Graduate thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Circulation and Medical Imaging

Halvor Dagssøn Landsverk

A Comparison of Two Methods, Urine Specific Gravity and Bioelectrical Impedance, to Measure Short-Time Effects of Underwater Work on Saturation Divers´ Hydration Status

Graduate thesis in Programme of Professional Study in Medicine
Supervisor: Jacky Lautridou
Co-supervisor: Ingrid Eftedal
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Contents

Norsk sammendrag – Summary in Norwegian

Metningsdykking er en veletablert metode som tillater dykkere å jobbe under vann over lengre perioder. En av utfordringene dykkerne står overfor under metningsdykking er å holde seg hydrerte. Det finnes flere metoder som måler hydreringsstatus, men ikke alle er egnet grunnet miljømessige utfordringer knyttet til metningsdykking.

I denne studien ble to metoder for å måle hydrering, urin spesifikk tetthet og bioelektrisk impedans, sammenlignet med tanke på hydreringsstatus hos dykkere.

Studien inkluderte 11 mannlige profesjonelle dykkere. De fulgte samme dykkerprotokoll, og jobbet i grupper på tre. Gruppene jobbet i kontinuerlig roterende 12-t skift i tre uker. Under et dykk er to dykkere i vannet, mens den tredje, kalt bellman, blir igjen i dykkerklokken for å overvåke og assistere dykkerne i vannet. Bellman er dermed ikke eksponert for stressfaktorene i vannet. Rollen som bellman roterer daglig mellom dykkerne i hver gruppe. Urin spesifikk tetthet og bioelektrisk impedans ble målt før og etter dykk og hver morgen på dager uten dykk.

Dykkere i dykkerklokken (bellman) opplevde en nedgang i impedans på 7.5 %, tilsvarende en økning på 4.8 % i hydreringsnivå etter et dykk sammenlignet med dykkerne i vannet. Denne endringen ble ikke sett for urin spesifikk tetthet. Dykkernes hydreringsgrad forble uendret gjennom hele testperioden. Det var ingen korrelasjon mellom urin spesifikk tetthet og bioelektrisk impedans.

Urin spesifikk tetthet og bioelektrisk impedans kan ikke brukes som to likeverdige metoder for å måle hydreringsgrad. Dataene fra studien indikerer at dykkernes hydreringsrutiner var effektive. Mest interessant var det at dykkere i dykkerklokken opplevde en økning i hydreringsnivå sammenlignet med dykkerne i vannet. Det vil være interessant for videre forskning innen metningsdykking å måle effektene av forskjellene i eksponering for disse to gruppene. I tillegg vil det være interessant å forstå hvilke fysiologiske forhold som slår ut på impedansmålinger, men ikke på urin spesifikk tetthet.

Abstract

Background

Saturation diving is a well-established procedure for enabling divers to work subsea for extended amounts of time. One of the challenges of saturation diving is ensuring that the divers stay hydrated. There are several ways of measuring hydration, but not all of them are suited for the work environment that the divers find themselves in.

In this study two methods of measuring hydration, urine specific gravity and bioelectrical impedance, were compared in the detection of changes in hydration status among divers.

Methods

The study included 11 male professional divers who were following the same dive protocol working in teams of three on continuously operating 12-hour shifts for three weeks. During bell-runs, two divers are in the water while the third – the so-called bell man – stays in the bell for support and backup. This third diver is thus not exposed to stressors from the in-water environment. The role as bell mall rotates daily between the divers in each three-man team. Urine specific gravity and bioelectrical impedance were measured before and after each dive and in the mornings on days without dives.

Results

Divers in the diving bell experienced a decrease of 7.5 % in impedance representing a 4.8 % increase in hydration level after a bell run compared to the in-water divers. This difference was not seen for specific gravity. Divers experienced no change in hydration status for the duration of the test period. There was no correlation between urine specific gravity and bioelectrical impedance.

Conclusion

Urine specific gravity and bioelectrical impedance cannot be used interchangeably as methods of measuring hydration. The data indicates that the hydration routines followed by the divers were effective. Most interestingly, divers working as the bellman during a bell run experienced a significant decrease in impedance measured, corresponding to an increase in hydration level, compared to the in-water divers. Moving forward, quantifying the effect of the difference in exposure for the two groups could be an interesting topic in the field of saturation diving. It may also be interesting to explore which physiological properties are impacting bioelectrical impedance analysis and not urine specific gravity.

Abbreviations and definitions

USG:	Urine specific gravity. The relative weight of urine compared to that of an equal volume of water.
BIA:	Bioelectrical impedance analysis.
TBW:	Total body water. In this study calculated from BIA.
ICW:	Intracellular water.
ECW:	Extracellular water.
Hyperbaric:	Increased ambient pressure.
Normobaric:	Normal pressure equal to that of sea level.
DSV:	Dive support vessel.
Heliox:	Gas mixture of oxygen and helium.

Introduction

Saturation diving

Saturation diving is one of the most advanced techniques used in commercial diving. Saturation divers stay under pressure until their tissues become saturated with hyperbaric breathing gases, normally heliox. This technique enables divers working at great depths to stay pressurized for a long time while limiting the risk of them developing ailments such as decompression sickness (DCS). Divers live in a pressurized chamber system when not in the water, thus facilitating several dives without requiring a lengthy decompression process in between dives.

The pressure chambers allow the divers to live at a pressure equal to the water depth where they are working while offering protection from the in-water exposure. However, they are still exposed to the elevated pressure from the environment and the elevated partial pressure of the breathing gases used (1).

One of the effects saturation diving has on the body is a change in fluid homeostasis. This has several causes. When diving in cold waters, divers use a hot-water suit to protect against the low temperatures at depth. Being immersed in hot water induces fluid loss through sweating, and salty sea water enhances this effect (2). There is also fluid loss caused by an increase in diuresis. Several factors may cause this. Immersion has been shown to cause a pressure-mediated fluid shift from tissue to the blood (3).The effect is greater in cold waters where the cold leads to vasoconstriction on its own (4). Immersion has also been linked to an increase in renal natriuresis (5).

Importance of hydration status in diving

Dehydration has been linked to decreased physical performance (6), although these findings may be influenced by other factors such as physical and thermal stress and fatigue (7). It is also theorized that dehydration increases the risk of venous gas bubble formation which in turn may lead to decompression sickness, a potentially life-threatening condition. Decompression sickness occurs when gases that have been saturated in the tissue form gas bubbles which enter the blood stream. This happens when the ambient pressure falls, for instance when a diver returns to the surface. The gas bubbles can enter small blood vessels where they will embolize. This can be life-threatening if it happens in the brain or lungs. The bubbles can also potentially damage endothelial function(8,9). Although animal studies have been inconclusive (10,11), experiments with military divers found that pre-dive hydration had a protective effect against venous bubble formation (12). While this theory is inconclusive, it may be because when hydrated, the blood has a lower viscosity, making gas exchange between capillary and alveolar membranes more effective. This ensures a higher rate of elimination of bubble- forming gases such as nitrogen.

The preferred way to measure dehydration status is with urine specific gravity (USG). The density of the urine is compared to the density to water and gives an estimate of the urine osmolality and it is unitless. While the specific gravity of water is 1.000, the renal system has a maximum concentrating capacity of 1.050 (13). A specific gravity exceeding 1.030 is defined as dehydration.

Bioelectrical impedance analysis

Bioelectrical impedance analysis (BIA) is another way to measure the water content of the body. By sending an electric current through the body, we can measure the impedance, which is the resistance to an alternating current. Whereas fat is low in water and a poor conductor, tissue like muscle is mostly water and conducts well. This means that total body water (TBW) is linked to the body´s fat-free mass (FFM), and TBW is found to be 73.2 % of FFM (14). TBW in turn consists of extracellular water (ECW) and intracellular water (ICW). These compartments are separated by a cell membrane.

Impedance can be seen as a combination of resistance, caused by TBW, and reactance, caused by the cell membrane´s capacitance. The membrane consists of a phospholipid bilayer, with hydrophilic heads pointing outwards and hydrophobic tails meeting in the middle. The membrane acts as a capacitor with the inner bilayer functioning as a thin insulator separating the conducting ECW and ICW. An insulator with a conductor on each side is called a capacitor, which has the ability to store electrons(15). At low frequencies, the membrane acts as an insulator with no current passing the membrane, and we get an estimate of the ECW. At high frequencies, the membrane acts as a capacitor and the current passes the membrane. The impedance measured then reflects both ECW and ICW.

In addition, by measuring changes in the reactance and resistance as the current alternates, an estimate of the phase angle can be obtained. The phase angle gives an indication of the membrane function and integrity, as leaks in the membrane leads to changes in the voltage. The phase angle therefore gives an indication of overall health, and has been shown to be a predictor of outcomes in terminal patients (16).

Study goal

The purpose of this study was to compare urine specific gravity and bioelectrical impedance in measuring hydration levels among saturation divers. Since bioelectrical impedance can be measured by the divers themselves within the pressure chambers, it would be beneficial to use bioelectrical impedance to monitor hydration status in hydration diving.

There is little research on methods of measuring hydration in saturation diving. Studying the relationship between two different tests for hydration status, may lead to improvements in the routines. Compared to urine specific gravity, BIA is a simpler procedure. It is non-invasive and can be performed within a matter of seconds. The equipment consists of a set of electrodes placed on the wrist and ankle and a device that registers the measurements. A small current is sent between the electrodes and the voltage is measured. The simplicity of this device means that the divers can perform the measurements themselves. These advantages make BIA an interesting option for future research and use in saturation diving.

