AALBORG UNIVERSITET
STUDENTERRAPPORT

Evaluation of Hydration Status in Patients with Intestinal Insufficiency or Intestinal Failure by Bioelectrical Impedance Measurements

10th semester short thesis

by

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Title: Evaluation of Hydration Status in Patients with Intestinal Insufficiency or Intestinal Failure by Bioelectrical Impedance Measurements

Semester: 10th semester, Medicine with Industrial Specialization, Translational Medicine

Project: 10th semester short thesis

Project period: February 2020 – May 2020

ECTS: 30 ECTS-points

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Study number: 20134221

Number of pages: 52

Number of appendices: 2

Number of appendixes pages: 6

Number of characters: 98480

Dansk resumé

Baggrund: Vand er et vigtigt næringsstof for liv og udgør en multifunktionel bestanddel af den menneskelige krop. Således medfører, især mangel på vand (dehydrering), men også overskydende mængder af vand (overhydrering) forskellige fysiologiske forstyrrelser i kroppen. Patienter med tarminsufficiens (INS) eller tarmsvigt (IF), der henholdsvis modtager oral ernæring (ON) eller hjemme parenteral ernæring (HPN), er i risiko for udvikling af forstyrrelser i kroppens vandbalance på grund af nedsat/ophørt tarmfunktion. Korrekt evaluering af patienternes hydreringstilstand er derfor et vigtigt aspekt af sygdomsmonitoreringen. Desværre findes der ikke én enkelt "gold standard" indenfor dette felt. Teknikker såsom bioelektrisk impedansanalyse (BIA) og bioelektrisk impedansvektoranalyse (BIVA) er blevet præsenteret som nye mulige alternativer til ældre teknikker, der anvendes i klinikken. Det er dog stadig ukendt hvor godt disse nye teknikker er i forbindelse med evalueringen af hydreringstilstanden hos patienter med INS eller IF.

Formål: At evaluere anvendelsen af BIA og BIVA som teknik til vurdering af hydreringstilstanden hos INS- og IF-patienter i forhold til referenceteknikker bestående af beregnet plasma-osmolaritet og 24-timers urinvolumen.

Metode: Hydreringstilstanden blev evalueret i 253 metabolisk stabile patienter med enten INS (n=125) eller IF (n=128) ud fra beregnet plasma-osmolaritet, 24-timers urinvolumen, heldkrops multifrekvens-BIA og BIVA. Patienterne blev klassificeret som enten dehydreret, euhydreret eller overhydreret i henhold til specifikke referenceintervaller for hver parameter. Korrelationen mellem parametrene blev vurderet ved Pearson's product-moment korrelation, mens pålideligheden blev testet ved brug af vægtet Kappa (κ_w) med lineære vægtning. Forskelle mellem ON- og HPN-patienter (dvs. patienter med henholdsvis INS og IF) blev beregnet for multiple demografiske og kliniske værdier samt for klassificeringen af hydreringstilstanden ved brug af Test of two proportions, Independent-samples T-test, og Chi-square test of homogeneity (r x 2)/Fisher's exact test (r x 2) med post hoc test, hvor det var relevant.

Resultater: En statistisk signifikant korrelation (p <0,05) blev kun fundet mellem plasma-osmolaritet og BIA-data samt BIVA-data, skønt korrelationerne var dårlige (korrelationskoefficient fra -0.150 til -0.245). Plasma-osmolaritet og 24-timers urinvolumen resulterede i en hydreringsklassificering, der var statistisk signifikant forskellig (p <0,05) mellem ON- og HPN-patienter. Post hoc-test kunne ikke bekræfte mellem hvilken hydreringsklasse forskellen eksisterede. Ingen af de andre teknikker kunne diskriminere mellem ON- og HPN-patienter ud fra hydreringsklassificeringen (p >0,05). Signifikant overensstemmelse (p <0,05) mellem teknikker blev kun fundet for plasma-osmolaritet og BIVA samt BIA, begge med en overensstemmelse mindre end dén forventet ved tilfældighed (κ_w <0,0).

Konklusion: Studiet demonstrerede, at hydreringsklassificeringen af INS- og IF-patienter varierede med valg af teknik, og at der ikke eksisterede nogen overensstemmelse bedre end dén forventet ved tilfældighed mellem standardteknikker (plasma osmolaritet og 24-timers urinvolumen) og nyere teknikker (BIA og BIVA). Det var ikke muligt at konkludere, om ON-patienter blev klassificeret oftere eller færre gange som dehydreret, euhydreret eller overhydreret i sammenligning med HPN-patienter. Yderligere studier med forbedret studiedesign anbefales for at verificere resultaterne.

Abstract

Background: Water is a vital nutrient of life and a multifunctional constituent of the human body thus, especially lack of water (dehydration), but also excessive amounts of water (overhydration) cause various functionally disturbances in the body. Persons with intestinal insufficiency (INS) or intestinal failure (IF) on respectively, oral nutrition (ON) or home parenteral nutrition (HPN) are at risk of abnormal water balance due to altered/impaired gastrointestinal functions. Thus, an important aspect of disease management is therefore proper evaluation of hydration status. However, no single gold standard exists. Techniques such as bioelectrical impedance analysis (BIA) and bioelectrical impedance vector analysis (BIVA) have been presented as new possible alternatives to older techniques used in clinical settings. Though, it is still unknown how well these techniques perform as hydration assessment methods in patients with INS or IF.

Objectives: To evaluate the performance of BIA and BIVA as hydration assessment techniques in INS- and IF-patients with calculated plasma osmolarity and 24-hour urine volume as reference techniques.

Methods: Hydration status was evaluated in 253 metabolic stable patients with either INS (n=125) or IF (n=128) according to calculated plasma osmolarity, 24-hour urine volume, whole-body multi-frequency-BIA and BIVA. Patients were classified by each parameter as either dehydrated, euhydrated, or overhydrated according to specific reference intervals. Correlation between parameters was assessed by Pearson's product-moment correlation while reliability was tested by weighted Kappa (κ_w) with linear weights. Differences between ON- and HPN-patients (i.e. patients with INS and IF, respectively) in regard to demographics and clinically values as well as hydration classification were investigated by Test of two proportions, Independent-samples T-test, and Chi-square test of homogeneity (r x 2)/Fisher's exact test (r x 2) with post hoc test where appropriate.

Results: A statistically significant correlation (p <0.05) was only found between plasma osmolarity and BIA-data as well as BIVA-data, although poor (correlation coefficient ranging from -0.150 to -0.245). Assessment by plasma osmolarity and 24-hour urine volume resulted in a hydration classification that was statistically significant different (p <0,05) between ON- and HPN-patients. Post hoc test could not confirm between which hydration status the difference existed. None of the other assessment techniques could discriminate ON-patients from HPN-patients based on hydration classification (p >0,05). Significant agreement (p <0,05) between techniques was only demonstrated for plasma osmolarity and BIVA as well as plasma osmolarity and BIA, both with an agreement less than the one expected by chance (κ_w <0.0).

Conclusion: The study demonstrated that hydration classification of INS- and IF-patients varied with choice of hydration assessment technique and that no agreement above the one expected by chance existed between standard techniques (plasma osmolarity and 24-hour urine volume) and novel techniques (BIA and BIVA). It was neither possible to conclude if ON-patients where classified more often or fewer times as dehydrated, euhydrated, or overhydrated than HPN-patients. Further studies are recommended with improved study design in order to verify the present study's results.

Acknowledgements

The author of the present study would like to thank all people who have been supportive and helpful during the study period. A special thanks to Medical Laboratory Technologist M. Køhler and Clinical Professor and Chief Physician H.H Rasmussen at Aalborg University Hospital. Thank you for competent supervising, guidance, comments, and for giving me the opportunity to work with you. Finally, a thanks to adjunct senior researcher Steven Brantlov at Aarhus University for kindly lending out A. Piccoli's BIVA software.

Abbreviations

A: cross-sectional area
ADH: antidiuretic hormone
BIA: bioelectrical impedance analysis
BIS: bioelectrical spectroscopy
BIVA: bioelectrical impedance vector analysis
BUN: blood urea nitrogen
Ca^{2+}: calcium
CET: Center for Nutrition and Bowel Disease, Aalborg University Hospital, Denmark
Cl^{-}: chloride
ECF: extracellular fluid
ESPEN: European Society for Clinical Nutrition and Metabolism
FFM: fat-free mass
H: height
$H_2PO_4^{-}$: dihydrogen phosphate
HCO^{3-}: bicarbonate
HPN: home parental nutrition
ICF: intracellular fluid
IF: intestinal failure
INS: intestinal insufficiency
K^{+}: potassium
L: length
MF-BIA: multi frequency bioelectrical impedance analysis
Mg^{2+}: magnesium
Na^{+}: sodium
ON: oral nutrition
PhA: phase angle (radian degree °)
PO_4^{3-}: phosphate
R: resistance (Ω)
RAA: renin-angiotensin-aldosterone
RXc-graf: resistance-reactance graph
SD: standard deviation
SF-BIA: single frequency bioelectrical impedance analysis
SO_4^{2-}: sulfate
TBW: total body water
V: volume
Xc: reactance (Ω)
Z: impedance (Ω)
Zs: Z score
κ_w: weighted kappa
ρ: specific resistivity

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1. Introduction and study aim

Water is a vital nutrient of life and a multifunctional constituent of the human body (Sawka, Cheuvront and Carter, 2005; Jéquier and Constant, 2010). It serves as a building material for cells, acts as a solvent, a reaction medium, a reactant, and a reaction product as well as a carrier, a lubricant, a shock absorber, and a thermoregulator (Jéquier and Constant, 2010). Depending on body composition (fat-free mass (FFM) and fat mass), about 60% of the human body weight is made of water (EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA), 2010; Jéquier and Constant, 2010). Of the total body water (TBW), 65% is intracellular fluid (ICF) and 35% are extracellular fluid (ECF) with the latter further divided into interstitial fluid and blood plasma. The distribution of water is not static but represents the effects of a dynamic ongoing exchange and regulation in the body (Sawka, Cheuvront and Carter, 2005). This regulation is often attributed to the kidneys though, another important, yet forgotten, participant is the gut (Michell, 2000; Chowdhury and Lobo, 2011; Hall, 2011, p. 303).

In the healthy human gut, approximately 98-99% of all water and electrolytes are reabsorbed, leaving only 150 ml of the 8-9 L of daily fluid passing through to be lost in the faeces (Allison, 2004; Macafee, Allison and Lobo, 2005; Chowdhury and Lobo, 2011). The flux of water and electrolytes in the small intestine is connected to the absorption of carbohydrates and in the large bowel to the absorption of short-chain fatty acids thereby linking the flux closely to nutrition (Macafee, Allison and Lobo, 2005). A breakdown in the integrity or absorptive function of the gut will not only have nutritional consequences but also effect water balance and hydration status. Depending on the underlying disease, large volumes of water may be pooled or lost (Allison, 2004; Macafee, Allison and Lobo, 2005).

For patients with intestinal insufficiency (INS) or intestinal failure (IF) this is a reality. Both conditions are characterized by a reduced function or a physical loss of the gut however, IF-patients are set apart from INS-patients by the need of intravenous nutritional supplementation and/or intravenous fluids for the maintenance of health and growth (Pironi *et al.*, 2015; Kappus *et al.*, 2016). Specially IF-patients on long-term parenteral nutrition are a risk of chronic dehydration which further has been linked to the development of renal dysfunction (Lauverjat *et al.*, 2006; Allan and Lal, 2018; Agostini *et al.*, 2019). Other consequences of dehydration are impaired cognition, altered mood status, and fatigue. Indeed, studies have shown that even milder levels of dehydration (lower than 2%

body mass loss) can impair memory and attention (Danone Nutricia Research for the Hydration for Health Initiative, 2018). Besides dehydration, oedema may also occur in INS and IF. It can be caused by malnutrition but also by excessive fluid infusion (Ahmed, Rahman and Cravioto, 2009; Pironi *et al.*, 2018). Thus, monitoring and evaluation of hydration status is a vital point in disease management.

Isotope dilution and neutron activation analysis techniques are widely accepted as the standards for assessment of TBW and body fluid spaces however, they are impractical, time-consuming and expensive in the daily clinical care (Armstrong, 2007). Other techniques include body mass change, plasma osmolality, urine osmolality, urine volume, urine color, and bioelectrical impedance measurements though, several published review papers claim that no single gold standard can be pointed out (Kavouras, 2002; Shirreffs, 2003; Armstrong, 2005, 2007). Instead it is highlighted that the choice of technique should be based on and tailored to the situation and population in which it should be used (Armstrong, 2007).

At the Center for Nutrition and Bowel Disease at Aalborg University Hospital in Denmark common approaches for evaluation of INS- and IF-patients' hydration status include laboratory test of blood for the calculation of plasma osmolarity and collection of 24-hour urine volume. This is done despite other novel approaches such as bioelectrical impedance analysis (BIA) and bioelectrical impedance vector analysis (BIVA) are available at the Center. Because the patient already undergoes bioelectrical impedance measuring for the estimation of FFM it would be convenient for the patient and clinician to use BIA or BIVA to determine the patient's hydration status. However, the performance of these hydration assessment techniques has not yet been examined in patients with INS or IF. Thus, the aim of the present study is to:

"Evaluate the performance of BIA and BIVA as hydration assessment techniques in INS- and IF-patients with calculated plasma osmolarity and 24-hour urine volume as reference techniques".

2. Theory

The current chapter is intended to give the reader a basic physiological knowledge of the different hydration assessment techniques that are used in the present study as well as an understanding of the execution of the various techniques.

