medium technical expertise required	Moderate	Non-invasive	9·5	35·8

*Data from Cheuvront *et al.*[69].

of hydration status such as urine specific gravity and urine colour. This is a sensitive method that can detect acute changes in hydration status such as moderate fluid losses of between 2 and 3 % of body weight[68]. Moreover, body weight change is commonly used to evaluate the severity of dehydration. Clinical symptoms depend on the severity of dehydration and the tolerance of individuals. Among these symptoms, the more common are an increased heart rate, a lengthening of capillary refill and a decreased systolic blood pressure. Even though no consensual definitions of acute and chronic dehydration exist, these two phenomena are very different. Acute dehydration mainly results from excess water loss due to pathological conditions such as diarrhoea or physical exercise, leading mostly to moderate-to-severe dehydration. On the other hand, chronic dehydration seems to be mainly linked to a lack of water intake as observed in the elderly. This kind of dehydration is often less serious and clinically more difficult to diagnose.

In physiological conditions, intra-individual variations in body weight rarely exceed 1·1 %[69]. Nevertheless, because of the significant inter-individual variations (26·6 %)[69] due to body composition change (e.g. fat mass, muscular mass, sex and age), a personal precise baseline is absolutely fundamental, but not always available. Moreover, changes in body composition, independently of hydration status changes, make this parameter unusable in studies of long duration. That is why this parameter is mainly used in acute experimental settings, based on sport activity or intense exercising, which greatly differ from free-living conditions from a physiological point of view. In addition, this parameter is relevant for one measurement at a given time point (e.g. after exercising), but cannot reflect hydration status during longer time periods (e.g. 24 h) as food ingestion, fluid intakes, faecal losses and urine production also affect body weight. Due to these reasons, determination of body weight change is not a suitable method for assessing hydration status in free-living condition sample studies.

Isotope dilution methods. The principle of isotope dilution methods is based on the distribution of a tracer substance after oral or intravenous administration[70]. These methods enable the measurement of TBW content, thanks to a tracer that gets distributed in all body fluid compartments. In this case, the most common tracers used are the stable isotopes of hydrogen and oxygen such as D_2O[71] and ^3H_2O[72]. Briefly, many hours after the administration of a precise quantity of a tracer, the tracer concentration achieved after equilibrium is measured in the plasma and/or urine. Its concentration allows to determine TBW content[70]. In the same way, tracers that get distributed only in extracellular compartments are used to measure extracellular water (ECW) content. Na, Cl and especially Br[73] isotopes are used in this case. The difference between TBW and ECW contents yields intracellular water (ICW) content[74]. Even though these methods are accurate, they cannot be used in large populations because of the significant technical conditions that are required.

Bioelectrical impedance analysis. Another method that can be used for assessing body composition, especially water content, is bioelectrical impedance analysis (BIA). Its principles have been widely described by Kyle *et al.*[75,76]. BIA is carried out based on the electrical properties of tissues. Indeed, tissues conduct electrical current differently depending on their water and electrolyte contents. Taking this property into account, equations have been developed to link body resistance to the electrical current of TBW, ECW and ICW. Sex, weight or age is usually taken into account in these equations. The referent isotope dilution method has been used to determine these equations. In the study carried out by Gudivaka *et al.*[77] the reference values obtained for TBW content were 44·2 (SD 6·3) kg for men (*n* 14) and 30·6 (SD 3·8) kg for women (*n* 13); for ECW content they were 15·7 (SD 3·2) kg for men and 12·2 (SD 1·8) kg for women; and for ICW content they were 28·5 (SD 3·7) kg for men and 18·4 (SD 2·5) kg for women.

