A novel method to determine lean body water using localized skin biopsies: correlation between lean skin water and lean body water in an over-hydration model.

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Running Title: Lean skin water correlates to lean body water.

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ABSTRACT

To determine the relationship between total body water (TBW) fraction and local water content measured in the skin (SW) this study assessed 8 anesthetized piglets in an over-hydration model. Total body water was assessed by deuterium oxide (D₂O) dilution and body mass measurements taken throughout the experiments, and by whole body carcass analysis at the end of each experiment. Additionally extracellular water and plasma volume were assessed using bromide dilution and Evan’s blue dilution, respectively. Skin water was assessed by tissue biopsies taken at 60 min intervals throughout the experiment. Lean body water (LBW) fraction and lean skin water (LSW) fraction were assessed by extracting the fat from the carcass and biopsy samples. A correlation does exist between TBW fraction and SW fraction with \( r^2 = 0.58 \) (P<0.05), however, the strongest correlation occurred between LBW fraction and LSW fraction with \( r^2 = 0.87 \) (P<0.05) and a standard error of prediction of 0.77%. These data demonstrate that LSW gives an accurate and precise estimate of LBW, and could therefore be used to determine the hydration index in appropriate research settings.

**Key words:** fat free percent water; hydration index; indicator dilution; skin biopsy
INTRODUCTION

Water is vital in the life of all mammals. It plays a central role in such functions as nutrient transport, waste removal, maintenance of cell volume, and thermal regulation. Assessment of hydration provides insight into basic biological processes, and therefore is an important clinical parameter in determining health status and treatment of disease. Both dehydration and over-hydration can have tragic consequences (8), thus maintaining a normal hydration state is a primary concern in all aspects of medicine. While there are no quantitative measurements of hydration currently being used in medicine, qualitative parameters such as skin turgor, urine production, and the presence of tears are commonly used to determine a patient’s hydration status. Researchers face a similar problem when studying models of disease. While the laboratory setting lends itself to more quantitative assessments, accurate measurements of hydration are often difficult and time consuming. A more readily attainable, accurate, and quantitative measure of hydration would provide scientists with a powerful tool in studying health and disease.

Body composition research aims to identify quantitative relationships between body components that are relatively constant under most circumstances. The water content or hydration of fat free mass (FFM), sometimes referred to as the hydration factor, is among the best known and most widely applied of the body composition constants (17). In healthy mature mammals, total body water (TBW) is a constant fraction of FFM, which, based on chemical analysis of mature animals ranging in body size from mice to cattle, has a magnitude of 0.74 with a range between 0.69 and 0.79 (20). Furthermore, whole body chemical analysis of 8 human cadavers gave a percent lean body water (LBW) of 73.7 ± 3.8 % (mean ± SD) (25), lending additional evidence for a quantitative normal hydration state for humans. Moreover, studying
many individuals with non-invasive techniques, several studies have demonstrated that this value remains constant in adults despite race, age, and level of body fatness (3, 13, 23).

There are essentially two “gold-standard” techniques for determining LBW to date, both of which are cumbersome and can be prone to errors. The first is whole-body chemical analysis which provides the most accurate results, however, requires homogenization of the carcass, and therefore is inappropriate in many situations. The second reference technique is isotope dilution. Despite a number of assumptions, this technique provides a reasonably accurate and precise measurement of TBW. However, the isotope can take up to 3 hours to equilibrate in an adult, and requires careful collection of fluid samples and extensive equipment for precise analysis. Furthermore, an assessment of FFM is still required in order to calculate LBW. As such, both these methods of assessing LBW have certain limitations. Another commonly used technique for determination of hydration status is bioelectrical impedance analysis (BIA). However, in order to collect the most accurate BIA results, body weight, age, and gender must be incorporated in the calculations (19). Additionally, results have generally been validated in healthy patients and appear to be most accurate in patients with stable water and electrolyte balance (12, 16), which may be limiting in clinical settings and/or in models of disease. Indeed, studies have demonstrated only weak correlations between BIA and weight change post-operatively (2, 21). In addition, the precision of the BIA varies according to the methodologies used to calculate extracellular volume (7, 15, 19). In fact, in critically ill patients only weight gains over 3 kg were reliably detected (18). An alternative technique which requires less equilibration time could provide scientists with a powerful new tool to determine body composition and hydration status.
Interestingly, increase in TBW has been previously demonstrated to increase skin thickness (1, 27), becoming visible to the human eye when the fluid increase in the skin reaches about 50%. Water is bound to hyaluronic acid, glycosaminoglycans and proteoglycans in the skin resulting in a disarrangement of collagen bundles (10). Hence skin turgor, while qualitative, can be a reliable method to determine large changes in whole body fluid content or distribution. The ability to quantitatively assess this relationship between fluid content of the skin and TBW could provide an accurate and sensitive measurement for fluid intake and balance.

