Narrative review

Intracellular Water and Cellular Hydration: Physiological Determinants, Measurement Limitations, and a Proposed Cellular Hydration Reserve Framework

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ABSTRACT

Background: Hydration is monitored using body mass, total body water, osmolality, and bioelectrical indices - all whole-body or extracellular quantities. Most body water is intracellular, and its distribution is governed by transmembrane solute gradients rather than by ingested volume. This review therefore summarises the physiological determinants of intracellular water (ICW), to examine how far routine measures reflect ICW, to assess whether intracellular hydration state and reserve are conceptually distinct, and to identify research priorities that can be tested. **Methods:** A structured narrative review of English-language, PubMed-indexed literature was undertaken. Five Boolean search lines covering intracellular water, cellular hydration, cell volume regulation, organic osmolytes, body cell mass, bioelectrical impedance, phase angle, potassium and magnesium homeostasis, muscle glycogen and dehydration were run, and the reference lists of key reviews were searched. Of 1,962 unique records, 332 were assessed on title and abstract, and 316 met the eligibility criteria. From these, and from 16 sources found by other methods, 38 sources were selected purposively, and each was verified against its database record. Interventions were graded according to how directly they had been tested against an intracellular endpoint. **Results:** Potassium distribution is the principal osmotic determinant of ICW; magnesium acts as a transport cofactor. Skeletal muscle and its glycogen-associated water form the largest reservoir, while dehydration, heat, ageing and short sleep impose regulatory demand. ICW varies considerably at equivalent muscle mass and is associated with muscle strength and physical function. No routine measure quantifies ICW directly, and dilution and impedance estimates remain method-, population- and device-dependent. Few interventions have been assessed against intracellular endpoints, and two direct studies of creatine were negative. **Conclusion:** Fluid intake alone does not determine cellular hydration. Cellular Hydration Reserve is proposed as a provisional latent construct requiring standardised challenge–response measurement and incremental validation.

***Key words*:** Body Water; Intracellular Fluid; Cell Size; Organism Hydration Status; Water-Electrolyte Balance; Potassium; Magnesium

In clinical and public health practice, hydration is discussed in terms of fluid volume, and the measures employed to monitor it - body mass, total body water, plasma osmolality, and urine osmolality - all describe whole-body or extracellular amounts. Although the greater part of the body's water is intracellular, its regulation is controlled by the distribution of solutes across cell membranes rather than by the volume of fluid consumed [1,2].Cell volume is actively regulated as the cells respond to osmotic disturbances by means of regulatory volume increase and regulatory volume decrease, these being achieved through the transport of inorganic ions and by either accumulating or releasing organic osmolytes [1,3,4].

Access this article online

Received – 15th September 2026

Initial Review – 16th September 2026

Accepted – 1st October 2026

Changes in cell volume also modify metabolic behaviour, with experimentally induced swelling and shrinkage producing opposing patterns of substrate handling and gene expression within minutes of a hormonal or nutrient stimulus [5]. Intracellular water (ICW) is thus a variable that is regulated and has functional implications, not just a passive by-product of fluid balance.The clinical observations support the distinction in question. In community-dwelling adults aged 75 years and over, ICW was found to be related to handgrip strength, gait speed, functional status and frailty after having taken into account muscle mass, and a large amount of variation in ICW was seen at the same level of muscle mass [6,7].

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A greater ratio of ICW to lean mass was also linked to greater strength and a lower risk of frailty, independently of lean mass [7], while a higher extracellular (ECW) -to- ICW ratio in the upper legs was negatively correlated with muscle strength and gait speed in older adults [8]. A systematic review of eleven observational studies concluded that imbalances in the distribution of body water are consistently linked with sarcopenia and with worse physical performance [9]. Although these are cross-sectional associations and do not prove causation, they show that the distribution of water carries information which is not provided by measures of total water and muscle mass.The factors which influence ICW have never been looked at together, and standard methods of assessment still regard hydration mainly as a matter of how much fluid the body contains rather than where that fluid is located. There is a practical significance to this distinction: in older people, where ICW decreases and thirst becomes less dependable, and among those who work outdoors or in industry and are subjected to repeated heat stress, a general overall body value gives the least satisfactory picture [6,8,10,11]. The present review was carried out to summarise the physiological factors that determine ICW and cell volume, to investigate how far commonly used measures of hydration and body composition reflect intracellular water, to evaluate whether the evidence supports separating the intracellular hydration state from the intracellular hydration reserve, and to outline research priorities that can be tested.

2. METHODOLOGY

A structured literature search was conducted in PubMed, with every retrieved record cross-checked against its publisher and DOI metadata. The search was restricted to English-language publications indexed with structured abstracts or full bibliographic records. Keywords including “intracellular water”, “cellular hydration”, “cell volume regulation”, “body cell mass”, “organic osmolyte”, “extracellular to intracellular water ratio”, “bioelectrical impedance”, “phase angle”, “potassium homeostasis”, “magnesium”, “muscle glycogen”, “dehydration” and “hydration assessment” were combined using Boolean operators, and the reference lists of retrieved reviews were hand-searched. The search was run using five search lines, which are reported in full in Supplementary Table S1. It returned 2,095 records, or 1,962 once duplicates were removed. A further 16 sources came from the reference lists of retrieved reviews and from targeted searches. In all, 38 sources were selected for inclusion.

