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Systemic Hydration: Relating Science to Clinical Practice in Vocal Health

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Abstract

Objectives—To examine the current state of the science regarding the role of systemic hydration in vocal function and health.

Study Design—Literature Review

Methods—Literature search spanning multiple disciplines, including speech-language pathology, nutrition and dietetics, medicine, sports and exercise science, physiology and biomechanics.

Results—The relationship between hydration and physical function is an area of common interest amongst multiple professions. Each discipline provides valuable insight into the connection between performance and water balance, as well as complimentary methods of investigation. Existing voice literature suggests a relationship between hydration and voice production, however the underlying mechanisms are not yet defined and a treatment effect for systemic hydration remains to be demonstrated. Literature from other disciplines sheds light on methodological shortcomings and in some cases offers an alternative explanation for observed phenomena.

Conclusions—A growing body of literature in the field of voice science is documenting a relationship between hydration and vocal function, however greater understanding is required to guide best practice in the maintenance of vocal health and management of voice disorders. Integration of knowledge and technical expertise from multiple disciplines facilitates analysis of existing literature and provides guidance as to future research.

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Introduction

Hydration and its relationship to health has long been the focus of experimental intrigue. Researchers from multiple disciplines have investigated the impact of more and less water on the functioning of the body for decades, with the general consensus being that a balance of fluids is required for optimum performance. Examination of voice production during superficial and systemic hydration challenges has revealed altered structure and function of the vocal folds, suggesting adequate hydration of the vocal tract to be essential for healthy phonation (1–3). Clinically, this has translated into recommendations regarding maintenance of systemic and surface hydration in both prophylactic and therapeutic regimes. Indeed, guidance on adequate and appropriate methods of hydration form an integral part of vocal hygiene education. Typically, recommendations include regular and adequate water consumption of approximately 8 eight-ounce glasses per day (64 fl.oz), avoidance of drying substances such as caffeine and alcohol, and using humidification or steam inhalation if exposed to drying environments such as air conditioning, smoke, or central heating (1, 4–6). However, recent reviews of the literature suggest further analysis of the relationship between hydration and phonation is required before clinicians may confidently prescribe such an approach (4), with the underlying physiological mechanisms of superficial and systemic vocal fold hydration still to be elucidated and no clear treatment effect of hydration on voice production yet demonstrated (1, 7).

This review encourages the integration of knowledge from several fields, including exercise physiology, medicine, speech-language pathology, nutrition and dietetics, in order to provide a framework for researchers and clinicians to analyse existing research into the relationship between hydration and vocal function, and to guide future investigations. To this end, information is first provided on the function and location of water within the body, the various categories of water excess and deficiency, the impact of water imbalance on performance, hydration assessment techniques, and hydration at the tissue and cellular level. The current literature concerning the impact of systemic hydration on vocal function is then reviewed in relation to these underlying principles. Readers are directed to previous reviews for coverage of surface hydration investigations (e.g., nasal breathing, nebulised substances) (1, 4, 7).

Water in the body

The human body is reliant on water for health and well-being. Its vital functions include that of building material, solvent for chemical reactions, medium of transport for nutrients and waste, thermoregulator, lubricant and shock-absorber(8). Water plays an integral role in each system in the body (circulatory, respiratory, digestive, endocrine, immune, lymphatic, muscular, nervous, reproductive, integumentary, skeletal and urinary), with regular replenishment required through intake of food and fluids to enable optimum function. Homeostatic mechanisms exist at both cellular and whole body levels in order to provide precise regulation of water balance. Under temperate conditions with moderate exercise, total body water (TBW) is maintained within 0.2% variation in any 24-hour period. This consistency, particularly in the volume and composition of extracellular fluid (ECF), facilitates cellular function (8). Fluid balance changes as small as a few hundred millilitres

result in alteration of the ionic concentration of the ECF, triggering signals controlling thirst and the volume and tonicity of urine (9). Through controlling both input and output, the body is able to achieve water balance between spaces and tissues, termed euhydration.

Body composition in terms of proportion of muscle, fat and water, typically follows a pattern through the lifespan, with the amount of fluid dependent on age and relative proportions of muscle and fat. In approximate terms, at birth water comprises 70% of body weight, with a fat proportion of 13%. With the rapid increase in fat in the first year of life to 20–25%, and the decline in ECF from 45% to 28% of body weight, TBW at 12 months approximates that of adults at 61% (10). Body composition continues to change through preadolescence and puberty, after which females typically stabilise (~50% TBW) while males tend to show slight decline in fat levels into adulthood (~60% TBW) resulting in a gender difference of approximately 10% (11). Following a plateau, aging processes (most rapid after ~60yrs, but commencing in middle age (12)) result in further changes due to a loss of fat-free mass, decreased thirst sensation and reduced ability of the kidneys to concentrate urine (10).

Alongside the relative proportion of body water, rate and volume of water input and output determine the susceptibility of an individual to alterations in water balance within the body. Water input occurs through ingestion of food (~30% water needs) and fluid (~60% water needs) and a small amount (10%) is produced through tissue catabolism (11). The main routes of water loss are through the kidneys, skin, respiratory tract and the digestive system, with a loss of approximately 2 to 3 litres per day for a sedentary adult (dependent on the environment) (8). While subtle hormonal changes assist in achieving water balance in the body, voluntary drinking of water is considered to be crucial for optimum function as thirst is not triggered in the hypothalamus until a loss of approximately 1% TBW. With increasing age, the thirst response is reduced, increasing the risk of altered fluid balance in the absence of voluntary drinking (10). Infants are also at greater risk of disturbed water balance levels than older children and adults due to the combination of a high body water percentage, a high turnover rate and comparatively high surface losses (proportionately large surface area) (8).

Hydration Balance, Excess and Deficiency

The term *hydration*, refers to the current state of water balance within an individual. It encompasses the terms euhydration, hypohydration and hyperhydration. *Euhydration* is defined in medical sources as the “normal state of body water content; absence of absolute or relative hydration or dehydration” (13). Rather than a specific point, euhydration follows a sinusoidal wave oscillating around an average basal level (14). The definition of ‘normal’ can be troublesome however, with lack of consensus in the literature as to the acceptable levels of variation. This paper will use the term euhydration to refer to the state of water balance in the body, when input and output are equal and fluids are maintained at desired osmolality, pH, temperature and composition. Having less water than euhydration is termed *hypohydration*, with *dehydration* used to refer to the process of uncompensated water loss reducing the TBW below the average basal value (14). Excess water in the body is variably

termed water intoxication, overhydration and hyperhydration in the literature (14). For direct comparison with hypohydration, this paper will use the term hyperhydration.