This study investigated the effect of fluid administration on TBW, LBW, and local percent water in lean tissue of the skin (LSW). We hypothesized that changes in LSW would reflect the fluid administration time line and that, after a short equilibration period, would correlate to TBW and LBW. Evaluation of the relationship between skin (SW) and the whole body parameters TBW and LBW will provide new information regarding fluid distribution during over-hydration, and could result in a new technique for determining hydration status and body composition.

**METHODS & MATERIALS**

*Experimental Design*

This study aimed to assess the relationship between LSW and LBW, and to quantify the potential ability to make a hydration measurement based on local skin biopsies. A stepwise over-hydration model was used to get a wide range of hydration levels to calibrate this method. Repeated hourly infusions of an isotonic solution were used to demonstrate the accuracy and precision of using LSW to predict LBW. While this method remains unsuitable for use in the clinical setting, improvements in technology
and non-invasive procedures may allow for the future assessment of regional hydration in the human population.

Eight female domestic piglets 4-6 weeks old, weighing 18.8 ± 0.9 kg (mean ± SE) were used in these experiments. Animals were weaned and were given water *ad libitum*. Each piglet fasted for 12 hours prior to commencement of experimentation. All experimental interventions were formally approved by the Institutional Animal Care & Use Committee for the United States Department of Agriculture.

Pre-anaesthetic medication (telozol/atropine) was given i.m. 30 min prior to the induction of anaesthesia. Each piglet was intubated and general anaesthesia was induced using 2% isoflurane in oxygen. After induction, catheters were inserted into a central artery and vein, and a Foley catheter was surgically inserted into the bladder. General anaesthesia was maintained by volume-controlled ventilation with isoflurane (2.0 vol%) delivered in 100% oxygen via veterinary anaesthesia ventilator (EMC model 2000, Hallowell, MA, USA). The protocol allowed adjustments of inspired isoflurane concentration in the order of 1-2 vol% according to reactions to standardized noxious stimuli or changes in blood pressure and heart rate. Ventilation (volume-controlled) was adjusted to produce normo-capnia (arterial carbon dioxide level about 40 mmHg) based on repeated arterial blood gas analysis and end-tidal carbon dioxide monitoring (NPB75, Nellcor Puritan Bennett, CA, USA).

After induction of general anaesthesia each piglet was allowed 30 min to stabilize. A low rate infusion of lactated Ringer’s solution (LRS; 130 mEq Na⁺, 109 mEq Cl⁻, 28 mEq lactate, 4 mEq K⁺, 3 mEq Ca²⁺) was commenced via the venous catheter and volume matched to fluid loss through urine output and blood sampling. All hemodynamic variables were measured continuously and recorded at regular intervals (20 min) throughout the experiment. Piglets were then given a bolus of the tracers
used to determine total body water, extracellular water, and plasma water content. Baseline measurements were recorded for 1 hour to ascertain stable resting values and equilibrium of tracers prior to the commencement of fluid administration.

The fluid therapy consisted of administration of 1.000 ± 0.075 litres of LRS, dosed with the indicators to prevent re-equilibration, in 20 min followed by 40 min of equilibration time. Additionally, a supplemental volume of LRS was given to compensate for the fluid lost from urine and blood sampling during the 40 min equilibration period.