Several bioimpedance methods have been developed since the 1970s. The first one is the single-frequency BIA (SF-BIA). In this method, a 50 kHz current is passed through the body through the electrodes placed on the hand and the ankle generally. This method enables to measure the sum of ICW and ECW contents, but does not allow determining TBW content. This kind of method has not yet been validated for use in altered hydration conditions[77]. The multiple-frequency BIA (MF-BIA) was developed in the 1990s to improve sensitivity and accuracy. In this method, electrical currents ranging from 0 to 500 kHz are used to evaluate TBW, ICW and ECW contents[78,79]. Nonetheless, frequencies below 5 kHz and above 200 kHz have poor conductivity reproducibility and their use should be avoided[80]. Shanholtzer & Patterson[81] found this method to be reproducible with the same technicians taking measurements. Gudivaka *et al.*[77] evaluated the validity of the MF-BIA in twenty-eight adults and found the maximum standard error estimates for TBW, ECW and ICW contents to be 2·4, 1·4 and 3·5 kg, respectively. Many studies have been realised to compare SF-BIA and MF-BIA. Patel *et al.*[82] demonstrated that better results could be obtained when using the MF-BIA for the determination of ECW content and that the SF-BIA is ideal for the determination of TBW content in critically ill patients. In another study, it was found that changes in ECW and ICW contents in elderly patients could not be detected using the MF-BIA[83].

The third method is bioelectrical spectroscopy (BIS). The main difference between BIS and classical BIA (SF-BIA and MF-BIA) is the use of mathematical modelling and mixture equations (e.g. Cole–Cole plots) to determine ECW or ICW content instead of the classical BIA equations. This method has been shown to be accurate and have a low bias in a non-physiological population[84]. Nevertheless, the authors do not agree with BIS variability results. Ward *et al.*[85] demonstrated a wide biological variation in a control population. Also, there is a debate regarding accuracy results because some authors have demonstrated accuracy improvement[86–88] with mixture equations, while others did not[89,90] and have even demonstrated worse accuracy[77]. Segmental BIA has also been developed, where two additional electrodes are placed on either side of the body to focus on well-defined body segments. Body segmentation is useful because it is less influenced by fat fraction or geometrical boundary conditions.

Another method developed using BIA is the bioelectrical impedance vector analysis, which was developed by Piccoli *et al.*[91–93]. In this method, results do not depend on equations or modelling and so the variability depends only on analytical errors and biological variations. Few clinical studies have been conducted using this method. Buffa *et al.*[94,95] demonstrated promising results, while Cox-Reijven *et al.*[96] demonstrated a low sensitivity, but a high specificity in detecting depletion.

The main advantage of using BIA methods is that they provide a rapid feedback. Moreover, these methods are relatively inexpensive, non-invasive and easy to perform[75]. Nonetheless, such methods exhibit a significant variability. The most important parameter is the choice of the equation or modelling used in the SF-BIA, MF-BIA or even in BIS leading to significant inter-BIA variations. Kyle *et al.*[76] reported more than twenty different equations for determining TBW content using BIA leading to various standard error estimates ranging from 0·88 to 3·8 litres when compared with a reference measure obtained with $^{2}H_2O$ or ^{18}O. With regard to ECW content, Kyle *et al.*[76] reported standard error estimates ranging from 0·98 to 2·2 litres when compared with the results obtained using the isotope dilution method in a review of twenty studies. With regard to ICW content, two studies have reported standard error estimates of 0·9 litres in an elderly population[97] and 1·9 litres in healthy men[98]. The chosen equation must be most relevant for the population studied, depending on ethnic group, age (elderly, adult or child), body shape abnormalities or fat mass distribution. For example, Cox-Reijven *et al.*[86] found BIS to lack sensitivity in an overweight population. Moreover, the reproducibility of BIA measurements depends on many factors. The change in electrode position has been underlined by Sinning & Morgan[99]. Roos *et al.*[100] and O'Brien *et al.*[101] also highlighted the change in electrolyte composition as a significant cause of variations in BIA measurements. Changes in skin and ambient temperatures are also responsible for variations in BIA measurements[102]. The development of improved BIA methods taking these factors into account is of great interest[103]. The placement of electrodes is important and could lead to variations in BIA measurements[104]. Lastly, standardised protocols that take into account all these parameters are essential for optimising BIA measurements[74].

Moreover, BIA seems to be insufficiently suitable for large-population studies in free-living conditions[105]. Kyle *et al.*[76] recommended that this method be used in only stable conditions. Furthermore, O'Brien *et al.*[101] underlined the difficulty in distinguishing fluid volume and electrolyte changes during acute hydration changes. Finally, the use of more accurate markers seems to be essential for assessing hydration status in a large-sample study.

