The Hydration Sweet Spot: Importance of Aquaporins

TAMARA HEW-BUTLER, KEVIN WEISZ

LEARNING OBJECTIVES

1. Recognize the regulated variables of water balance (osmolality and volume)
2. Describe the short-term regulation of water balance
3. Explain long-term adaptations to chronic dehydration and overhydration
4. Recognize the limitations and advantages for using body weight and urine color to determine hydration status

ABSTRACT

In both hyponatremia and hypernatremia, the sodium value (numerator) appears the most deranged. However, it is the changes in water content (denominator) that is most responsible for the pathophysiology, morbidity and mortality associated with dysnatremia. Therefore, fluid homeostasis involves both short-term and long-term water regulation at the molecular level to accommodate simultaneous and continuous changes in both fluid intake and output. Behavioral manipulation of long-term renal adaptations appear counter-intuitive in healthy individuals (i.e. water-loading induces dehydration – not water retention - in athletes seeking to "weigh-in light"), but do offer protection against behavioral (polydipsia) and environmental (dehydration) extremes. This mini-review summarizes both the short-term and long-term renal response to water intake, from the molecular perspective of the aquaporin-2 water channel. Then, four frequently asked questions will be addressed from this perspective: 1) What are the clinical consequences of drinking too much and 2) What are the clinical consequences of drinking too little? 3) How do we measure hydration? And 4) How much water do we need? We will close with practical advice on how humans should best maintain normal blood sodium concentrations (normonatremia, with preferential cellular size) from the perspective of thirst, vasopressin and small - but powerful - armies of aquaporin channels.

ABBREVIATIONS: [Na^+] – sodium concentration, ADH - anti-diuretic hormone, AVP – arginine vasopressin, AQP – aquaporin, AQP2 – aquaporin-2 water channel, V2R – vasopressin 2 receptors, SIADH - syndrome of anti-diuretic hormone secretion, UOsm - urine osmolality, USG – urine specific gravity,

INDEX TERMS: Fluid recommendations, thirst, aquaporin water channels, fluid balance, hydration guidelines

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Short-term Regulation of Water Intake

Dehydration: Short-term regulation of water lack, with or without water loss, includes osmotic and volemic stimulation of the anti-diuretic hormone, arginine vasopressin (AVP). In brief, AVP stimulates vasoconstriction by binding to vasopressin 1 receptors (V1R) within the smooth muscle of the vasculature.[1] Simultaneously, AVP stimulates anti-diuresis (reabsorption of water into the bloodstream) by binding to vasopressin 2 receptors (V2R) located in the kidney collecting duct.[2] Stimulation of the V2R triggers a complex intracellular molecular cascade that results in the insertion of aquaporin (AQP) water channels into the lumen of the

collecting duct, thereby increasing collecting duct water permeability and facilitating water movement from the kidney back into the circulation. This is why AVP is also called "anti-diuretic hormone/ADH," since its main effect is at the kidney, serving to retain body water. Essentially, aquaporin channels act like little straws (with a diameter equaling the size of a single water molecule) that "poke" through cell membranes and provide transient "escape routes" for water to flow.

The main aquaporin channel that is activated by AVP, through stimulation of the V2R, is the aquaporin-2 water channel (AQP2).[2,3] Rat models demonstrate that AVP can increase collecting duct permeability within 40 seconds of activation of the V2R.[4] Furthermore, water permeability of the collecting duct is increased by 50% (of maximal) within 9 minutes,[4] which is close to the 10-20 minute half-life of circulating AVP.[2] Quantification studies suggest there are ~12 million AQP2 channels per collecting duct cell,[3] which underscores the molecular strength of the anti-diuretic response. Thus, when the concentration of solutes (namely sodium) in the blood increases, AVP is osmotically (and linearly) stimulated, serving to limit urinary water loss and promote fluid retention within seconds.