Materials and Methods

Data source

All data was collected from commercial saturation divers working on the UK continental shelf in the summer of 2021. The experiment was conducted aboard a TechnipFMC Diving Support Vessel (DSV) in the North Sea (Figure 1).

Ethical approval

The study protocol was approved by the Norwegian Regional Committee for Medical and Health Research Ethics, approval reference ID 117404.

Information about the experiment was given out prior to the experiment, with information regarding experimental procedures and use of private data. All divers who wanted to participate in the experiment were asked to sign a declaration of consent.

Subjects

Eleven healthy professional male divers were included in this study. All divers were certified for diving in Norwegian and/or UK waters. The divers´ physical characteristics are included in Table 1.

Table 1. Diver characteristics (mean ± SD)

Subjects	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
11	46.2 ± 11.6	184.6 ± 8.6	93.6 ± 17.0	27.26 ± 3.23

Diving procedures

The subjects were grouped into four teams of three. The teams were working overlapping 12-hour shifts, with a 6-hour interval between each team, ensuring a continuous underwater activity. The divers worked the same shift for the entirety of the experiment, meaning that the groups had different circadian rhythms. They were diving every day, except for when the ship was sailing back and forth between port in the UK. The divers were living in pressurized chambers on the DSV between shifts. See Figure 2 for a layout of the diving system aboard the ship. From here they were transported to the ocean floor via a pressurized diving bell during shifts. Both living chamber and dive bell were continuously monitored by a crew on the DSV to ensure the safety of the divers. The dive bell was connected to the DSV via an umbilical equipped with hoses for pressurization and decompression, power supply, gas reclaim, hot water for the diving suits and cables for communications. During a dive, two of the divers would be working in the water and the third would remain in the diving bell to monitor and assist the divers in the water. The role as in-water diver and bellman would rotate so that every diver took a turn as bellman every three days. A bell dive takes up to 8 hours, with the in-water divers working in the water for a maximum of 6 hours, including a mandatory 30 min restitution and rehydration break in the bell between the third and fourth hour of work.

For this study, the divers worked on the UK side of the North Sea, where a full saturation may last up to 28 days. Daily measurements of hydration status pre- and post dives were performed for 18 or 19 days during the bottom phase of the saturation (19 days for one team, 18 for the three other), when the divers were held at a storage depth of 63 m, and performed daily dives to a depth of 63-64 m.

Daily dives were cancelled on either one or two days (depending on team number) when the vessel was moving for dock-side crew shifts.

Figure 1. Diving support vessel used during the project (Photo curtesy of TechnipFMC).

The subjects were trained in use of the bioimpedance device and in taking urine samples. Height, weight and blood pressure were measured before the experiment. Baseline values for urine specific gravity (USG) and bioelectrical impedance (BIA) were measured at surface level in a normobaric environment before starting the dive protocol. Entering the chamber blow down, or compression, was then started. The subjects were tested before and after each dive, and once on days without dives. They were instructed to do the pre dive measurements straight after waking up, before their breakfast. Urine samples were collected from the second void of the day, as close in time as practically achievable to the BIA measurements. The post dive measurements were to be taken shortly after bell runs. The impedance values were transmitted via Bluetooth from the device to a mobile phone outside the chamber. Urine samples were sent through a chamber port and analysed using a refractometer in the vessel hospital.

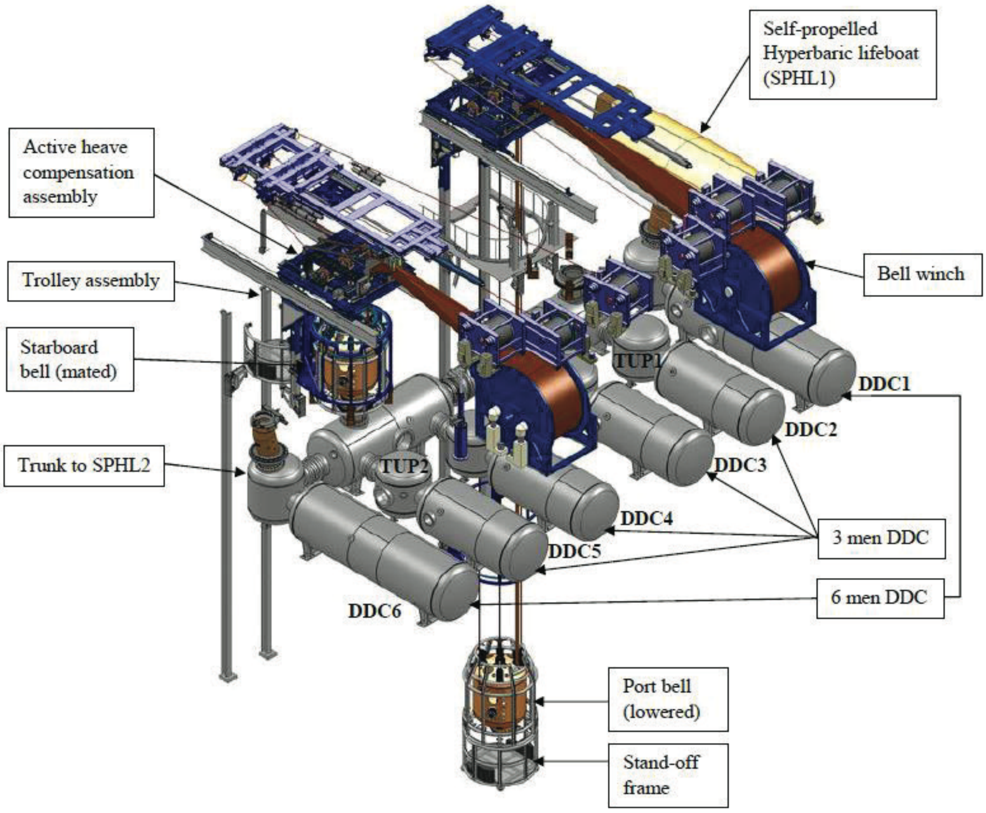

Figure 2. Schematic of diving system layout onboard the diving vessel (17).

Apparatus for bioelectrical impedance analysis

Bioelectrical impedance was measured using the BiodyXpert^{TM} by Aminogram (La Ciotat, France). The device is handheld and connected to an app where the measurements are displayed. Electrodes are on both sides of the grip and on the front. The measurement was performed as follows:

1) The divers would be correctly positioned (Figure 3).
2) A small amount of electrode gel was applied to all electrodes.
3) The right hand was holding the device. Three fingers were placed on one grip electrode and the thumb on the other.
4) The front electrode was placed on the right ankle, below the malleoli.

Figure 3. Showing the impedance device and the correct position for measuring impedance (18).

The impedance values for frequencies at 5 Hz, 20 Hz, 50 Hz, 100 Hz and 200 Hz (n=5) proved consistently reliable, with Cronbach´s alpha = 0.995.

Assessing hydration status

Urine specific gravity, impedance and total body water (TBW) were included as assessments of hydration status. Total body water was calculated from the BIA-data using the formula by Kushner et Schoeller (1986), by recommendation from the producer of the BIA-device (Aminogram):

$$TBW = (0.382 + (0.014 * Sex)) * (H^2 / Z50) + (0.105 + 0.038) * W + 8.315 + 0.084 * Sex$$

TBW = Total body water in litres
Z50 = impedance value at 50 Hz
Sex, where male = 1 and female = 0
H = Height in cm
W = Weight in kg

As this formula is meant for impedance values at 50 Hz, total body water was only calculated from this frequency. The other frequencies were still useful for checking the accuracy and variance for the impedance meter across frequencies.

In addition to providing a good comparison of urine specific gravity and BIA, this would also give a good picture of the overall hydration status for the saturation divers involved.

The two testing methods, urine specific gravity and BIA, were checked for:

1) Correlation between data from the two methods, using all values obtained from all divers. Both correlation between absolute values and between percentage change from post-dive values to pre-dive values were performed.
2) Change in hydration status over time, by sorting the measurements into four time periods. The first value was the baseline value taken before decompression. The other three values were the mean pre-dive and post-dive values of days 1-6, 7-12 and 13-19 for each diver respectively. In addition, the mean values for all pre-dive and post-dive values were calculated and compared against each other and the baseline.
3) Percentage change from pre-dive to post-dive value between bellman and in-water diver. To compare if there were different effects between the groups, the change from pre dive measurement to post dive measurement was calculated as the percentage change from pre- dive to post-dive with pre dive as baseline. As the number of Diver and Bellman measurements were uneven, means were calculated. The means for Diver and Bellman percentage change in value were then matched as pairs for each diver.

Statistical analyses

All data were analysed using the statistical program Prism by GraphPad and SPSS version 27. The data was checked for normality by visual inspection of Q-Q plots and Shapiro-Wilk´s test for normality. In case of non-normality non-parametric analysis was applied. Statistical significance was set a priori at $p < 0.05$ for all tests.

Correlation between methods of measuring hydration

Spearman rank correlations were used to investigate the relationship between changes in urine specific gravity and changes in measures obtained through BIA (total body water and impedance). USG-values were compared to BIA-values for all frequencies, in addition to body mass and percentage change in USG- and BIA-values between post and pre dive measurements.