These comprised 18 narrative and physiological reviews, 5 human experimental and validation studies, 6 observational studies, 4 randomised controlled trials, 2 European Society for Clinical Nutrition and Metabolism guideline documents, 1 systematic review, 1 meta-analysis and 1 biophysical in vitro study. No date restriction was applied to foundational cell-physiology literature, because the mechanisms concerned were established before 2010; empirical and interventional evidence was prioritised from 2000 onward, and 26 of the 38 included sources were published between 2010 and 2026. Non-peer-reviewed and non-indexed sources were excluded, and animal or in vitro studies were eligible only when equivalent human data were unavailable. Selection was purposive rather than exhaustive; no formal risk-of-bias appraisal or quantitative synthesis was undertaken, so selection bias cannot be excluded. Sources were retained based on relevance, methodological rigour and contribution to understanding the determinants and measurement of intracellular water. Titles were screened first, and records that passed were assessed for eligibility on their title and abstract. A record was eligible if it reported human data, or mammalian physiology applicable to human cells, and if it dealt directly with ICW, cell volume regulation, a determinant of ICW, the measurement of body water compartments, or an exposure or intervention with a compartmental outcome. Case reports, letters and commentaries were not eligible. One record judged out of scope at the abstract stage was retained on review because it explains the magnesium-dependent loss of cellular potassium. Of the 1,962 records screened on title, 1,630 were excluded. Of the 332 assessed on title and abstract, 16 were excluded (outcome not related to intracellular water, 10; mechanism outside scope, 2; not applicable to human physiology, 2; disease-specific with no transferable finding, 2), leaving 316 eligible records. Because the aim was a narrative synthesis, 22 of these were selected purposively as the most direct evidence for each point, and the remaining 294 were not cited. Adding the 16 sources from other methods gave 38 included sources. The selection process is reported as a PRISMA 2020 flow diagram [12] (Figure 1).

3. RESULTS

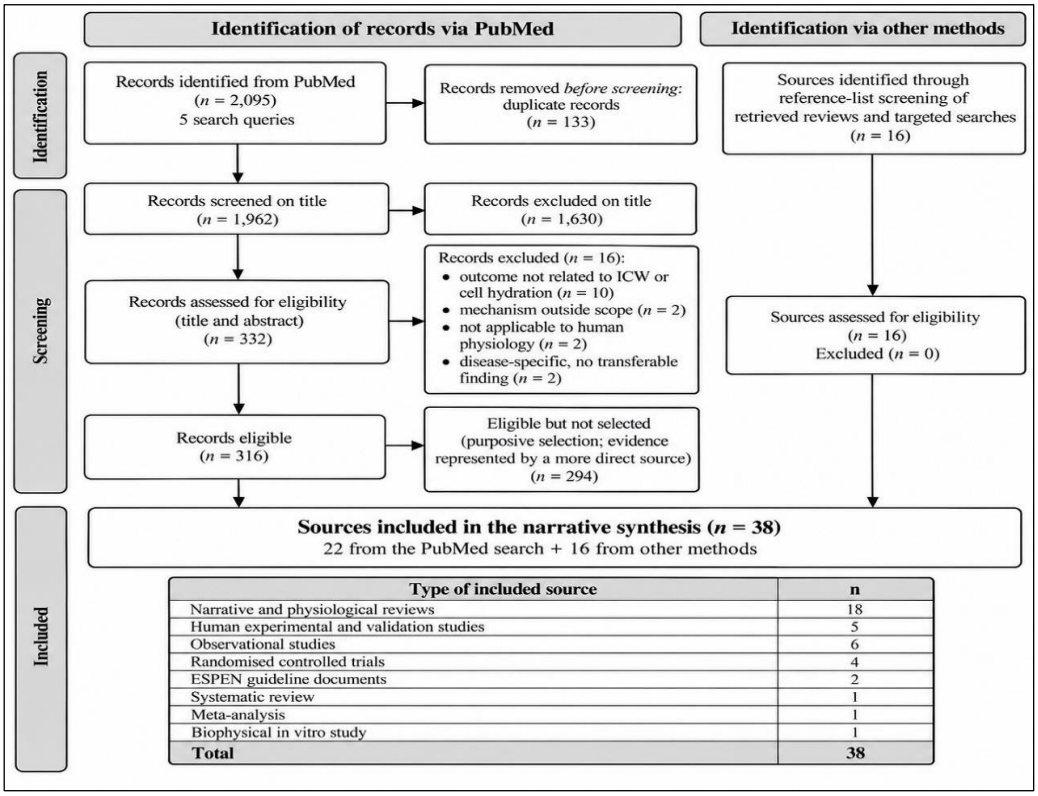
3.1 Operational definitions

Because these terms are used interchangeably in the literature, they are defined here before the evidence is presented and are used consistently throughout the review.