Three main types of imbalance exist within both hypo- and hyper-hydration, distinguished according to the extracellular sodium concentration which influences the direction of water flow between intracellular and extracellular environments (Table 1). Istonic hypohydration, equal net depletion of electrolytes and water, is considered to be the most common form of fluid imbalance in humans(8). Each category of water imbalance requires specific targeted intervention, and if left unchecked can result in life-threatening consequences (Table 2). At lesser degrees of imbalance, negative changes in all body systems can be observed, with similar symptoms being present for both hyper- and hypo-hydration (e.g., headache, dizziness, confusion, restlessness, muscle cramping or twitching, and vomiting) (Table 2). Greater differentiation exists in the condition of mucus membranes, skin turgor and urine output.

Prevalence of Water Imbalance

Research involving athletes has provided valuable information on everyday hydration status in healthy individuals. Measures taken during sporting camps and practices have revealed a substantial portion of youth athletes to present with hypohydration prior to involvement in physical activity, and for this to remain or worsen across days (15–18). More than half of adult athletes also tend to be hypohydrated, whether they are involved in recreational sport (19), or in collegiate (18) or professional teams (15, 20, 21). Some evidence exists that hypohydration is more common in male athletes than female (22).

Prevalence and incidence rates of hypohydration have been investigated in at-risk populations of the young and the elderly. Hypohydration as a result of gastroenteritis is considered a common paediatric condition, with estimates of 30 million children being affected each year in the United States alone. Of these, 1.5 million require outpatient care and 200 000 are hospitalized (23). It is not clear however, the proportion of children who are in a state of fluid deficit when they are otherwise healthy. Hypohydration in older adults living in the community is widely considered to be common (24), although some contention exists within the literature as to the accuracy of these beliefs (10). Hyperhydration is considered rare in otherwise healthy individuals, with the majority of reports of excess body fluid due to polydipsia (intake >3 liters fluid per day) associated with mental illness (25, 26) or those undergoing medical care as in diabetes and dialysis (27–29).

Influence of hydration on physical performance

Examination of the literature surrounding athletes is a fertile source of information regarding the potential of mild-moderate hypohydration to influence performance. Deficits in hydration levels of as little as 1–2% have been shown to have detrimental effects on endurance, thermoregulatory capability and motivation, and to increase fatigue and perceived effort (9, 30, 31). Heart rate is increased, muscle power is decreased, physiological strain is greater and cognitive performance is reduced (9, 14, 32). Activities requiring high-intensity and endurance have been reported to be particularly susceptible to fluid deficits (9, 32).

The potential to further improve on usual function by fluid loading prior to exercise has also been investigated. An early experiment by Moroff and Bass (33) demonstrated increased perspiration in combination with reduced pulse rate and internal temperatures in men who overhydrated (2 liters water) prior to walking in addition to replacing fluid losses during the task. Contemporary investigations have attempted to overcome the rapid clearance of excess fluid by the body, through the use of metabolites such as glycerol which enhance fluid retention through osmotic gradients. However, a recent review of their effectiveness noted equivocal results in terms of thermoregulatory, cardiovascular or overall performance advantage (34). In adequately hydrated individuals then, consensus is lacking on the potential for enhanced function through the provision of additional fluids (9).

Assessment of Body Hydration

Measurement of hydration levels remains a controversial topic within the scientific literature. The recommended gold standard for accurate determination of an individual's hydration status varies depending on the source, with recent accounts suggesting that no single valid measure is currently available which is suitable across hydration assessment requirements (9, 14). This is largely due to the dynamic nature of water balance within the body, with fluid moving through a complex matrix of interconnected compartments (14). A recent review by Armstrong (14), analyzed 13 hydration assessment techniques in terms of measurement resolution, accuracy and validity. Armstrong noted that while the evidence base for hydration assessment techniques is growing, difficulties remain with gaining an accurate representation of the volume and location of TBW, particularly during daily activities or when fluid balance is perturbed. In order to achieve an accurate assessment of whole body hydration, a combination of measures was recommended, with due consideration given to the timing of administration.

Measures of hydration range from simple ratings of thirst or examination of urine color to stable isotope dilution and neutron activation analysis. Plasma osmolality is reported to provide an accurate ($\pm 1-2\%$) assessment of extracellular fluid, and in turn hydration status particularly when combined with a measure of TBW through isotope dilution (14). In laboratory conditions (controlled posture, activity, diet and environment), this combination of measures currently represents the most accurate assessment of TBW and concentration of body fluids (14). However, the complex, time consuming and invasive nature of these analyses render them impractical in all but the most controlled settings. Simple measures of change in body weight are considered an accurate representation of TBW when taken in close proximity, however are not sensitive over a longer period of time (14). Bioelectrical impedance is reported to have reduced reliability and accuracy and is not able to measure changes of less than one litre (14, 35). Ultrasonic assessment has been suggested as an alternative, providing convenient, noninvasive measurements with acceptable accuracy (35). Examination of urine is commonly cited, including measurements of osmolality, 24-hour volume and specific gravity (USG) alongside color comparisons. However, while considered indicative of body fluid balance, urine indices are more reflective of the recent volume of fluid intake than an accurate representation of overall hydration status (14, 36) and predominately detect hypertonic hypohydration (35).

Timing of analysis is crucial in order to gain an accurate representation of body hydration, with consideration of physiological processes particularly important when attempting to document rehydration following a period of hypohydration. The ingestion of a large volume of water has been shown to result in the excretion of diluted urine by the kidneys even when the body has an overall water deficit (8). Similarly, plasma osmolality is not returned to baselines even following ingestion of large volumes of fluid(36) and tissue rehydration may occur over a period of days (35). In addition, any measures taken during and immediately after exercise reflect an altered balance in each fluid compartment (intracellular fluid, interstitial fluid and plasma) due to temporary adjustments in circulatory and renal function (14).