Change in hydration status

A repeated measures analysis of variance (ANOVA) was performed to investigate whether hydration status changed during the experiment. Post hoc comparisons using Tukey´s test were performed to identify differences when a statistically significant difference was indicated by the ANOVA. If the assumption of sphericity was not met, as assessed by Mauchy´s test of sphericity, this was corrected by using epsilon; Greenhouse & Geisser correction.

Bellman vs Diver comparison

Differences between the Diver and Bellman groups were checked using a paired t-test for normally distributed data and the Wilcoxon matched pairs test for non-normal data.

Results

The main results were that there was a significant decrease in hydration levels among the in-water divers compared to the bellmen as measured by bioelectrical impedance, and that there was no meaningful correlation between the urine specific gravity and bioelectrical impedance measurements. In addition, there was no change in hydration status for the divers during the length of the experiment.

Correlation between methods of measuring hydration

Although the correlation between urine specific gravity and impedance proved significant, the correlation was weak. In addition, the correlation pointed to the opposite relationship than what was expected. The correlation coefficients between the USG and BIA values were negative. *See Appendix E for statistics.*

Change in hydration status

Urine specific gravity

There were no statistically significant differences between any of the timepoints compared. *See Appendix A for statistics.*

Bioelectrical impedance

There was a statistically significant difference between the baseline and pre-dive timepoints, $p < 0.001$. Post hoc analysis with Tukey´s test showed a statistically significant increase in impedance from baseline to each of the three time periods. The increase was 6.6% for days 1-6, $p = 0.009$, 7.1% for days 7-12, $p = 0.006$ and 7.8% for days 13-19, $p = 0.006$.

There was a statistically significant difference between the baseline and post-dive timepoints, $p = 0.014$. Post hoc analysis with Tukey´s test showed a statistically significant increase in impedance from baseline to days 1-6, $p = 0.04$ and baseline to days 7-12, $p = 0.03$. The increase was 4.6% and 4,1% respectively.

There was a statistically significant difference between the baseline, mean pre-dive and mean post-dive values, $p = 0.001$. Post hoc analysis with Tukey´s test showed a statistically significant difference between all three categories, baseline, mean pre-dive and mean post-dive. Impedance increased with 7.1% from baseline to mean pre-dive, $p = 0.003$, increased with

4.2% from baseline to mean post-dive, *p* = 0.03 and decreased with 2.7% from mean pre-dive to mean post-dive, *p* = 0.003. *See Appendix B for statistics.*

Total body water

There was a statistically significant difference between the baseline and pre-dive timepoints, *p* = 0.002. Post hoc analysis with Tukey´s test showed a statistically significant decrease in total body water from baseline to days 7-12, *p* = 0.02 and baseline to days 13-19, *p* = 0.01. The decrease was 3.4% and 3.5% respectively.

There were no statistically significant differences between the baseline and post-dive timepoints.

There was a statistically significant difference between the baseline, mean pre-dive and mean post-dive values, p = 0.007. Post hoc analysis with Tukey´s test showed a statistically significant decrease in total body water from baseline to mean pre-dive, *p* = 0.01, with a decrease of 3.5%. There was also a significant increase in total body water from mean pre-dive to mean post-dive, *p* = 0.003, with an increase of 1.6%. *See Appendix C for statistics.*

Bellman vs Diver comparison

There were no significant differences in urine density between bellmen and in-water divers. However, there were statistically differences between the two groups for the BIA and, thus in extension, for total body water.

Bioelectrical impedance

A Wilcoxon matched pairs test showed that the bellman group had a median change between post-dive value and pre-dive value of -7.581 compared to the Diver group´s 0.022, *p* < 0.001.

Total Body Water

A Wilcoxon matched pairs test showed that the bellman group had a median change between post-dive and pre-dive value of 4.842 compared to the Diver group´s 0.715, *p* < 0.001.

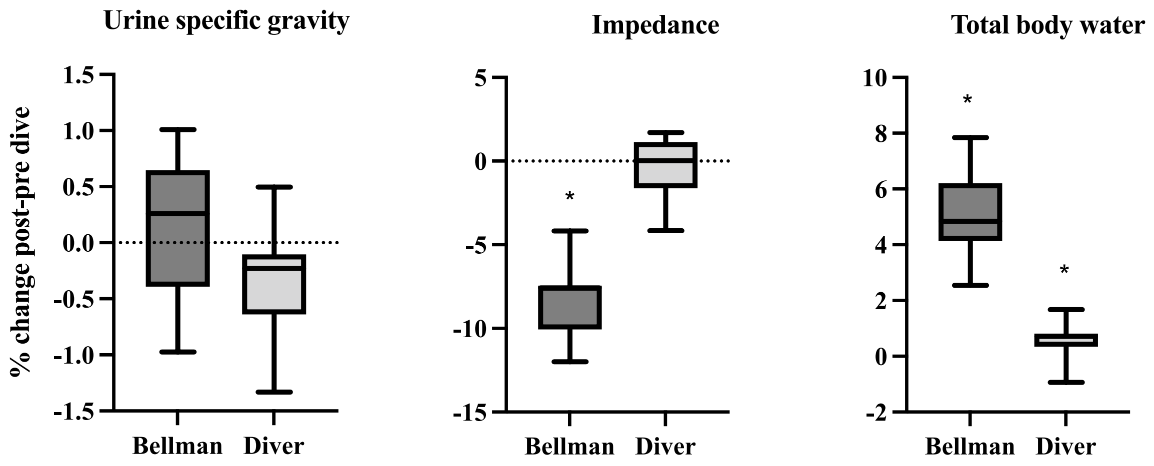

Figure 6. Box plot showing percentage changes from pre-dive values to post-dive values in urine specific gravity, bioelectrical impedance at 50 Hz and total body water post-dive for bellmen and in-water divers. Results are expressed as median with interquartile ranges (25th – 75th percentiles) and minimum/maximum values. Stars over boxes represent statistically significant changes. Impedance and total body water were calculated using 50 Hz data from the body impedance meter. Urine specific gravity was measured by refractometry.

Discussion

Correlation between methods of measuring hydration

The main purpose of this study was to investigate if there is a relationship between urine specific gravity and BIA when measuring hydration status in divers. While urine specific gravity an established lab standard, BIA is a rather novel and unproven method with potential upside. Compared to specific gravity, which requires a urine sample to be analyzed, BIA is noninvasive and quick to perform.

The data indicated that there was no significant correlation between the two measurements, neither in corresponding measurements nor in registered change after dives. While both methods detected changes in hydration after the dives, the changes were unrelated. The trend was even pointing towards a slight negative correlation between the two measurements, which is counterintuitive given that both methods show dehydration on a rising scale.

An earlier study assessing hydration status in marathon runners also found no relationship between specific gravity and BIA as biomarkers (19), pointing to an inherent difference between the two methods. BIA has been shown to lag behind urine specific gravity in detecting hydration status (20). It was suggested that exercise may increase the resistance in the body. Increased blood flow to muscles leads to a rise in tonicity that may affect the impedance analysis (21). Research on saturation diving and hyperbaric exposure have also pointed to physiological changes that may affect analysis. Changes have been observed in both hematological variables (22) and body composition (23). Immersion is also known to induce a fluid shift from the extremities to the central organs(1).

In addition to the differences between the two methods, the test environment was challenging. Working deep beneath the surface is tough for instruments as well as humans. Several common medical instruments are not usable in a hyperbaric environment. This could be because they pose a danger to the divers or as a result of poor functionality under these extreme conditions (24). For instance, instruments powered by an alternating current may create sparks. This can be lethal in a closed environment with gases such as oxygen and hydrogen. Instruments therefore need to be powered by a low voltage current.

The urine was taken up to the surface for analysis, so the hyperbaric environment did not have an impact on the analysis itself. The BIA measurement was done in a pressurized environment, but there is no evidence to conclude that this influenced the devices, and they were used throughout the experiment without any issue.

Change in hydration status

Effect of time under pressure

The difference between the two methods is again apparent. The USG measurements showed no significant change between any of the four time points, while the BIA measurements showed a significant increase from baseline to dive values. The main difference in BIA values therefore seems to stem from the transition from a normobaric to a hyperbaric environment.

With the data available in this study, one can only speculate about the reasons for this difference. First, it is another illustration of the difference and incompatibility between the two methods. Furthermore, while the two methods both give a measure of hydration status, they do so by measuring different parameters. As only BIA detects a change between baseline and values under hyperbaric saturation, one might speculate that the change in pressure affects the body mechanisms measured by the BIA more than those measured by specific gravity.

The main takeaway, however, is that time under pressure doesn't seem to affect the hydration status of saturation divers. As hyperbaric exposure is known to cause dehydration, this finding points to effective hydration routines among the divers. Additionally, baseline values should be taken in the hyperbaric environment to get a correct reference value.

Diver vs Bellman role

The most interesting finding from the study was the difference in hydration values between the in-water divers and the bellmen. Compared to the in-water divers, the bellmen experienced an approximately eight percent drop in impedance and almost five percent increase in total body water measured from pre-dive to post-dive, meaning they were more hydrated after the bell-run. The in-water divers showed no significant change in hydration after the dive. Assuming the validity of this result, one must look at the difference between the diver and bellman roles. As previously stated, the bellman stays inside the diving bell during the dive to monitor the in-water divers. They are therefore sheltered from the exposure to water, which effects a pressure on the body, and increases diuresis (1). In addition, the bellmen don´t wear the hot water suit which have been shown to facilitate water loss through sweating (1,25). They are also avoiding the physical strain that the divers experience. Exercise may also lead to a fluid shift, with extravascular fluid being absorbed from inactive tissue into the blood stream (26).