If anti-diuresis is maximal and plasma osmolality/sodium concentration ($[Na^+]$) continues to rise (or, if circulating plasma volume decreases beyond ~8-10%) the sensation of thirst will be centrally stimulated to bring water back into the body.[2,5,6] The intake of fluids (in response to physiologically-regulated behavioral thirst) combined with AVP-induced maximal anti-diuresis collectively serves to dilute plasma osmolality/$[Na^+]$ back into the normal physiologic range while assisting in the restoration of circulating blood volume. The reproducibility and linear strength of osmotically-driven thirst and the psychobiology of thirst awareness has been elegantly reviewed elsewhere.[5,6]

Overhydration: Short-term regulation of water excess, with or without abnormal fluid retention, includes osmotic and volemic suppression of both AVP and thirst. Fluid that is administered or ingested beyond need will dilute body solutes (namely, sodium) within the bloodstream, which in turn will cause all of the cells within the body to swell.[2,6] This dilution of blood solutes will trigger the loss of excess body water via suppression the AVP secretion from the posterior pituitary gland.[2] In the absence of circulating AVP, the AQP2 water channels will remain inside the cytoplasm of kidney collecting duct cells, thereby making the lumen of the collecting duct impermeable to water. With no "escape route" channels, all of the water previously filtered by the kidney is then promptly excreted as urinary water. Thus, urinary electrolyte-free water losses serve to "concentrate" plasma solutes/$[Na^+]$ back towards the normal physiological range via suppression of AVP and sequestration of AQP2 water channels within the confines of the cell.

When excess fluid enters the body, physiologically regulated thirst will also be suppressed. Unregulated drinking (i.e. fluids consumed above the dictates of thirst) often occurs in response to: increased palatability (sugar-sweetened beverages),[7] social factors (drinking with friends, routine morning coffee)[2] or psychological compulsion to drink more fluids (psychogenic polydipsia or belief that fluid is advantageous to health).[8-12] It is important to note that the onset of thirst (set-point) and/or the slope of the linear relationship between plasma osmolality/$[Na^+]$ vs. thirst appreciation may be altered in compulsive water drinkers[13] or in patients with the syndrome of inappropriate anti-diuretic hormone secretion (SIADH),[14] but is rarely abolished outside of organic brain disease.[15,16]

Long-term Adaptations to Water Intake

Dehydration: It is questioned whether or not man can adapt to chronic water deprivation like other desert creatures, such as the kangaroo rat who can conserve water by excreting a solid urine.[17] However, advances in molecular technology suggest that humans have the ability to transiently adapt to 1-3 days of water deprivation.[3,18] Molecular adaptation to chronic (>24 hours) water deprivation occurs via upregulation of AQP2 water channel expression in kidney collecting duct cells, thereby maximizing anti-diuresis and urine concentration above baseline (ad libitum drinking) levels.[3,18] Rodent models demonstrate a five-fold increase in both AQP2

expression and water permeability within the kidney collecting duct following 24-hours of water restriction.[3,7] This increase in AQP2 expression appears to be directly mediated by vasopressin, since vasopressin infusion has been shown to increase AQP2 mRNA.[3]

Human studies corroborate these animal findings, with healthy men demonstrating higher urine osmolalities, greater tubular reabsorption of free water, and decreased urine volume following 3-days without liquid food or water.[18] This augmentation of urine concentration (with decreased urine flow) was compared with baseline "ad libitum" fluid intakes and systematically evaluated after a vasopressin (Pitressin™) challenge.[18] Furthermore, no changes in either glomerular filtration rate or urea excretion accompanied this 25-50% enhancement of urinary water conservation above baseline levels.[18] Thus, the long term (>24 hours) adaptation to fluid deprivation appears to be an enhanced capacity for renal water reabsorption via AVP-mediated upregulation of AQP2 (and AQP3) water channels within collecting duct epithelium.

The practical translation of this long-term "conditioning" effect[3] suggests that prior dehydration would enhance water retention. Such advice would be contrary to the popular belief that athletes should drink large amounts of fluids before exercise to prevent dehydration.[19,20] From a regulatory perspective, the upregulation of AQP2 channels secondary to chronic water deprivation is compatible with evolutionary pressures to conserve water during unexpected periods of drought. Thus, to maximize fluid retention during competitive exercise, athletes should consider drinking less - not more - in the days leading up to the race. Figure 1 summarizes the water conservation mechanism at the collecting duct.

