Determination of muscle mass, fat mass and body water. Is bioimpedance with Impedimed SFB7 consistent with reference methods?

- A cross sectional validation study

Degree Project in Medicine

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Table of Contents

Abbreviations .................................................................................................................. 3
Abstract .............................................................................................................................. 4
Background ......................................................................................................................... 6
Aim and research questions .................................................................................................. 11
  Aim .................................................................................................................................. 11
  Research questions ......................................................................................................... 11
Material and Methods ........................................................................................................ 12
  Population and data collection ....................................................................................... 12
  Measurements ............................................................................................................... 12
  Statistical methods and variable analyses ................................................................... 16
  Data analysis ................................................................................................................. 18
Ethics ................................................................................................................................ 19
Results ................................................................................................................................ 20
  TBSMM estimates, a comparison between DXA and SFB7 ............................................. 20
  FM estimates, a comparison between DXA, ADP and SFB7 ........................................ 21
  TBW estimates, a comparison between $^3$H-dilution and SFB7 .................................... 22
  ECW estimates, a comparison between Br-dilution and SFB7 ........................................ 22
  ICW estimates, a comparison between Dilution Techniques and SFB7 and
  between TBK and SFB7 ............................................................................................... 23
Discussion ........................................................................................................................ 24
  Findings ......................................................................................................................... 24
  Adjacent studies ............................................................................................................ 25
  Methodological considerations ...................................................................................... 26
    Strengths and Weaknesses ......................................................................................... 27
    Clinical significance and improvements ....................................................................... 27
    Transferability to the ill ............................................................................................... 28
Conclusions and Implications ......................................................................................... 29
Populärvetenskaplig sammanfattning – (på svenska) ...................................................... 31
Acknowledgements .......................................................................................................... 33
References ........................................................................................................................ 33
Figures and Tables ............................................................................................................. 43
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>3H</td>
<td>Tritium</td>
<td>IQR</td>
<td>Interquartile Ranges</td>
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<td>ADP</td>
<td>Air Displacement Plethysmography</td>
<td>LSTM</td>
<td>Lean Soft Tissue Mass</td>
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<td>ALST</td>
<td>Appendicular Lean Soft Tissue</td>
<td>MNA</td>
<td>Mini Nutritional Assessment</td>
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<tr>
<td>BCM</td>
<td>Body Cell Mass</td>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>BMI</td>
<td>Body Mass Index</td>
<td>MUST</td>
<td>Malnutrition Universal Screening Tool</td>
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<tr>
<td>Br</td>
<td>Bromide</td>
<td>NaBr</td>
<td>Sodium Bromide</td>
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<tr>
<td>CF</td>
<td>Calibration Factor</td>
<td>NRS-2002</td>
<td>Nutritional Risk Screening</td>
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<tr>
<td>CPS</td>
<td>Counts Per Second</td>
<td></td>
<td>2002</td>
</tr>
<tr>
<td>CT</td>
<td>Computerized Tomography</td>
<td>rP</td>
<td>Pearson correlation</td>
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<tr>
<td>DXA</td>
<td>Dual-energy X-ray Absorptiometry</td>
<td>SFB7</td>
<td>Impedimed SFB7 bioimpedance device</td>
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<tr>
<td>ECW</td>
<td>Extracellular Water</td>
<td>SAT</td>
<td>Subcutaneous Adipose Tissue</td>
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<tr>
<td>ESPEN</td>
<td>The European Society of Clinical Nutrition and Metabolism</td>
<td>TBK</td>
<td>Total Body Potassium</td>
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<td></td>
<td></td>
<td>TBSMM</td>
<td>Total Body Skeletal Muscle Mass</td>
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<td>FFM</td>
<td>Fat Free Mass</td>
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<tr>
<td>FM</td>
<td>Fat Mass</td>
<td>TBW</td>
<td>Total Body Water</td>
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<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<td>ICW</td>
<td>Intracellular Water</td>
<td>VAT</td>
<td>Visceral Adipose Tissue</td>
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<td>IMAT</td>
<td>Intermuscular Adipose Tissue</td>
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Abstract

Determination of muscle mass, fat mass and body water. Is bioimpedance with Impedimed SFB7 consistent with reference methods?

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Background: Malnutrition and fluid imbalance are common among hospitalized patients. Bioimpedance is a manageable and inexpensive method to estimate Total Body Skeletal Muscle Mass (TBSMM), Fat Mass (FM), Total Body Water (TBW), Extracellular Water (ECW) and Intracellular Water (ICW). However, further validations against reference methods are required before introducing it to everyday use in clinical practise.

Aim: To validate SFB7 bioimpedance device’s ability to measure TBSMM, FM, TBW, ECW and ICW against reference methods in healthy subjects.

Methods: 50 healthy adults (men, n=25; women, n=25) were measured with SFB7 and reference methods: Dual-energy X-ray Absorptiometry (DXA), Air Displacement Plethysmography (ADP), Dilution Techniques and Total Body Potassium (TBK). Paired-Samples T-Test, linear regression and Bland Altman plots were used to compare SFB7 estimates for TBSMM, FM, TBW, ECW and ICW with reference methods.
**Results:** The correlations between estimates by SFB7 and reference methods in men were high for TBSMM ($r_P=0.914$) and ECW ($r_P=0.931$), however significant differences in mean and systematic bias were found in both variables. The correlations between SFB7 and reference methods for FM, TBW and ICW were not impressive.