Plasma osmolality. Because plasma osmolality reflects intracellular osmolality, it has historically been considered to be a good marker standard for assessing hydration status[106], although some limitations have been underlined[15,22]. The most important regulated variable in the central nervous system to control human fluid–electrolyte balance is indeed extracellular osmolality. Consequently, plasma osmolality cannot validly represent chronic hypohydration as the brain is constantly affected by a change in plasma osmolality (i.e. moving it towards a set point or shifting it to exist within an acceptable range). Osmolality is measured using either a freezing-point depression osmometer or, more rarely, a vapour pressure-depression osmometer. Neuroendocrine regulation of plasma osmolality is such that normal values rarely deviate by more than 1–2 % from a basal value of 287 mOsm/kg in healthy, well-hydrated individuals. Intra-individual and inter-individual variations are indeed very low (1·3 and 1·5 %, respectively)[69]. Moreover, its measure is highly reproducible with an analytical CV $< 0{\cdot}4\,\%$[69]. Because of this small deviation window, a cut-off of 290 mOsm/kg is commonly used to define the limit between euhydration and dehydration[107–109].

When plasma osmolality measurement is not possible, in physiological conditions, it could be replaced with osmolality calculation. Indeed, plasma osmolality depends on plasma solute concentrations. Na is the most abundant electrolyte in ECW and is mainly responsible for plasma osmolality, in association with its matched anions, urea and glucose. The calculated osmolality is defined as follows: osmolality $= 2 \times [Na^+] +$ [urea] + [glucose]. Apart from pathological conditions, such as hyperglycaemia (diabetes) and the terminal stage of chronic kidney disease with increased uraemia, Na concentration is highly correlated with plasma osmolality, and its measurement could be an alternative for plasma osmolality measurement[110].

Although methods based on plasma osmolality are not expensive, plasma osmolality may not be the most suitable parameter for field studies because collecting blood samples is considered to be invasive for subjects. Due to this reason, this parameter is not suitable for a large-sample hydration assessment study, especially in children[111]. Moreover, plasma osmolality is more or less relevant, depending on the context. In case of acute changes, especially during physical exercise, plasma osmolality has been described to change, while in a chronic dehydration context, such as in low drinkers or during progressive dehydration, e.g. in case of fluid deprivation, plasma osmolality is preserved, while only urinary indices change because of kidney adaptation[112,113]. Considering all these factors, the use of an indirect marker thereby seems essential for large-sample studies of long duration, and urinary markers appear to be good alternatives for assessing hydration status in such contexts.

Urinary indices. As has been described previously, the kidney is the main regulator of water loss in response to an elevation of plasma osmolality[10]. During hypertonic dehydration conditions, AVP is secreted, leading to water reabsorption in the collecting duct, without electrolyte reabsorption. This mechanism leads to a decreased urine output with an increased urine concentration. It is essential for scientists to assess hydration status by measuring urine concentration. There are three urinary markers that are widely used for assessing urine concentration: urine osmolality; urine specific gravity; urine colour.

Urine osmolality. Urine osmolality is the concentration of osmotic solutes present in the urine. It is measured, as has been described previously for plasma osmolality, using a freezing-point or vapour pressure-depression osmometer. Urine osmolality depends on two parameters: the quantity of

solutes and the volume of water. Regarding the quantity of Na, K and urea are the most abundant solutes in the urine. In physiological conditions, their amounts mainly depend on the diet, with daily osmole elimination in urine being closely related to daily osmole intake.

In a dehydrated healthy individual, a small volume of highly concentrated urine will be produced and will be reflected by an elevated urine osmolality, while in an individual with a high fluid intake, a large amount of urine will be produced, resulting in a low urine osmolality. Thus, urine osmolality reflects the capacity of the kidney to appropriately respond to variations in body water balance. Urine osmolality ranges from 50 to 1400 mOsm/kg. Few rare pathological conditions, such as diabetes insipidus, syndrome of inappropriate AVP secretion and preterminal stage of chronic kidney diseases, could disturb the concentration capacity of the kidney. In these rare cases, urinary indices cannot be used.