**Monitoring of Hemodynamic Variables**

Heart rate was obtained using surface ECG electrodes. Systemic arterial pressures were monitored continuously using a fluid-filled catheter introduced into the aorta, connected to a pressure transducer, and linked to a monitor (HP model 78534B monitor & terminal, Hewlett Packard, CA, USA). Oxygen saturation was monitored using a pulse oximeter (NPB75, Nellcor Puritan Bennett, CA, USA) placed on the nose or tongue of the piglet. Core body temperature was monitored using an esophageal probe and peripheral body temperature was monitored using a K-type thermocouple (Omega Engineering Inc., CT, USA) affixed to the forehead and chest. Arterial blood samples were collected at 20 min intervals to monitor total hemoglobin (HemoCue, CA, USA), indicator concentrations, and blood biochemistry (Irma ABG model 436303, Diametrix Medical Inc., NM, USA). Blood samples used to determine indicator concentrations were spun at 5200 g, the plasma removed, and the samples frozen in dry ice and then stored at -20°C until further analysis. The indwelling Foley’s catheter allowed for continuous monitoring of diuresis. Urine output was recorded every 20 min and samples were collected for analysis of indicator
concentrations. Additionally, body weight was monitored continuously using a GP-100ks AND balance with a 101 kg capacity and 1.0 g resolution (A&D Weighing, CA, USA) which automatically recorded any change in weight greater than 30g. Upon completion of the experiment the whole carcass was frozen at -20°C until further analysis.

**Indicator Dilution Techniques**

In addition to whole carcass chemical analysis, total body water was measured using the deuterium oxide dilution technique. An improved spectroscopic method for quantification of deuterium oxide in blood plasma or urine has been established by Jennings, et. al. (9). Deuterium oxide distributes throughout the body in the same manner as water. However, the H-O and D-O stretching energies in the mid-infrared are readily distinguishable from each other. This protocol establishes an analytical method to determine the total body water volume for a living subject. Piglets were given a 5 ml/kg bolus of a 10% deuterium oxide solution at the start of experimentation. Blood samples were collected prior to administration of the bolus to determine background levels of deuterium oxide in the blood, then at 20 min interval throughout the experiment to establish equilibrium and determine total body water (equilibrium established in 1-2 hrs). Samples were analysed as previously described (9). At a given time point after the bolus injection, the total deuterium oxide loss in the urine and blood prior to sampling was compensated for in the calculation of total body water.

Extracellular volume was determined using the sodium bromide dilution technique. A spectrophotometric method for quantification of bromide ions in serum was improved by Trapp and Bell (22), and has been reported to be adequate to
quantify the concentration of bromide ions in plasma and urine. Briefly, fluorescein reacts with oxidized bromide ions to produce a color that can be measured in the visible spectrum at 520 nm. Piglets were given a 5 ml/kg bolus of a 30 g/L sodium bromide solution at the start of experimentation. Blood samples were collected prior to the bolus being given to determine background levels of bromide ions in the blood, then at 20 min interval throughout the experiment to establish equilibrium and determine extracellular water volume (equilibrium establishes in ~ 1 hr). Samples were analysed as previously described (22). At a given time point after the bolus injection, the total bromide ion loss in the urine and blood prior to sampling was compensated for in the calculation of extracellular water volume.

Plasma volume was quantified using the Evan’s Blue technique (28). Evans Blue binds to the plasma albumin, thus remains primarily in the vascular space. It absorbs strongly at 620 nm and the concentration of Evans Blue in a plasma sample can be determined spectrophotometrically. Piglets were given a 5 ml/kg bolus of a 42.5 mg/L Evan’s Blue solution at the start of experimentation. Blood samples were collected prior to the bolus being given, then at 20 min interval throughout the experiment to establish equilibrium and determine plasma volume (equilibrium establishes in a few min). Samples were analysed as previously described (28). After the 60 min time point, plasma volume was assessed using changes in the percent plasma protein to ensure greater accuracy.