ICW is the quantity of water within the cellular compartment, expressed in litres or relative to lean or muscle mass. It is a measurable quantity, although only indirectly [13,14].

Cell volume is the physical volume of an individual cell or tissue compartment. It changes with osmotic and metabolic conditions and is the variable subject to regulatory volume increase and decrease [1,3].

Cellular hydration denotes the physiological state arising from cell volume and its regulation, including the signalling consequences of volume change [5]. It is a state description rather than a single number.



Type of included source	n
Narrative and physiological reviews	18
Human experimental and validation studies	5
Observational studies	6
Randomised controlled trials	4
ESPEN guideline documents	2
Systematic review	1
Meta-analysis	1
Biophysical in vitro study	1
Total	**38**

Figure 1. Flow diagram of source identification, screening, and inclusion, adapted from the PRISMA 2020 template. ESPEN: European Society for Clinical Nutrition and Metabolism.

Cellular Hydration Reserve (CHR), denotes the capacity of a cellular or tissue compartment to maintain or recover intracellular volume when challenged. It is defined dynamically and is not equivalent to any of the three preceding terms.

3.2 The physiological basis of ICW and cell volume

Water easily passes through most cell membranes, and thus the quantity of water contained within a cell is mainly determined by the balance of osmotically active solutes on both sides of the membrane rather than by the amount of water present. This balance is controlled by a relatively small number of interacting factors, all of which are well explained in the physiological literature [1–5]. These factors are the intracellular effective osmoles, primarily potassium salts; the extracellular osmolality; the membrane's permeability to water; ion transport, in which the sodium–potassium adenosine triphosphatase (Na^+/K^+-ATPase) has a major role; organic osmolytes such as taurine, betaine and myo-inositol; the macromolecular content and intracellular crowding; the metabolic state of the cell; hormonal regulation; and, at the whole-body level, the kidney's regulation of tonicity. Figure 2 shows how these elements are arranged across the cell membrane. which water moves in response to this gradient.

Organic osmolytes enable cells to build up osmotic activity at high concentrations without causing as much disruption to protein function as inorganic ions would. Both the macromolecular content and intracellular crowding affect cell water content and are themselves affected by changes in cell volume; intracellular crowding has also been suggested as one of the signals by which cells sense their own volume. On the other hand, hormonal and renal regulation mainly exert their effects on the extracellular compartment, focusing on maintaining systemic tonicity rather than directly regulating cell volume [1,2,4]. Cell volume regulation is an active and bidirectional process; regulatory volume decrease is mainly due to potassium-chloride cotransport and taurine efflux, while regulatory volume increase is achieved through sodium-hydrogen exchange and sodium-potassium-chloride cotransport [1,4].

Secondly, skeletal muscle does not act as a perfect osmometer since its volume changes by an amount less than that which would be expected from the corresponding change in extracellular osmolality and, after the initial disturbance, cell volume is partly restored by the regulated transport of ions and water [15]. Thirdly, the transmembrane ion gradient is not self-sustaining and depends continuously on ATP hydrolysis by the Na^+/K^+-ATPase, the activity of which in skeletal muscle is influenced by contraction, training status and hormonal signals [16]. ICW thus depends on two closely related factors: the amount of solute available to the cell and its metabolic ability to maintain the intracellular solute gradients. These factors are demonstrated in Figure 2.