2.1 Plasma osmolality

In clinical practice, the concept of water balance refers to the relationship between TBW and body solutes. Thus, one of the most widely used hematological markers of hydration status is plasma osmolality with osmolality defined as milliosmoles of solute per kilogram of solution (mOsm/kg) (Armstrong, 2005; Jéquier and Constant, 2010; Baron *et al.*, 2015; Shah and Mandiga, 2020). In the human body, the cell membrane separates the TBW into ICF and ECF with the latter further separated by the capillary membrane into plasma and interstitial fluid (Allison, 2004). The distribution of water occurs by osmosis, i.e. diffusion of water across a semipermeable membrane from an area of low solute concentration to an area of high solute concentration, until an equilibrium is reached (Cooper and Moore, 1999; Raimann *et al.*, 2017). This means that:

1. In steady state, the osmolality is equal between the compartments.
2. The total amount of solutes in each compartment determines the distribution of TBW (Raimann *et al.*, 2017; Roumelioti *et al.*, 2018).

Certain solutes are distributed almost exclusively in one compartment (i.e. effective osmoles), while others are distributed in all the TBW (i.e. ineffective osmoles) (Cooper and Moore, 1999; Rasouli, 2016; Raimann *et al.*, 2017). Because plasma and interstitial fluid have similar electrolyte contents, the exchange of water between these two compartments is mainly driven by hydrostatic pressure and oncotic or colloid osmotic pressure. The latter due to differences in protein concentration with protein molecules largely remaining within the capillary network (Cooper and Moore, 1999; EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA), 2010). In contrast to this, the exchange of water between ECF and ICF is due to different distribution of effective solutes, mediated by the cell membrane's permeability and transport pumps (Cooper and Moore, 1999; Allison, 2004; Roumelioti *et al.*, 2018). Table 1 gives the approximate concentration of osmolar substances in the ECF and ICF (note that concentration is given as mOsm/L H_2O, i.e. osmolarity and not osmolality. However, in dilute solutions the two terms can be used synonymously since the differences are small (Hall, 2011, p. 291)).

Table 1. Approximate concentration of osmolar substances in extracellular fluid and intracellular fluid. After Hall, 2011, p. 288.

Solute	**Extracellular fluid (mOsm/L H_2O)**		**Intracellular fluid (ICF) (mOsm/L H_2O)**
	Plasma fluid	Interstitial fluid	
Sodium (Na^+)	142	139	14
Potassium (K^+)	4.2	4.0	140
Calcium (Ca^{2+})	1.3	1.2	0
Magnesium (Mg^{2+})	0.8	0.7	20
Chloride (Cl^-)	108	108	4

Bicarbonate (HCO^{3-})	24	28.3	10
Phosphate (PO_4^{3-}) and dihydrogen phosphate ($H_2PO_4^-$)	2	2	11
Sulfate (SO_4^{2-})	0.5	0.5	1
Phosphocreatine	-	-	45
Carnosine	-	-	14
Amino acids	2	2	8
Creatine	0.2	0.2	9
Lactate	1.2	1.2	1.5
Adenosine triphosphate	-	-	5
Hexose monophosphate	-	-	3.7
Glucose	5.6	5.6	Very low
Protein	1.2	0.2	4
Urea	4	4	4
Others	4.8	3.9	10
Total	301.8	300.8	301.2
Corrected osmolar activity**	282.0	281.0	281.0
** = correction has been made because cations and anions exert interionic attraction that can cause a slight elevation in osmotic "activity" of the dissolved substance (Hall, 2011, p. 291).			

The plasma osmolality is set around the point of 280-290 mOsm/kg H_2O with a basal mean of 287 mOsm/kg H_2O in well-hydrated individuals. The osmolality rarely varies by more than 2% due to a complex interaction of regulatory processes, enzymes, receptor responses and hormones (Cooper and Moore, 1999; Armstrong, 2005; Jéquier and Constant, 2010). The renal regulation of sodium (Na^+) and water is one of the most important mechanisms which involves vasopressin or antidiuretic hormone (ADH) released from the hypothalamus, the renin-angiotensin-aldosterone (RAA) system, and the atrial natriuretic hormone (Cooper and Moore, 1999). The control is so tight that a rise in plasma osmolality of only 1-2% stimulates the hypothalamus to secrete ADH which in turn stimulates thirst and the urge to drink as well as the reabsorption of water from the renal distal tubules and collecting ducts (Cooper and Moore, 1999; Grandjean and Campbell, 2004; Baron *et al.*, 2015).

2.1.1 Measured plasma osmolality

Direct measurement of plasma osmolality in the laboratory is done by use of a freezing point depression osmometer or, more rarely a vapor pressure depression osmometer (Armstrong, 2005; Baron *et al.*, 2015). The cut-off value for dehydration may variate between laboratories. Some define the limit between euhydration and dehydration as 290 mOsm/kg H_2O (Baron *et al.*, 2015) however, many Danish hospitals uses 300 mOsm/kg H_2O as cut-off (Arre, 2017; Bergstedt, 2018; Ladefoged, 2020; Region Sjælland, 2020).

2.1.2 Calculated plasma osmolarity (osmolality)

If plasma osmolality is unable to be measured directly, it can be replaced with calculated plasma osmolarity (Baron *et al.*, 2015; Raimann *et al.*, 2017). In contrast to osmolality (mOsm/kg), osmolarity is defined as milliosmoles of solutes per liter of solution (mOsm/L) and can be calculated

by the arithmetic summation of concentrations of osmotically active solutes (Rasouli, 2016; Raimann *et al.*, 2017). Plasma osmolality and osmolarity is related by plasma water as:

$$Osmolality = \frac{Osmolarity}{plasma\ water} = \frac{Osmolarity}{0.93},$$

since the content of water in plasma is approximately 0.930 kg water/L plasma (Rasouli, 2016). In clinical practice the numerical values of osmolarity do not differ significantly from those of osmolality and the 2 terms are used synonymously (Gennari, 1984; Šklubalová and Zatloukal, 2010).

In plasma the osmolar concentration is primarily defined by 5 major osmoles: sodium (Na^+), chloride (Cl^-), bicarbonate (HCO_3^-), glucose, and urea (Koeppen and Stanton, 2013; Rasouli, 2016). Though, Cl^- and HCO_3^- are rarely used, instead only Na^+ is used because sodium ions are counter balanced by the chloride and bicarbonate anions (Rasouli, 2016).

Throughout the years more than 37 equations have been developed for the calculation of plasma osmolality. This has contributed to variation and uncertainty when comparing research results (Choy *et al.*, 2016; Raimann *et al.*, 2017). Thus, in the last 10 years several studies have been conducted in order to find the best equation and thereby enhance harmonization (Fazekas *et al.*, 2013; Siervo *et al.*, 2014; Hooper, Abdelhamid, Ali, *et al.*, 2015; Martín-Calderón *et al.*, 2015; Choy *et al.*, 2016). The following equation by Khajuria and Krahn (Khajuria and Krahn, 2005) has been pointed out as a superior equation in several studies (Heavens *et al.*, 2014; Siervo *et al.*, 2014; Hooper, Abdelhamid, Ali, *et al.*, 2015; Martín-Calderón *et al.*, 2015) and is further recommended by European Society for Clinical Nutrition and Metabolism (ESPEN) in their 2019 guideline on clinical nutrition and hydration in geriatrics (Volkert *et al.*, 2019):

$$Plasma\ osmolarity = 1.86 \times (Na^+ + K^+) + 1.15 \times glucose + urea + 14\ (all\ measured\ in\ mmol/L)$$

2.3 Urine volume

Every day the body loses water which must be regained in order to stay euhydrated (Danone Nutricia Research for the Hydration for Health Initiative, 2017). As illustrated in figure 1, the main water input comes from ingestion of fluids (and food) while water loss is due to several mechanisms. Off all these mechanisms (insensible water loss, sweat, stools, and urine), urine is by far the most controlled and active player in the regulation of the body's water balance. In fact, water that are lost by evaporation, sweat, and stools are unregulated and happens irrespectively of the body's water status. In contrast to this, the amount of excreted urine is the result of the kidneys 2 major functions; excretion of solute

wastes and regulation of body fluid volumes (Kavouras, 2002; Hall, 2011, p. 345; Danone Nutricia Research for the Hydration for Health Initiative, 2017).

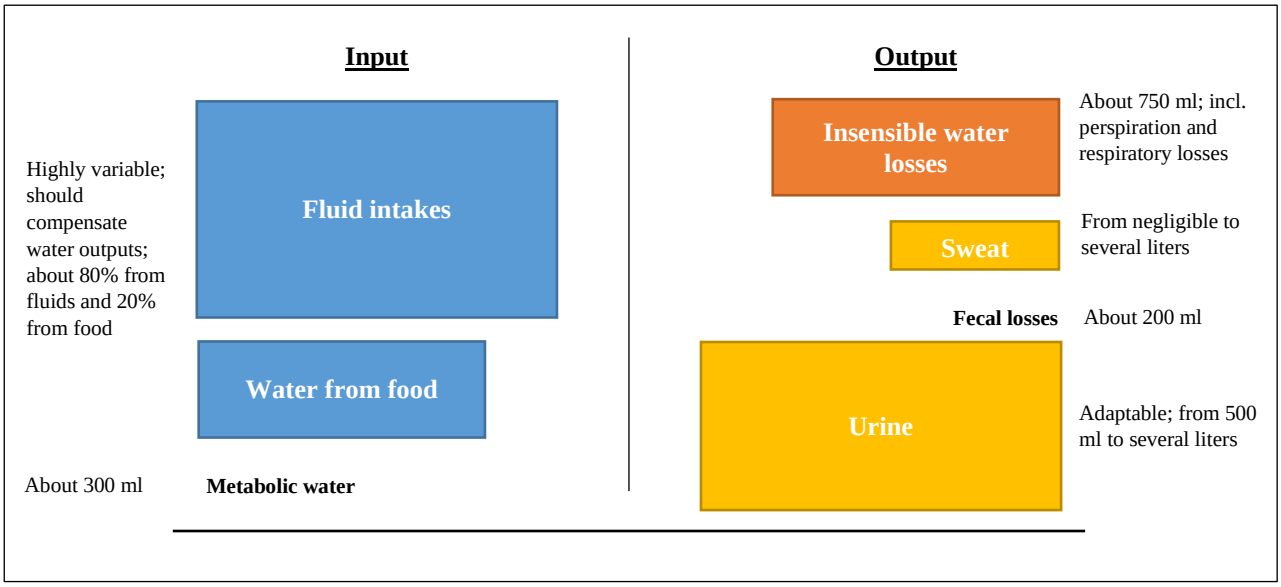

Figure 1. Typical water inputs and outputs per day in a healthy adult. Made with inspiration from Danone Nutricia Research for the Hydration for Health Initiative, 2017.

Under normal circumstances the kidneys filter more than 150 L fluid per day though, less than 1% is actually secreted into the urine leading to an urine volume of only 1.5 L/day (Kavouras, 2002; Tack, 2010). In large excess of water, the kidneys are able to excrete as much as 20 L of urine per day while in cases of low water supply, the kidneys will conserve water and only excrete a minimal obligatory volume (about 500 ml) in order to get rid of excess solutes (Hall, 2011, pp. 345–347; Danone Nutricia Research for the Hydration for Health Initiative, 2017). This high range in urine volume is due to the kidneys ability to produce urine with a concentration ranging from as low as 50 mOsl/L to as high as 1200-1400 mOsm/L (Danone Nutricia Research for the Hydration for Health Initiative, 2017). A high level of ADH and a high osmolarity of the renal medullary interstitial fluid are the basic requirements for the formation of concentrated urine (Hall, 2011, pp. 347–348). ADH's connection to plasma osmolality/osmolarity has already been explained in section *2.1 Plasma osmolality*, however, other factors such as low blood volume, blood pressure, nausea, morphine, and nicotine do also stimulate the secretion of ADH (Hall, 2011, p. 357). The formation of high osmolarity of the renal medullary interstitial fluid is due to the function of the countercurrent mechanism. This mechanism depends on the special anatomic arrangement of different parts of the kidney (Hall, 2011, p. 348) which will not be discussed further since it is out of the present rapport's scope.

A urine volume about 100 ml/hour indicates a hydrated state while higher outputs of 300-600 ml/hour and lower outputs under 30 ml/hour most likely indicates excess fluid intake and dehydration, respectively (Grandjean and Campbell, 2004).

The use of urine volume as a marker of hydration is an inexpensive method though, it has been criticized for its inconvenience of 24-hour collection as well as the potential sample loss. Additionally, it may mirror recent volume of consumed fluid rather than hydration status and because of age decline in renal functions, it may not be well suited in older adults (Grandjean and Campbell, 2004; Armstrong, 2007; Jéquier and Constant, 2010).

2.4 Bioelectrical impedance analysis (BIA)

BIA is a technique that measures tissue conductivity and exploits that electrolyte-rich water has a lower resistant to the passage of an electrical current. This means that conductivity will be proportional to water-rich tissue and TBW. Explained in other words; lipid-rich adipose tissue and bones are poor conductors due to low water content whereas lean tissue is a good electrical conductor because of high water content (about 73%) (Heymsfield *et al.*, 2005, p. 81; Fosbøl and Zerahn, 2015; Buckinx *et al.*, 2018; Kuriyan, 2018; Dyhre-Petersen, 2019).

BIA is carried out by attaching surface electrodes to the body (often in a tetrapolar arrangement). In order to avoid gravity pooling body water in the legs while standing, the subject being measured is asked to lay down in a supine position (Heymsfield *et al.*, 2005, p. 81). A "detector" electrode is placed at the wrist and at the ipsilateral ankle while a "current" electrode is placed near each detector thereby allowing an alternating electrical current to enter the body and to be detected. A minimum of 4-5 cm between each "current" electrode and "detector" electrode is preferred to avoid electrical interference (Heymsfield *et al.*, 2005, p. 81; Fosbøl and Zerahn, 2015; Dyhre-Petersen, 2019). This setup is the most common but other setups are possible to (Earthman, 2015).