**EQUATION FOR CALCULATION OF PERCENT PLASMA VOLUME:**

\[
\text{% Plasma Volume } (t) = \frac{[P_{v60} - (P_{v60} \times F_{w60})]}{1 - F_{w(t)}}
\]

- \( P_{v60} \): Plasma volume at 60 min as calculated by Evan’s Blue
- \( F_{w60} \): Water fraction by weight of 100 µl of blood at 60 min
- \( F_{w} \): Water fraction by weight of 100 µl of blood at time point \( t \)
Skin Biopsies and Percent Water in Lean Tissue of the Skin

Skin biopsies were collected in triplicate at 20 min intervals throughout the experiment using a circular 5 mm diameter blade with a 3 mm depth limit. Biopsies were collected from the abdominal region between the middle of the sternum and the upper line between the iliac crests. At each time point biopsies were collected from various areas of the abdomen to help account for regional variability. Samples were immediately frozen in dry ice and stored at -20°C for future analysis. To determine LSW, samples were thawed and accurately weighed to within 0.001 g. Samples were then lyophilized for at least 3 days in a Freezone 6 lyophilizer (Labconco Corporation, MO, USA) then re-weighed until no further weight change could be ascertained. The fat content was then determined using a modification of the Folch method (5) which excluded water from the extraction process. Briefly, an organic solvent was used to extract lipids, a hydrophobic substance, from dry or lyophilized tissue samples between 10 and 100 mg in weight. The lipid containing organic solvent was removed, washed with a solvent, and evaporated with the fat remaining. Samples were re-weighed and the final weight was the fat free, dry tissue weight. This result was then used to calculate both the LSW fraction of the sample.

Equation for Calculation of Lean Skin Water:

The averages of the following 2 equations were used to negate for extractions errors:

\[
\% \text{LSW} = \left[ 1 - \frac{(M_d - M_f)}{(M_w - M_f)} \right] \times 100
\]

\[
\% \text{LSW} = \left[ 1 - \frac{M_{ff}}{(M_w - M_d - M_{ff})} \right] \times 100
\]

- \(M_w\): Wet mass of skin sample
- \(M_d\): Dry mass of skin sample
- \(M_f\): Mass of fat extracted from skin sample
- \(M_{ff}\): Fat free mass of skin sample
Whole Carcass Analysis

Samples of the whole carcass homogenate were dehydrated and the fat was extracted as per described for the tissue biopsies. Additionally, three 1.0 kg samples of the homogenate were collected for further processing. Samples were analysed for water, protein, carbohydrate, fat, and ash content by an external laboratory (Anresco Laboratories, CA, USA). TBW and LBW were then back-calculated from the recorded weight based on the assumption the weight change was primarily water gain/loss.

EQUATION FOR CALCULATION OF TOTAL BODY WATER:

\[
\% \text{ TBW}(t) = \left( \frac{(F_w - 1) \times M_c + M(t)}{M(t)} \right) \times 100
\]

\(F_w\): Fraction of water in the carcass (at end of experiment)
\(M_c\): Mass of carcass (at end of experiment)
\(M(t)\): Mass of animal at time point (t)

EQUATION FOR CALCULATION OF LEAN BODY WATER:

\[
\% \text{ LBW}(t) = \left( \frac{(F_w - 1) \times M_c + M(t)}{M(t) - (M_c \times F_f)} \right) \times 100
\]

\(F_w\): Fraction of water in the carcass (at end of experiment)
\(M_c\): Mass of carcass (at end of experiment)
\(M(t)\): Mass of animal at time point (t)
\(F_f\): Fraction of fat in the carcass (at end of experiment)

Statistics

All statistical comparisons were made using one- or two-way ANOVA tables, as appropriate, with significance set at the P<0.05 level. Specific within group differences were located with Tukey/Kramer’s post hoc comparison. All data
statistics were compared using MatLab v. 7.0.4 (MathWorks, MA, USA) software package and data are reported as mean ± SE.

RESULTS

Animal Characteristics

The animal characteristics (n = 8) are presented in Table 1. Initial baseline values were measured on the morning of each experiment after induction of general anaesthesia but before the commencement of fluid administration. Final body weights were measured after the final equilibrium period just prior to termination of the experiment.

Body composition for each of the piglets was determined by whole carcass chemical analysis at the end of experimentation (after the addition of ~5L of LRS). Results established whole body water, protein, fat, and ash content (Table 2). These data allowed for the determination of FFM and LBW based on standard equations.