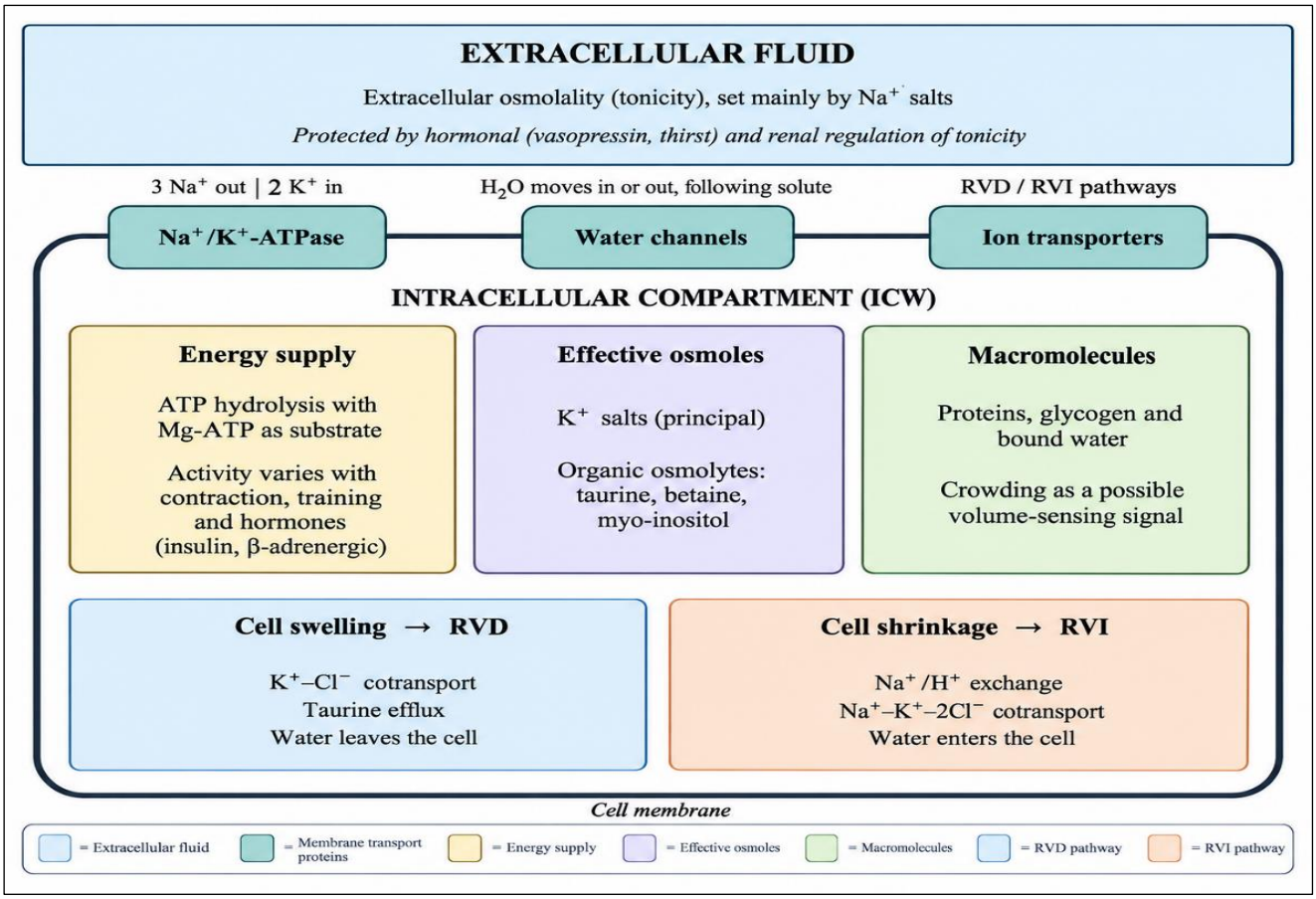


Figure 2. Physiological basis of intracellular water and cell volume. Water crosses the membrane in response to the gradient between intracellular effective osmoles (mainly potassium salts, organic osmolytes and macromolecule-associated solutes) and extracellular osmolality. The Na^+/K^+-ATPase, using Mg-ATP, maintains the gradient. Regulatory volume decrease (RVD) and regulatory volume increase (RVI) restore volume after swelling or shrinkage, while hormonal and renal mechanisms act on extracellular tonicity

3.3 The factors that determine ICW

The three domains in which determinants are considered are derived from the physiology, namely the availability of osmotically active solute, the tissue reservoir in which that solute and its water are stored, and the regulatory demand imposed on the system (Figure 3). These domains are not independent of one another, and the evidence in support of each varies both in type and in strength.

3.3.1 Solute availability

Potassium is the most common intracellular cation, and the way it is distributed across the cell membrane is the main factor in establishing the transmembrane ionic gradient [17,18]. The distribution of potassium inside the cell is controlled by insulin and β-adrenergic signalling, with skeletal muscle acting as the primary buffer; long-term balance is maintained by the kidneys [17,19]. Magnesium has a distinct role in this system: it is an essential cofactor for the hydrolysis of ATP by Na^+/K^+-ATPase and affects pump kinetics as well as the conductance of potassium channels [20,21], and magnesium deficiency causes renal potassium loss by releasing the magnesium-dependent inhibition of the renal outer medullary potassium channels [22]. Its effect on ICW is therefore indirect and occurs through its influence on potassium handling.

3.3.2 Cellular and tissue reservoirs

Skeletal muscle contains the largest amount of ICW [16,19] and some of this water is linked to glycogen [23]. This link is dynamic, as, after 72 hours of carbohydrate loading, the distribution of both ECW and ICW in different muscle segments changed in a manner that corresponded to the muscle glycogen levels as measured by spectroscopy [24]. The size of the reservoir can therefore be altered to some extent, although the relationship between glycogen and the water associated with it is only approximate and not a fixed stoichiometric ratio [23]. The functional relevance of reservoir hydration, as distinct from reservoir size, is supported by the observation that ICW and the ICW-to-lean-mass ratio are associated with strength and physical function after adjustment for muscle mass [6,7], and by the finding that ICW predicts handgrip strength, calf circumference and physical performance in 1,009 community-

dwelling older adults. The authors stated that such indirect estimates should not be interpreted as direct indicators of cellular integrity [25].