Hydration of tissues and cells

Examination of hydration at the individual tissue and cellular level provides greater insight into the precise and complex relationship that exists in order to maintain homeostasis and the physiological basis of the negative impact of water imbalance in the body. Water is the main component of the majority of soft tissues (~70–80%), with molecular components including proteins, organic and inorganic compounds contributing the remainder (35). Water then, plays an integral role in structure and function, with changes in water content necessarily resulting in alterations to performance. Even skin, which has a comparatively low water content of 30%, shows variation in thickness, elasticity and density depending on hydration levels (37). Excess hydration is known to negatively influence the behaviour of the cornea through creation of surface epithelial irregularity and alteration of the tear film-air interface (38). Investigations into the biomechanical properties of soft tissues have supported this notion, with behaviour linked with extracellular matrix (ECM) hydration both in vivo and in vitro (39). The viscoelastic properties of articular cartilage for example, have been shown to alter depending on hydration, with reduced ability to dissipate energy and resultant increased likelihood of rupture in a hyperhydrated state (as in osteoarthritis) (40). At the cellular level, the properties of elastin have shown significant stiffening with dehydration, resulting in reduced fatigue resistance to cyclic loading particularly at high frequency (39).

In order to document change in biomechanics as a function of hydration, researchers have typically dehydrated or hyperhydrated excised tissues through immersion in hypertonic / hypotonic solutions and/or exposure to dry / humid air. Measurement of the change in the weight and/or thickness of specimens is typically employed to infer water deficit, euhydration and hyperhydration. Ergometers (stress-strain) have been used to provide information on tissue elasticity, while rheometry measures also yield information on the viscous properties of the tissue (viscoelasticity). A sizeable proportion of the current knowledge regarding the impact of hydration on viscoelasticity of body tissues has stemmed from investigation of animal and human vocal folds.

Impact of alterations of hydration level on voice

Hydration, and its relationship with voice, has been a popular topic of investigation in both clinical and physiological investigations. Recent reviews of the relationship between

hydration and vocal fold function report a growing body of evidence that systemic and superficial dehydration alters the viscoelastic properties of the mucosa, having detrimental impacts on aerodynamic and acoustic measures of phonation (1, 4, 7). However, the benefit of therapeutic hydration regimes remains unclear, with a recent meta-analysis of hydration treatment outcomes on phonation threshold pressure (PTP) (a suggested indirect measure of vocal effort) revealing substantial variation across studies with no statistically significant treatment effect (41). The authors noted that methodological differences between investigations hampered comparison, calling for increased clarity on the amount, type and duration of hydration intervention to be defined in order to best guide clinical practice.

Critical appraisal of the existing literature according to key underlying principles is of benefit in order to determine the current evidence base for systemic hydration effects in vocal health and guide future investigations. Consideration should be given to the hydration status of the individuals or tissues involved, the method and timing of hydration assessment, how hydration change was induced and the measures of vocal function employed. Tables 3–7 provide a summary of in vivo human systemic hydration investigations to date according to these factors (2, 3, 42–58). The reader is also referred to the summary (subjects, challenges and outcome) of in vivo (human) and ex vivo (animal) investigations provided by Leydon and colleagues (7) in their review of surface hydration. A portion of these studies induced changes to systemic hydration (water + mucolytic drug(2, 49–51)) in combination with environmental changes to humidity levels(42, 43, 49–51).

Research into the relationship between hydration and voice production in vivo has generally taken a similar approach to the broader literature in this area (physical and cognitive impacts), attempting to compare performance in a hypohydrated state (through fluid restriction and/or inducement of heat stress or high activity) with that of a euhydrated state brought about through the provision of water sufficient to overcome water loss (9) (Tables 3,4 and 6). Overall, positive effects have been documented following ‘reversal’ of dehydration. However, while this methodology allows comparison of vocal function following relative increases and decreases to individual fluid intake, it does not lend itself to accurate determination of the hydration status of the vocal tract, nor the individual as a whole. Indeed, studies have typically not employed direct measurements of hydration, relying instead on subject determined usual water intake and activity levels to estimate the balance of water within the body. Given the prevalence of hypohydration in otherwise healthy individuals (15–22), ‘usual’ intake and exercise may in fact represent an already imbalanced system, with induced changes to water intake and/or exercise further exacerbating dehydration or alternatively inducing euhydration (rather than the intended hyperhydration). In addition, the category (hypotonic, hypertonic or isotonic) of hyper- or hypo-hydration of the participant during the experiment is not able to be determined without accurate measurement and therefore the impact of more or less water on the underlying physiology can not be determined. Further confounding the existing literature is the tendency to examine vocal function directly following an induced hydration challenge when the fluid compartments are unlikely to be in equilibrium and attempts at rehydration through rapid ingestion of large volumes of water which, in theory, do not immediately rehydrate body tissues and render urine analysis inaccurate (14).

Greater control of hydration intervention and accuracy of hydration measurement is possible in excised tissues. Direct and indirect measures of vocal fold biomechanics, including PTP, rheology, traction tests, electroglottography, acoustics and laryngeal imaging, have demonstrated dehydration of tissue to result in changes in viscoelastic properties (59–63), altered epithelial barrier function (64), and reduced amplitude of VF motion (65). However, difficulty exists in the generalisation of these findings to typical voice production given the severity of dehydration induced in these studies, generally far exceeding physiological levels expected in vivo. For example, Hanson and colleagues (66) recently reported on the reduced ability of vocal fold lamina propria to regain water balance (measured by volume) following 70% dehydration as compared to 30% dehydration. Such levels of fluid depletion are not generally compatible with life. Clinically, patients experience alterations in plasma osmolality at only $\pm 1\%$ TBW, 2% depletion in TBW is known to result in exercise performance deficits, and dry mucous membranes are a sign of moderate dehydration of 3–9% (10, 23). In addition, the potential for varied impacts of type of hypohydration (according to tonicity) on lamina propria has not been fully explored.

The impact of increased fluid within the vocal folds also requires further examination. Finkelhor and colleagues (60) reported the effects of immersing excised canine larynges in hypertonic, hypotonic and isotonic solutions on threshold pressures required to induce phonation. Interestingly, greater fluid volume within the vocal folds was shown to require less air pressure than the other conditions, leading the researchers to question the impact of oedema on vocal fold viscosity, suggesting that it may in fact be beneficial. Similarly, human studies of PTP have shown ‘wet’ conditions (mix of superficial and systemic hydration changes) to reduce threshold pressures (49, 50) (Table 6) and fluid removal from hypervolemic individuals undergoing dialysis has been reported to increase PTP and perceived effort (48) (Table 7), and result in transient hoarseness in some cases (48, 57). In contrast, individuals with laryngeal oedema report difficulty in initiating phonation and demonstrate increased subglottal pressures and reduced pitch, indicative of increased tissue viscosity (67, 68). The exact relationship between vocal fold viscosity and the volume, composition and location of excess fluid remains unclear. In order to generalise these findings to clinical cases then, research examining finer increments of hydration change (in both directions) is required.