Overall, measuring urine osmolality has many advantages (Table 1). First, it is a non-invasive and cheap method that can be performed in large populations in everyday-life conditions. This method permits to detect the trend to dehydration easily because osmolality increases in parallel with hypertonic dehydration[114,115]. In addition, it is sensitive enough to detect small changes in the hydration status. For 1-unit variation in plasma osmolality, there is a 100-unit variation in urine osmolality showing a larger deviation window[16]. For instance, Armstrong *et al.*[114] showed that urine osmolality reflects dehydration more accurately than blood indices. Moreover, among all the urinary markers, urine osmolality has the best sensitivity (91 %), which is almost equal to that of plasma osmolality (90 %)[69].

Intra-individual variation in urine osmolality is significant with a 28·3 % variation and even more for inter-individuals with a 57·9 % variation coefficient[69]. Manz & Wentz[16] showed that the mean 24 h urine osmolality varies from 360 mOsm/kg in Poland to 860 mOsm/kg in Germany, mainly because of the cultural differences in dietary fluid and osmole intakes.

A large number of studies have shown that urinary osmolality increases in response to dehydration[116–120]. Nevertheless, defining a cut-off value for euhydrated and dehydrated subjects is difficult. These authors have suggested the use of a population-specific cut-off value that would be equal to the mean maximal value minus 2 SD. In Europe, this cut-off value would be 830 mOsm/kg[16]. Grant & Kubo[106] defined dehydration as a urine osmolality above 1000 mOsm/kg. In 1994, Amstrong *et al.*[114] defined dehydration as a urine osmolality exceeding 1052 mOsm/kg. Oppliger *et al.*[115] first set a dehydration cut-off value at 700 mOsm/kg for evaluating a hypohydrated group and then decided to set the cut-off value at 800 mOsm/kg to increase the correlation with the results obtained for plasma osmolality. Cleary *et al.*[121] used a 700 mOsm/l threshold; Peacock *et al.*[122] used 900 mOsm/kg as the cut-off value.

To conclude, it is clear that no consensus has been reached regarding the dehydration cut-off value[123]. However, in line with the results reported by Manz & Wentz[16,46] and conclusions drawn by the EFSA, an osmolality over 800 mOsm/kg could be a relevant cut-off value to define the limit between a euhydrated and a slightly dehydrated status. This cut-off value is not generalisable because of the great variability between different kinds of populations according to their dietary habits.

Urine specific gravity. Urine specific gravity corresponds to the measure of urine density, defined as the weight of urine compared with that of an equal volume of distilled water. The specific gravity of plain water is equal to 1·000, whereas that of normal urine samples usually ranges from 1·013 to 1·029. To prevent weight loss by dehydration in weight category sport, the National Collegiate Athletic Association[124] has decided that dehydration would be defined by a urine specific gravity value over 1·020–1·025. Armstrong *et al.*[123] reported that these limits reflect the upper range of a euhydrated state. This cut-off value is in accordance with the results of numerous studies exhibiting a real consensus state regarding urine specific gravity measurements[121,125,126]. In physiological conditions, intra-individual variation in urine specific gravity is effectively negligible with only a 0·4 % variation coefficient. Inter-individual variation is also very low with a 1·0 % variation coefficient, making the measurement very robust and reliable[69]. Urine specific gravity is measured using a refractometer, which yields results immediately with low technical requirements. Numerous studies have shown that urine osmolality and urine specific gravity are strongly correlated, indicating that the measurements of both these parameters are consistent[114,115].

A single gravity test strip could be used to determine urine specific gravity. The major advantage of this is that patients, especially stone formers, or volunteers can use it themselves. Its benefit has been underlined in old institutionalised people[127]. Although a German study analysing 340 first morning urine samples demonstrated a reasonably good correlation between refractometry and single test strip results[128] and a study analysing 174 urine samples demonstrated refractometry measurement to lack accuracy, refractometry measurement remains the 'gold standard' to define urine specific gravity[129].

Urine specific gravity measurement has one main disadvantage: both the number and size of the particles in the solution affect it. Indeed, urine specific gravity can vary when unusual quantities of larger molecules such as glucose, proteins and urea are present in the urine, generating falsely elevated values that suggest highly concentrated urine. This phenomenon also occurs during urine osmolality measurement where glucose and urea also have an osmotic effect.