3.3.3 Regulatory demand

The third determinant domain involves conditions that involve repeated or continuous osmotic stress. Dehydration induced by exercise in heat reduced knee-extensor muscle volume measured by magnetic resonance imaging (MRI) by 3.9% at 3% and 5.9% at 5% loss of estimated total body water, with no change in non-exercising musculature, and intermittent fluid replacement prevented the decline [26]. Acute mild hypohydration producing 1.9% body-mass loss reduced estimated cell volume by 2.9% and lowered muscle water content, with elevated copeptin, although glycaemic regulation was unaffected [27]. Occupational exposure to heat is linked to adverse kidney outcomes, and heat stress affects renal physiology in a manner that is influenced by exercise, dehydration, acclimation and age [10,28]; whether these exposures produce sustained change in ICW has not been established. The ageing process leads to a decrease in both total and ICW, as well as to a diminished sense of thirst and to altered responses to fluid regulation [6,11]. Short sleep duration has been associated with higher odds of inadequate hydration defined by urine concentration in United States and Chinese adults, with adjusted odds ratios of 1.59 and 1.42 for six versus eight hours [29]; this is a cross-sectional association assessed by a renal measure, and no intracellular endpoint was examined.

3.4 What do the commonly used measures quantify?

For the determinants mentioned above to be studied, they must be measurable; each of the commonly used measures is physiologically significant for the quantity it describes, and none of them is a direct measure of intracellular hydration (Table 1).

Plasma osmolality measures extracellular tonicity rather than ICW itself; it is therefore physiologically relevant to ICW but cannot serve as a direct measure of intracellular hydration [2]. Urine concentration describes the renal response to tonicity and is further removed. Dilution techniques using deuterium oxide and bromide quantify total body water and ECW, with ICW obtained by subtraction, and these remain the reference procedures; multifrequency bioelectrical impedance spectroscopy correlates closely with them at the group level, but individual agreement is limited, with reported standard deviations for ICW differences of approximately 2 to 3 litres in healthy participants [14]. Meta-analysis indicates that multifrequency approaches do not significantly overestimate total body water against reference methods, which supports their use for group-level estimation rather than individual interpretation [30]. European guidance states explicitly that determining changes in body cell mass, ECW, and ICW requires further methodological work using a model that prevents extracellular change from corrupting the intracellular estimate, and that application in altered hydration states requires further validation [13,31]. Phase angle is associated with fluid distribution and cellular characteristics and carries prognostic information across clinical and athletic populations, but it is a bioelectrical tissue property rather than a measure of ICW [32–34]. Thus, a standardised, validated, clinically interpretable measure of intracellular hydration and reserve is still lacking.

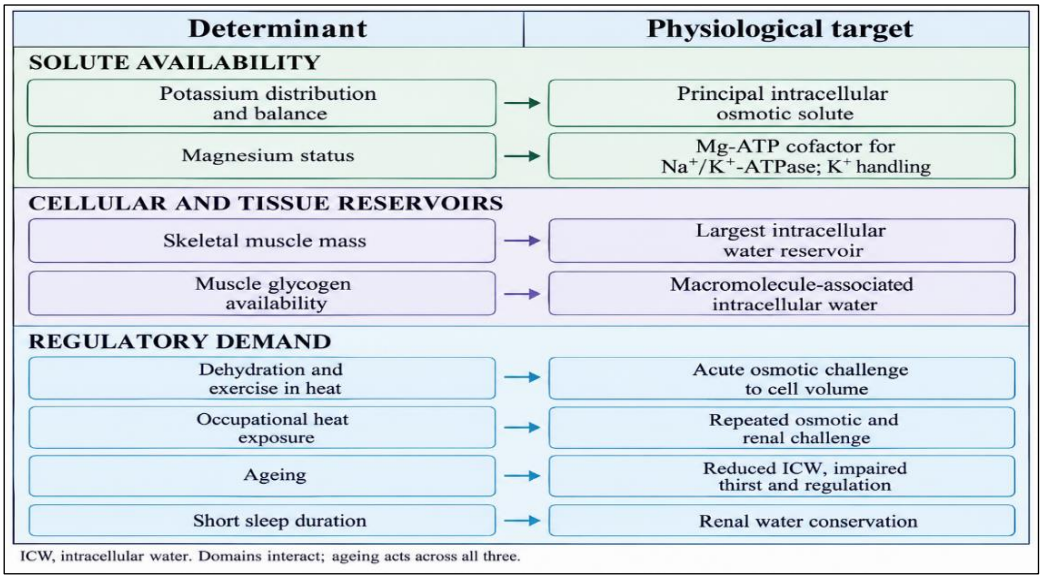


Figure 3. Determinants of intracellular water by physiological domain. Determinants are grouped by the mechanism through which they act: solute availability, cellular and tissue reservoirs, or regulatory demand. Ageing acts across all three domains.
Table

1. Physiological meaning and limitations of commonly used measures relevant to ICW.

What is measured	What it indicates	Principal limitation as an index of ICW
Body weight, total body water	Total body water content	Aggregates compartments; insensitive to redistribution [2]
Plasma osmolality	Extracellular tonicity	Physiologically relevant to ICW but not a direct measure of it [2]
Urine osmolality or specific gravity	Renal concentrating response	Indicates renal handling, not cellular water [2]
Extracellular-to-ICW ratio	Compartment distribution at rest	Equation-, population- and device-dependent; no validated cut-off points [8,13]
Phase angle	Bioelectrical tissue properties	Associated with cell mass and distribution; not ICW-specific [32–34]
Body cell mass	Metabolically active cellular mass	Quantity of cellular tissue, not its water status or resilience [35]
Dilution (H_2O, bromide)	Total body and extracellular water; ICW by subtraction	Reference method, but limited individual agreement with impedance [14,30]
Proposed CHR	Capacity to maintain or recover intracellular volume	Proposed, not validated

3.5 From the intracellular hydration state to the CHR

The measures in Table 1 describe what a compartment contains when it is measured.