The correlations between estimates by SFB7 and reference methods in women were high for FM ($r_{P_{DXA}}=0.898$, $r_{P_{ADP}}=0.890$) and ECW ($r_P=0.907$). SFB7 underestimated FM and overestimated ECW significantly. The correlations between estimates by SFB7 and reference methods for TBSMM, TBW and ICW were not impressive.

**Conclusions:** Bioimpedance by Impedimed SFB7 by proprietary software and equations does not correctly predict TBSMM, FM, TBW, ECW and ICW in healthy men and women.

**Key words:** Bioimpedance; body water; total body skeletal muscle mass; fat mass; validation
Background

Malnutrition is a condition where a deficiency or imbalance of energy, protein and other nutrients has caused measurable and adverse changes in body composition, function or an individual's course of disease (1).

Malnutrition is common among hospitalized patients globally, as well in Europe (2), Asia (3, 4), Australia (5), Africa (6, 7) and America (8, 9). It results in high costs (10), longer hospitalization and correlate with infections and worse prognoses (11, 12). Considerable loss of body cell mass as a result of inadequate nutritional therapy has been seen in patients in surgical gastrointestinal and orthopaedic wards (13). Parts of the problem tend to be lack of physician awareness (12) and education (13). A Danish study shows that patients at nutritional risk rarely are evaluated regarding nutrition and therefore lack proper planning and monitoring (14).

There are several screening tools to detect malnutrition recommended by The European Society of Clinical Nutrition and Metabolism (ESPEN) (15). Malnutrition Universal Screening Tool (MUST) investigate the presence of malnutrition based on Body Mass Index (BMI), unplanned weight loss and presence of acute disease (16). Nutritional Risk Screening 2002 (NRS-2002) takes BMI, percent recent weight loss, change in food intake, severity of disease and age into account (17). The Mini Nutritional Assessment (MNA) takes height/weight, weight loss, questions related to lifestyle, medications, mobility and food intake as well as the patients subjective evaluation of his or hers health and nutrition into consideration (18).
Meanwhile almost 40 % of the adult population is considered either overweight or obese (19).

BMI is a simple method to classify underweight, overweight and obesity in adults. Body weight in kilograms is divided by the square height in metres (kg/m$^2$). Underweight is defined as BMI < 18.5, normal range is defined as BMI 18.5 – 24.99, overweight is defined as BMI ≥ 25 and obese is defined as BMI ≥ 30 (20). However, BMI does not say to what extent the weight consists of fat, muscles or fluids, increasing the need for more precise methodology for determination of body composition (21).

Dehydration is also common among hospitalized patients and correlates with poor outcomes, such as death (22, 23). Fluid overload is seen among patients with dialysis (24), decompensated heart failure (25) and septic chock (26). Fluid overload in dialysis patients is associated with higher risk of cardiovascular death (27). In acute conditions, it may lead to pulmonary oedema (28).

Hydration can be approximated by repeated body weight measurements (29), blood tests (haemoglobin, haematocrit, electrolytes and urea-to-creatinine ratio), urine samples (osmolality, electrolytes, fluid rate) and clinical observations (blood circulation, skin turgor and pitting oedema) (30).

Body composition can be described in different ways. Body weight can be defined as fat mass (FM) + fat free mass (FFM), where FFM on a molecular level includes water, proteins, glycogen, minerals and essential lipids. On a cellular level total body
water (TBW) is divided into intracellular water (ICW) and extracellular water (ECW). On a tissue-system level BW can be divided into adipose tissue, skeletal muscle, bone, viscera and blood. Body composition can also be described on an atomic level and a whole body level, the latter concerning body size, shape, exterior and physical characteristics (31).

Anthropometry comprises several classical ways to measure a subject, including simply measuring height and body weight. Different body parts breadths, circumferences and areas are also of interest as well as skinfold thickness and body volume (32).

In addition, there are more resource-demanding methods to measure body composition. Imaging techniques such as magnetic resonance imaging (MRI) and computerized tomography (CT) are considered the most accurate. Neutron activation is a highly valued method where tissues are depicted by a measurable decay product arisen when exposing chemical elements to a neutron flux. However, these methods require very expensive equipment and are limited to research applications. In addition, CT and Neutron activation exposes the subject to radiation (33). Air Displacement Plethysmography (ADP), Dual-energy X-ray absorptiometry (DXA), Dilution Techniques, Whole body counting for Total Body Potassium (TBK) and bioimpedance are other methods (32).