The bioelectrical impedance device measures 4 primary components; resistance (*R*), reactance (*Xc*), impedance (*Z*) and phase angle (*PhA*) (Ceniccola *et al.*, 2019; Lukaski *et al.*, 2019). Impedance is determined by the relationship between resistance and reactance according to: $Z^2 = R^2 + Xc^2$. The frequency of the electrical current determines the values of *R* and *Xc*. As illustrated in figure 2, the value of *Z* will equal *R*, and *Xc* will be zero if the frequency is low. Because there are different current pathways within the body and some of these retards the current more than others, a reactance will occur as frequency increases. I.e. the value of *Xc* will increase with increasing frequency until at a

specific frequency at which *Xc* will reach its maximum. This specific frequency depends on the conductor (i.e. the subject's body). As the frequency continues to increase, *Xc* will fall and *Z* will again be equal to *R* only. When the frequency changes from low to high, an angle is created between *Z* and *R*. This angle is called *PhA* and is the arctangent of the ratio of resistance, i.e.: $\tan^{-1}(\frac{XC}{R}) \times (\frac{180}{\pi})$ expressed in radian degrees (Heymsfield *et al.*, 2005, pp. 79–80; Dyhre-Petersen, 2019; Lukaski *et al.*, 2019).

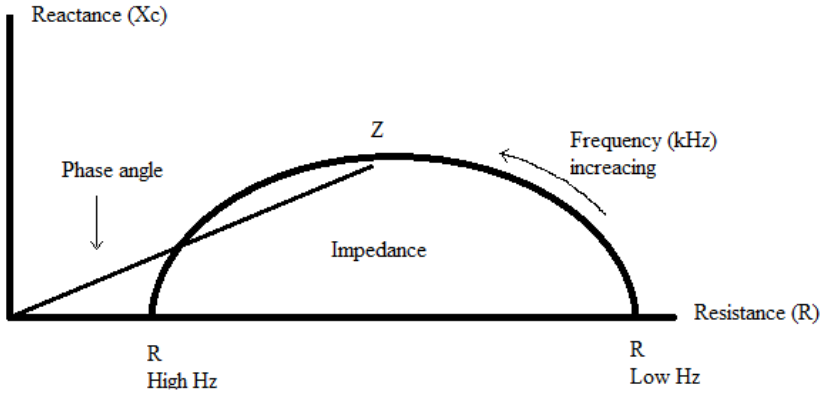

Figure 2. Impedance (Z) plot curve of resistance (R) and reactance (Xc) with frequency. After Heymsfield et al., 2005, p. 79, in Dyhre-Petersen, 2019.

The use of BIA in body hydration assessment is due to the existence of geometrical relationships between a conductor's shape and *R* (Heymsfield *et al.*, 2005, p. 80; Lukaski *et al.*, 2019). The length of the conductor, its cross-sectional area, and material type will determine *R* (Khalil, Mohktar and Ibrahim, 2014). The material type is described by a specific resistivity that is an electrical property of a homogenous conductor independently of the conductor's length and cross-sectional area. A long conductor will have a greater *R* than a short one because the resistance is proportional to the length of the conductor. Furthermore, *R* is inversely proportional to the conductor's cross-sectional area. This means that a conductor with a small cross-sectional area will have the greatest *R*. This is evident by the following equation: $R = \rho \times \frac{L}{A}$, where ρ is the specific resistivity, *L* is the length, and *A* is the cross-sectional area (Heymsfield *et al.*, 2005, p. 80; Dyhre-Petersen, 2019). The equation of the volume of a conducting cylinder can be applied if the human body is seen as a cylinder with a uniform cross-sectional area and a homogenous composition (Heymsfield *et al.*, 2005, p. 80; Fosbøl and Zerahn, 2015; Lukaski *et al.*, 2019):

$$\text{Conductor volume (V)} = \text{length } (L) \times \text{area } (A)$$

$$A = \frac{V}{L}$$

$$\text{Resistance } (R) = \rho \times \frac{L}{A} = \rho \times L \times \frac{L}{V}$$

$$V = \rho \times \frac{L^2}{R}$$

Since the human body violates the assumptions for the use of the above equation, it cannot be used directly to calculate the volume of TBW. Instead, the impedance index ($\frac{L^2}{R}$) is used with standing height (H) as a biological surrogate for L in combination with other anthropometric information (weight, age, and gender) to format multiple regression prediction equations for TBW (Heymsfield *et al.*, 2005, p. 80; Fosbøl and Zerahn, 2015; Lukaski *et al.*, 2019).

Today, different types of bioimpedance devices exist; single-frequency BIA (SF-BIA), multi-frequency BIA (MF-BIA), and bioelectrical spectroscopy (BIS) (Teigen *et al.*, 2017; Dyhre-Petersen, 2019). As indicated by their names, SF-BIA uses a single frequency to measure impedance while MF-BIA uses multiple frequencies. The two are primary set apart by the ability to distinguish the distribution of body water into ICF and ECF – an ability that only belongs to MF-BIA (Heymsfield *et al.*, 2005, p. 84; Dyhre-Petersen, 2019). BIS uses an entire spectrum of frequencies from 5-1200 kHz that, instead of being applied to a regression equation, undergoes complex modeling and thereafter is applied to complex algorithms in order to predict TBW and ECF (Lukaski and Piccoli, 2012; Teigen *et al.*, 2017).

BIA has been appreciated as a non-invasive, safe, practical, simple, and less-expensive technique in comparison to other approaches such as isotope dilution and neutron activation analysis (Martinoli *et al.*, 2003; Armstrong, 2007; Jaffrin and Morel, 2008; Lukaski and Piccoli, 2012). However, the technique is indirect and suffers from both technical and biological limitations (Jaffrin and Morel, 2008; Lukaski *et al.*, 2019). Possible sources of error have been pointed out: validity (accuracy and precision) of the impedance measurement (see Appendix 1 for a list of key items in order to enhance validity and standardization of impedance measurements), electrical-volume errors, inter-individual differences (biological variability in the diameter of body segments, limb lengths, and body fatness), and last but not least error of prediction from the regression equation (Lukaski *et al.*, 2019). Because regression equations are made by regressing the impedance index (and other variables such as age, weight, sex etc.) against TBW that has been obtained from a reference method (Fosbøl and Zerahn,

2015), the regression equations yield errors from the reference method (Lukaski *et al.*, 2019). Furthermore, this exclude the use of the equations in any sample or individual differing in characteristics from the original sample in which the equations were made (Jaffrin and Morel, 2008; Norman *et al.*, 2012; Lukaski *et al.*, 2019). Thus, the use of BIA in point-of-care individual assessments of hydration has been doubted (Lukaski *et al.*, 2019).

2.5 Bioelectrical impedance vector analysis (BIVA)

BIVA is the direct use of raw bioimpedance measurements in a resistance-reactance (*RXc)* graph; an approach developed by Piccoli et al. (Piccoli *et al.*, 1994) (Norman *et al.*, 2012). This method enables classification and ranking of changes in hydration as well as soft tissue mass by comparing vector position to a healthy ethnicity-, age-, and sex-matched population (Lukaski *et al.*, 2019).

In practice, the *RXc*-graph is made by plotting the impedance parameters *R* and *Xc* as a bivariate vector normalized by the subjects height (i.e. $\frac{R\ (\Omega)}{H(m)}$ and $\frac{Xc\ (\Omega)}{H(m)}$). The length of the vector is inversely related to TBW, and in combination with the vector's direction, defined by the *PhA*, the vector will provide information about hydration status and body cell mass (see figure 3) (Lukaski and Piccoli, 2012; Norman *et al.*, 2012; Lukaski *et al.*, 2019). Tolerance ellipses are plotted in the graph, representing 50%, 75% and 95% of reference values thereby allowing a subject's vector to be ranked and classified immediately (Norman *et al.*, 2012). A vector within the 50% tolerance ellipse indicates normal hydration while lengthening/shortening of the vector from 51% to 75% and >76% percentile tolerance ellipses in the upper/lower range indicates, respectively a moderate and severe dehydration/fluid overload (Lukaski *et al.*, 2019). Migration of the vector sideways indicates a decrease or increase in mass of soft tissue (Norman *et al.*, 2012). Besides classification of a single individual, a group of subjects can also be portrayed in the *RXc*-graph as a mean vector. Instead of tolerance ellipses, a 95% confidence ellipse is plotted in order to describe the mean vector (Norman *et al.*, 2012).

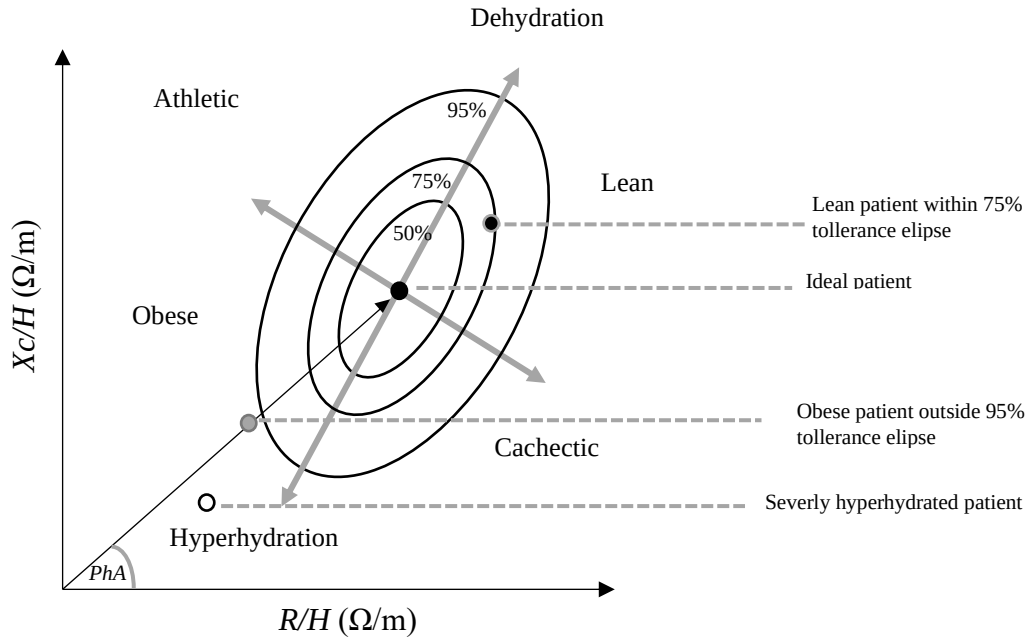

Figure 3. Example of RXc-graf with 50%, 75%, and 95% tolerance ellipses. Different vector positions indicate different body compositions however, they can theoretically produce the same phase angle (PhA). Longitudinal changes in hydration and cell mass are thus interpreted more reliably by BIVA than PhA alone. The graph is adapted from Norman et al., 2012.

In addition to the *RXc*-graph it is also possible to create a *RXc*-score graph (see figure 4). By transforming *R/H* and *Xc/H* to bivariate Z-scores (here denoted *Zs* to avoid confusion with the accepted symbol of impedance (*Z*)):

$$Zs(R) = \frac{R/H - mean}{SD} \text{ and } Zs(Xc) = \frac{Xc/H - mean}{SD},$$

where mean and SD is the one of the *R/H* and *Xc/H* of the reference population. Tolerance and confidence ellipses are based on standard reference intervals thus, allowing the graph to be used with any analyzer in any population (Piccoli and Pastori, 2002).

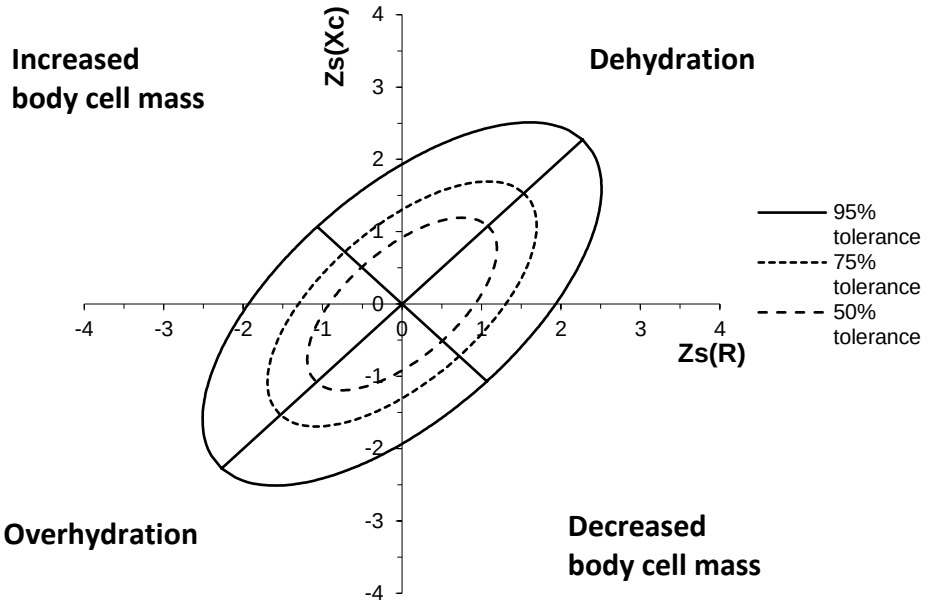

Figure 4. Example of RXc-score graph with tolerance ellipses of the 50th, 75th, and 95th percentile standard reference intervals. Long or short lengths of the vector are related to dehydration (upper right quadrant) and overhydration (lower left quadrant), respectively. Migration sideways of the vector is related to increased body cell mass (upper left quadrant) and decreased body cell mass (lower right quadrant). The graph is made with inspiration from Brantlov et al., 2019.

The error of BIVA is only associated with bioelectrical impedance measurement and reproducibility (1-2%). Furthermore, this approach has the advantage of being independent of regression equations. Also, the detection and ranking of changes in hydration status by BIVA has been found to be <500 ml in real-time (Lukaski *et al.*, 2019). Thus, BIVA has emerged as a promising tool for assessment and monitoring of patients (Norman *et al.*, 2012).