3.5.1 CHR as a latent construct

CHR is suggested to be considered as a latent construct. It cannot be observed directly and must be inferred from a set of indicators, just as frailty is inferred from measurable components such as weight loss, weakness, a slow gait, and low levels of physical activity [36]. This is in accordance with the general principle of construct validation, whereby a construct is defined by the pattern of observations it is expected to predict rather than by any one measurement [37]. Since no single measurement and no arithmetic combination of the measures listed in Table 1 can be regarded as a measurement of CHR, because each of these measures refers to the resting state and several of them are dependent on the method and the device used [13,14], CHR is not presented here as a composite index but instead as an operational framework meant to assist in the design of future studies.

3.5.2 Static state versus dynamic reserve

The distinction between resting state and reserve is the main conceptual contribution of this review. All measures in Table 1 are static: they describe a compartment at a single point in time [2,13]. Reserve, by contrast, is a property expressed only under load. Skeletal muscle is known for its ability to actively keep its volume when it comes to osmotic or contractile stress [15,16]. Many human studies have already shown this phenomenon. In the study of exercise-induced dehydration, muscle volume fell progressively with water loss in exercising muscle but not in resting muscle, and the decline was abolished by fluid replacement [26]; carbohydrate loading redistributed segmental water in parallel with glycogen accretion [24]; and skeletal muscle shows partial volume recovery after an initial osmotic perturbation rather than behaving as a passive osmometer [15].

3.5.3 Novelty relative to existing constructs

Body cell mass quantifies the metabolically active, potassium-rich cellular component of the body and is classically estimated from total body potassium [35]; muscle mass quantifies tissue quantity; the extracellular-to-ICW ratio and the ICW-to-lean-mass ratio describe resting distribution [7,8]. CHR overlaps substantially with all of these. The principal empirical motivation for treating reserve separately is the observation of considerable variability in ICW at equivalent muscle mass [6], together with associations between ICW indices and function that persist after adjustment for lean or muscle mass [6,7,25]. It is therefore an empirical question whether reserve contains any information beyond these established measures, and the framework to be described below has been constructed to test this.

3.6 A proposed measurement framework for future studies

The framework organises measurement into three levels rather than a single score (Table 2, Figure 4). Each level answers a different question, and only the last one addresses reserve directly.

3.6.1 Level 1: state

The first level is state: compartment volumes obtained by dilution or, where validated, by multifrequency bioelectrical impedance, expressed relative to lean or muscle mass [13,14]. Indexing matters here, because ICW varies considerably between individuals of similar muscle mass [6], and an unindexed value cannot show whether a given compartment is well or poorly hydrated for its size.

3.6.2 Level 2: determinants

The second level is determinants: indicators of solute availability, reservoir size and regulatory demand, including potassium status, muscle mass and glycogen availability [17,23]. These correspond to the three domains shown in Figure 3. They help to explain why two people with the same resting ICW might respond quite differently to the same challenge, but they do not measure that response.

3.6.3 Level 3: challenge response

The third level, and the only one specific to reserve, is challenge response: change in ICW or muscle volume during a standardised osmotic, thermal or exercise challenge, and the rate and completeness of recovery afterwards [15,24,26,27]. Only this level tests reserve. The first two characterise the conditions under which reserve is expressed and are necessary for interpreting the third, not substitutes for it.

A standardised challenge would require a defined and reproducible stimulus, a pre-specified intracellular endpoint, and measurement at both perturbation and recovery. The existing studies indicate what is feasible: fluid restriction after a controlled heat exposure [27], graded loss of estimated total body water during exercise in heat with imaging of both exercising and non-exercising muscle [26], and carbohydrate manipulation with spectroscopic measurement of glycogen and segmental water [24]. None was designed to quantify reserve, and the endpoints, stimuli, and measurement intervals differ between them, which is precisely the gap that standardisation would close.

A three-level measurement framework for CHR measurement is shown in Figure 4 and Table 2 shows the recommendations for future studies.