Percent Change in Body Weight

The percent change in body weight throughout the experiment (Figure 1) demonstrates that the fluid administration and maintenance protocol was effective at increasing the total body water. As expected, during each 20 min fluid administration period there was a dramatic increase in body weight, followed by stable weight during the 40 min equilibrium period. After only the initial fluid administration step there was a significant increase (P<0.05) in weight which was maintained throughout the course of the experiment. The fluid administration protocol was repeated 5 times and resulted in a total of a 23.4 ± 2.1 % increase in body weight.
Fluid therapy performed in this study administered the LRS directly to the vascular compartment. This resulted in an initial plasma volume expansion that equilibrated with the interstitial volume during the following 40 min recovery period. This plasma volume expansion was also reflected in the pattern of total hemoglobin fluctuations (Figure 2). After each fluid administration period there was a dramatic decrease in total hemoglobin (P<0.05), followed by a recovery period as the fluid moved from the vascular compartment into the interstitial space. This is further demonstrated in Figure 3a where, following each fluid therapy step, there is an initial increase in plasma volume, as measured by the Evan’s Blue. Comparatively, there is a delayed response to the fluid administration in the interstitium, which starts to increase only during the recovery period (assessed using sodium bromide dilution). Unfortunately, due to the larger standard error associated with the deuterium oxide data, the observable upward trend in the absolute value of the TBW did not reach statistical significance (Figure 3a). However, when assessed as percent change from baseline, both TBW and LBW demonstrated increases (P<0.05) following the initial fluid administration step (Figure 3b). A similar trend was observed in measurements of skin water from the tissue biopsies (Figure 4). Though not statistically significant (P=0.76), there was an upward trend in the water content of the skin (SW). When this was expressed as a function of lean tissue (LSW) the increase (P>0.05) in water content was very similar to that of the whole body (LBW).

It is interesting to note that the variance for TBW is increased compared to that of LBW (6.26 vs. 3.84, respectively), indicating that LBW is a much more consistent measure across different subjects than TBW. This attenuation of variance is also seen in the comparison between SW and LSW (9.99 vs. 5.30, respectively), demonstrating
that the water content in lean tissue is more stable throughout a population. This observation is consistent with previously published data (20, 23, 25).

Relationship Between Total Body Water and Local Skin Water

The definitive upward trend observed in both TBW and SW indicates a similar response to the fluid administration protocol. Thus it is not surprising that there is a strong positive correlation ($r^2=0.58$, $P<0.05$) between these two parameters (Figure 5a). As such, these data quantitatively demonstrate that skin turgor is a valid test for determining hydration status in a clinical setting. Even more convincing, however, is the stronger correlation between LBW and LSW ($r^2=0.87$, $P<0.01$), expressed in Figure 5b. The high variability in fat content increases the standard error associated with the water measurements taken both systemically and locally. The elimination of this source of variability provides compelling evidence that LSW is an accurate and precise indicator of LBW.

DISCUSSION

This study is the first study to investigate a possible quantitative relationship between LBW and LSW. Previously, skin turgor has been used as a clinical marker of hydration status, but this has been qualitative at best. The results from this study demonstrate that LSW correlates with LBW in an over-hydration model, and that this correlation can be used to provide a meaningful quantitative assessment of LBW.

Clinical assessment of growth, nutritional status, and health is enhanced by accurate measurement of body composition, whether one is assessing normal development or physical condition parameters, monitoring the natural course of a chronic disease, or evaluating responses to diet or drug interventions (4, 11, 14). The
use of a hydration constant such as LBW, which is minimally affected by age or gender in the adult population (23), can eliminate systematic errors in body composition methods. Furthermore, age and gender specific constants have been developed for children from birth to 10 years of age (6, 26). Additionally, measurement of body composition is important for optimum clinical care during hospitalization in order to maintain appropriate hydration during treatment and recovery. While LSW is currently not appropriate for clinical use, it could provide tremendous advantage to researchers investigating animal models of growth and development, or of various diseases. Of note, regional variability in skin tissue hydration is a concern. Local edema and/or skin pathologies would alter the relationship between LSW and LBW, thereby rendering the measurement less accurate. Additionally, gravitational effects which can alter skin thickness and water content of the skin must be taken into consideration. Thus such areas are contraindicated for this technique. Choosing a site like the torso and ensuring the absence of any skin anomalies, including mammary glands and scar tissue or wrinkles in the vicinity of the navel, allow for accurate detection of both LSW and LBW. Moreover, the data from the torso biopsies demonstrated excellent precision, allowing for reliable tracking of changes in hydration over the course of the experiments. The ability to use LSW to determine LBW will allow future research a simple and accurate measure for a parameter that is at present difficult to assess.