To better understand what leads to the difference in hydration status between divers and bellmen, there is a need for more studies to investigate the effect of each environmental factor that the divers are exposed to.

Again, illustrating the difference between the two measuring methods, the difference in hydration status was not present in the USG measurements. This could again point to the difference in lag times between the specific gravity and BIA measurements discussed earlier. This theory could be supported by the fact that the pre-dive values for both diver and bellman were similar, meaning that any changes seen after each dive, were transient and had reset before the next dive. The fact that the USG values showed no difference between diver and bellman could mean that the effects of the dive had already disappeared, since specific gravity has been shown to be ahead of BIA in detecting hydration status. Another possible explanation is that the divers started hydrating before performing the measurements, as specific gravity has been found to better pick up acute fluid intake than BIA (27).

Limitations

Sample size

One of the main limitations of the study is the small sample size of eleven divers, which limited the ability to find statistically significant differences. This was further exacerbated by the high variability between subjects.

As the sample size for the study was quite small, some assumptions had to be made to be able to perform the statistical analyses.

For the comparison between the two dive roles, diver and bellman, it was assumed that the effect of each role would subside in time for the next dive. As all divers rotated between being diver and bellman, it was impossible to compare the two roles with independent populations. The assumption of no lasting effect from one dive to another can be considered strengthened as all pre dive values were in the same range, independent of the role in the previous dive.

In addition, the frequency ratio between being diver and bellman was approximately 2:1, resulting in unequal sample size, which may affect the analysis.

Variation in sampling

While the measurements were scheduled to be conducted at specific time points, this was difficult to achieve in practice. The pre-dive measurements were meant to be taken before breakfast, but this was not always done. As for the post-dive measurements, these were in most cases performed in the living chamber straight after each bell run. There would however, in some cases take some time before the divers were available to do the measurements, due to other tasks taking precedence. Given the fact that there appeared to be a time lag associated with the BIA data relative to urine data, variation in time of day of the measurements could contribute to some of the variability in the readings. However, as such variability has also been reported by others, we find it likely that the findings are real.

Circadian rhythm

As the divers were working in teams that did shift work, each team had a different circadian rhythm. While it is possible that this influences hydration status(28), this was not tested for in the experiment.

Premature end of experiment

Finally, the study had to be stopped before the divers underwent decompression. While this was unavoidable, it is regrettable, as it would have been interesting to see investigate the measurements during and after decompression.

Conclusion

Both urine specific gravity and bioelectrical impedance analysis are known to express changes in hydration, they do so inconsistently of each other. However, the data in this study does not show a meaningful correlation between the results from the two methods. In fact, the data indicates a weak negative correlation. Therefore, it is not feasible as of now to replace the standard urine specific gravity test with the simpler bioelectrical impedance measurement. More research needs to be conducted looking at the acute changes in renal and cardiovascular systems and in body water composition during hyperbaric exposure. This would help in identifying the lag times theorized for the two methods.

While the two methods did not show a relationship in measured changes in hydration, they nevertheless both indicated that the divers stayed hydrated during time under hyperbaric exposure. This would indicate that the dive protocol being used is effective in keeping divers hydrated.

Most interestingly, divers working as the bellman during a bell run experienced a significant decrease in impedance measured, corresponding to an increase in hydration level, compared to the in-water divers. Moving forward, quantifying the effect of the difference in exposure for the two groups could be an interesting topic in the field of saturation diving. It may also be interesting to explore which physiological properties are impacting bioelectrical impedance analysis and not urine specific gravity.

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Appendices

Appendix A: ANOVA tables, multiple comparisons plots and bar charts for change in urine specific gravity over time

F (1.361, 13.61) = 0.2712	p = 0.6820	SE	Mean 1	Mean 2	Mean difference	Adjusted p-value	95% CI of differences
Day 1-6 vs Baseline		0.0022	1.018	1.018	-0.0005	0.9965	-0.0073 to 0.0064
Day 7-12 vs Baseline		0.0021	1.019	1.018	0.0006	0.9906	-0.0058 to 0.0071
Day 13-19 vs Baseline		0.0022	1.019	1.018	0.0009	0.9779	-0.0058 to 0.0075
Day 7-12 vs Day 1-6		0.0008	1.019	1.018	0.001	0.4978	-0.0012 to 0.0034
Day 13-19 vs Day 1-6		0.0006	1.019	1.018	0.001	0.1955	-0.0005 to 0.0032
Day 13-19 vs Day 7-12		0.001	1.019	1.019	0.0002	0.9948	-0.0027 to 0.0032

ANOVA of baseline and pre-dive values for urine specific gravity with multiple comparisons and post hoc adjustment with Tukey´s test.

F (1.338, 13.38) = 0.3174	p = 0.6471	SE	Mean 1	Mean 2	Mean difference	Adjusted p-value	95% CI of differences
Day 1-6 vs Baseline		0.0029	1.017	1.018	-0.0012	0.9739	-0.0101 to 0.0077
Day 7-12 vs Baseline		0.0028	1.017	1.018	-0.0017	0.9264	-0.0104 to 0.0069
Day 13-19 vs Baseline		0.0027	1.017	1.018	-0.0018	0.9048	-0.0102 to 0.0065
Day 7-12 vs Day 1-6		0.0008	1.017	1.017	-0.0005	0.9310	-0.0031 to 0.0021
Day 13-19 vs Day 1-6		0.0010	1.017	1.017	-0.0006	0.9217	-0.0037 to 0.0024
Day 13-19 vs Day 7-12		0.0011	1.017	1.017	-0.0001	0.9995	-0.0035 to 0.0032

ANOVA of baseline and post-dive values for urine specific gravity with multiple comparisons and post hoc adjustment with Tukey´s test.

F (1.185, 11.85) = 0.476	p = 0.5352	SE	Mean 1	Mean 2	Mean difference	Adjusted p-value	95% CI of differences
Pre value vs Baseline		0.0021	1.019	1.018	0.0003	0.9865	-0.005 to 0.006
Post value vs Baseline		0.0028	1.017	1.018	-0.0016	0.8353	-0.009 to 0.006
Post value vs Pre value		0.0011	1.017	1.019	-0.0019	0.2396	-0.005 to 0.001

ANOVA of baseline and mean pre and post values for urine specific gravity with multiple comparisons and post hoc adjustment with Tukey´s test.