For the use of BIVA important technical concerns must be considered (Lukaski *et al.*, 2019). Proper derivation and implementation of BIVA requires all measurements to be obtained from a phase-sensitive bioelectrical impedance device since measurements from a non-phase-sensitive instrument can cause an 8-10% repositioning of vectors. In addition to this, the use of high-impedance electrodes has been found to lead to misclassification of hydration status (Lukaski *et al.*, 2019).

2.6 Advantages and disadvantages of techniques

The table below gives a resumé of advantages and disadvantages of the aforementioned hydration assessment techniques:

Table 2. Advantages and disadvantages of selected hydration assessment techniques.

Hydration Assessment Techniques	Advantages	Disadvantages	References
Calculated plasma osmolarity	Quick; No need of osmometer.	Invasive; Indirect measurement; Results depend upon used equation.	(Hooper, Abdelhamid, Ali, *et al.*, 2015)
24-hour urine volume	Inexpensive; Appropriate for field research.	Inconvenience of 24-hour collection; Potential sample loss; Less suitable in older adults due to age decline in renal functions; May mirror recent volume of consumed fluid rather than hydration state; High within subject variation.	(Grandjean and Campbell, 2004; Armstrong, 2007; Jéquier and Constant, 2010)
Bioelectrical impedance analysis (BIA)	Non-invasive; Quick, Portable.	Protocol-related difficulties (for example fasting, optimal position of electrodes, and effect of posture); Indirect; Results are dependent on model assumptions and population specific equation.	(Thomas, Ward and Cornish, 1998; Vaché *et al.*, 1998; Kyle *et al.*, 2004a; Fosbøl and Zerahn, 2015)
Bioelectrical impedance vector analysis (BIVA)	Non-invasive; Quick; Portable; In comparison to BIA it is only affected by impedance measurement errors and biological variability of subjects.	Same protocol-related difficulties as for BIA; Allows only classification and ranking of hydration and not quantification of fluid volume; Need of population references.	(Kyle *et al.*, 2004a; Lukaski *et al.*, 2019)

3. Methods

3.1 Study design

The present study was a retrospective, comparative, analytical, cross-sectional, database study of consecutively recruited, clinically stable INS- and IF-patients on oral nutrition (ON) and home parenteral nutrition, respectively. The study was conducted in collaboration with the Center for Nutrition and Bowel Disease (CET), Aalborg University Hospital, Denmark, during the period of February to May 2020.

3.2 Study population

The study population consisted of 253 patients selected from a pseudonymized patient data base obtained by CET at Aalborg University Hospital in Denmark. The data base included a total of 540 metabolically stable INS- and IF-patients that had been recruited consecutively during the period of 2010-2019. Figure 5 shows the participant screening and enrollment in the present study.

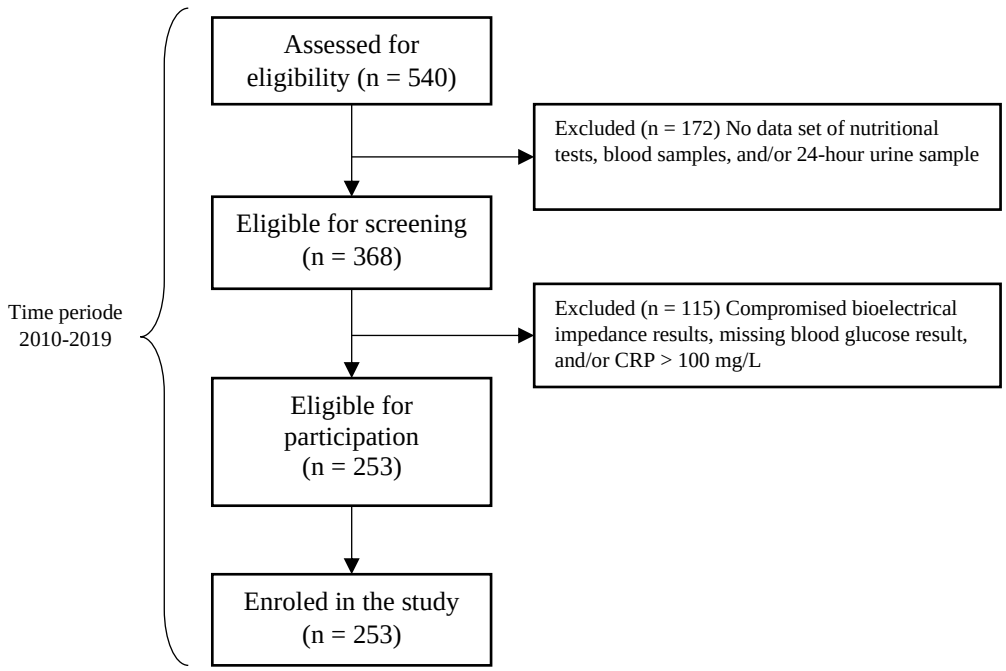

Figure 5. Participant screening and enrollment.

3.3 Ethical concerns

Informed consent of participants was not required since all data originated from a pseudonymized data base thus, maintaining the patients' confidentiality. Furthermore, the participation in the present study did not involve any additionally tests or measurements. The data collection, storage and analyzing was approved by the Danish Data Protection Agency, Northern Denmark Region, (journal no.: 2019-49).

3.4 Anthropometry

Body weight and height were measured prior to bioelectrical impedance measurements by trained personnel. Weight was measured by digital electronic scale (Seca 701) to the nearest 0.1 kg with light indoor clothes and no shoes. Standing height was measured by a wall-mounted stadiometer (Seca 222) to the nearest 0.1 cm, barefooted. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2).

3.5 Biochemistry

Natrium-, sodium-, potassium-, glucose-, and urea carbamide-values were derived from venous blood samples by accredited hospital biomedical personnel. All samples were analyzed by standard methods

by use of Roche-Cobas 6000/8000 (Roche Diagnostics, Basel, Switzerland). Plasma osmolarity was calculated by the equation of Khajuria and Krahn:

$Plasma\ osmolarity = 1.86 \times (Na^+ + K^+) + 1.15 \times glucose + urea + 14\ (all\ measured\ in\ mmol/L)$ (Khajuria and Krahn, 2005).

Twenty-four-hour urine volume was collected by the patient at home according to instructions provided by the Department of Clinical Biochemistry at Aalborg University Hospital, Denmark.

Blood samples and 24-hour urine volume were obtained within 1 month of anthropometric and bioimpedance measurements.

3.6 Bioelectrical impedance measurement

Bioelectrical impedance measurements were obtained by a whole-body multi-frequency analyzer Bio-Scan 920-II (Maltron, Essex, UK) the same day as anthropometric assessments and by the same trained researcher in order to ensure accuracy across patients. Eight-hour retaining from physical activity, minimum 4-hours of fasting (water allowed until 2 hours before measurement), and voided bladder was required of the patient before measurement. The assessment was performed with the patient laying down in a supine position with legs separated approximately 45° and arms approximately 30° away from the torso at a non-conducting bed. Adhesive electrodes were placed in a standard tetra-polar arrangement on the patient's right side, on the surface of the dorsal hand, wrist, foot, and ankle. A 10 minutes rest on the bed was given before start of measurement to allow body water to accumulate evenly in the body (Dyhre-Petersen, 2019). Raw impedance data (*Z*, *PhA*, *R*, and *Xc*) were measured at 50 kHz.

3.6.1 BIA

Fat mass, FFM, TBW, ECF, and ICF were determined by the multi-frequency analyzer BioScan 920-II (Maltron, Essex, UK) according to undisclosed proprietary calculations of the manufacture.

3.6.2 BIVA

Raw bioelectrical impedance data measured at 50 kHz (*PhA*, *R*, and *Xc*) were used to generate *RXc*-graphs and *RXc*-score graphs by use of BIVA Software, developed by A. Piccoli and G. Pastori (Piccoli and Pastori, 2002). For the *RXc*-graph, *R* and *Xc* were normalized by the subject's height (*R/H* and *Xc/H*, both in Ω/m) and plotted as an individual impedance vector (a point) in the *RXc*-graph with 50th, 75th, and 95th percentile tolerance ellipses. For male and female subjects the tolerance

ellipses were based on respectively, an Italian male reference population of 354 white males, age 16-85, BMI 16-31, and an Italian female reference population of 372 white females, age 16-85, BMI 16-31 (Piccoli *et al.*, 1995). To ease the interpretation and allow all vectors to be plotted together independently of sex, normalized vector components (*R/H* and *Xc/H*) were transformed into bivariate Z-scores (*Zs*, no unit) using the mean and SD of the sex-specific reference population (i.e. *Zs(R)* = (*R/H* – 371.9)/49 if female and (*R/H* – 298.6)/43.2 if male, and *Zs(Xc)* = (*Xc/H* – 34.4)/7.7 if female and (*Xc/H* - 30.8)/7.2 if male). The vectors where then plotted in a *RXc*-score graph with tolerance ellipses of 50th, 75th, and 95th standard reference intervals (Piccoli and Pastori, 2002).

3.7 Statistical data analysis

Descriptive statistics were expressed as number and percentages or mean ± SD where appropriate. Unless other described, a level of 0.05 was used as statistically significant level i.e. stating statistically significance at the $p = < 0.05$ level. Differences in demographics and clinically characteristics between ON- and HPN-patients were determined by the test of two proportions (chi-square test for homogeneity) when the dependent variable was a dichotomous variable and by the independent-samples T-test when the dependent variable was a continuous variable. A Pearson product-moment correlation was applied to determine the existence of a linear relationship between hydration assessment parameters (plasma osmolarity, 24-hour urine volume, TBW (%), TBW (L), *PhA*, *R/H*, and *Xc/H*). Evaluation and classification of patients' hydration status was done by a 3-point system (1 = dehydration, 2 = euhydration, 3 = overhydration) according to reference values of each specific hydration assessment technique (see table 5 in section *4.3 Classification of hydration status*). Distribution differences of dehydrated, hydrated, and overhydrated between ON- and HPN-patients were assessed by a Chi-square test of homogeneity (r x 2) or Fisher's exact test (r x 2) if the assumption of minimum expected counts was violated. The agreement of hydration classification between the different hydration assessment techniques was evaluated by Weighted kappa (κ_w) with linear weights with post hoc test where appropriate. All statistical analyses were done by the software IBM SPSS Statistics version 26 for Windows (SPSS Inc, Chicago, IL) with all test assumptions being met otherwise stated. An elaboration of each statistical analysis and its assumptions is given below:

Test of two proportions (chi-square test for homogeneity):

The test of two proportions requires a sufficiently large sample size to produce a valid result. Although it is not strictly an assumption, it was checked by making a cross table with expected frequencies. If all cells of the 2 x 2 cross table had an expected frequency greater than or equal to 5, the sample size was considered to be sufficient (Hollander and Wolfe, 1999; Laerd Statistics, 2016a).

Independent-samples T-test:

The assumption of no significant outliers was tested by visual inspection of a boxplot with values greater than 1.5 box-lengths from the edge of the box defined as outliers (Laerd Statistics, 2015). Normal distribution of the dependent variable for each group of the independent variable (i.e. ON- and HPN-patients) was determined by inspection of Q-Q plots and Shapiro-Wilk's test with a statistically significance of $p < 0.05$ indicating violation of normality (Laerd Statistics, 2015). The assumption of homogeneity of variances was assessed by Levene's Test of Equality of Variances with a statistically significance of $p < 0.05$ indicating violation of the assumption (Laerd Statistics, 2015). The modified t-test, i.e. the Welch t-test, was used when the assumption of homogeneity of variances was not met.

Pearson product-moment correlation:

The assumption of linear relationship between continuous variables was determined by visual inspection of a scatterplot along with identification of outliers. Normal distribution was tested by inspection of Q-Q plots and Shapiro-Wilk's test with a statistically significance of $p < 0.05$ indicating violation of normality (Laerd Statistics, 2018). Pearson's correlation coefficient value was reported to indicate the strength and direction of the association between variables. A value of +1 or -1 was considered to indicate a perfect positive or negative association, respectively, while a value of zero indicated no association at all. I.e. the closer to +1 or -1, the stronger the association (Laerd Statistics, 2018).

Chi-square test of homogeneity (r x 2)/Fisher's exact test (r x 2):

The sample size adequacy assumption was evaluated by checking that no more than 20% of the cells of the produced contingency table had expected frequencies of 5 or less and that no cells had expected frequencies less than 1 (Cochran, 1954; Laerd Statistics, 2017). If the assumption was not met the Fischer's exact test (r x 2) was performed instead. Because both test types were omnibus tests, post hoc testing was carried out for cases that were statistically significant. The post hoc test called z-test of two proportions was used for Chi-square test of homogeneity (r x 2) while the multiple Fisher's exact tests (2 x 2) was used for Fisher's exact test (r x 2) (Laerd Statistics, 2017). Bonferroni adjustment was applied to both post hoc tests to correct for multiple comparisons. Thus, statistical significance was declared if $p < 0.16667$ instead of $p < 0.05$ because the number of pairwise comparisons was 3 in the present study. This may lead to a conservative and overly stringent *p*-value in order to decrease the risk of making a Type I error (i.e. "false" statistically significant result) (Laerd Statistics, 2017).

Weighted kappa (κ_w):

The weighted kappa test was carried out with linear weights meaning that penalties for disagreement between categories (i.e. in the present study: dehydrated, euhydrated, and overhydrated) were equally weighted. The value of weighted kappa (κ_w) was reported together with 95% confidence intervals and *p*-value. The minimal possible value of κ_w (-1) was interpreted as no observed agreement, negative values were interpreted as less than the agreement expected by chance, and a value of zero as an agreement no better than chance. Increasingly values greater than zero indicated increasing better-than-chance agreement with +1 as maximum, indicating perfect agreement (Laerd Statistics, 2016b).