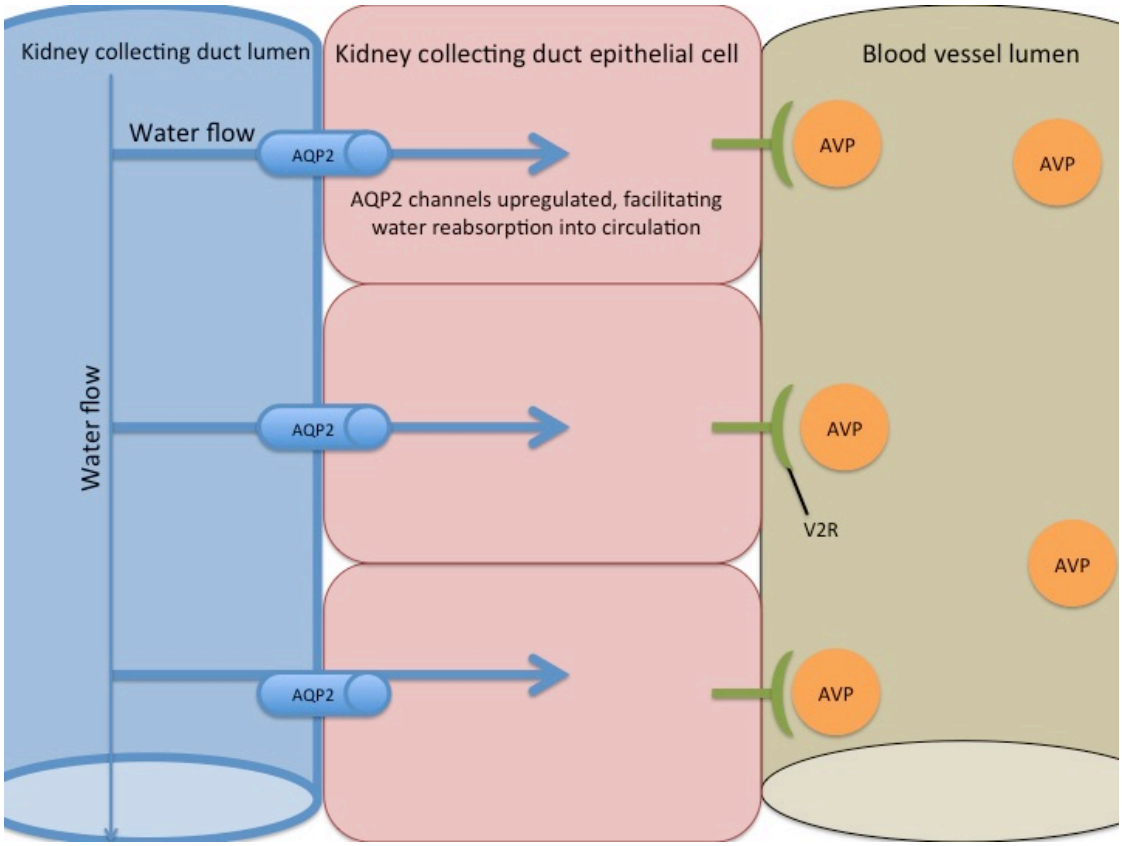

Figure 1. Schematic diagram of the kidney collecting duct during water deprivation. Sustained (> 24-hours) elevated levels of AVP bind to V2R, stimulating upregulation of AQP2 to increase water permeability of collecting duct epithelial cells and promoting water conservation. See text for details.

Overhydration: Rats water-loaded for 24-hours (given free access to sweetened water, which stimulates drinking above thirst) demonstrate suppressed osmotic water permeability at the kidney collecting duct.[7] Water-loaded rats also demonstrate a significant reduction in intra-membrane particle clusters (presumptive AQP2 channels) at the kidney collecting duct, which supports AQP2 downregulation from chronic AVP suppression.[3,7] Therefore, sustained water intake that is in excess of need will dilute plasma osmolality/[Na^+] which in turn will: 1) suppress AVP; 2) trigger sustained urinary free water excretion; 3) downregulate AQP2 water channels; and 4) ultimately result in decreased permeability of the kidney collecting duct to subsequent stimulation by AVP (i.e. animals cannot maximally concentrate urine with increased kidney water losses). It is of interest to note that Battleboro rats lack the ability to produce vasopressin and therefore in constant diuresis (aka diabetes insipidus) and can upregulate AQP2 water channels in collecting duct epithelium when exposed to chronic vasopressin infusion.[21,22] However, it takes three days to restore maximal urinary concentrating ability and five days before maximal expression of AQP2 channels are observed in these previously vasopressin-deficient rats.[22] More practically speaking, 3-5 days may be necessary to fully reverse this otherwise "dehydrating" adaptive renal response to chronic AVP suppression.