Although some measurement techniques are more accurate and precise than others, there is no gold standard for body composition and hydration assessment in vivo that can be readily performed. All methods incorporate assumptions that do not hold true in all cases; however, the importance of such assumptions can be minimized. Previous studies have demonstrated that approximately half of the
variability in LBW was due to methodology, indicating that true biological variability in healthy individuals is relatively low (24, 26). The experimental error associated with current methods of assessing LBW limit its usefulness in accurately determining body composition. However, our results demonstrate that LSW can be used as an accurate and precise measure of LBW, and that experimental error is minimal.

Regression analysis is traditionally used in validation studies to determine the relationship between the actual and predicted variables, with standard error of prediction (SEP) used to describe the prediction accuracy. We found a high correlation coefficient between LBW and LSW, and the SEP was 0.77%. Subsequent calculation of total error (TE=0.80), a measure of the variability of the prediction errors around the line of identity, was nearly identical to our SEP (X compared to Y), signifying almost no systematic differences between LBW determined using whole body measurements and LSW. Consequently, it might be concluded that from these analyses that LSW is an appropriate measurement for the estimation of LBW within our sample population.

In this study we have validated the use of LSW against chemical carcass analysis. Complete carcass recovery and uniform homogenization of the resulting dry matter was achieved, which we consider a necessary prerequisite for acceptance of chemical analysis as a gold standard. We were able to show that LSW measurements were highly correlated with chemical analysis. Additionally we found a high degree of correlation with indicator dilution techniques and LSW, providing further evidence for a significant correlation between LSW and LBW.

These collective data provide useful information and insight into the relationship between skin water content and whole body water. The strong correlation coefficient and low standard error of prediction indicate that changes in these parameters during
overhydration are remarkably congruent. We conclude, therefore, that LSW gives an accurate and precise estimate of LBW.
ACKNOWLEDGEMENTS

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DISCLOSURES

This work was sponsored in full by Nellcor Puritan Bennett, a subsidiary of Tyco Healthcare.
REFERENCES


TABLE LEGEND

Table 1: Animal characteristics. Values are for n = 8 piglets.

Table 2: Carcass composition. Values are for n = 8 piglets.
FIGURE LEGEND

Figure 1. Percent weight change from baseline with fluid administration. Vertical arrows indicate the addition of ~1 L of water.
* Denotes differences (P<0.05) compared with baseline.

Figure 2. Blood hemoglobin content during fluid administration protocol. Vertical arrows indicate the addition of ~ 1 L of water.
* Denotes a difference (P<0.05) compared with baseline.

Figure 3. Water content of the different compartments during the fluid administration protocol as measured by indicator dilution. Vertical arrows indicate the addition of ~ 1 L of water.
* Denotes a difference (P<0.05) compared with baseline values.

Figure 4. Changes in skin water (SW) and lean skin water (LSW) during the fluid administration protocol measured by tissue biopsies. Vertical arrows indicate the addition of ~ 1 L of water.
* Denotes a difference (P<0.05) compared with baseline values.

Figure 5. Correlation between skin water and whole body water in whole and lean tissues during fluid administration.
### TABLE 1: Animal Characteristics

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<td>Initial MAP (mmHg)</td>
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**TABLE 2: Carcass Composition**

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FIGURE 1: Percent weight change from baseline with fluid administration.
**FIGURE 2:** Blood hemoglobin content during fluid administration.
FIGURE 3: Change in water content with fluid administration.

(a) Change in total body water (TBW), extracellular water (ECW), interstitial water, and plasma water with fluid administration over time. Bars indicate standard error of the mean.

(b) Change in lower body water (LBW) and total body water (TBW) with fluid administration over time. Bars indicate standard error of the mean.
FIGURE 4: Changes in local skin water with fluid administration.
**Figure 5:** Correlation between skin water and whole body water.

### a

- **R²** = 0.5852
- P < 0.05

### b

- **R²** = 0.8653
- P < 0.05