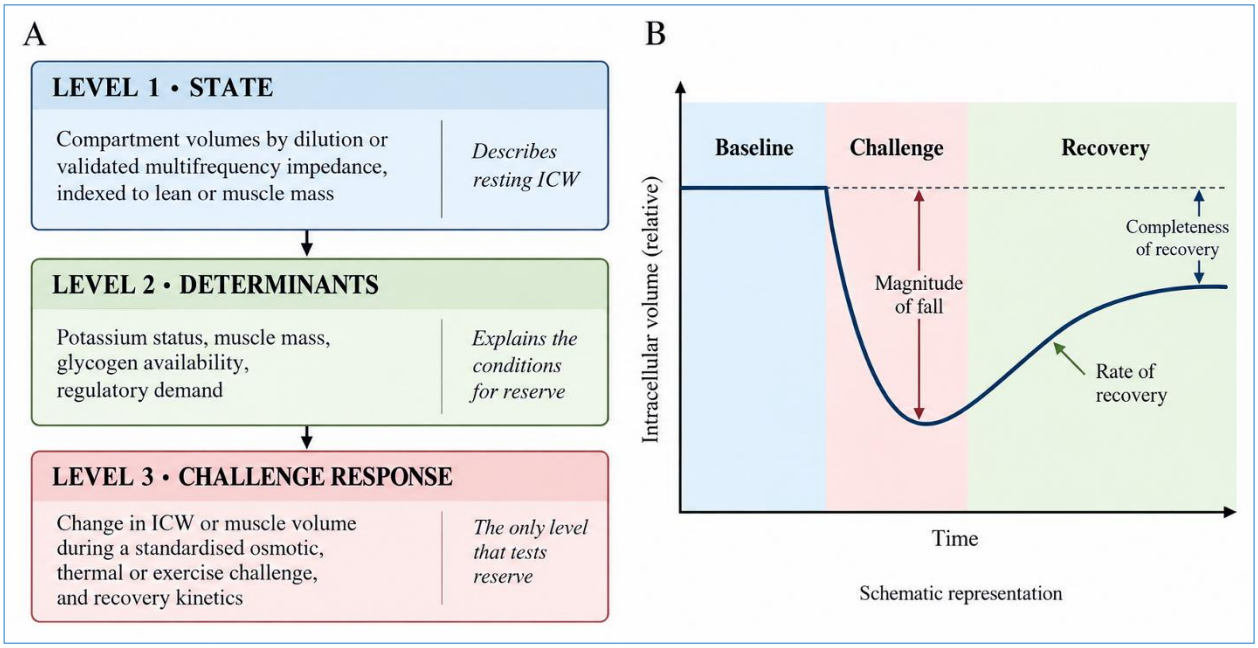


Figure 4. Proposed three-level measurement framework for the CHR. (A) State and determinant measures describe the resting compartment and the conditions that shape it; only the challenge–response level tests reserve. (B) Schematic time course of intracellular volume during a standardised challenge, showing the three quantities a challenge–response study would record: the baseline, the magnitude of the fall, and the rate and completeness of recovery. Panel B is illustrative and is not derived from data.

Table 2. Proposed three-level measurement framework for future studies.

Level	Measurement	Purpose	Status
1. State	Compartment volumes by dilution or validated multifrequency impedance, indexed to lean or muscle mass	Characterises resting ICW	Available; requires standardisation [13,14]
2. Determinants	Potassium status, muscle mass, glycogen availability, regulatory demand	Characterises conditions under which reserve is expressed	Available; indirect [17,23]
3. Challenge response	Change in ICW or muscle volume during a standardised osmotic, thermal or exercise challenge, and recovery kinetics	The only level that tests reserve	Demonstrated in small studies; not standardised [24,26,27]

3.7 Interventions and the directness of the available evidence

Evidence is graded by directness: D1 shows ICW, muscle water or cell volume measured in humans; D2: whole-body water or a physiological surrogate measured in humans with intracellular effects inferred; and D3: mechanistic or compositional data without a human hydration outcome (Table 3). Resistance training is the only intervention shown to raise ICW relative to muscle mass over a sustained period. Over 16 weeks in 30 men and 34 women, total body water increased by approximately 7.5% and ICW by 8.2% in men and 11.0% in women, against smaller increases in skeletal muscle mass of 4.2% and 3.9% [38]; ICW therefore rose proportionally more than muscle mass, although the estimates were impedance-derived in a single uncontrolled cohort (D1, low to moderate certainty). Carbohydrate availability alters ICW through glycogen-associated water, demonstrated with spectroscopic measurement, but the effect is transient (D1, moderate) [24].

Two direct studies of creatine returned negative findings and are reported as such. Creatine supplementation increased total body water without altering the distribution between compartments [39], and in resistance-trained men it did not change the ratio of ICW to skeletal muscle mass (both D1, moderate) [40]. The frequent description of creatine as cell-volumizing is therefore not supported by the studies that measured the relevant variable.

Beverage composition has been characterised at the whole-body level only. In a randomised trial of thirteen beverages, oral rehydration solution and both full-fat and skimmed milk produced smaller four-hour urine output than water, with beverage hydration indices at two hours of approximately 1.5; orange juice, sports drink, cola, tea, coffee, beer and sparkling water did not differ from water(D2, moderate) [41]. Fluid replacement during exercise in heat prevented loss of exercising-muscle volume, which is a direct intracellular observation within a dehydration model rather than a general rehydration recommendation [26]. The sleep association is D2 and cross-sectional [29]. Dietary potassium and magnesium adequacy rests on established physiology [17,19,20,22] without human trials using intracellular endpoints (D3).

Interventions with the most direct evidence have the smallest supporting studies, and the direct evidence that does exist for a widely promoted supplement is negative. This pattern, rather than any single result, is the practical conclusion of this section

Table 3. Interventions graded by directness of evidence for ICW.