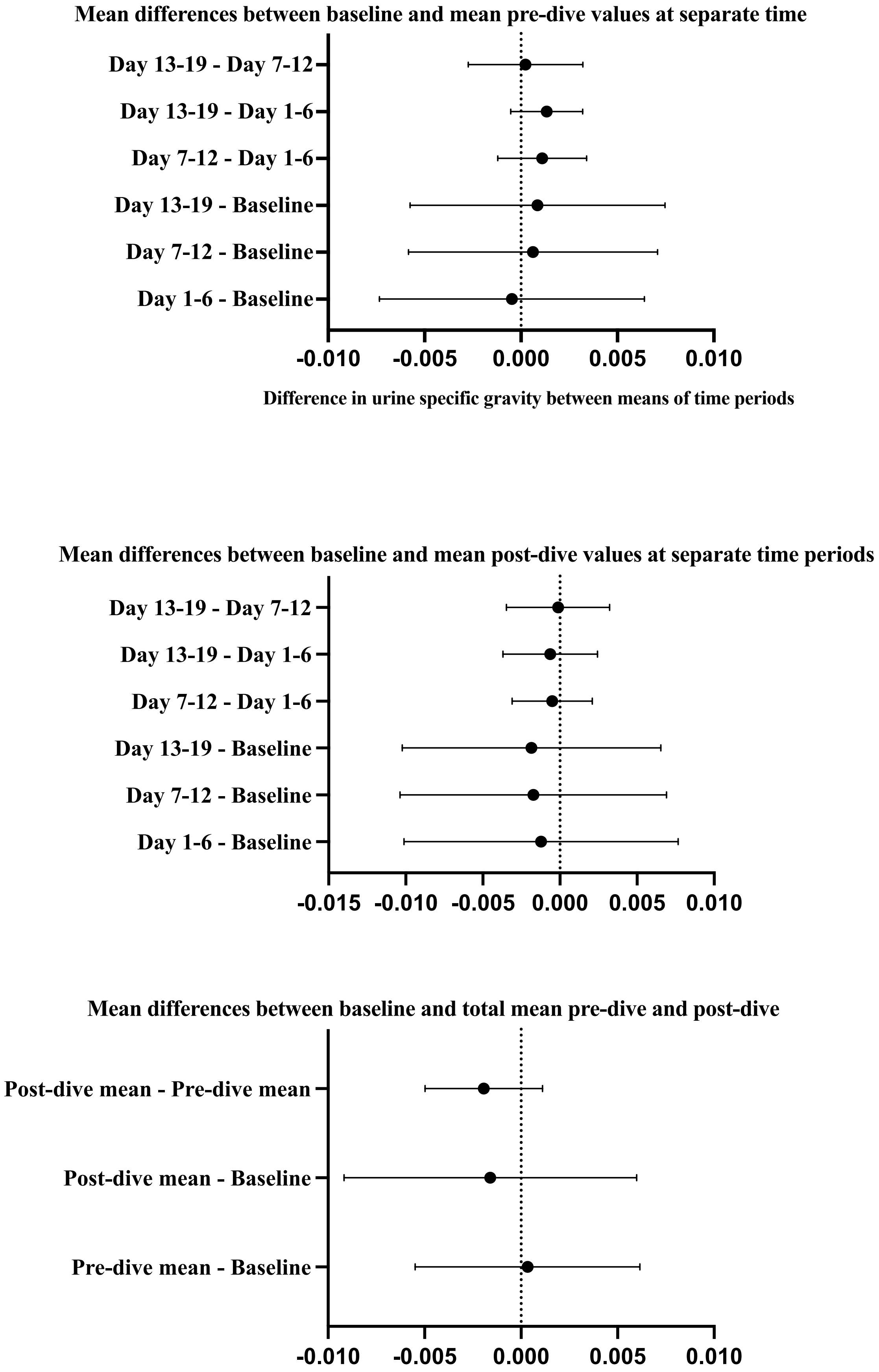
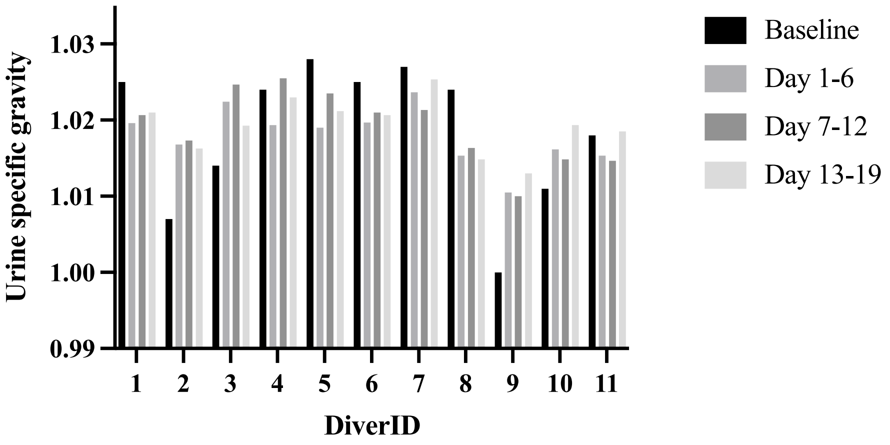
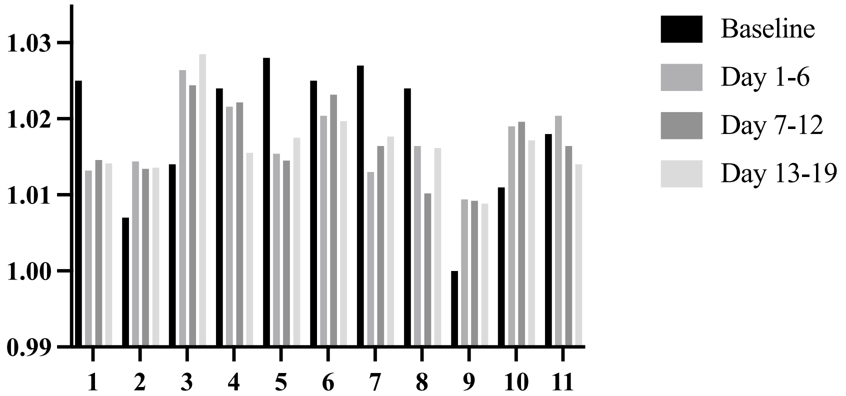


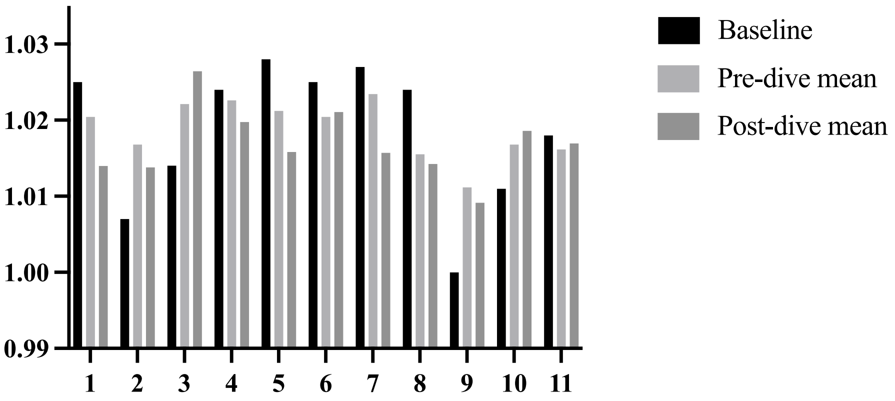
Figure 7. Multiple comparisons plot for urine specific gravity at separate time periods. Dots represents mean difference and whiskers are 95 % confidence intervals.



Mean pre-dive USG at different time periods



Mean post-dive USG at different time periods



Mean USG at different time periods

Figure 8. Graphs showing baseline and mean urine specific gravity values for each diver.

Appendix B: ANOVA tables, differences plot and bar charts for change in impedance at 50 Hz over time.

F (1.538, 15.38) = 16.18	p = <0.001	SE	Mean 1	Mean 2	Mean difference	Adjusted p-value	95% CI of differences
Day 1-6 vs Baseline		7.132	478.4	448.8	29.55	0.0091	7.727 to 51.36
Day 7-12 vs Baseline		7.116	480.6	448.8	31.82	0.0055	10.05 to 53.59
Day 13-19 vs Baseline		7.862	483.9	448.8	35.09	0.0056	11.04 to 59.14
Day 7-12 vs Day 1-6		3.387	480.6	478.4	2.273	0.9057	-8.090 to 12.64
Day 13-19 vs Day 1-6		3.701	483.9	478.4	5.545	0.4733	-5.777 to 16.87
Day 13-19 vs Day 7-12		2.656	483.9	480.6	3.273	0.6221	-4.854 to 11.40

ANOVA of baseline and pre dive values for bioelectrical impedance at 50 Hz with multiple comparisons and post hoc adjustment with Tukey´s test.

F (1.790, 17.90) = 5.747	p = 0.0139	SE	Mean 1	Mean 2	Mean difference	Adjusted p-value	95% CI of differences
Day 1-6 vs Baseline		6.440	469.5	448.8	20.64	0.0397	0.9331 to 40.34
Day 7-12 vs Baseline		5.357	467.3	448.8	18.45	0.0271	2.066 to 34.84
Day 13-19 vs Baseline		8.014	466.4	448.8	17.55	0.1913	-6.972 to 42.06
Day 7-12 vs Day 1-6		3.618	467.3	469.5	-2.182	0.9288	-13.25 to 8.886
Day 13-19 vs Day 1-6		4.129	466.4	469.5	-3.091	0.8752	-15.72 to 9.540
Day 13-19 vs Day 7-12		5.004	466.4	467.3	-0.909	0.9977	-16.22 to 14.40

ANOVA of baseline and post-dive values for bioelectrical impedance at 50 Hz with multiple comparisons and post hoc adjustment with Tukey´s test.

F (1.246, 12.46) = 16.03	p = 0.001	SE	Mean 1	Mean 2	Mean difference	Adjusted p-value	95% CI of differences
Pre value vs Baseline		7.070	480.8	448.8	32.00	0.0029	12.62 to 51.38
Post value vs Baseline		6.241	467.8	448.8	19.00	0.0304	1.893 to 36.11
Post value vs Pre value		2.828	467.8	480.8	-13.00	0.0026	-20.75 to -5.246

ANOVA of baseline and mean pre and post values for bioelectrical impedance at 50 Hz with multiple comparisons and post hoc adjustment with Tukey´s test.