Human studies support these animal findings. In one study, eight healthy young males drank 5-6 L of water per day for three days[18] while in another study, two males drank ~10 L of water per day for 11 days.[23] In both trials, the ability to concentrate urine, in response to a vasopressin challenge, at the end of each study trial was "grossly impaired."[18,23] These findings would support downregulation of AQP2 water channels, in response to chronic AVP suppression via sustained water intake, as a protective mechanism against the development of water intoxication. This "physiologic nephrogenic diabetes insipidus" appears characteristic of compulsive water drinkers, as hypothesized elsewhere.[24]

Interestingly enough, the practical translation of this long-term "conditioning"[3] effect is currently being practiced by athletes looking to "weigh-in light" or "cut weight" before competition.[25,26] This "water loading" regimen, commonly used by body builders and mixed martial artists, entails drinking two gallons of water per day for 5-14 days before competition and then limiting water intake 24-hours prior to weigh-in to maximize "weight" loss through dehydration. Enhanced water weight loss has also been documented in compulsive water drinkers whereas during a 24-hour water restriction protocol, normal individuals will lose 1-2% body weight while compulsive water drinkers will lose ~5% of body weight from this mechanism previously described as a "washing out" of the renal medulla.[11] Thus, while the efficacy of this regime remains largely undocumented outside of lay reports, the safety and long term consequences of this adaptive technique does warrant further investigation. Figure 2 summarizes the electrolyte-free water loss mechanism at the collecting duct.

What are the Clinical Consequences of Drinking too Much?

The perils of water intoxication (and resulting hyponatremia) from drinking fluids either in excess of need or beyond the ability to excrete any excess fluid have been previously described in the current Focus section paper on "Hyponatrema." In addition to the potentially fatal consequences of dilutional hyponatremia, people who drink high volumes of water due to central diabetes insipidus,[27] nephrogenic diabetes insipidus,[28,29] psychogenic polydipsia,[30] or social polydipsia[8] may develop dilation of the urinary tract and non-obstructive hydronephrosis with or without renal damage. In most cases, the drinking-induced urinary tract dilation and hydronephosis were reversed with a return to more moderate fluid intakes.[8,27,30,31] Of particular note, one 53-year-old female developed bilateral hydronephrosis from drinking 4.5-5.5 L of fluid daily for three years thinking that this practice was "healthy."[8] After being advised to limit her intake to less than two liters per day, complete reversal of her hydronephrosis and hydrouretor was noted within six weeks.[8]

Another potential deleterious consequence of excessive fluid intake is proteinuria in association with polyuria.[12] A reduction (0.41 vs. 0.16 g/24