4. Results

4.1 Demographics and clinically characteristics

In total 253 metabolically stable INS- and IF-patients from the Center for Nutrition and Bowel Disease at Aalborg University Hospital in Denmark participated in the study. The patients' age ranged from 15-86 years, with a mean of 59.7 ± 15.3 years. The patients were divided into two groups based on their nutrition, i.e. ON and HPN. The distribution of patients in the ON-group versus patients in the HPN-group is presented in table 3 along with demographics and clinically characteristics. Numbers in bold with a * indicate a statistically significant difference between the 2 groups.

Table 3. Demographics and clinically characteristics of study participants. Data are presented as mean ± Standard Deviation (SD) or percent (%). Differences in proportions between participant on oral nutrition (ON) and on home parenteral nutrition (HPN) were assessed for all parameters. Statistically significant cases and violation of test assumptions are highlighted – consult table description.

Demographics	**Oral nutrition (ON) (n = 125)**	**Home parenteral nutrition (HPN) (n =128)**	**Total (n = 253)**
Female	70 (56.0%)	71 (55.5%)	141 (55.7%)
Male	55 (44.0%)	57 (44.5%)	112 (44.3%)
Total	125 (49.4%)	128 (50.6%)	253 (100%)
Age (years)	60.2 ± 15.7 [a]	59.2 ±15.0 [a, b]	59.7 ± 15.3
Height (cm)	169.6 ± 9.7	168.6 ± 9.3	169.1 ± 9.5
Weight (kg)	**66.2 ± 16.3 *** [a, b]	**61.1 ± 14.8 *** [a, b]	63.6 ± 15.6
BMI (kg/m^2)	**22.9 ± 4.6 *** [a, b]	**21.4 ± 4.1 *** [a, b]	22.1 ± 4.4
Fat mass (kg)	**18.8 ± 8.9 *** [a, b]	**16.1 ± 8.6 *** [a, b]	17.5 ± 8.8
Fat mass (% of weight)	**29.7 ± 8.5 *** [a, c]	**23.5 ± 7.6 *** [a, b, c]	26.6% ± 8.7
FFM (kg)	47.4 ± 10.0 [a, b]	45.0 ± 9.3 [a]	46.2 ± 9.7
FFM (% of weight)	**70.4 ± 8.4 *** [a, c]	**76.8 ± 7.3 *** [a, b, c]	73.6 ± 8.5
Blood data			
Na^+ (mmol/L)	**139.2 ± 3.8 *** [a, b]	**137.7 ± 3.7 *** [a]	138.5 ± 3.8
K^+ (mmol/L)	4.0 ± 0.5 [a, b]	4.0 ± 0.5	4.0 ± 0.5
Glc (mmol/L)	**6.3 ± 1.7 *** [a, b]	**6.7 ± 1.6 *** [a, b]	6.5 ± 1.7
Urea Carb (mmol/L)	7.0 ± 4.1 [a, b]	7.9 ± 5.3 [a, b]	7.4 ± 4.8
Plasma osmolarity (mOsm/L)	294.6 ± 7.7 [b]	293.2 ± 8.1 [a, b]	293.9 ± 7.9
Urine data			
24-hour urine volume (ml)	1315.1 ± 820.1 [a, b]	1481.5 ± 797.6 [a, b]	1399.3 ± 811.5
BIA data			
TBW (L)	34.7 ± 7.3 [a, b]	33.0 ± 7.6 [a, b]	33.8 ± 7.7
TBW (% of weight)	**51.0 ± 6.4** *	**56.3 ± 6.3 *** [a, b]	53.7 ± 6.8
ECF (L)	16.0 ± 3.3 [a, b]	15.3 ± 4.7 [a, b]	15.6 ± 4.2
ECF (% of TBW)	**46.6 ± 3.2 *** [a, c]	**44.9 ± 3.4 *** [a, b, c]	45.7 ± 3.4
ICF (L)	18.8 ± 4.2 [a, b]	18.0 ± 4.7 [a, b]	18.4 ± 4.5
ICF (% of TBW)	**53.4 ± 3.2 *** [a, c]	**54.6 ± 3.9 *** [a, b, c]	54.0 ± 3.6
ECF/ICF (L)	0.85 ± 0.1 [a]	0.85 ± 0.1 [a, b]	0.85 ± 0.1

BIVA data			
Z (Ω)	**582.5 ± 103.2 ***	**619.9 ± 107.2 ***	601.4 ± 106.7
PhA (degrees)	**7.2 ± 1.3 ***	**6.7 ± 1.3 ***	7.0 ± 1.3
R (Ω)	**578.1 ± 102.8 ***	**614.3 ± 113.2 *** [b]	596.4 ± 109.5
Xc (Ω)	72.4 ± 16.8	72.6 ± 17.6	72.5 ± 17.2
R/H (Ω/m)	**343.2 ± 70.2 ***	**366.8 ± 76.7 *** [b]	355.1 ± 74.4
Xc/H (Ω/m)	42.9 ± 10.7 [a]	43.2 ± 11.0	43.1 ± 10.8
Zs(Xc)	**0.5 ± 1.2 ***	**0.1 ± 1.3 *** [a, b]	0.3 ± 1.3
Zs(R)	**1.6 ± 1.5 *** [a, c]	**1.2 ± 1.2 *** [b, c]	1.4 ± 1.3

[a] = not normally distributed; [b] = one or more outliers; [c] = equal variances not assumed, i.e. violation of assumption of homogeneity of variances; Numbers in bold with a * = statistically significant difference at the *p*-value < 0.05; BMI = body mass index; FFM = fat-free mass; Na^+ = sodium; K^+ = potassium; Glc = glucose; Carb = carbamide; BIA = bioelectrical impedance analysis; TBW = total body water; ECF = extracellular fluid; ICF = intracellular fluid; BIVA = bioelectrical impedance vector analysis; *Z* = impedance; *PhA* = phase angle; *R* = resistance; *Xc* = reactance; *Zs* = Z score; H = height; ON = oral nutrition; HPN = home parenteral nutrition.

4.2 Correlation between hydration assessment parameters

A Pearson product-moment correlation was carried out to determine if a linear relationship existed between the reference parameters (plasma osmolarity and 24-houre urine volume) and any of the essential parameters of BIA (TBW (%) and TBW (L)) and BIVA (*PhA*, *R/H*, and *Xc/H*). Pearson's correlation coefficient is listed in table 4 with statistically significant correlation coefficients highlighted in bold with *.

Table 4. Correlation coefficient for Pearson's product moment correlation of hydration assessment techniques. Statistically significant cases and violation of test assumptions are highlighted – consult table description.

Correlation coefficient for Pearson's product moment correlation							
	Plasma osmolarity (mOsm/L) [a]	24-hour urine volume (ml) [a]	TBW (%) [a]	TBW (L) [a]	*PhA* (degrees)	*R/H* (Ω)	*Xc/H* (Ω) [a]
Plasma osmolarity (mOsm/L) [a]		-0.010 [b]	**-0.150*** [b]	0.012 [b]	-0.122 [b]	**-0.185*** [b]	**-0.245*** [b]
24-hour urine volume (ml) [a]	-0.010 [b]		0.103 [b]	0.102 [b]	-0.105 [b]	-0.114 [b]	**-0.173*** [b]

[a] = not normally distributed; [b] = one or more outliers; Numbers in bold with a * = statistically significant correlation at the *p*-value < 0.05; TBW = total body water; *PhA* = phase angle; *R/H* = resistance/height; *Xc/H* = reactance/height.

4.3 Classification of hydration status

Each patient's hydration status was evaluated according to plasma osmolarity, 24-hour urine volume, TBW by BIA, and BIVA. A score of either 1, 2, or 3 was given to the patient, indicating the status of dehydration, euhydration, or overhydration, respectively. The score was given according to the reference values of the individually hydration technique. Table 5 shows the results of hydration classification in ON- and HPN-patients by the different techniques together with reference values. Please be aware of plasma osmolarity and TBW that are figured two times in the table but as: 1)

plasma osmolarity with an upper limit of >300 mOsm/L and 2) plasma osmolarity with an upper limit of >295 mOsm/L, and 1) TBW as percentage of body weight and 2) TBW in liter, respectively. Furthermore, the classification of hydration status by BIVA was based on interpretation of *RXc*-score graphs (See appendix 2).

Table 5. Classification of hydration status according to different hydration assessment techniques.

		Classification of hydration status by plasma osmolarity (reference values nr.1)			
Nutrition		1 Dehydrated [>300 mOsml/L]	2 Euhydrated [275-300 mOsml/L]	3 Overhydrated [<275 mOsml/L]	Total
ON*	Count (%)	30 (24.0%)	93 (74.4%)	2 (1.6%)	125 (100.0%)
HPN*	Count (%)	16 (12.5%)	110 (85.9%)	2 (1.6%)	128 (100.0%)
Total	Count (%)	46 (18.2%)	203 (80.2%)	4 (1.6%)	253 (100.0%)
		Classification of hydration status by plasma osmolarity (reference values nr.2)			
Nutrition		1 Dehydrated [>295 mOsml/L]	2 Euhydrated [275-295 mOsml/L]	3 Overhydrated [<275 mOsml/L]	Total
ON	Count (%)	59 (47.2%)	64 (51.2%)	2 (1.6%)	125 (100.0%)
HPN	Count (%)	52 (40.6%)	74 (57.8%)	2 (1.6%)	128 (100.0%)
Total	Count (%)	111 (43.9%)	138 (54.5%)	4 (1.6%)	253 (100.0%)
		Classification of hydration status by 24-hour urine volume			
Nutrition		1 Dehydrated [<720 ml/day]	2 Euhydrated [720-7200 ml/day]	3 Overhydrated [>7200 ml/day]	Total
ON*	Count (%)	30 (24.0%)	95 (76.0%)	0 (0.0%)	125 (100.0%)
HPN*	Count (%)	16 (12.5%)	112 (87.5%)	0 (0.0%)	128 (100.0%)
Total	Count (%)	46 (18.2%)	207 (81.8%)	0 (0.0%)	253 (100%)
		Classification of hydration status by TBW (%)			
Nutrition		1 Dehydrated [Male: <45%] [Female: <40%]	2 Euhydrated [Male: 45-70%] [Female: 40-60%]	3 Overhydrated [Male: >70%] [Female: >60%]	Total
ON	Count (%)	5 (4.0%)	114 (91.2%)	6 (4.8%)	125 (100.0%)
HPN	Count (%)	8 (6.3%)	109 (85.2%)	11 (8.6%)	128 (100.0%)
Total	Count (%)	13 (5.1%)	223 (88.1%)	17 (6.7%)	253 (100%)
		Classification of hydration status by TBW (L)			
Nutrition		1 Dehydrated [Male: <35 L] [Female: <25 L]	2 Hydrated [Male: 35-46 L] [Female: 25-33 L]	3 Overhydrated [Male: >46 L] [Female: >33 L]	Total
ON	Count (%)	17 (13.6%)	83 (66.4%)	25 (20.0%)	125 (100.0%)
HPN	Count (%))	26 (20.3%)	89 (69.5%)	13 (10.2%)	128 (100.0%)
Total	Count (%)	43 (17.0%)	172 (68.0%)	38 (15.0%)	253 (100.0%)
		Classification of hydration status by BIVA			
Nutrition		1 Dehydrated [Outside 75% tolerance ellipse in upper right quadrant of *RXc*-score graph]	2 Euhydrated [Within 75% tolerance ellipse]	3 Overhydrated [Outside 75% tolerance ellipse in lower left quadrant of *RXc*-score graph]	Total
ON	Count (%)	40 (32.0%)	80 (64.0%)	5 (4.0%)	125 (100.0%)
HPN	Count (%)	53 (41.4%)	73 (57.0%)	2 (1.6%)	128 (100.0%)
Total	Count (%)	93 (36.8%)	153 (60.5%)	7 (2.8%)	253 (100.0%)

Letters in bold with * = statistically significant difference in proportions at the *p*-value < 0.05 with all post hoc pairwise comparisons (with a Bonferroni corrected *p*-value < 0.016667) being not statistically significant; ON = oral nutrition; HPN = home parenteral nutrition; TBW = total body water; BIVA = bioelectrical impedance vector analysis; *RXc*-score graph = resistance-reactance-score graph.

4.4 Differences in hydration status between ON- and HPN-patients

In order to determine whether the distribution of dehydration, euhydration, and overhydration was equal in the ON-group and HPN-group, a Chi-square test of homogeneity (r x 2) (or Fisher's exact

test (r x 2) if assumption of expected counts was violated) was performed. Plasma osmolarity (with reference values nr.1) and 24-hour urine volume were the only hydration assessment techniques that showed a statistically significant result ($p = 0.043$ and $p = 0.022$, respectively) (see table 6). However, the following post hoc analyzes with pairwise comparisons and use of Bonferroni correction were not statistically significant ($p > 0.016667$). Thus, it was not possible to determine within which hydration class (i.e. dehydrated, euhydrated, and overhydrated) the difference in proportions existed.

Table 6. p-value of chi-square test of homogeneity and Fisher's exact test for difference in proportions between patients on oral nutrition (ON) and on home parenteral nutrition (HPN) by different hydration techniques. Also, p-value for post hoc test with pairwise comparisons of hydration status "dehydrated", "euhydrated", and "overhydrated".