Intervention	Directness	Certainty	What was measured	Direction of finding
Resistance training	D1	Low–moderate	ICW and skeletal muscle mass over 16 weeks [38]	ICW increased proportionally more than muscle mass
Carbohydrate loading	D1	Moderate	Segmental ECW and ICW with muscle glycogen [24]	ICW changed with glycogen; transient
Fluid replacement during exercise in heat	D1	Moderate	Muscle volume by MRI [26]	Prevented loss of exercising-muscle volume
Creatine supplementation	D1	Moderate	Total body water and compartment distribution [39]; ICW-to-muscle-mass ratio [40]	Negative for redistribution in both studies
Electrolyte- and nutrient-containing beverages	D2	Moderate	Urine output and beverage hydration index [41]	Oral rehydration solution and milk only
Adequate sleep duration	D2	Low	Urine concentration, cross-sectional [29]	Association only
Dietary potassium and magnesium adequacy	D3	—	Established transport physiology [17,19,20,22]	Mechanistic; no intracellular endpoint tested

D1: intracellular water, muscle water or cell volume measured in humans; D2: whole-body or surrogate human measure with intracellular effect inferred; D3: mechanistic or compositional data only.

4. DISCUSSION

4.1 Principal findings and practical relevance

The amount of water that a cell contains is determined both by the solutes it can hold and by the energy it expends in holding them, which means that fluid intake is necessary for keeping cells hydrated but is not in itself sufficient to achieve this [1,16,17]. Most of the water is stored in skeletal muscle, and the observational evidence indicates that the degree to which this tissue is hydrated contains information regarding strength and function that cannot be obtained from muscle mass alone [6,7,25]. On the other hand, the methods currently in use still only measure either the entire body or the extracellular portion, and even the more advanced techniques agree only poorly when applied to individuals [13,14]. The limited number of interventions that have been tested against an intracellular

endpoint yield conflicting results: resistance training and fluid replacement during exercise in hot conditions seem to be of help, while creatine, which is often advocated for this purpose, did not affect water distribution [26,38-40].

In hot climates, people who work outdoors are subjected to repeated heat and fluid stress [10,28], and older individuals have both a lower amount of ICW and a diminished thirst response [11]. For both groups, recommendations focusing solely on the quantity of fluid taken will not be enough. The evidence examined in this review supports a more comprehensive and mainly non-pharmacological strategy centred on maintaining muscle mass through resistance exercise, ensuring adequate intake of dietary potassium and magnesium, getting sufficient sleep, and making appropriate use of fluids and electrolytes when exposed to heat. It must be remembered, though, that only some of these measures have been tested against an intracellular endpoint (Table 3).

4.2 Limitations

Most of the evidence connecting ICW was based on cross-sectional studies; reverse causation and confounding factors such as inflammation, oedema or adiposity cannot be excluded; a low ICW index might result from reduced physical activity or from subclinical fluid overload rather than being the cause of weakness. The compartment estimates vary according to the method used, the population studied and the device employed, and there is still only limited agreement at the individual level between impedance and dilution measurements, which means that every intracellular estimate mentioned here has to be interpreted with caution [13,14,30]. Several determinants are supported by mechanistic rather than human intracellular evidence and are labelled accordingly. Part of the observational evidence derives from overlapping research groups, which limits independence [6,7,9,25]. The direct interventional evidence base is small: of the five studies that measured an intracellular endpoint, two returned negative findings [39,40]. CHR is a proposed latent construct that has not been operationalised, derived, or validated in any dataset, and no clinical application is implied or recommended.

4.3 Priorities for future research

For future studies, the standardisation of compartment measurement is required, involving device- and population-specific validation using dilution methods and the published reference ranges according to age and sex [13,14]. More challenge-response studies involving a defined osmotic, thermal, or exercise load should be applied, and the change in ICW and its recovery should be measured to assess reserve [26,27]. Incremental-validity analyses should be carried out to determine whether challenge-response measures can predict strength, physical performance, and clinical outcomes beyond body cell mass, muscle mass, and resting ICW [6,35]. More randomised controlled trials on modifiable determinants, potassium adequacy, resistance training and glycogen availability, using intracellular endpoints instead of whole-body surrogates should be carried out to establish the findings of the present study [24,38].

CONCLUSION

Fluid intake alone does not determine cellular hydration, which depends on the distribution of solutes across the cell membrane and the metabolic work that sustains it, and no routine measure yet quantifies ICW directly. CHR is therefore proposed as a provisional latent construct, not a clinical index, to be tested through standardised challenge-response measurement and incremental validation against body cell mass, muscle mass and resting ICW.

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How to cite this article: Shobhitha TB, Yadhushree PV, Poojary Y, Sujatha KJ, Bindu GV, Shetty P. Intracellular Water and Cellular Hydration: Physiological Determinants, Measurement Limitations, and a Proposed Cellular Hydration Reserve Framework. Indian J Integr Med. 2026; Online First.

Funding: None; *Conflicts of Interest: None Stated*