A common clinical adage is for patients to avoid ‘dehydrating’ substances, such as caffeine, alcohol, aspirin, antihistamines, decongestants and diuretic medications, based on the premise that such agents have a drying effect (e.g., through secretion reduction or diuresis) on the body (53, 54). So strong is this notion, that these substances are often reported as controlled in existing investigations into hydration effects on voice (e.g. all participants avoid caffeine prior and during testing). However examination of the literature reveals little evidence that such substances do indeed result in vocal change (Table 5). Verdolini and colleagues (2) found equivocal results in their investigation of a diuretic and antihistamine on PTP and PPE ratings, concluding that the respiratory system may retain fluids longer than other regions of the body during dehydration, PPE may not be a reliable indicator of hydration status and PTP changes may in fact be due to alterations in neuromuscular function. Similarly, the action of anticholinergic drugs on vocal function also remains unclear, with detrimental effects on PTP, PPE, and frequency range reported post

glycopyrrolate injection in 20 healthy men, but no clear change to other acoustic, aerodynamic or strobolaryngoscopic parameters (52). Atropine is said to dehydrate the larynx through reduced laryngeal gland secretion (55), however no perceptible change in voice quality was noted in 4 healthy men post injection despite participant report of a 'dry throat' and alterations to frequency per unit change in transglottal pressure (dF/dP) at low frequencies. The few studies that have investigated caffeine in isolation have shown no clear detrimental impact on PTP, PPE (54), frequency irregularity (53), perceived vocal quality, fundamental frequency, jitter or shimmer (58). Further research is needed to elucidate the impact of such drugs on phonation and the underlying mechanisms involved. Several possibilities exist for the lack of significant impact of caffeine on phonation, including the sensitivity of measures to vocal change, the relatively low number of participants involved (ranging from 8–25), or that the ingestion of caffeine did not produce a drying effect (in the larynx or the body as a whole) in these individuals as expected. As participant hydration status was not measured in these studies, the latter remains unknown, however examination of the broader literature suggests that caffeine may in fact not have a diuretic effect in individuals who regularly drink caffeinated beverages (69).

Clinical implications and future directions

Research suggests that a substantial proportion of individuals in the general population are likely to have some form of water imbalance. In otherwise healthy individuals this is likely to be a variant of hypohydration. In theory, the young and the old are at increased risk of water imbalance, however given the reported prevalence in healthy adults involved in sport (~ 50%), the possibility should be considered across the lifespan. Along with general physical and cognitive effects, the literature supports the clinical adage that a relationship between hydration and voice production exists. However, while animal and human studies are beginning to elucidate the underlying physiological and functional impact of water imbalance on voice production, numerous questions remain and warrant further investigation.

A common prescription in vocal hygiene is increased systemic hydration through regular ingestion of water. Approaching this from a biomechanical and physiological perspective requires consideration of several factors, including: the current hydration status of the individual client (euhydrated or one of six categories of water deficit or excess), which fluid would best remediate a deficit /excess if present (dependent on tonicity) and what dosage and frequency would be required (dependent on age, gender, environment, body composition and activity level). For those clients who are euhydrated, will additional fluids reach the vocal tract, and if so is it likely to result in improved performance? Research from the exercise physiology literature is equivocal on the ability of induced hyperhydration (through fluid ingestion) to result in improvements in physical activity, and clients may in fact void the excess fluid prior to it reaching the intended target.

The concept of euhydration and its application to vocal fold physiology is promising. Both hypohydration and hyperhydration are known to alter vibratory characteristics of the vocal folds suggesting that optimum function lies somewhere along the hydration spectrum. This notion, raised twenty-five years ago by Finkelhor and colleagues (60), still requires further

investigation. Similarly, do the different forms of water excess and deficit induce different changes to the biomechanics of the vocal folds? Further research investigating vocal fold biomechanics in varying degrees and categories of hydration would be beneficial in this area.

Muscular function is known to vary according to hydration status, with increased fatigue and decreased rapidity of movement resulting from water deficit (32). Does this effect exist in laryngeal muscles and could hydration status contribute to muscular dysfunction and/or fatigue in the vocal tract? Certainly fatigue effects have been noted in hypohydrated individuals (42, 43, 46), however investigators have attributed this to alterations in vocal fold composition, with little or no consideration of potential muscular involvement.

In order to further scientific understanding of the role of hydration in phonation and to best guide clinical practice, future investigations would benefit from the integration of knowledge and techniques from multiple disciplines to guide rigorous experimental design. In addition to ensuring adequate power and validity of experiments (larger participant numbers and randomized, controlled designs where possible), researchers are encouraged to measure and report hydration status of participants using valid techniques and at appropriate time points. The nutrition and exercise physiology literature is replete with recommendations in this regard. Documentation of alterations in vocal function as a result of hydration requires selection of sensitive, yet clinically relevant experimental measures. Including measurement of cognitive and muscular performance, along with discrete vocal fold measures, would seem advantageous, as both have been shown to fluctuate with hydration and have the potential to influence perceived phonatory effort and phonation in general.

Conclusions

Adequate hydration is essential for health and well-being, playing an integral role in body systems, tissues and cells. A growing body of literature in the field of voice science is documenting the impact of altered water balance, both in vivo and ex vivo, on the structure and function of the vocal folds and voice production as a whole. Integration of knowledge and research techniques from multiple disciplines enables critical appraisal of the existing literature and provides guidance for future investigations. Greater understanding of the role of hydration in vocal health is required to guide best practice in the maintenance of vocal health and management of voice disorders.