	***p*-value**					
Test type	Plasma osmolarity (reference values nr.1)	Plasma osmolarity (reference values nr.2)	24-hour urine volume	TBW (%)	TBW (L)	BIVA
Chi-square test of homogeneity	-	-	**0.018** *	0.326	0.054	-
Fisher's exact test	**0.043 ***	0.522	-	-	-	0.217
Post hoc test with pairwise comparisons with Bonferroni corrected *p*-value <0.016667						
ON versus HPN Dehydrated	0.022	-	0.018	-	-	-
ON versus HPN Hydrated	0.027	-	0.018	-	-	-
ON versus HPN Overhydrated	1.00	-	No overhydrated	-	-	-

Numbers in bold with * = statistically significant difference in proportions; TBW = total body water; BIVA = bioelectrical impedance vector analysis; ON = oral nutrition; HPN = home parenteral nutrition.

4.5 Reliability testing

The reliability of the different hydration assessment techniques was evaluated by Weighted kappa (κ_w) with linear weights. Table 7 shows the strength of agreement (i.e. value of κ_w) together with 95% confidence intervals and *p*-value. The reliability of the individually techniques were tested with plasma osmolarity (reference values nr.1 and nr.2) and 24-hour urine volume as reference techniques. A statistically significant agreement in classification of patients' hydration status was only found with plasma osmolarity as reference technique. However, the strength of agreement for all statistically significant cases between plasma osmolarity and the novel techniques (TBW (%) and BIVA) was less than the agreement expected by chance (see table 7).

Table 7. Reliability testing of hydration assessment techniques by Weighted kappa with linear weighting.

Reliability between plasma osmolarity (reference values nr.1) and…	**Weighted kappa (κ_w) (Linear weighting)**	**P-value**	**Lower 95% confidence interval**	**Upper 95% confidence interval**
Plasma osmolarity (reference values nr.2)	**0.478** *	0.001	0.380	0.576

24-hour urine volume	-0.009	0.878	-0.125	0.106
TBW (%)	-0.070	0.068	-0.101	-0.040
TBW (L)	-0.025	0.557	-0.103	0.053
BIVA	**-0.104** *	0.043	-0.196	-0.011
Reliability between plasma osmolarity (reference values nr.2) and…				
24-hour urine volume	-0.020	0.698	-0.118	0.079
TBW (%)	**- 0.060** *	0.029	-0.107	-0.012
TBW (L)	-0.070	0.074	-0.143	0.004
BIVA	**-0.134** *	0.020	-0.243	-0.025
Reliability between 24-hour urine volume and…				
TBW (%)	0.018	0.638	-0.063	0.099
TBW (L)	-0.15	0.723	-0.093	0.064
BIVA	-0.070	0.186	-0.169	0.029

Numbers in bold with * = statistically significant at the *p*-value < 0.05; TBW = total body water; BIVA = bioelectrical impedance vector analysis;

5. Discussion

The aim of the present study was to evaluate the performance of BIA and BIVA as hydration assessment techniques compared to calculated plasma osmolarity and 24-hour urine volume in patients with INS or IF. The study population consisted of 253 patients divided into an ON-group and an HPN-group. The ON-group corresponded to patients with INS while the HPN-group corresponded to patients with IF. The 2 groups were similar in sample size, sex, and height however, statistically significant different regarding body composition with the ON-group having a higher weight, BMI, fat mass and lower FFM (%) than the HPN-group. Statistically significant difference in mean values were also found between the 2 groups regarding biochemical-, BIA-, and BIVA-data. No statistically significant good correlation and agreement were found between any of the reference techniques and novel techniques. Only reference techniques found a statistically significant different hydration classification between ON- and HPN-patients but because of no agreement between these 2 techniques, the result is doubtful.

Based on the study's results, the following discussion will focus on the suitability of plasma osmolarity and 24-hour urine volume as reference techniques, likely causes of the obtained reliability results for BIA and BIVA, the impact of having the "right" reference intervals, and difference in hydration classification between ON- and HPN-patients. Furthermore, recommendations and ideas will be addressed for future studies and the present study's limitations will be summarized at the end.

5.1 Plasma osmolarity and 24-hour urine volume as reference techniques:

The mean value of the calculated plasma osmolarity in the present study did not differ statistically significant between the ON-group and HPN-group though, the use of plasma osmolarity (reference values nr.1) resulted in a statistically significant different hydration classification of ON-patients when compared to HPN-patients. The same situation was true for 24-hour urine volume which also resulted in a statistically significant difference in hydration classification despite no significant difference in mean urine volume. Furthermore, the percentage of ON- and HPN-patients classified as either dehydrated, euhydrated, or overhydrated was almost identical between these 2 techniques, though, surprisingly the agreement between them was poor and not significant (i.e. the techniques failed to classify the same patients as dehydrated, euhydrated, and overhydrated) Also, the correlation between plasma osmolarity and 24-hour urine volume was weak and not significant. Overall, this may question the use of plasma osmolarity and 24-hour urine volume as suitable reference techniques in the present study since it is uncertain which one of the techniques that reflects the "true" hydration status of the patients.

Calculated plasma osmolarity is usually considered a fair way to assess dehydration. Indeed it has achieved a recommendation of grade B with a strong consensus (94% agreement) by ESPEN for the screening of low-intake dehydration in older persons (Volkert *et al.*, 2019). However, drawbacks related to the use of plasma osmolarity as stand-alone-assessment technique in the present study must be discussed. If water and salt are lost equally, the plasma osmolarity will not change thus, plasma osmolarity will be within the normal range as well will the concentration of Na^+ in the plasma (Grandjean and Campbell, 2004; Powers, 2007). I.e. by use of plasma osmolarity one is unable to detect isotonic dehydration/hypovolemia (Cheuvront *et al.*, 2013; Armstrong *et al.*, 2016). A hypotonic dehydration may also be overlooked since it will result in a decreased plasma osmolarity that may be interpreted as overhydration and not dehydration. In case of hypotonic dehydration water loss is accompanied by excessive salt loss thus, plasma osmolarity will be lower than normal as well will the concentration of Na^+ (Powers, 2007). This state will cause an osmotic shift of water from the ECF to the ICF which can lead to cell swelling and cerebral oedema (Oster and Singer, 1999; Grandjean and Campbell, 2004). Overall, this means that plasma osmolarity as stand-alone-assessment is most suitable for detection of hypertonic dehydration (i.e. water loss exceeding salt loss). A state that is reflected by an increased plasma osmolarity and an increased Na^+ concentration causing the water to shift from the ICF to the ECF (i.e. the cells shrink) (Grandjean and Campbell,

2004; Powers, 2007). However, increased osmolarity can also occur due to ineffective osmoles in the blood that only contribute to an elevation in osmolarity but not tonicity. Thus, a high calculated plasma osmolarity may not always indicate dehydration (Cheuvront *et al.*, 2013).

A study by Johnson et al. 2015, investigated markers of hydration process during fluid volume modification and found that assessment of 24-hour urine volume reflected the applied water intervention (i.e. urine volume increased significant with increased total water intake and vice versa) (Johnson *et al.*, 2015). Furthermore, 24-hour urine volume was able to discriminate between subjects with low and high fluid intake while serum osmolality was unable to do the same. Additionally, serum osmolality did not change significant during water intervention. Thus, 24-hour urine volume was more useful in detecting low fluid intake than osmolality (Johnson *et al.*, 2015). These results speak for the use of 24-hour urine volume as an early test for the prevention of dehydration. However, the study sample consisted of healthy college-aged females (Johnson *et al.*, 2015) therefore raising question about the validity of the results in males, subjects of older age, and diseased subjects.

Regarding age, a large systematic review with meta-analyses of clinical symptoms, signs, and test for identification of impeding and current water-loos dehydration in older people, concluded that urine volume was not useful, and should not be relied on as stand-alone-test for assessing presence or absence of dehydration in older people (Hooper, Abdelhamid, Attreed, *et al.*, 2015). Thus, it might have been misleading to use 24-hour urine volume in the present study as reference technique since the mean age of the total study sample was no more than approximately 6 years from the definition of old age (≥ 65 years (Volkert *et al.*, 2019)).

Finally, 24-hour urine volume is known to variate considerable within subjects. To minimize this variation and possible errors like incomplete voiding, incomplete sampling and spillage of urine, the mean value of 3 consecutive 24-hour urine volume collections is favorable (Heymsfield *et al.*, 2005, p. 208). However, in the present study only 1 single collection was used.

Collectively, the use of plasma osmolarity and 24-hour urine volume may not have been appropriate as reference techniques for the evaluation of BIA and BIVA as hydration assessment techniques.

5.2 Reliability of BIA and BIVA in classification of hydration status

Because the outcome variable of plasma osmolarity, 24-hour urine volume, BIA, and BIVA differed, it was not possible to compare the techniques directly. Instead each technique's ability to classify

patients' hydration status was compared. Surprisingly, the reliability between each reference technique (plasma osmolarity and 24-hour urine volume) and the novel techniques (BIA and BIVA) was very poor. Indeed, a statistically significant reliability was only obtained between the following:

- Plasma osmolarity (reference values nr.1) and BIVA.
- Plasma osmolarity (reference values nr.2) and BIA-derived TBW (%).
- Plasma osmolarity (reference values nr.2) and BIVA.

All with the strength of agreement being less than the one expected by chance. The poor reliability was further supported by very weak correlations that were only statistically significant for the correlation of plasma osmolarity with TBW (%) and the BIVA-data (*R/H* and *Xc/H*), as well as the correlation between 24-hour urine volume and *Xc/H*. In the following sections, different factors that may have influenced BIA's and BIVA's reliability, will be discussed.

5.2.1 Time interval between measurements

The most likely cause of the above described results is the fact that measurements were not done on the same day. Indeed, several days and even weeks may have passed between the different measurements. This time interval is too large when considering the human body's constant strive after water balance as well as possible alterations in subjects' disease state. A subject may very well have been euhydrated at the time of 24-hour urine volume measurement and then, days after dehydrated at the bioelectrical impedance-assessment. This is a major drawback in the study design, and it can only be recommended not to be repeated in future studies. Optimally, for future studies, the different measurements should be collected at the same day however, the require of fasting prior to both impedance- and plasma glucose-measurement makes it impossible, i.e. fasting may contradict the 24-hour urine volume assessment. However, a full data set of measurements within 2-3 days seems achievable thus, minimizing the risk of significant altered hydration status between measurements.

5.2.2 Factors affecting impedance measurement

Measurement of bioelectrical impedance has been praised as easy however, multiple factors can influence the measurement and create noise (Heymsfield *et al.*, 1997). A study by Nescolarde et al., 2016, found a large variability in intrinsic resistance and reactance values of different commercial electrodes. The variability was so large that it produced statistically significant displacement of bioimpedance vector positions in healthy adults in a *RXc*-graph (Nescolarde *et al.*, 2016).

Furthermore, a disposition of electrodes from their traditional place on the wrist and ankle in the direction of the trunk within 1 cm increments can create a drop of 10 Ω per cm (Sergi *et al.*, 2017). Another factor is body position where failure to abduct extremities has been found to affect resistance measurements by 2-3%, and skin-to-skin contact with crossed legs and hands at the waist affected measurements with 18% and 43%, respectively (Kushner, Gudivaka and Schoeller, 1996; Earthman, 2015; Dyhre-Petersen, 2019). Additionally factors include physical activity, alcohol, skin surface, skin and core temperature, ambient temperature, etc. (Earthman, 2015). Earthman, 2015, and Kyle et al., 2004b, has each provided an extensive list of recommendations for optimal impedance measurement in adults. The lists can be viewed in appendix 1 (Kyle *et al.*, 2004b; Earthman, 2015; Dyhre-Petersen, 2019).

Additionally, it is important to remember that whole-body impedance measurement is based on the assumption that the human body is a single, symmetrical cylinder with a uniform cross-sectional area and homogenous composition – an assumption that is not physiologically correct (Mulasi *et al.*, 2015). In 1989, Fuller and Elia, reported that the forearm contributed with 25% to whole-body impedance although only accounting for 1.3% of the body weight. They also found that the trunk, accounting for about 50% of the body weight, only contributed to the impedance with approximately 10% (Fuller and Elia, 1989; Dyhre-Petersen, 2019). Thus, the question arises whether the measurements are representative to the total body (Matthie, 2008)

Despite many possible influencing factors it is mentionable that the measurements in the present study were obtained according to Aalborg University Hospital's standard protocol for bioelectrical impedance measurement *and* executed by the same educated staff member in order to reduce as many as possible errors. Also, the participants were not obese and had no amputations, making it less likely that the assumption of the human body as a single, symmetrical cylinder with a uniform cross-sectional area and homogenous composition should have added to significant errors (Kyle *et al.*, 2004b; Matthie, 2008).

5.2.3 Selection of BIA equation

Beside factors associated with the measurement and assumptions of impedance, an additional factor influences the BIA-results when the impedance measurements undergo analysis. The fact that BIA cannot measure body volumes directly but must use statistically derived, population-specific

equations that often have been validated in healthy subjects under controlled conditions, enhances the risk of errors (Mulasi *et al.*, 2015). In 2004, Kyle et al. presented more than 20 different equations for TBW with standard error estimates ranging from 0.88-3.8 liters in compare to isotope dilution technique (Kyle *et al.*, 2004b; Baron *et al.*, 2015). Thus, the validity of BIA is highly dependent on the selection of suitable equations that matches the study population (Fosbøl and Zerahn, 2015; Mulasi *et al.*, 2015; Dyhre-Petersen, 2019). However, many BIA devices do not specify the equations that is programmed into their software which results in a "black box" approach (Mulasi *et al.*, 2015). Likewise, the equations used in the present study is unknown because it is kept as proprietary information of Maltron, Essex, UK. It is therefore unknown how suitable the equations are in INS- and IF-patients as well which additionally parameters (i.e. weight, height, sex, etc.) the equations include. This is a substantial factor, that must be included in the interpretation of the present study's results as well when comparing the results to other studies.