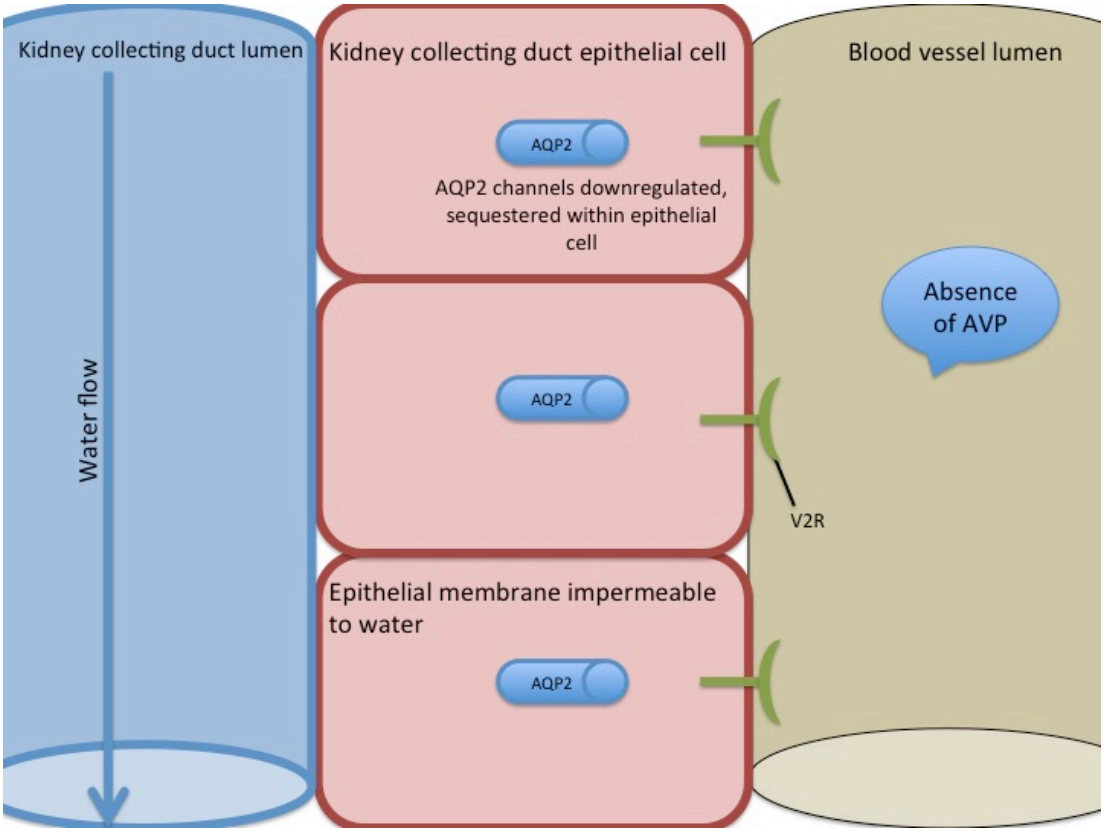

Figure 2. Schematic diagram of the kidney collecting duct during water loading. Chronic (> 24-hours) of AVP (and lack of binding to V2R), causes downregulation of AQP2 to decrease water permeability of collecting duct epithelial cells, and results in electrolyte-free water losses. See text for details.

hours) in proteinuria was documented in a cohort of 56 individuals after reducing fluid ingestion from ~4 L/day to ~2 L/day for one week.[12] These individuals originally drank large quantities of fluid (~4 L/day) as a "healthy lifestyle choice." Whether or not proteinuria from water loading is harmful to the kidney remain unclear.[12,32]

What are the Clinical Consequences of Drinking too Little?

The perils of dehydration (and resulting hypernatremia) from under-replacement of fluids or fluid deprivation (thirsting) have been previously described in the current Focus section paper on "Hypernatremia." In addition to the potentially fatal effects of hypernatremia, dehydration has been implicated in the development of heatstroke, muscle cramps, and performance decrements in athletes.[33] In hospitalized patients, dehydration (defined by urine specific gravity >1.020, osmolality >600 mOsmol/kg H_2O; and dark color) predicted 30-day mortality in elderly patients admitted into the hospital for acute care[34] and increased post-operative complications following hip fracture surgery by four-fold.[35] Voluntary dehydration (urine osmolality >800 mOsmol/kg H_2O) in schoolchildren demonstrated a trend towards decreased cognitive function when compared to children with lower urine osmolalities.[36] With regards to disease prevention, drinking >2 L of water per day may benefit patients with chronic kidney disease or kidney stones.[37] However, most carefully conducted studies demonstrate little to no health benefit from drinking eight glasses of water per day.[38]

How do we Measure Hydration?

All mammals regulate extracellular fluid around a set-point of 300 mOsmol/kg H_2O,[6] which represents a blood sodium concentration ($[Na^+]$) of ~140 mmol/L. This set-point is fiercely protected by the body[2,5,6] to protect cellular size and function. However, because drawing blood is invasive and

measurement of plasma osmolality/blood [Na^+] requires expensive clinical equipment, alternative indicators of "hydration status" are commonly utilized. The easiest and cheapest surrogate markers to assess hydration status are body weight and urine (color and density). The pros and cons of each are summarized below:

Body weight: The use of body weight changes (from baseline) to estimate water loss as a guide for fluid replacement (fluid lost = fluid replaced) is a widely accepted clinical strategy to assess hydration status both at rest[39,40] and during exercise.[33,41] This strategy is beneficial because it is more individualized[33,41] when compared to previous "blanket" (600-1200 ml/hour) guidelines.[42] However, during exercise, the use of body weight as a marker of hydration status and guide for fluid replacement is confounded by exercise-induced substrate utilization, metabolic water production, and variable electrolyte composition of sweat.[43] Furthermore, because body weight is not a physiologically regulated variable, runners greatly underestimate sweat water losses[44,45] and cannot subjectively gauge how much fluid they need to ingest to prevent dehydration during exercise.[44] Thus, it appears that the use of body weight as a marker of hydration status may be more useful under steady-state (non-exercise, resting) conditions and less useful as a guide for monitoring plasma osmolality or volume during exercise.[46]

Urine: Urine color, specific gravity (USG), and osmolality (UOsm) are routinely used to assess hydration status, with a variety of cut-off values used to define "dehydration."[33,34,36,47] Studies supporting the use of "spot" urine samples as valid markers of hydration status are those which document significant correlations between urine vs. urine parameters such as: urine color vs. USG,[47] urine color vs. UOsm,[48] and USG vs. UOsm.[49] Studies which refute urine as an accurate index of hydration status contend that urine is confounded by fluid intake, diet, metabolism, exercise, and non-osmotic stimuli to AVP secretion.[34,41] Studies evaluating urine variables vs. plasma osmolality fail to document significant linear relationships in elderly patients admitted to the VA hospital[50] or in physically fit males exercising in the heat.[49] No significant correlations were found between urine indices vs. body weight losses in children treated in the hospital for gastroenteritis.[51] Thus, although assessment of urine color, osmolality and specific gravity are cheap, easy, and widely utilized, it is important to emphasize that urine represents the renal *response* to fluid homeostasis (i.e. preservation of plasma osmolality and volume) and not necessarily a *reflection* of real-time hydration status with regards to osmoregulation or volume status.

How Much Water do we Need?

Because water is essential to life, evolutionary pressures to maintain water balance and preserve osmolality are encoded within the DNA of all vertebrates (and some invertebrate) species.[52] The amount of water that each individual needs at any given time, in any situation, is centrally dictated in real-time by linear alterations in the physiological sensation of thirst in response to often subtle changes in plasma osmolality.[5,6] The more water that the body needs, the thirstier animals become (and vice versa).[5] The dire consequences associated with both underdrinking and overdrinking have been sensationalized and are rare. However, misguided drinking (not in response to thirst) can have fatal consequences if unexpected variables (heat, stress, infection, drugs, getting lost in the desert or at sea) exceed maximum compensatory capabilities associated with habitual tendencies (often from well-intentioned advice). Therefore, hydration advice should be simple: drink to thirst. Any overshoot or under-replacement of fluid will be continuously corrected, without conscious thought, by millions of renal aquaporin channels shuttling in and out of luminal kidney collecting duct cells, as orchestrated by vasopressin. So, why not trust in our physiology?

REFERENCES

1. Holmes CL, Landry DW, Granton JT. Science review: Vasopressin and the cardiovascular system part 1--receptor physiology. Crit Care 2003;7:427-34.
2. Verbalis JG. Disorders of body water homeostasis. Best Pract Res Clin Endocrinol Metab 2003;17:471-503.
3. Knepper MA. Molecular physiology of urinary concentrating mechanism: regulation of aquaporin water channels by vasopressin. Am J Physiol 1997;272:F3-12.
4. Wall SM, Han JS, Chou CL, Knepper MA. Kinetics of urea and water permeability activation by vasopressin in rat terminal IMCD. Am J Physiol 1992;262:F989-98.
5. McKenna K, Thompson C. Osmoregulation in clinical disorders of thirst appreciation. Clin Endocrinol (Oxf) 1998;49:139-52.