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Table 1

Physiology of water imbalance

	Deficiency/Excess	Cellular Fluid Shift	Extracellular Volume	Intracellular Volume	Blood Serum Osmolality
Hypohydration					
Isotonic	↓ sodium ↓ water	none	↓	-	-
Hypotonic	↓↓ sodium ↓ water	blood → cells	↓	↑	↓
Hypertonic	↓↓ water ↓ sodium	cells → blood	↓	↓	↑
Hyperhydration					
Isotonic	↑ water ↑ sodium	none	↑	-	-
Hypotonic	↑ water	blood → cells	↑	↑	↓
Hypertonic	↑↑ sodium ↑ water	cells → blood	↑	↓	↑

Table 2

Causes, symptoms & treatment of water imbalance

	Common Causes	Symptoms	Treatment
<i>Hypohydration</i>			
Isotonic	Vomiting, diarrhoea, diuretics, peritonitis, burns, sedative & carbon monoxide intoxication, sunstroke, haemorrhage	Thirst, fatigue, fainting, collapse, vomiting, hypotonia, muscle cramps, rapid pulse	Isotonic salt solutions
Hypotonic	Inadequate sodium intake after vomiting, diarrhoea, sweating. Increased sodium losses due to adrenal failure, chronic diuretic therapy, diarrhoea, heat exhaustion.	Fatigue, fainting, hypotonia, vomiting, collapse, fever, muscle cramps, rapid pulse, depressed consciousness level, 'shock', confusion	Sodium chloride +/- isotonic saline
Hypertonic	Inadequate water intake, sweating, watery diarrhoea, osmotic diuretics, hyperventilation, chronic nephropathy, polyuric phase of acute renal failure, diabetes insipidus, prolonged nil by mouth, tube feeding with inadequate water	Decreased skin turgor, dry mucous membranes, thirst, fever, restlessness, delirium, coma	Pure water or glucose solution
<i>Hyperhydration</i>			
Isotonic	Excessive administration of isotonic infusion solutions in oliguric or anuric states, cardiac failure, nephrotic syndrome, chronic uraemia, acute glomerulonephritis, liver cirrhosis	Oedema, effusions, hypertension dyspnoea	Sodium chloride Fluid restriction Diuretics Osmotic therapy
Hypotonic	Excess administration of salt-free solutions, gastric lavage with water, increased ADH activity, liver failure	Weakness, nausea, vomiting, dyspnoea, confusion, loss of consciousness, headache, photophobia, muscle twitching, hyperirritability, polyuria, convulsions	Fluid restriction Dialysis Sodium chloride if tendency to alkalosis
Hypertonic	Drinking sea water, overactivity of renal cortex in Conn's syndrome, Cushing's syndrome, administration of steroids, hypertonic tube feeding (no flush), hypertonic enemas, excessive administration of sodium chloride.	Vomiting, diarrhoea, labile blood pressure, pulmonary oedema, restlessness, changes in central venous pressure	Sodium chloride & fluid restriction Diuretics Osmotic therapy

Table 3

Investigations of systemic hydration induced voice changes in vivo with altered fluid intake

Ref	Patients	Hydration Inducement	Hydration Measures	Hydration Status	Vocal Function Measures	Main Results	Conclusions
Solomon et al. (2003) (43)	4 M 19–29 yr healthy	<p><i>Method</i></p> <p>1 Typical hydration</p> <p>Usual amount non-caffeinated fluids</p> <p>2 Low hydration</p> <p>25% typical amount non-caffeinated fluids + repeated dry swallows</p> <p>3 High hydration</p> <p>75% more than typical amount non-caffeinated fluids + repeated ingestion of 30ml water</p> <p><i>Timing</i></p> <p>2 days prior to Ax patient altered fluid intake</p> <p>Every 5 min during reading tasks - fluid intake (high) or dry swallow (low)</p>	<p><i>Method</i></p> <p>Self-report nutrition & hydration log</p> <p><i>Timing</i></p> <p>Prior to initial Ax & during</p>	<p><i>Pre</i></p> <p>Not measured</p> <p><i>Post</i></p> <p>Not measured</p>	<p><i>Measures</i></p> <p>PTP</p> <p>10, 50, 80 PPE</p> <p>VAS-PTP</p> <p>Lx imaging closure, supraglottic activity, mucus, color, mucosal wave, symmetry & amplitude</p> <p><i>Timing</i></p> <p>Pre ✓ Post ✓</p>	<p>PTP ↓ (>2SDs) high cf low hydration 2/4 patient at selected pitches only</p> <p>PTP (>2SDs) from typical for 88% of trials (both low & high hydration conditions)</p> <p>PPE correlated with PTP across all pitches, patient & sessions</p> <p>Lx imaging - characteristics varied inconsistently across hydration & fatigue conditions 2/6 patients displayed sustained changes in 50% parameters in low-hydration</p>	<p>Drinking water should not be expected to lower PTP</p> <p>Findings did not support the prediction that drinking water would attenuate detrimental effects of strenuous phonation task</p> <p>Ability to match PPE with PTP varies amongst individuals</p> <p>PTP & PPE increase after prolonged loud phonation</p>
Solomon et al. (2000) (42)	4 F 22–29 yr healthy	<p><i>Method</i></p> <p>24–32% humidity, no liquid prior</p> <p>Typical Low Hydration 16oz water/day</p> <p>High hydration 5x16oz water/day</p> <p><i>Timing</i></p> <p>2 days prior to baseline & experimental sessions - monitor intake & abstain from caffeine, alcohol, high-sodium foods, dehydrating substances, strenuous voice use</p>	<p><i>Method</i></p> <p>Self-report nutrition & hydration log</p> <p><i>Timing</i></p> <p>Prior to initial Ax</p>	<p><i>Pre</i></p> <p>Not measured</p> <p><i>Post</i></p> <p>Not measured</p>	<p><i>Measures</i></p> <p>PTP</p> <p>10, 50, 80, conv PPE</p> <p>VAS-speaking</p> <p>Lx imaging</p> <p>Vibratory closure pattern</p> <p><i>Timing</i></p> <p>Pre ✓ Post ✓</p>	<p>PTP</p> <p>↑PPE with loud reading in each hydration condition 1/4 patients</p> <p>↓PTP in high hydration condition prior to loud reading</p> <p>PTP generally returned to pre-loud reading levels after 15 min rest</p> <p>Laryngeal imaging</p> <p>No diff. between hydration conditions</p>	<p>PTP can be used as a repeatable measure & is seen to vary with prolonged loud phonation</p> <p>Drinking water appeared to attenuate or delay increased PTP in prolonged reading</p>
Hamdan et al. (2007) (44)	28 F 21–45 yr healthy	<p><i>Method</i></p> <p>1 Fasting</p> <p>Abstain from all food & drink</p> <p>2 Non-fasting</p>	<p><i>Method</i></p> <p>Not measured</p> <p><i>Timing</i></p> <p>Not measured</p>	<p><i>Pre</i></p> <p>Not measured</p> <p><i>Post</i></p> <p>Not measured</p>	<p><i>Measures</i></p> <p>PPE -4-point scale</p> <p>Acoustics</p> <p>F₀, RAP, NHR, shimmer, turbulence index, habitual pitch, MPT</p> <p>Lx imaging</p>	<p>Patient perception post fast-vocal fatigue (53.6%)</p> <p>deepening of voice (21.4%)</p> <p>harshness (10.2%)</p> <p>PPE sig. ↑ during fasting</p>	<p>Fasting has an effect on voice, most perceived by the individual as an ↑ in phonatory effort</p> <p>Acoustic analysis is not revealing, nor is laryngeal videostroboscopy</p>