5.2.4 Factors affecting BIVA

BIVA has been considered as an attractive alternative to BIA because it does not depend on regression equations however, it is not flawless (Matthie, 2008; Norman *et al.*, 2012; Mulasi *et al.*, 2015). BIVA uses the raw impedance data which may be flawed due to already discussed factors associated with the measurement of impedance. Additional factors that can compromise BIVA results include use of an unsuitable reference population, "reading" of the *RXc*-graph, and use of single frequency impedance measures (Matthie, 2008; Bronhara, Piccoli and Pereira, 2012; Mulasi *et al.*, 2015).

The present study used the inherent Italian reference population of the BIVA Software though, it is possible that better results could have been obtained if a Danish reference population had been used. Unfortunately, such one does not exist at the current moment.

When it comes to the interpretation of the *RXc*-graph, the present study had to create clear cut-offs in order to classify patients. This is however not correct since the boundaries between the BIA body patterns (i.e. dehydration, body cell mass decrease, overhydration, and body cell mass increase. See figure 4 in theory chapter) are more fluent than clear-cut. The closer a vector is positioned to a given boundary line, the more uncertain becomes the classification (Bronhara, Piccoli and Pereira, 2012). Therefore, in the present study, the classification of dehydrated and overhydrated patients is vitiated with an uncertainty and the "real" number of dehydrated and overhydrated patients may be higher or lower thus, compromising the reliability of BIVA.

Finally, the raw BIVA impedance data (*Z*, *PhA*, *R*, and *Xc*) were measured at a single 50 kHz current. This means that the current could not penetrate all cells to capture TBW because this only happens fully at a higher current (Matthie, 2008). Thus, it is questionable how well the *R/Xc*-graph can detect whole body water losses that result in intracellular or extracellular dehydration (Matthie, 2008; Cheuvront and Kenefick, 2014).

5.3 Impact of reference intervals and selected parameters

As stated in the previous section *5.2.4 "Factors affecting BIVA"*, the choice of reference and cut-off values affects the results of hydration classification. This is true for all the parameters that have been tested in the present study. It is clearly demonstrated by the agreement between plasma osmolarity with reference values nr.1 and plasma osmolarity with reference values nr.2. The only difference between these 2 parameters was the upper limit value indicating dehydration, i.e. >300 mOsm/L and >295 mOsm/L, respectively. However, this small difference of only 5 mOsm/L meant that a total of 65 patients classified as euhydrated by plasma osmolarity with reference values nr.1, were classified as dehydrated by plasma osmolarity with reference values nr.2 – a difference of 25.7%. This was further reflected by a weighted kappa value of only 0.478, $p = 0.001$, between the 2 parameters. Also, the use of >295 mOsm/L as cut-off value resulted in a statistically significant agreement with 2 of the novel hydration techniques (BIVA and BIA-derived TBW (%) with a κ_w of -0.134 and -0.060, respectively) as compared to only 1 single statistically significant agreement (BIVA with a κ_w of -0.04) with the use of >300 mOsm/L as cut-off. Thus, the choice of reference intervals affects not only the classification of hydration status but also the reliability between hydration techniques.

Of course, reference intervals should be selected based on evidence however, the scientific and clinical community is not always agreeing upon which intervals to use. This is also the reason why plasma osmolarity was represented in the present study with 2 different reference intervals. Some researchers and clinicians report the use of >300 mOsm/L as cut-off (Arre, 2017; Bergstedt, 2018; Ladefoged, 2020; Region Sjælland, 2020) while others use >295 mOsm/L (Baron *et al.*, 2015).

Regarding 24-hour urine volume it was even more difficult to assign numerical values of dehydration, euhydration, and overhydration because of lacking references in the scientific literature. A study by Armstrong et al., 2010, reported 95% confidence intervals (675-3000 ml/day) though, they were only valid for men with a weight of 75.1 kg (Armstrong *et al.*, 2010). Instead the present study used the references reported in a review by Grandjean and Campbell, 2004, however, the use of a cut-off value of >7200 ml/day as indicator of overhydration may be too high.

The same difficulties existed for BIA. Because intervals for ECF and ICF were to uncertain, only TBW was tested as a hydration parameter in the present study. Deducing the hydration status from TBW (L) alone has been pointed out to be inappropriate because absolute water volume varies with height, weight, and body composition (Park, Jo and Lee, 2018). Thus, TBW as percentage of body weight was included in the present study. The inappropriateness of TBW (L) is supported by the fact that the present study only found a statistically significant correlation and agreement for TBW (%) and not TBW (L). The use of TBW (L) may only be useful when comparing repeated measures for a single patient and not as a tool for comparison of TBW between patients.

5.4 Differences in hydration status between ON- and HPN-patients

The mean value of the calculated plasma osmolarity and 24-hour urine volume in the present study did not differ statistically significant between ON- and HPN-patients. Though, both plasma osmolarity (with reference values nr.1) and 24-hour urine volume resulted in a statistically significant different hydration classification of ON-patients in compare to HPN-patients. With the use of a Bonferroni corrected *p*-value it was not possible to determine within which hydration status (i.e. dehydration, euhydration, and overhydration) the difference occurred. However, at the *p*-value of < 0.05, a significant difference was seen in the proportions between ON- and HPN-patients classified as dehydrated and euhydrated, i.e. more ON-patients were classified as dehydrated and fewer as euhydrated in compare to HPN-patients. However, the reliability of this result is questionable since the agreement between plasma osmolarity and 24-hour urine volume was poor and not significant. In other words, these 2 techniques *did* show that ON- and HPN-patients were not classified in a similar way, but they failed to classify *the same* patients as being dehydrated, euhydrated, and overhydrated.

Regarding BIA and BIVA, multiple of the mean value data where statistically significant different between ON- and HPN-patients (i.e. TBW (%), ECF (% of TBW), ICF (% of TBW), *Z*, *PhA*, *R*, *R/H*, *Zs (Xc)*, and *Zs (R)*) though, no significant difference was seen in the hydration classification by these 2 techniques.

Thus, collectively the present study cannot confirm or reject any differences in hydration classification between ON- and HPN-patients.

5.5 Considerations when choosing a hydration assessment technique

The choice of hydration assessment technique should be based on *and* tailored to the situation and population in which the technique should be used (Armstrong, 2007). This means that many questions

must be addressed before any decision making, for example questions about practicality, quickness, environmental settings, the imprecision of the technique's measurement, and how easily the measurement is confounded (need of controls) (Cheuvront and Kenefick, 2014). These questions are frequently asked, however more infrequently asked questions include what type of hydration status that is most likely, magnitude of dehydration or overhydration that is wanted for detection, the desire of good measurement sensitivity, specificity, or both, whether within- and between-person variation is known, and whether it is possible to calculate a reference change value (Cheuvront and Kenefick, 2014).The aim of the present study may therefore be interpreted as a simplification of that decision making that is related to the selection of a hydration assessment technique. The present study investigated only the reliability between standard and novel techniques however, according to the above-mentioned questions, many other aspects could and should have been investigated. Thus, based on these considerations and the results of the present study several proposals to future studies have been fostered. These ideas and recommendations will be discussed in the following chapter.

5.6 Future studies

In any kind of study that compares the classification of hydration status between 2 or more techniques, it is highly recommended that all measurements are obtained within a minimal time interval. Thus, reducing the risk of altered hydration status between measurements. It is further recommended to explore what type of dehydration (i.e. intracellular-, extracellular-, or mixed-dehydration) that is most common because not all techniques can detect all types of dehydration. Considering BIA, the use of a prediction equation that is developed in a population similar to the study population is preferred over the use of manufacture's (often) unknown equation.

Furthermore, reliable reference intervals should be investigated for ECF (%), ICF (%) or ECF/ICF so that these measurements could be included in the BIA hydration assessment. Likewise, reliable reference intervals should be explored for *PhA* and impedance ratio (i.e. *Z* measured at low frequency divided with *Z* measured at high frequency) in order to investigate whether these parameters could be used as stand-alone assessments for hydration status. It is recommended to use biological variation analysis in the investigation of reference values since variation is present both within and between subjects (Cheuvront and Kenefick, 2014). In case of high variation, multiple measurements and/or stratification by age, sex, disease etc. may improve the trustworthiness of the reference intervals (Cheuvront and Kenefick, 2014).

Additionally, Danish population standard references should be created for the use in BIVA. Finally, the desired clinically measurement sensitivity and specificity should be determined in ON- and HPN-patients together with the consequences associated with inaccurately determination of hydration status.

5.7 Study limitations

The most obvious limitation of the present study is the use of hydration measurements that were not obtained at the same time or within a narrow time interval. Also, 24-hour urine volume was based on 1 single collection instead of the mean value of 3 consecutive collections. The use of the inbuilt equations in the bioelectrical impedance device contributed "black box" parameters and the selected Italian reference population for BIVA may not have been appropriate for the study sample. Lastly, the study sample consisted of patients with INS and IF which are "umbrella" terms of multiple pathophysiologic states like short bowel, intestinal fistula, intestinal dysmotility, mechanical obstruction, or extensive small bowel mucosal disease, that may be caused by acquired or congenital, gastrointestinal or systemic, benign or malignant diseases (Pironi et al., 2015(Dyhre-Petersen, 2019)). Thus, the health/disease status of the study sample was not homogeneous although, all patients were considered as metabolic stable.

6. Conclusion

The present study demonstrated a poor reliability of BIA and BIVA as hydration techniques in INS- and IF-patients. This result is however doubtful because the reliability between the reference techniques was poor too. Thus, the study can only conclude that hydration classification of INS- and IF-patients varies with choice of hydration assessment technique. Future studies are recommended to verify the present study's results since these most likely have been compromised by too large time intervals between the different measurements.

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Appendix 1

Table X gives general recommendations in order to enhance validity and standardization of bioelectrical impedance measurements while table Y gives recommendations under specific clinical and disease conditions.

Table X. Recommendations for Optimizing Whole-Body Bioimpedance Measurements in an Adult by (Earthman, 2015)*, "Body Composition Tools for Assessment of Adult Malnutrition at Bedside: A Tutorial on Research Considerations and Clinical Applications".*

Protocol Parameter	Recommendation
Preparing for the measurement	
Food/beverage and activity	Individual should fast (nil per os except water) and avoid alcohol, caffeine, and exercise at least 8 hours prior to measurement in the morning (research settings); shorter time frames and other times of day may be acceptable in the clinical setting – note time of day for consistency in follow-up measures.
Void bladder	Individual should void bladder prior to measurement
Clean skin surface	Clean skin surface well with alcohol; individual should not use lotion or oils on the skin prior to measurement; avoid placing electrodes on broken skin.
Device calibration	Calibrate the bioimpedance devise according to manufacturer's recommendations prior to measurement
Height and weight	Obtain an accurate measure of height and weight
Testing conditions and considerations	
Device placement	Place device on nonmetal surface, at least 1 m away from electronic or magnetic devices.
Ambient temperature	Avoid excessively warm or cool ambient temperatures
Electrodes and leads	Use electrodes with sufficient surface area (≥4 cm^2); store electrodes in sealed bag away from heat; use device-specific leads provided by manufacturer.
Electrode placement	Place electrodes at least 5 cm apart, if possible; proximal electrodes should never be moved from standard anatomical site placement; if necessary, the distal electrodes may be moved to achieve at least 3 cm of separation; the most important thing is to measure and record distance between electrodes to ensure placement consistency for follow-up measurements.
Side of body	If using standard tetrapolar placement of electrodes, measure on the same side of the body as previous measures; in individuals with amputations, muscle atrophy, or other abnormal conditions, use the nonaffected side, if possible; be consistent on side of measurement for follow-up. Right-side measurements are commonly used in the literature.
Body positioning and limb separation	Body position should be supine, except for stand-on scale devices, with arms separated ≥30° from the trunk and legs separated by ~45°; in individuals with overweight and obesity, separate arms from trunk and legs from each other using rolled cotton towels/blankets.
Fluid and electrolyte status	Note if serum electrolytes are abnormal; it is best to conduct bioimpedance measurements only when serum electrolytes are normal. Note if oedema is present; causes lower resistance values.
Menstrual cycle in females	Note menstrual cycle; be consistent in terms of timing for follow-up measurements.
Timing of measurement	If individual is ambulatory, individual should assume a supine position for 5-10 minutes; standardize the timing for measurements by noting the time when the individual assumes the supine position and the time when you take the measurement (e.g. at 10 minutes), and ensure consistency of timing for all follow-up measurements, Note if individual is confined to bed.
Repeat measurements	Repeat measures recommended for research studies.

Table Y. Recommendations for clinical application of bioelectrical impedance analysis by (Kyle *et al.*, 2004b)*, "Bioelectrical impedance analysis – part II: utilization in clinical practice".*

	Definition/comments	**Recommendations**
Instruments/material		
Generator	Consistent signal of reproducible amplitude	Calibration of electrical equipment.
	Batteries	Battery-p0wered to avoid interference with current variations. Autonomy for >20 measurements.
Analyzer	Measures of *R* or impedance and *Xc* or phase angle	Regular calibration against known ohmmeter. Identify type of signal measured (i.e. impedance or *R* or *PhA* or *Xc*).
	Automatic verification of skin resistance	Identify abnormal skin resistance, in cases of excessive resistance (e.g. pachydemia).
Cables	Length	Appropriate for length of subject. Height (up to 2 m)
	Diameter/isolation	Meets manufacturer's recommendation
Electrodes	Surface size	Meet instruments requirements (>4 cm^2).
	Integrity of gel	Keep electrodes in sealed bag. Protect against heat.
Statiometer	Calibrated to 0.5 cm	Use tape measure for subjects who are unable to stand and for knee-ankle height or arm span.
Scale	Calibrated to 0.1 kg	Regular cross-calibration with other scales.
Subjects		
Height and weight	Measure height (0.5 cm) and weight (0.1 kg) at the time of the BIA	Self-reports are not valid.
Food, drink, alcohol	Fasting/no alcohol for >8 h recommended	Shorter periods may be acceptable for clinical practice (versus research).
Bladder voided		Subject has voided before measurement.
Physical exercise		No exercise for >8 h.
Timing	Note time of measurement	For longitudinal follow-up, perform measurement at the same time of day. Note menstrual cycle in females.
Skin condition	Temperature	Ambient temperature.
	Integrity	No skin lesions at the sight of electrodes. Change site of electrodes if lesions.
	Cleaning	Clean with alcohol.
Electrode position	Note body side of measurement	Always measure same body side.
	Distance between electrodes	Minimal of 5 cm between electrodes. If needed, move proximal electrode
Limb position	Abduction of limbs	Arms separated from trunk by about 30° and legs separated by about 45°.
Body position	Supine, except for "scale" type BIA instruments	Ambulatory subjects supine for 5-10 min. For research protocol, standardize time. Note if subject is confined to bed.