Lastly, this method is considered to be as accurate as urine osmolality measurement[114,130], with the same specificity (91 %) and an almost equivalent sensitivity (89 %)[69]. Urine specific gravity measurement could even present the advantage of a low inter-individual variability when compared with urine osmolality measurement. Urine specific gravity could thus be recommended to be used for assessing hydration status in large-population studies.

Urine colour. Urine colour is the third common urinary marker used for assessing hydration status. A urine colour chart has been developed to assess urine concentration in healthy humans[116]. Briefly, this chart has a standardised colour scale ranging from 1 (pale yellow, corresponding to diluted urine) to 8 (dark brown, corresponding to concentrated urine). The general admitted value for a cut-off

definition between euhydration and dehydration is mainly set at 4 units[109,121].

This method has several advantages: it is cheap and non-invasive; it does not require technical expertise and gives immediate results. Moreover, this method has the best specificity (97 %) among all the methods based on urinary markers and the analytical variation is negligible[69]. Armstrong *et al.*[116] found that there is a linear relationship between urine colour, specific gravity and osmolality, showing that all these urinary markers are suitable for assessing hydration status. However, the main disadvantage of using urine colour chart is its lack of sensitivity (81 % with a 5·5 cut-off value)[69]. Moreover, it can be affected by dietary factors, illness and medications[131], leading to significant intra-individual and inter-individual variability (30·9 and 47·4 %, respectively)[69].

In conclusion, urine colour seems to be less sensitive, but more specific than urine osmolality or specific gravity to assess hydration status and may not be the most suitable marker for large-sample studies.

Validity of urinary indices for assessing hydration status. Because urine is stored in the bladder before excretion, urine can be collected at different time points. Usually, urine is collected either in the morning before ingesting any food or fluid (fasting morning urine) or over a 24 h time period (24 h urine). Urinary indices of morning urine are not always correlated with those of 24 h urine samples[123]. Several studies have shown that morning urine is more concentrated than 24 h urine samples[21,132]. Indeed, during night, there is a lack of fluid intake and accumulation of urine in the bladder. Collection of 24 h urine samples provides concentrated morning urine and diluted urine corresponding to the periods of rehydration during the day. First morning urine assessments give information about water balance at a single time point, while 24 h urine collection reflects the whole-day body water balance[133]. In the absence of excessive extra renal water losses by sweating, hydration status (reflected by urine concentration) mainly depends on water intake so that daily repartition of water intake will greatly influence urine concentration. Thereby, urinary measurements should be interpreted relative to the type of urine collection performed.

If the bladder is properly voided before the water load, urine dilution, as judged from urine osmolality, may be observed as early as 30–60 min after a water load. It should be stressed that most studies addressing the impact of water intake on urine osmolality or specific gravity were performed under very specific conditions of acute dehydration elicited by physical exercise. Under these conditions, ingestion of less than 1·0 litres of hypotonic fluid was found to have only a limited effect on urine osmolality in the hour following ingestion[116,134]. Kovacs *et al.*[120] showed that nearly 3 h are required to normalise urine osmolality and colour after an acute 3 % dehydration period. In contrast, ingestion of a large amount of fluid within a short period of time during rehydration was found to induce a rapid increase in urinary output even when the subjects were dehydrated[120].

The majority of researchers consider 24 h urine collection as the gold standard for urinary hydration markers in daily life[133]. Nevertheless, collecting 24 h urine samples is a heavy procedure that is difficult to perform in large-sample studies on hydration. Perrier *et al.*[130] demonstrated that afternoon urine collection could be a good representative of 24 h urine collection and become a suitable alternative for 24 h urine collection. Moreover, the use of urine osmolality:urine creatinine ratio has been discussed for assessing hydration status and seems to be reproducible in individuals aged >5 years[135]. Nevertheless, further studies would be needed to validate this marker and its correlation with other accurate hydration status markers.