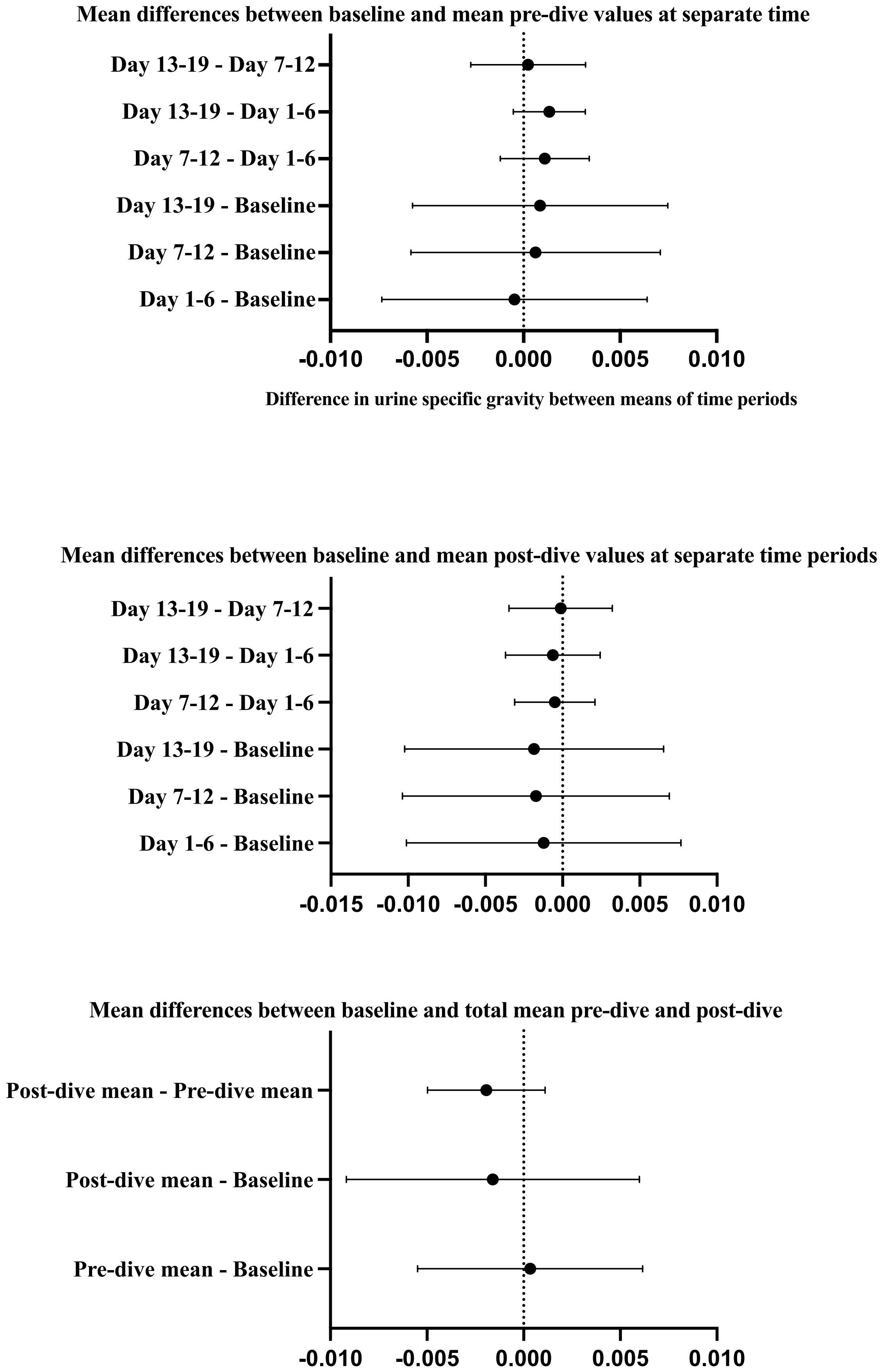
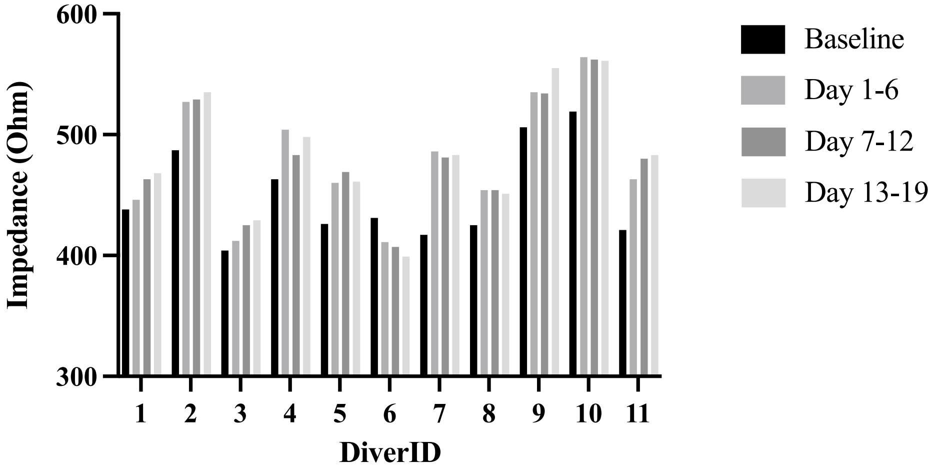
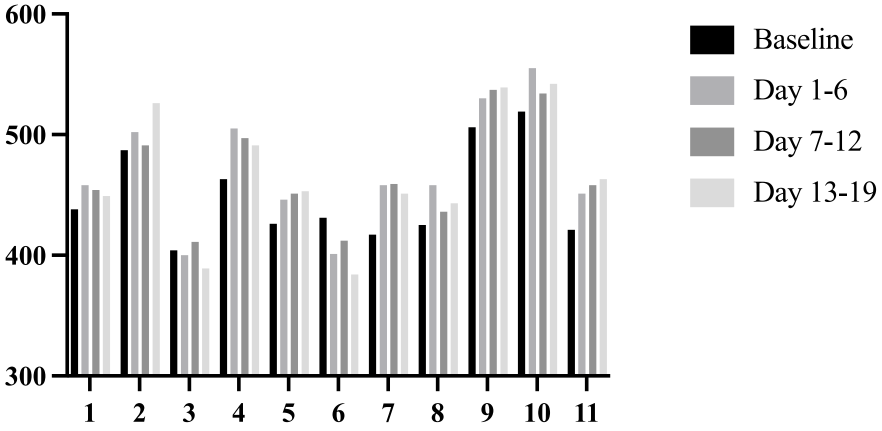


Figure 9. Multiple comparisons plot for bioelectrical impedance (50 Hz) at separate time periods. Dots represents mean difference and whiskers are 95 % confidence intervals.

Mean pre-dive impedance at 50 Hz at different time periods



Mean post-dive impedance at 50 Hz at different time periods



Mean impedance at 50 Hz at different time periods

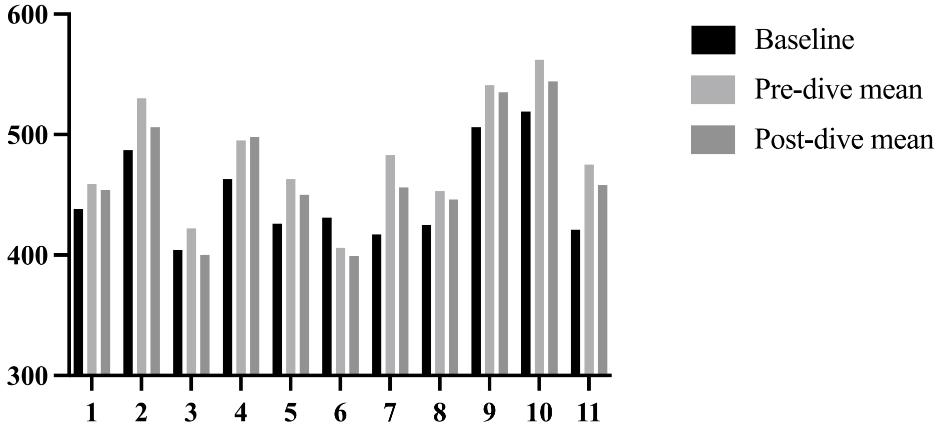


Figure 10. Graphs showing baseline and mean impedance at 50 Hz for each diver.

Appendix C: ANOVA tables, differences plot and bar chart for change in total body water over time

F (1.931,19.31) = 9.138	p = 0.0018	SE	Mean 1	Mean 2	Mean difference	Adjusted p-value	95% CI of differences
Day 1-6 vs Baseline		0.5735	52.09	53.82	-1.727	0.0539	-3.482 to 0.02733
Day 7-12 vs Baseline		0.5012	52.00	53.82	-1.818	0.0203	-3.352 to -0.2847
Day 13-19 vs Baseline		0.4759	51.91	53.82	-1.909	0.0111	-3.365 to -0.4533
Day 7-12 vs Day 1-6		0.3426	52.00	52.09	-0.091	0.9930	-1.139 to 0.9571
Day 13-19 vs Day 1-6		0.3521	51.91	52.09	-0.182	0.9532	-1.259 to 0.8953
Day 13-19 vs Day 7-12		0.2113	51.91	52.00	-0.091	0.9719	-0.7372 to 0.5554