Ref	Patients	Hydration Inducement	Hydration Measures	Hydration Status	Vocal Function Measures	Main Results	Conclusions
Hamdan et al. (2011) (45)	26 M 22-50 yr healthy	Usual intake Timing Testing occurred 1-2 hrs prior to sunset to ensure at least 14hrs fast	As per (44)	As per (44)	Symmetry & amplitude mucosal wave periodicity closure Timing Pre ✓ Post ✓	Acoustic sig. ↓ max phonation time (~2.7/sec) Lx imaging - no changes	↑ phonatory effort likely secondary to dehydration & an element of muscular fatigue at respiratory & phonatory levels
		As per (44)	As per (44)	As per (44)		Patient perception post fast - vocal fatigue not sig. different Sig. ↑ PPE during fasting Acoustic - Sig. diff habitual pitch, voice turbulence index, NHR Lx imaging - no changes	Fasting results in increased effort in males which may be due to dehydration or decreased muscular endurance
Fluid intake + rest							
Yiu & Chan (2003) (46)	10 M 10 F 20-25 yr healthy Amateur singers previous fatigue post karaoke singing	Method 1 Hydration & voice rest (HVR) rest + 100ml water 2 Non-hydration & (non HVR) no rest & no fluids Timing Ingestion of fluid following each song	Method Not measured Timing Not measured	Pre Not measured Post Not measured	Measures Acoustics Fo, jitter, shimmer, NHR Perceptual roughness & breathiness (VAS) Phonetogram Timing Pre ✓ Post ✓	Significant difference ↑ time till fatigue HVR group ↑ jitter in speech non HVR male patients after 10 songs (returned to normal) ↓ highest Hz for female non HVR patients after 10 songs (returned to normal)	Some evidence that hydration & vocal rest reduces vocal fatigue Hydration & vocal rest should be recommended for singers as preventative measure to reduce vocal fatigue & negative effects of prolonged voice use

Table 4
Investigations of systemic hydration induced voice changes in vivo employing rapid reversal of dehydrated state

Ref	Patient	Hydration Inducement	Hydration Measures	Hydration Status	Vocal Function Measures	Main Results	Conclusions
Franca & Simpson (2009) (47)	19 F 18-35 yr healthy	<i>Method</i> 1 Abstain from water & food intake prior to testing	<i>Method</i> Patient report intake of tobacco, caffeine, alcohol & medications	<i>Pre</i> Not measured	<i>Measures</i> Acoustics jitter & shimmer	Improved jitter & shimmer in rehydrated condition, but not for all participants	Hydration has a positive impact on voice Results may be applied in prevention, counseling & treatment of voice disorders for individuals & employers
		<i>Timing</i> 2 Ingest 1l water 14 hr fast prior to testing 1l water in 20 min Test 90mins post ingestion	<i>Post</i> Not measured	<i>Pre</i> X Post ✓ Initial Ax in dehydrated state post fast			
Selby & Wilson (n.d.) (56)	8 M 18-31 yr healthy	<i>Method</i> All patients abstain from alcohol & caffeinated drinks. Testing at 20°C no air conditioning	<i>Method</i> Patient report intake of alcohol, caffeine, & water	<i>Pre</i> Patient water intake .25-.2l/day 50% often thirsty	<i>Measures</i> Laryngograph Lx imaging Acoustics jitter, Fo mode, range, irregularity	Lx imaging 1/8 patients excluded due to sulcus vocalis No diff. between smokers & non-smokers Acoustics Sig. ↑ modal Fo in conversation post rehydration	Hydration status does not have a marked influence on Fo mode, range & regularity
		<i>Timing</i> 1 Dehydration Abstain from all fluids & food prior to testing 2 Rehydration Ingest 2l electrolytic fluid	<i>Timing</i> Bioelectric impedance reported inconsistent & unable to track small changes in TBW <i>Post</i> Not measured	<i>Pre</i> X Post ✓ Initial Ax in dehydrated state post abstinence			
Fujita et al. (2004) (3)	6 M 28-36 yr healthy PVTU Working in low humidity	<i>Method</i> Remain in workplace environment (except transit) & abstain from systemic medications, coffee, alcoholic drinks & diet products	<i>Method</i> Not measured	<i>Pre</i> Not measured	<i>Measures</i> Lx imaging Videokymography quotient	No statistical analysis completed on results ↓ open phase time/closed phase time post hydration in 80% of patient, but ↑ in remaining 20% patient 5/6 patient presented with affections of the VF mucosa After hydration	Videokymography is an objective method of Ax to determine abnormalities of VF mucosa pre- & post-hydration Reduced quotient open phase time/closed phase time following hydration
		<i>Timing</i> 1 Dehydration Abstain all liquids 2 Rehydration Ingest 200ml room temperature aqueous solution with	<i>Timing</i> Not measured	<i>Pre</i> X Post ✓ Initial Ax in dehydrated state post abstinence			

Ref	Patient	Hydration Inducement	Hydration Measures	Hydration Status	Vocal Function Measures	Main Results	Conclusions
		<p>electrolytes + inhalation 0.9% saline electrolytes + inhalation 0.9% saline</p> <p><i>Timing</i> Unknown general abstinence period, no fluid 4hrs prior to testing Fluid ingestion immediately following initial Ax Saline inhalation 10mins</p>				<p>↓ appearance of viscosity of mucus & bright VFs ↑ amplitude of VF mucosa wave vibration</p>	

Table 5
Investigations of systemic hydration induced voice changes in vivo employing pharmaceuticals