6. Bourque CW. Central mechanisms of osmosensation and systemic osmoregulation. Nat Rev Neurosci 2008;9:519-31.
7. Lankford SP, Chou CL, Terada Y, Wall SM, et al. Regulation of collecting duct water permeability independent of cAMP-mediated AVP response. Am J Physiol 1991;261:F554-66.
8. Maroz N, Maroz U, Iqbal S, Aiyer R, et al. Nonobstructive hydronephrosis due to social polydipsia: a case report. J Med Case Rep 2012;6:376.
9. Barlow ED, De Wardener HE. Compulsive water drinking. Q J Med 1959;28:235-58.
10. Lee S, Chow CC, Koo LC. Altered state of consciousness in a compulsive water drinker. Br J Psychiatry 1989;154:556-8.
11. Walls LL, Supinski CR, Cotton WK, McFadden JW. Compulsive water drinking: a review with report of an additional case. J Fam Pract 1977;5:531-3.
12. Clark WF, Kortas C, Suri RS, Moist LM, et al. Excessive fluid intake as a novel cause of proteinuria. CMAJ 2008;178:173-5.
13. Thompson CJ, Edwards CR, Baylis PH. Osmotic and non-osmotic regulation of thirst and vasopressin secretion in patients with compulsive water drinking. Clin Endocrinol (Oxf) 1991;35:221-8.
14. Smith D, Moore K, Tormey W, Baylis PH, et al. Downward resetting of the osmotic threshold for thirst in patients with SIADH. Am J Physiol Endocrinol Metab 2004;287:E1019-23.
15. Cuesta M, Gupta S, Salehmohamed R, Dineen R, et al. Heterogenous patterns of recovery of thirst in adult patients with adipsic diabetes insipidus. QJM 2016;109:303-8.
16. Hammond DN, Moll GW, Robertson GL, Chelmicka-Schorr E. Hypodipsic hypernatremia with normal osmoregulation of vasopressin. N Engl J Med 1986;315:433-6.
17. Edholm OG. Does man in a hot climate adapt to water deprivation? Isr J Med Sci 1965;1:1046-7.
18. Epstein FH, Kleeman CR, Hendrikx A. The influence of bodily hydration on the renal concentrating process. J Clin Invest 1957;36:629-34.
19. Luff C. How to Stay Hydrated Before, During, and After Your Runs. verywell [2016] Available from: URL:https://www.verywell.com/hydration-and-running-2911541
20. Brant J. Drink This: The eight rules of fluid replacement, and how they'll energize your running. Runner's World [2003] Available from: URL:http://www.runnersworld.com/hydration-dehydration/drink-this
21. DiGiovanni SR, Nielsen S, Christensen EI, Knepper MA. Regulation of collecting duct water channel expression by vasopressin in Brattleboro rat. Proc Natl Acad Sci U S A 1994;91:8984-8.
22. Kishore BK, Terris JM, Knepper MA. Quantitation of aquaporin-2 abundance in microdissected collecting ducts: axial distribution and control by AVP. Am J Physiol 1996;271:F62-70.
23. De Wardener HE, Herxheimer A. The effect of a high water intake on the kidney's ability to concentrate the urine in man. 1957. J Am Soc Nephrol 2000;11:980-7.
24. Terris J, Ecelbarger CA, Nielsen S, Knepper MA. Long-term regulation of four renal aquaporins in rats. Am J Physiol 1996;271:F414-22.
25. Trout C. Cutting Weight and Rehydrating. Juggernaut: Be unstoppable [2014] Available from: URL:http://www.jtsstrength.com/articles/2014/01/27/cutting-weight-rehydrating/
26. Kobilansky G. How to Cut Weight the Right Way. Breaking Muscle HQ [2016] Available from: URL:http://breakingmuscle.com/mma/how-to-cut-weight-the-right-way
27. Friedland GW, Axman MM, Russi MF, Fair WR. Renal back pressure atrophy with compromised renal function due to diabetes insipidus. Case report. Radiology 1971;98:359-60.
28. Streitz JM, Jr., Streitz JM. Polyuric urinary tract dilatation with renal damage. J Urol 1988;139:784-5.
29. Zender HO, Ruedin P, Moser F, Bolle JF, et al. Traumatic rupture of the urinary tract in a patient presenting nephrogenic diabetes insipidus associated with hydronephrosis and chronic renal failure: case report and review of the literature. Clin Nephrol 1992;38:196-202.
30. Blum A, Friedland GW. Urinary tract abnormalities due to chronic psychogenic polydipsia. Am J Psychiatry 1983;140:915-6.
31. Himeno Y, Ishibe T. Bilaterally dilated upper urinary tract and bladder induced by diabetes insipidus. Int Urol Nephrol 1990;22:129-32.
32. Viberti GC, Mogensen CE, Keen H, Jacobsen FK, et al. Urinary excretion of albumin in normal man: the effect of water loading. Scand J Clin Lab Invest 1982;42:147-57.
33. Sawka MN, Burke LM, Eichner ER, Maughan RJ, et al. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc 2007;39:377-90.
34. Johnson P, Waldreus N, Hahn RG, Stenstrom H, et al. Fluid retention index predicts the 30-day mortality in geriatric care. Scand J Clin Lab Invest 2015;75:444-51.
35. Ylinenvaara SI, Elisson O, Berg K, Zdolsek JH, et al. Preoperative urine-specific gravity and the incidence of complications after hip fracture surgery: A prospective, observational study. Eur J Anaesthesiol 2014;31:85-90.
36. Bar-David Y, Urkin J, Kozminsky E. The effect of voluntary dehydration on cognitive functions of elementary school children. Acta Paediatr 2005;94:1667-73.
37. Clark WF, Sontrop JM, Huang SH, Moist L, et al. Hydration and Chronic Kidney Disease Progression: A Critical Review of the Evidence. Am J Nephrol 2016;43:281-92.
38. Valtin H. "Drink at least eight glasses of water a day." Really? Is there scientific evidence for "8 x 8"? Am J Physiol Regul Integr Comp Physiol 2002;283:R993-1004.
39. Lavagno C, Camozzi P, Renzi S, Lava SA, et al. Breastfeeding-Associated Hypernatremia: A Systematic Review of the Literature. J Hum Lact 2016;32:67-74.
40. van DP, van Wouwe JP, Breuning-Boers JM, van BS, et al. Reference chart for relative weight change to detect hypernatraemic dehydration. Arch Dis Child 2007;92:490-4.
41. Hew-Butler T, Rosner MH, Fowkes-Godek S, Dugas JP, et al. Statement of the Third International Exercise-Associated Hyponatremia Consensus Development Conference, Carlsbad, California, 2015. Clin J Sport Med 2015;25:303-20.
42. Convertino VA, Armstrong LE, Coyle EF, Mack GW, et al. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc 1996;28:i-vii.
43. Maughan RJ, Shirreffs SM, Leiper JB. Errors in the estimation of hydration status from changes in body mass. J Sports Sci 2007;25:797-804.
44. O'Neal EK, Caufield CR, Lowe JB, Stevenson MC, et al. 24-h fluid kinetics and perception of sweat losses following a 1-h run in a temperate environment. Nutrients 2014;6:37-49.