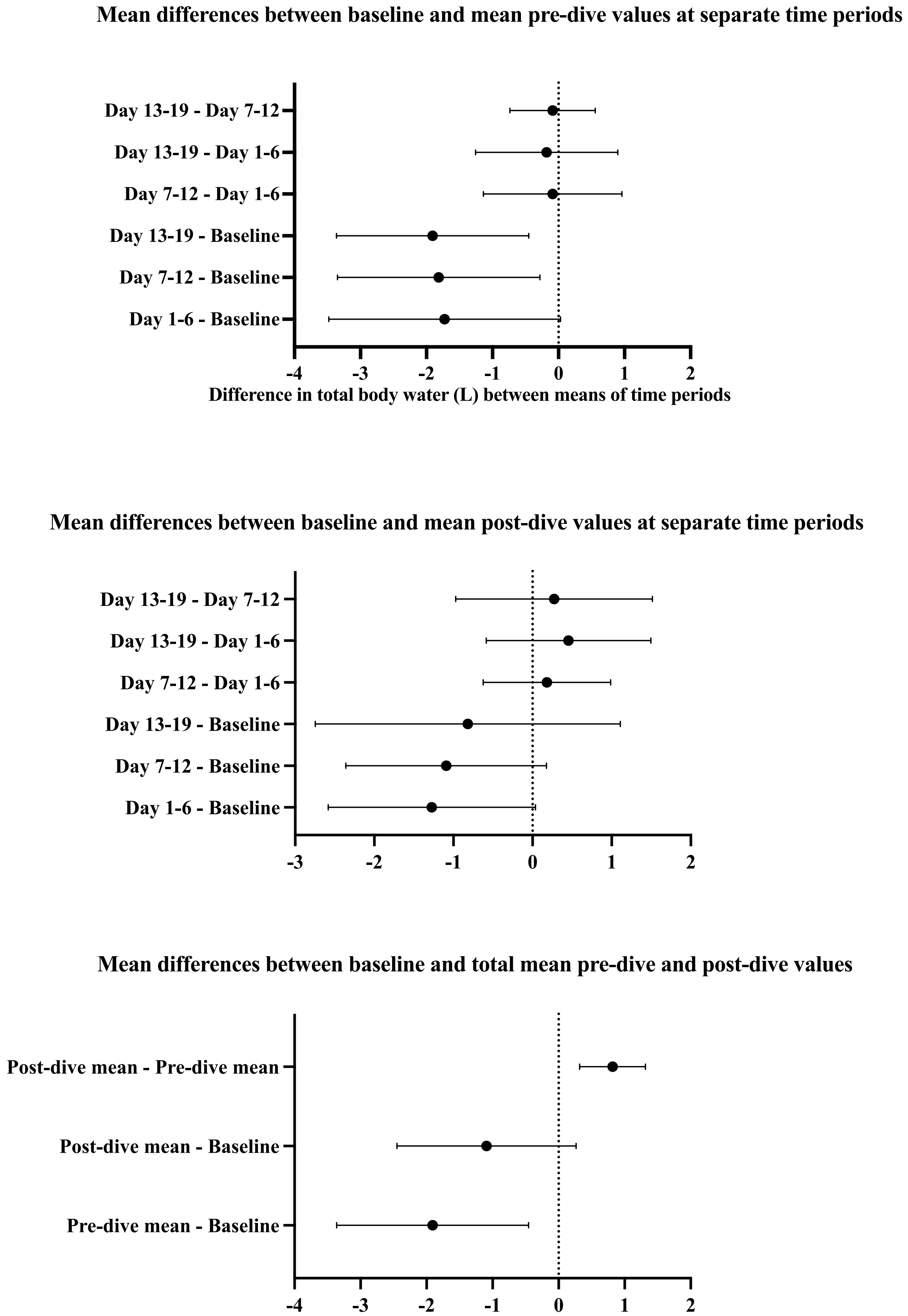
ANOVA of baseline and pre-dive values for total body water calculated from impedance at 50 Hz with multiple comparisons and post hoc adjustment with Tukey´s test.

F (1.756, 17.56) = 3.441	p = 0.0600	SE	Mean 1	Mean 2	Mean difference	Adjusted p-value	95% CI of differences
Day 1-6 vs Baseline		0.4283	52.55	53.82	-1.273	0.0575	-2.583 to 0.03770
Day 7-12 vs Baseline		0.4146	52.73	53.82	-1.091	0.0982	-2.359 to 0.1775
Day 13-19 vs Baseline		0.6298	53.00	53.82	-0.818	0.5837	-2.745 to 1.109
Day 7-12 vs Day 1-6		0.2635	52.73	52.55	0.182	0.8986	-0.6243 to 0.9879
Day 13-19 vs Day 1-6		0.3402	53.00	52.55	0.455	0.5626	-0.5861 to 1.495
Day 13-19 vs Day 7-12		0.4066	53.00	52.73	0.273	0.9057	-0.9711 to 1.517

ANOVA of baseline and post-dive values for total body water calculated from impedance at 50 Hz with multiple comparisons and post hoc adjustment with Tukey´s test.

F (1.184, 11.84) = 9.852	p = 0.0069	SE	Mean 1	Mean 2	Mean difference	Adjusted p-value	95% CI of differences
Pre value vs Baseline			51.91	53.82	-1.909	0.0122	0.456 to 3.362
Post value vs Baseline			52.73	53.82	-1.091	0.1184	-0.265 to 2.447
Post value vs Pre value			52.73	51.91	0.818	0.0030	-1.317 to -0.320

ANOVA of baseline and mean pre and post values for total body water calculated from impedance at 50 Hz with multiple comparisons and post hoc adjustment with Tukey´s test.

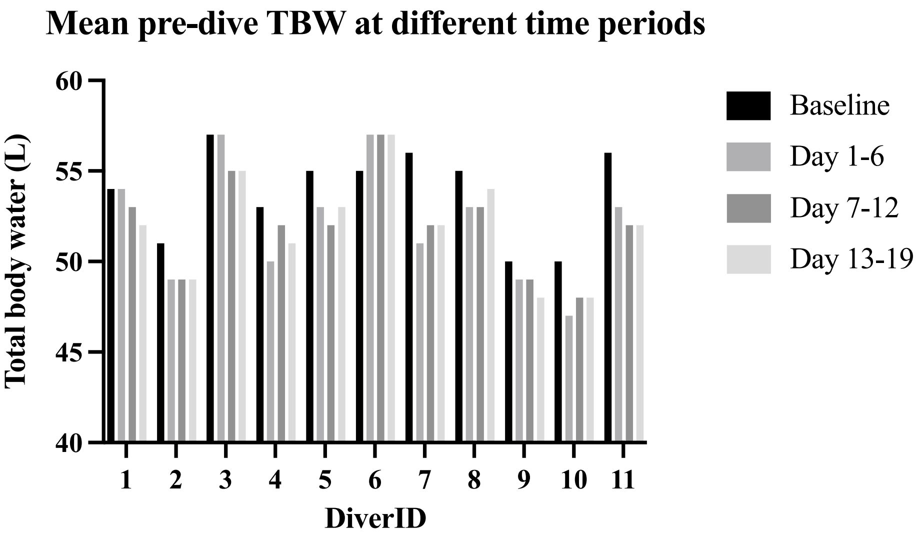


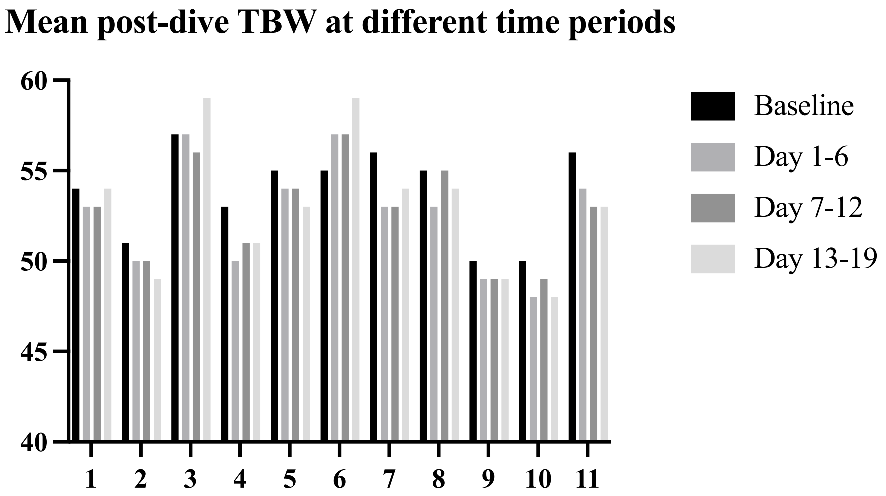
Mean differences between baseline and mean pre-dive values at separate time periods

Mean differences between baseline and mean post-dive values at separate time periods

Mean differences between baseline and total mean pre-dive and post-dive values

Figure 11. Multiple comparisons plot for total body water calculated from 50 Hz BIA at separate time periods. Dots represents mean difference and whiskers are 95 % confidence intervals.





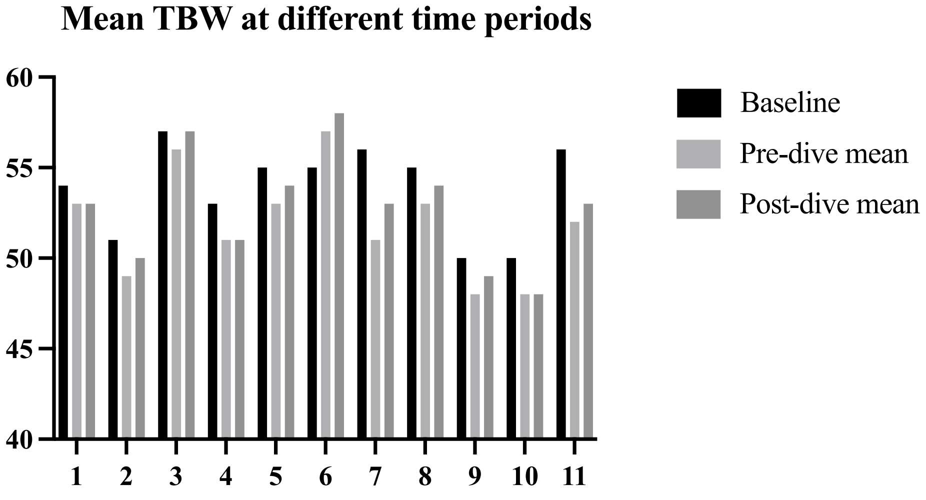
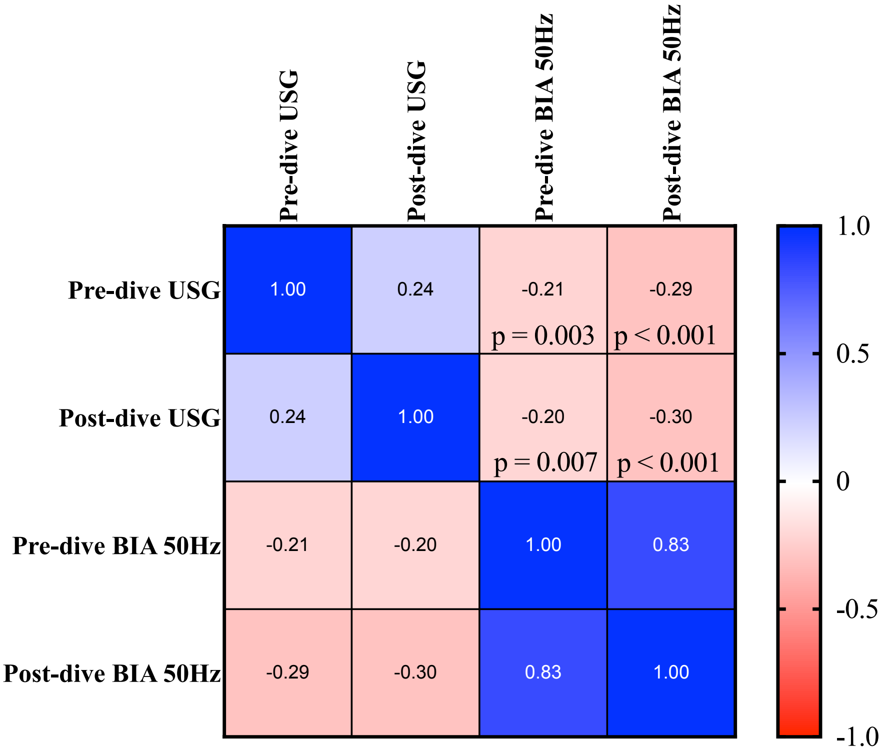