Ref	Patient	Hydration Inducement	Hydration Measures	Hydration Status	Vocal Function Measures	Main Results	Conclusions
Roh et al. (2006) (52)	20M 21–24 yr healthy	<i>Method</i> Avoid caffeine, alcohol, high sodium food, drugs, dehydrating substances, excessive eating or drinking of water, strenuous voice use 1 Xerostomia intramuscular injection 0.3mg (1.5ml) glycopyrrolate 2 Control intramuscular injection 1.5ml saline <i>Timing</i> Timing for general avoidance not specifically stated. Baseline, injections, post measure + 3hrs	<i>Method</i> Saliva volume by modified swab Pt perception dry mouth (VAS) <i>Timing</i> Before & every 30mins for 3hrs post injection	<i>Pre</i> Not measured <i>Post</i> Not measured	<i>Measures</i> Acoustic Fo, jitter, shimmer NHR, voice range profiles Aerophone II max phonation time, average airflow, subglottal pressure PTP PPE - VAS post reading 20mins Lx imaging VAS vibratory closure pattern, supraglottic activity, presence of mucus, color, mucosal wave, amp & symmetry <i>Timing</i> Pre ✓ Post ✓	Saliva flow rates ↓ ~50% post glycopyrrolate injection post 30–60mins, lowest 90–120mins post injection Sig. ↑ dry mouth 30mins post highest level 120mins. ↑ PPE & PTP both groups post 3hrs, sig higher in treatment group. Voice range profile sig. ↓ pitch & loudness in treatment group No sig. change Max phonation time, average airflow, videostroboscopy ratings	Glycopyrrolate may induce decreases in mucosal wetness of VFs as well as oral mucosa. Salivary hypofunction may be closely related to vocal changes A change in vocal function should be considered for irradiated patients - current data is similar to previous investigation in head & neck cancer patients
Akhtar et al. (1999) (53)	8M 4F 27–55yr healthy	<i>Method</i> 250mg pure caffeine (5x Propulus tablets) <i>Timing</i> Post baseline measures	<i>Method</i> Caffeine (mg/l) blood <i>Timing</i> Pre & 1hr post ingestion	<i>Pre</i> Not measured <i>Post</i> Not measured	<i>Measures</i> Laryngograph Irregularity of Fo Free speech Reading passage 'Happy Birthday' <i>Timing</i> Pre ✓ Post ✓	Sig. effects btw patient, not within subjects (pre & post) in all 3 conditions Caffeine mg/l varied between patients Reading - substantial Fo variation in each task across patient prior to caffeine ingestion.	Considerable individual variability in response to caffeine ingestion Mean percentage of irregularity increased over time suggesting an effect on the VFs caused by caffeine ingestion
Erickson-Levendoski & Sivasankar (2011) (54)	8 M 8 F 18–27 yr 8 prior vocal training	<i>Method</i> 1 Caffeine 2x coffees (~480mg) 2 Control - 2x decaf coffee (~24mg) 70% humidity <i>Timing</i> 2 sessions at same time on 2 consecutive days	<i>Method</i> Not measured <i>Timing</i> Not measured	<i>Pre</i> Not measured <i>Post</i> Not measured	<i>Measures</i> PTP 10 80 PPE - VAS - 'Happy Birthday' 50% pitch range Measures taken post 35min & 70min vocal loading task <i>Timing</i> Pre ✓ Post ✓	No sig. effects of caffeine on PTP or PPE Ingestion of caffeine did not worsen the effects of vocal loading on PTP or PPE Vocal loading sig. ↑ PTP but not PPE	A high dose of caffeine does not adversely affect PTP or PPE measures

Ref	Patient	Hydration Inducement	Hydration Measures	Hydration Status	Vocal Function Measures	Main Results	Conclusions
Ahmed et al. (2012) (58)	25 adults healthy	<p>Method</p> <p>1 Caffeinated coffee ~400mg +routine water</p> <p>2 De-caffeinated coffee ~ 4–8mg +routine water</p> <p>3 Water only - 2l/day</p> <p>Additional carbonated drinks, if same caffeine category but not permitted in water group No alcohol for any group Timing Ingestion of fluid for 2 days</p>	<p>Method</p> <p>Drink diary Timing 48h between measures</p>	<p>Pre</p> <p>Not measured</p> <p>Post</p> <p>Not measured</p>	<p>Measures</p> <p>Perceptual rating GRBAS Acoustics Fo, jitter, shimmer Timing Pre ✓ Post ✓</p>	<p>No statistical diff. in any measure of voice quality between groups pre- or post-intervention</p>	<p>Caffeinated coffee does not have a sig. detrimental effect on voice quality</p>
Tanaka et al. (2001) (55)	4 M 26–35 yr healthy	<p>Method</p> <p>Intravenous injection of atropine sulfate 0.5mg Timing 10mins prior to testing</p>	<p>Method</p> <p>Not measured Timing Not measured</p>	<p>Pre</p> <p>Not measured</p> <p>Post</p> <p>Not measured</p>	<p>Measures</p> <p>Change in frequency per unit change in transglottal pressure (dF/dP) Timing Pre ✓ Post ✓</p>	<p>Pt perceived dry throat post atropine No perceived hoarseness dF/dP ↓ at lower Fo but not higher Fo</p>	<p>Relationship between dF/dP & Fo reflects length-versus-depth adjustments for Fo control</p>

Table 6
Investigations of systemic hydration induced voice changes in vivo employing pharmaceuticals plus humidification