To conclude, urinary indices allow to accurately assess hydration status during mild dehydration. Among the methods based on these indices, those based on urine specific gravity and colour are easy to be performed, while those based on urine osmolality require technical expertise. Collection of urine samples is non-invasive and cheap. High technical expertise is not required to perform these two measurements, and these measurements can be carried out quickly. These measurements are thus very well suited for field studies. However, these measurements may be less accurate in some situations such as during rehydration, isotonic dehydration (loss of water and Na at the same concentrations as in the plasma) and hypotonic dehydration (loss of Na). In spite of its specificity, urine colour is certainly the least sensitive urinary marker, but urine specific gravity, with good specificity and sensitivity, could easily be used in a large-sample study.

Saliva parameters. Similar to urine, saliva is another easily accessible fluid. Saliva flow rate is a very important parameter among the salivary parameters. In physiological unstimulated conditions, saliva flow rate has been evaluated to be about 0·46 (SD 0·2) ml/min and 0·32 (SD 0·2) ml/min, respectively[136,137]. It has been shown that 24 h dehydration is associated with decreased saliva flow rates in a small sample of healthy young and older adults[138]. During metabolic rehydration of these subjects, unstimulated saliva flow rate increased, but remained significantly lower than the baseline levels. Saliva osmolality is also an important parameter. Cheuvront *et al.*[69] defined normal values of 71 (SD 15) mOsm/kg in an eighteen-person euhydrated population. A further investigation carried out by Walsh *et al.*[139] showed that during acute mild dehydration (3 % body weight change), saliva flow rate decreases (from about 0·5 to 0·2 ml/min), while saliva osmolality (from 50 to about 100 mOsm/kg) and total protein concentration (from 0·7 up to 1·8 mg/ml) increase. These variations are correlated with body weight change, urine osmolality and plasma osmolality[139]. In the study carried out by Pross *et al.*[113], following 24 h of fluid deprivation, saliva osmolality was found to increase slowly when compared with urine specific gravity and colour modifications, which indicates the lack of sensitivity of this parameter. Moreover, values of salivary parameters returned to baseline levels >1 h after the ingestion of a rehydration solution[139], suggesting that the measurement of these parameters is more relevant for assessing dehydration status than for assessing hydration status during rehydration period. Moreover, Singh & Peters[140] found a lack of correlation between saliva osmolality and urine osmolality and specific gravity in multiday events.

In addition, the large inter-individual variability highlighted by Ely *et al.*[141] and Walsh *et al.*[139] and measured by Cheuvront *et al.*[69] (35·8 % inter-individual variation coefficient) does not enable the assessment of hydration status in a large population as a baseline value should be determined for each subject to set his or her own euhydration reference value. Moreover, this baseline seems difficult to assess because of a significant intra-individual variability[69] (9·5 %), probably linked to the profound effect of oral intake[141]. Saliva osmolality is the least sensitive (81 %) and specific (83 %) hydration status marker[69].

In conclusion, even though saliva osmolality measurement is non-invasive and cheap, it cannot be used in subjects with progressive dehydration in large-sample studies because of its lack of accuracy, its need for technical expertise, and its significant inter-individual and intra-individual variability.

Recently, tear osmolality has also been evocated as a hydration status marker[142,143], with a good correlation with plasma osmolality, but further studies are required to validate its utilisation.

Conclusion

To date, no ideal and consensual method has been developed to assess hydration status, especially in large-sample studies. Body weight change seems difficult to assess in such a context because of the necessity of a baseline value. Because plasma osmolality directly reflects intracellular osmolality, it constitutes a good marker to assess acute hydration changes, but cannot represent chronic hydration status because it changes constantly. Moreover, venepuncture is considered to be invasive for subjects and is not suitable for a large-sample hydration assessment study. Urine concentration reflects renal response to changes in plasma osmolality and is in most cases well correlated with plasma osmolality. Among the urinary markers, urine colour is probably the least sensitive marker. Urine osmolality and especially urine specific gravity could be used easily to assess hydration status in a large-sample study. Although 24 h urine collection is the gold standard to assess urine concentration, it is a demanding procedure that is difficult to use in large-sample studies. First morning urine or afternoon urinary spot samples can be used, with the former being easier to standardise and the latter being more representative of the whole-day water balance. Knowledge about the daily repartition of fluid intake is required to analyse urinary markers.

Understanding the advantages and limitations of using each hydration status marker is a key point to conducting large-sample studies concerning hydration status. These large studies will probably be of great interest in the near future in the field of preventive medicine.

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