Figure 12. Bar charts showing baseline and mean values of total body water calculated from 50 Hz BIA for each diver.

Appendix D.
Range of measurements of urine specific gravity, BIA and total body water at 50 Hz for each diver. Values include <u>all</u> measurements for each diver.

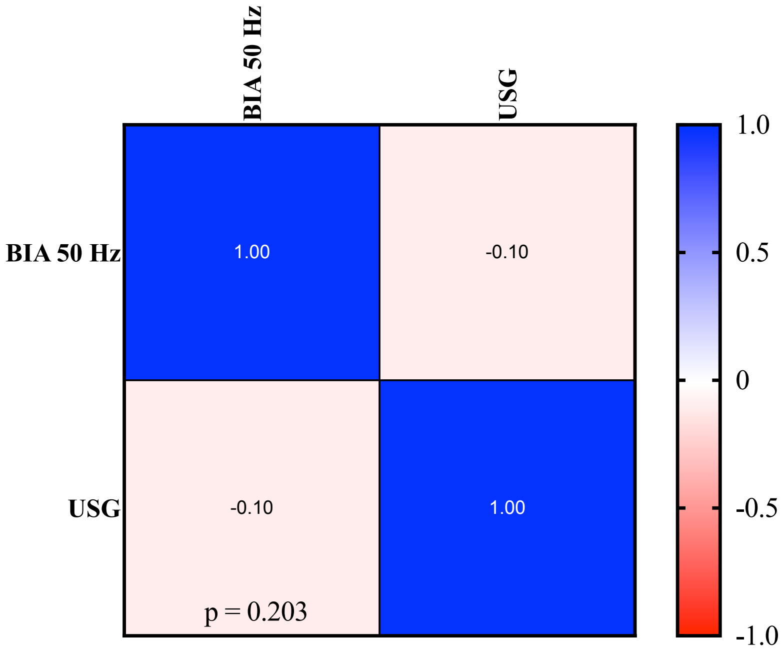
Diver ID		USG: Pre-Dive	USG: Post Dive	BIA 50 Hz: Pre-Dive	BIA 50 Hz: Post Dive	TBW 50 Hz: Pre-Dive	TBW 50 Hz: Post Dive
1	Mean with SD	1.020 (0.005)	1.015 (0.009)	464.33 (7.594)	453.53 (19.253)	52.608 (0.525)	53.395 (1.280)
	Upper 95% CI	1.023	1.020	468.54	464.20	52.950	54.077
	Lower 95% CI	1.018	1.010	460.13	442.87	52.391	52.713
	Median	1.021	1.012	463.00	461.00	52.718	52.911
2	Mean with SD	1.017 (0.006)	1.014 (0.004)	528.93 (16.25)	516.33 (29.844)	49.005 (0.782)	49.678 (1.559)
	Upper 95% CI	1.021	1.016	537.93	532.86	49.422	50.508
	Lower 95% CI	1.014	1.012	519.93	499.81	48.588	48.847
	Median	1.020	1.014	529.00	525.00	48.832	49.174
3	Mean with SD	1.021 (0.007)	1.025 (0.006)	427.69 (9.086)	400.31 (23.813)	55.334 (0.788)	57.545 (2.101)
	Upper 95% CI	1.025	1.029	433.18	414.70	55.754	58.665
	Lower 95% CI	1.016	1.021	422.20	385.92	54.914	56.426
	Median	1.023	1.024	427.00	410.00	55.402	56.666
4	Mean with SD	1.024 (0.005)	1.019 (0.006)	497.93 (11.744)	495.43 (19.658)	50.540 (0.680)	50.781 (1.123)
	Upper 95% CI	1.027	1.022	504.71	506.58	50.903	51.379
	Lower 95% CI	1.021	1.015	491.15	484.08	50.178	50.182
	Median	1.025	1.017	496.00	502.00	50.694	50.420
5	Mean with SD	1.022 (0.004)	1.016 (0.008)	463.69 (13.068)	450.25 (24.001)	52.724 (0.813)	53.619 (1.617)
	Upper 95% CI	1.024	1.020	470.65	463.04	53.142	54.451
	Lower 95% CI	1.020	1.011	456.72	437.46	52.306	52.788
	Median	1.023	1.015	466.50	458.00	52.527	53.041
6	Mean with SD	1.021 (0.004)	1.020 (0.007)	404.67 (13.004)	388.20 (12.941)	56.885 (0.997)	58.287 (1.336)
	Upper 95% CI	1.023	1.024	411.87	395.37	57.416	58.999
	Lower 95% CI	1.018	1.016	397.47	381.03	56.354	57.575
	Median	1.021	1.019	404.00	394.00	56.956	57.856
7	Mean with SD	1.023 (0.004)	1.016 (0.103)	485.56 (10.211)	455.38 (23.343)	51.450 (0.863)	53.288 (1.568)
	Upper 95% CI	1.025	1.021	491.00	467.81	51.910	54.124
	Lower 95% CI	1.021	1.010	480.12	442.94	50.990	52.453
	Median	1.023	1.012	488.50	460.50	51.173	52.879
8	Mean with SD	1.015 (0.008)	1.014 (0.007)	452.94 (14.475)	445.63 (24.251)	53.447 (1.046)	53.957 (1.681)
	Upper 95% CI	1.020	1.018	460.65	458.55	54.004	54.853
	Lower 95% CI	1.011	1.011	445.22	432.70	52.889	53.061
	Median	1.012	1.012	455.00	447.00	53.472	53.777
9	Mean with SD	1.011 (0.006)	1.009 (0.005)	543.44 (13.692)	535.19 (25.372)	48.514 (0.643)	48.782 (1.243)
	Upper 95% CI	1.015	1.012	550.73	548.71	48.856	49.444
	Lower 95% CI	1.008	1.007	536.14	521.67	48.171	48.119
	Median	1.013	1.010	540.50	540.00	48.545	48.499
10	Mean with SD	1.016 (0.007)	1.019 (0.007)	561.00 (9.709)	543.50 (20.196)	47.537 (0.436)	48.368 (0.950)
	Upper 95% CI	1.020	1.022	566.17	554.26	47.769	48.875
	Lower 95% CI	1.013	1.015	555.83	532.74	47.305	47.862
	Median	1.019	1.017	559.50	546.00	47.614	48.222
11	Mean with SD	1.017 (0.003)	1.016 (0.006)	478.20 (10.718)	460.80 (15.612)	52.019 (0.916)	53.094 (1.270)
	Upper 95% CI	1.019	1.019	484.14	469.45	52.508	53.771
	Lower 95% CI	1.015	1.013	472.26	451.15	51.531	52.418
	Median	1.018	1.016	477.00	463.00	51.940	52.815

Appendix E. Correlation matrices for urine specific gravity and bioelectrical impedance.



	Pre-dive USG	Post-dive USG	Pre-dive BIA 50Hz	Post-dive BIA 50Hz
Pre-dive USG	1.00	0.24	-0.21 p = 0.003	-0.29 p < 0.001
Post-dive USG	0.24	1.00	-0.20 p = 0.007	-0.30 p < 0.001
Pre-dive BIA 50Hz	-0.21	-0.20	1.00	0.83
Post-dive BIA 50Hz	-0.29	-0.30	0.83	1.00

Matrix showing correlation between absolute values of urine specific gravity and bioelectrical impedance. The number in the box equals the Spearman correlation coefficient r.



	BIA 50 Hz	USG
BIA 50 Hz	1.00	-0.10
USG	-0.10 p = 0.203	1.00

Matrix showing correlation between urine specific gravity and bioelectrical impedance for percentage change between post-dive and pre-dive values. The number in the box equals the Spearman correlation coefficient r.

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