#	Pt	Hydration Inducement	Hydration Measures	Hydration Status	Vocal Function measures	Main Results	Conclusions
Verdolini-Marston et al. (1990) (49)	3M 3F 25–46 yr healthy 4 singers 2 non-singers	<i>Method</i> 1 Dry	<i>Method</i> Not measured <i>Timing</i> Not measured	<i>Pre</i> Not measured <i>Post</i> Not measured	<i>Measures</i> PTP low mid high <i>Timing</i> Pre ✓ Post ✓	Minimal diff. between dry, control and wet conditions at speaking pitch ↑ pitch = ↑ PTP across conditions PTP lowest in wet condition at high pitch Slight ↑ PTP in dry condition compared with control condition	Validate the relationships between PTP & pitch, and PTP & VF viscosity Relative proportion of variance due to humidity & to systemic hydration is unclear
		2 Wet	3tsp decongestant (Dimetapp)				
		3 Normal	85–100% humidity + water 2×2tsp mucolytic (Robitussin)				
Verdolini-Marston et al. (1994) (51)	6F 18–33 yr otherwise healthy VF nodules or polyps 6m–5yrs 8m post onset 2 no voice training 4 singers 5 no therapy 1 therapy	<i>Method</i> 1 Hydration	<i>Method</i> Pt report of fluid intake <i>Timing</i> Hydration prospective log Control retrospective report General questioning prior to	<i>Pre</i> Not measured <i>Post</i> Not measured	<i>Measures</i> PTP low mid high PPE Lx imaging 5-point scale Perceptual rating 5-point scale SIN ratios <i>Timing</i> Pre ✓ Post ✓	Overall improved performance following both placebo & hydration conditions, with hydration reported to be superior PTP - no sig. diff., trend present at high pitch only Lx scope - Less severe rating (sig.), but less than 1 point Perceptual Rating no sig. diff. Acoustics - treatment effects present, but no clear hydration effect	Hydration may be of benefit in the treatment of vocal nodules & polyps Benefits of treatment may be present only while undertaking treatment Hydration effects appear to vary between individuals
		2 Control	8x 160z water + 90–100% humidity 3×1 tsp (Robitussin) mucolytic				
		3×1 tsp cherry syrup 8 sets of 20 forefinger flexions 30–40% humidity + scented candles					
		Restrictions-limit heavy voice use, alcohol & caffeine intake, smoke exposure					

#	Pt	Hydration Inducement	Hydration Measures	Hydration Status	Vocal Function measures	Main Results	Conclusions
Verdolini et al. (2002) (2)	2M 2F 21-28 yr healthy	<p><i>Timing</i> Medications 6 hourly Each treatment for 5 consecutive days</p> <p><i>Method</i> 1 Diuretic Lasix +4oz fluid/hr</p> <p>2 Anti-histamine +4oz fluid/hr diphenhydramine hydrochloride</p> <p>3 Placebo sugar pills +8oz fluid/hr</p> <p>Fluid & food control in between testing sessions <i>Timing</i> 4 days (1 daybreak), 16 hrs/day Active drug given 3 hours post arrival (following 4 pretreatment measures) Sugar pills 3-4/ day</p>	<p><i>Method</i> Body weight Saliva viscosity Pt report of health <i>Timing</i> Measures taken each hour for 16 hours per day for 4 days</p>	<p><i>Pre</i> Not measured <i>Post</i> Inferred from weight change and saliva viscosity</p>	<p><i>Measures</i> PTP high PPE <i>Timing</i> Pre ✓ Post ✓</p>	<p>Inconsistency across pt</p> <p>↑ PTP 5-12hrs post diuretic Antihistamine did not result in salivary change or PTP effects ↓ PPE at midday in placebo cond'n but ↑ for antihistamine No clear relationship of phonatory effort with other measures Saliva viscosity did not show reliable change across treatments or days</p>	<p>Respiratory system may retain fluids longer than other body parts during dehydration Changes in PTP may be due to VF viscosity and/or neuromuscular function PPE may not be reliable indicator of hydration status</p>
		<p><i>Method</i> Testing at 27°C</p> <p>1 Hydration 90% humidity +++water 2x 2tsp mucolytic (Robitussin)</p> <p>2 Dehydration 10-20% humidity +no fluid 2x2tsp decongestant (Dimetapp)</p> <p>3 Control 50% humidity + no fluid control 2x2tsp cherry syrup</p> <p><i>Timing</i> 4hr exposure to humidity Mucolytic at start & 30mins prior Decongestant 60mins prior Control 120mins prior</p>	<p><i>Method</i> Pt report of fluid intake <i>Timing</i> During testing</p>	<p><i>Pre</i> Not measured <i>Post</i> Not measured</p>	<p><i>Measures</i> PTP 10 conv. 80 PPE <i>Timing</i> Pre ✓ Post ✓</p>	<p>An inverse relationship between PTP & hydration level Sensitivity of PTP to hydration level progressively greater with increasing pitch sig. ↑PPE in dry condition vs the control & wet conditions No diff. between the control & wet conditions</p>	<p>Changes in PTP with hydration level are pitch dependent, with the greatest impact seen at high pitches PPE is less sensitive to changes in hydration level than PTP It would be better to base PPE on PTP task than conversational speech</p>

Table 7

Investigations of voice changes post fluid removal in vivo

#	Pt	Hydration Inducement	Hydration Measures	Hydration Status	Vocal Function measures	Main Results	Conclusions
Fisher et al. (2001) (48)	6M 2 F 40–85 yr 7pts end stage renal disease 1M healthy	<i>Method</i> Dialysis treatment including sodium modeling Able to drink 60z liquids + saline IV Fluid reversal fluid consumption according to pt customary habits <i>Timing</i> Fluid consumption overnight	<i>Method</i> Weight Fluid removed & delivered Blood pressure Temp. Heart Rate <i>Timing</i> Weight pre-dialysis Vitals every 15min	<i>Pre</i> presumed to be hypervolemic <i>Post</i> Not measured	<i>Measures</i> PTP 30 PPE Singing 'Happy Birthday' 11 point scale PPVQ rating 11 point scale <i>Timing</i> Pre ✓ Post ✓	↓ body fluid correlated with ↑PTP & ↑PPE Fluid loss = 16–64% variance in PTP Phonatory changes were not considered to interfere with activity, were improved 6hrs post, and not present in all cases. 2 pts showed no reliable treatment response	Voice deterioration can result from rapid & severe hydration challenges Removal of extracellular water in a hypervolemic state to induce a normovolemic state can result in voice changes Phonation can occur despite large-volume & fast fluid removal from the body
Ori et al. (2006) (57)	11 M 5 F 35–78yr Chronic Hemodialysis 1.5–15yr	<i>Method</i> Dialysis session <i>Timing</i> 4 hours	<i>Method</i> Dry weight Weight Blood pressure <i>Timing</i> Before & after dialysis	<i>Pre</i> hypervolemic <i>Post</i> 3/8 pt = drywgt 2/8 pt > drywgt 3/8 pt < drywgt	<i>Measures</i> Lx imaging Measurement of VFs area, length, width PPVQ hoarseness mild or sig. <i>Timing</i> Pre ✓ Post ✓	Post dialysis VF thickness ↓ in 13/16pts, but ↑ in 3/16pts ↓ weight & blood pressure 62% pt perceived post dialysis hoarseness No relationship between perceived hoarseness & VF thickness changes	Pts may experience transient hoarseness when undergoing chronic hemodialysis ↓ VF thickness is observed during dialysis, probably as a result of hydration removal