45. O'Neal EK, Davis BA, Thigpen LK, Caufield CR, et al. Runners greatly underestimate sweat losses before and after a 1-hr summer run. Int J Sport Nutr Exerc Metab 2012;22:353-62.
46. Hew-Butler T, Collins M, Bosch A, Sharwood K, et al. Maintenance of plasma volume and serum sodium concentration despite body weight loss in ironman triathletes. Clin J Sport Med 2007;17:116-22.
47. Mentes JC, Wakefield B, Culp K. Use of a urine color chart to monitor hydration status in nursing home residents. Biol Res Nurs 2006;7:197-203.
48. Kavouras SA, Johnson EC, Bougatsas D, Arnaoutis G, et al. Validation of a urine color scale for assessment of urine osmolality in healthy children. Eur J Nutr 2016;55:907-15.
49. Popowski LA, Oppliger RA, Patrick LG, Johnson RF, et al. Blood and urinary measures of hydration status during progressive acute dehydration. Med Sci Sports Exerc 2001;33:747-53.
50. Wakefield B, Mentes J, Diggelmann L, Culp K. Monitoring hydration status in elderly veterans. West J Nurs Res 2002;24:132-42.
51. Steiner MJ, Nager AL, Wang VJ. Urine specific gravity and other urinary indices: inaccurate tests for dehydration. Pediatr Emerg Care 2007;23:298-303.
52. Beets I, Temmerman L, Janssen T, Schoofs L. Ancient neuromodulation by vasopressin/oxytocin-related peptides. Worm 2013;2:e24246.