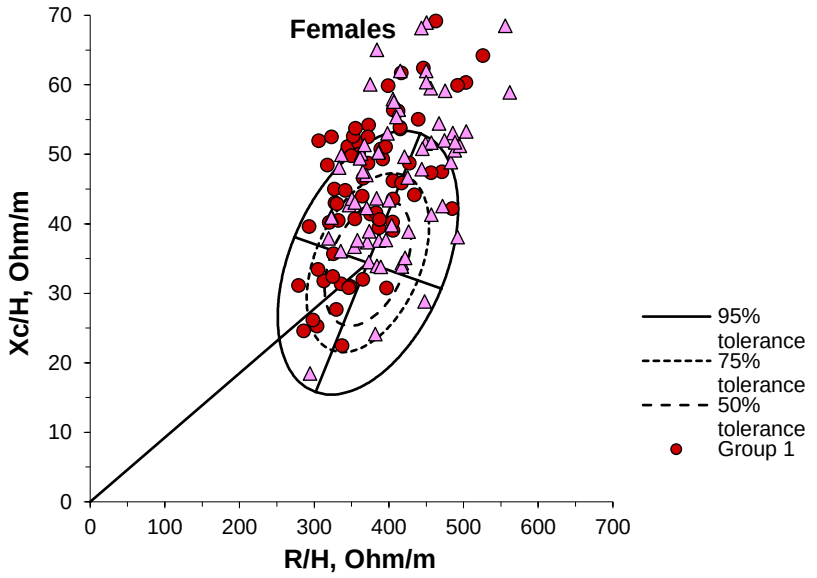
Environment	Electrical interference	No contact with metal frame of bed. Neutral environment (no strong electrical or magnetic fields).
Body shape	Note body abnormalities	Note measurement validity (e.g. *R* or *Xc* outside of expected range of subject). Consider validity of measurement when interpreting results (e.g. abnormally low *R* suggest oedema).
	Amputation	Measure non-affected limb. Not valid for research, but permits determination of body compartments because error is consistent.
	Atrophy, hemiplegia	Measure non-affected side.
	Abnormal limb of trunk (e.g. scoliosis)	Note abnormal condition.
	Dystrophy (HIV, Cushing's syndrome etc.)	Limited validity in conditions of abnormal body compartment distribution.
	Obesity	Use electricity-isolating material (e.g. towel) between arm and trunk, and between thighs.
Ethnic group		Note race. Use race-specific BIA equation if applicable.
Disease conditions		
Cardiac insufficiency	Oedema interferes with measurement	Measure patient in stable condition.
Liver failure	Ascites/oedema interferes with measurement accuracy	Consider segmental BIA measurement.
Kidney failure	Oedema/altered ion balance interferes with measurement	Consider segmental BIA measurement.
Abnormal serum electrolyte concentrations	Affects BIA measurement	Perform BIA when serum electrolytes are within normal range. Compare BIA results when serum electrolyte concentrations are similar.
Hypothyroid	Pachydermia	May invalidate measurement because of high skin resistance.
Treatments		
IV/Electrolyte infusions	Peripheral oedema interferes with measurement	Body composition assessment invalid if patient is abnormally hydrated.
Drugs that affect water balance	Steroids, growth hormone, diuretics	If patient is stable, measurement should be effected at the same time after medication administration.
Dialysis	Hemo-, peritoneal dialysis	Use special protocols.
Ascites puncture		Use special protocols.
Orthopedic prosthesis/implants (metal)	E.g. hip prothesis	Measure non-affected side. Note prosthesis/implants
Pacemakers and defibrillators		No interference is anticipated. However, there are known incidents. Therefore, monitor for cardiac activity.

1

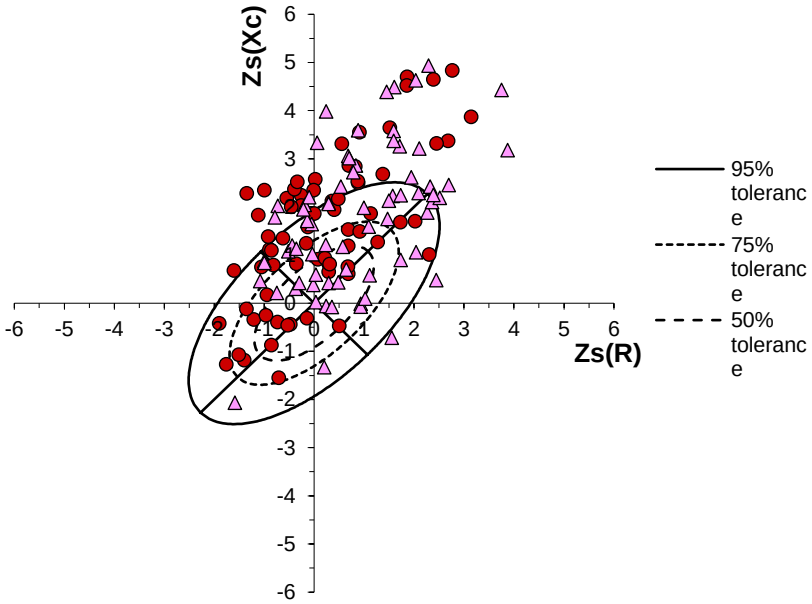
Appendix 2

BIVA-graphs made with BIVA Software 2002.

Graph 1 depicts the *RXc*-graph of females divided according to nutrition. Reference ranges of the female reference population are indicated as 50th, 75th, and 95th percentile tolerance ellipses. Graph 2 shows the same sample of females but plotted as a *RXc*-score graph with 50th, 75th, and 95th percentile standard reference intervals.

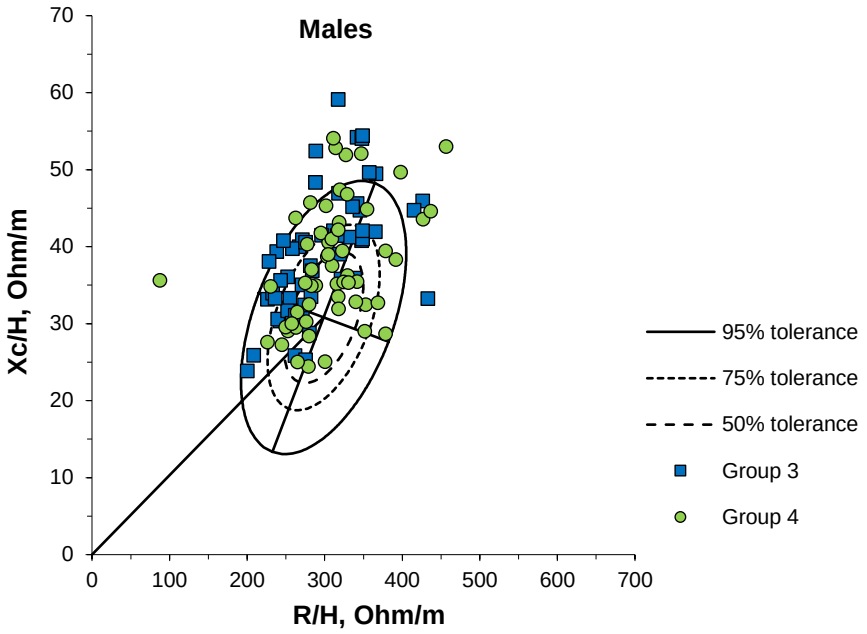


Graph 1. RXc-graph of females divided in groups according to nutrition together with 50th, 75th, and 95th tolerance ellipses. Group 1 = oral nutrition; Group 2 = home parenteral nutrition.

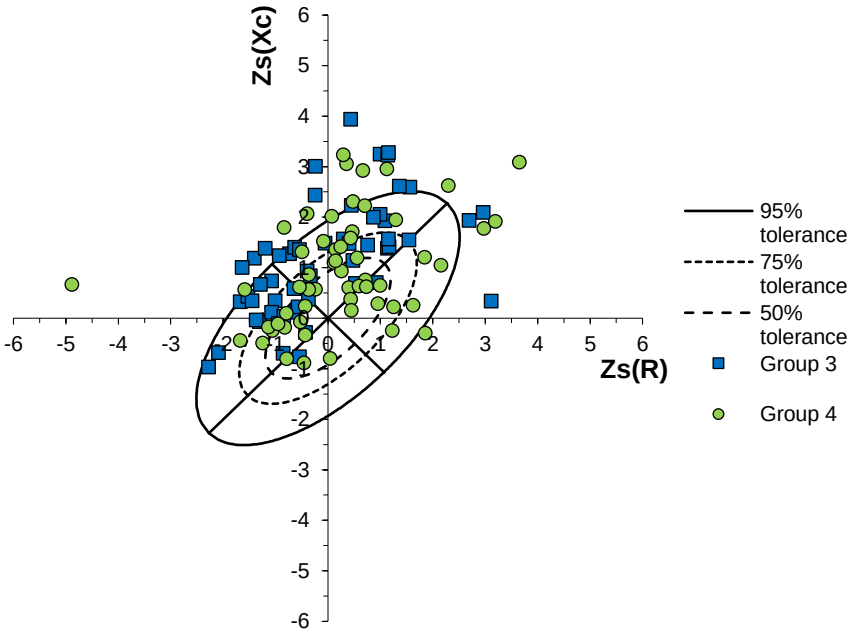


Graph 2. RXc-score graph of females divided in groups according to nutrition together with 50th, 75th, and 95th standard reference intervals. Group 1 = oral nutrition, Group 2 = home parenteral nutrition.

Graph 3 depicts the *RXc*-graph of males divided according to nutrition Reference ranges of the male reference population are indicated as 50th, 75th, and 95th percentile tolerance ellipses. Graph 4 shows the same sample of males but plotted as a *RXc*-score graph with 50th, 75th, and 95th percentile standard reference intervals.

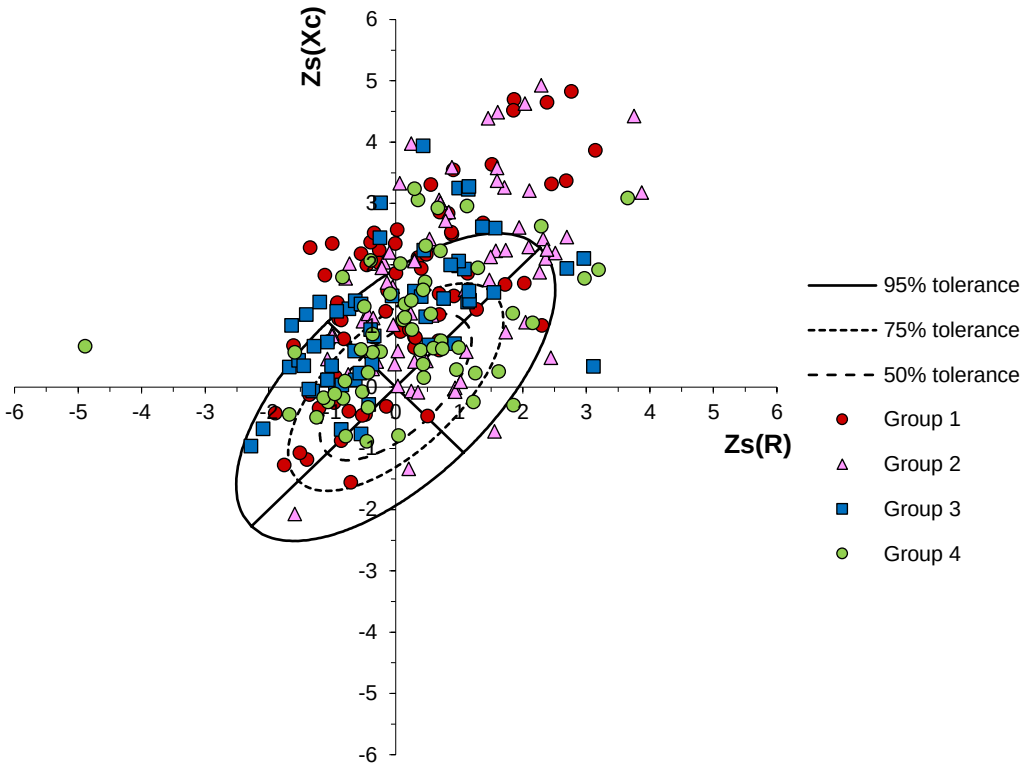


Graph 3. RXc-graph of males divided in groups according to nutrition together with 50th, 75th, and 95th tolerance ellipses. Group 3 = oral nutrition; Group 4 = home parenteral nutrition.



Graph 4. RXc-score graph of males divided in groups according to nutrition together with 50th, 75th, and 95th standard reference intervals. Group 3 = oral nutrition; Group 4 = home parenteral nutrition.

Graph 5 depicts a jointed *RXc*-score graph of females and males with 50th, 75th, and 95th standard reference intervals.



Graph 5. RXc-score graph of females and males divided in groups according to nutrition and sex together with 50th, 75th, and 95th standard reference intervals. Group 1 = females on oral nutrition; Group 2 = females on home parenteral nutrition; Group 3 = males on oral nutrition; Group 4 = males on home parenteral nutrition.