ADP, a form of densitometry, estimates body volume by calculations of the pressure changes that occur when a subject is put in a closed chamber. By also weighing the subject the density will be given. FM can be derived from the density (34).
For DXA measurements, X-rays with two discrete photon energy levels pass through the body. Different tissues have different attenuation for each energy level, hence the remaining photons measured by detectors after passage will reflect an image of body composition. DXA divides the body into three components: FM, bone mineral (BMC) and lean soft tissue mass (LSTM) (35). Equations based on LSTM on arms and legs, called appendicular lean soft tissue (ALST), predominantly consisting of muscles, are used to predict Total Body Skeletal Muscle Mass (TBSMM) (36).

Dilution Techniques can measure TBW and ECW. To measure TBW the subject is administered a specific amount of labelled water (e.g. tritium ($^3$H)). To measure ECW the subject is administered a specific amount of bromide (Br) ions. The tracers ($^3$H and Br) will distribute in each intended compartment. The concentration of $^3$H and Br in blood after an equilibration time will provide the volume of TBW and ECW by Fick’s dilution principle ($c_1V_1=c_2V_2$) (32). ICW is obtained by subtracting ECW from TBW (31).

TBK is measured using a whole-body counter. The whole-body counter uses the fact that radioactive $^{40}$K, natural existent in the body, decays producing gamma rays. A detector system intercepts the gamma rays and a value for TBK can further be derived from the activity of $^{40}$K in the body (32). 98% of all potassium is located within the cells (37). Body cell mass (BCM), consisting of muscle mass, visceral organs, blood and brain can be derived from TBK (32). BCM can be recalculated to ICW (38).
Bioimpedance measures TBW and ECW by allowing an alternating current to pass through the body water at different frequencies. The current will encounter impedance: a combination of resistance and reactance (capacitance arisen from cell membranes). At zero frequencies the current does not penetrate the cell membranes, thus giving a value for ECW. At high frequencies the current on the contrary does penetrate the cell membranes, providing a value for both ECW and ICW together becoming TBW (39). Equations to estimate total body skeletal muscle mass (TBSMM) (40), FM and FFM (41) have been developed.

Dilution Techniques, whole body counting for TBK, ADP and DXA are considered reference methods in this context (39). However, they have several drawbacks. The methods would not allow bedside measurements. Whole-body counters are expensive and difficult to access. Dilution Techniques are invasive and require advanced equipment for analysis. DXA and dilution with labelled water expose the subject to radiation (32). For these reasons, interest has been directed towards measurements, which allows inexpensive, non-invasive, manageable bedside measurement where test results can be obtained quickly. However bioimpedance, being an indirect method requires predictive equations, which has its weaknesses (39, 42).

Measurement with bioimpedance has been shown to be consistent with reference methods regarding body water in healthy individuals (43, 44), including changes in ECW during both dehydration and rehydration (45). Bioimpedance is able to estimate FM (46) as well as Subcutaneous Adipose Tissue (SAT) and skeletal muscle mass in haemodialysis patient, indicating that it could be used to assess nutritional status (47). However, bioimpedance lack the ability to estimate TBW and ECW in the overweight
and obese (48) as well as in patients undergoing severe weight loss due to gastric reduction surgery (49). Estimating errors have been seen among patients with cancer (50), haemodialysis (51), other internal medicine disorders (52) and gastrointestinal conditions (53). Whilst bioimpedance may provide values consistent with reference methods at a group level, individual differences may occur, as seen in end-stage renal disease patients (54).

In this study, we aimed to investigate if previous corresponding values between bioimpedance and reference methods (Dilution Techniques, TBK) for estimating body water in healthy individuals were reproducible when using an Impedimed SFB7 bioimpedance device (Impedimed SFB7, Australia), further referred to as SFB7. We also aimed to compare the ability of SFB7 to measure TBSMM compared to DXA, using external predictive equations. Furthermore we aimed to compare the FM estimate provided by SFB7 with DXA and ADP. The study was meant to serve as pilot study for possible further research in the ill.

**Aim and research questions**

**Aim**

To validate SFB7’s ability to determine TBSMM, FM, TBW, ECW and ICW in healthy subjects in comparison with reference methods (DXA, ADP, ³H/Br-dilution, TBK).

**Research questions**

- Will SFB7 provide values for TBSMM consistent with DXA?
- Will SFB7 provide values for FM consistent with DXA and ADP?
• Will SFB7 provide values for TBW consistent with $^3$H-dilution?
• Will SFB7 provide values for ECW consistent with Br-dilution?
• Will SFB7 provide values for ICW consistent with Dilution Techniques and whole body counting for TBK?

**Material and Methods**

**Population and data collection**

During the fall of 2016 volunteers were asked orally or by posting to participate in this study. Posters were pinned to billboards at the University of Gothenburg and Chalmers university of Technology in Sweden. Between October 6th and November 14th 2016, 50 healthy subjects over the age of 20, divided in 25 men and 25 women had their body composition measured by SFB7, Dilution Techniques, DXA, ADP and whole body counting for TBK. Healthy was defined as the absence of symptoms and regular medication. People with claustrophobia were excluded because of the narrow spaces entailed by the whole body counter and the ADP. Pregnant females were excluded because of the exposure to radiation. People with BMI > 34kg/m² or < 16kg/m² were excluded because of the bioimpedance’s minor ability to measure these (42).

**Measurements**

Each subject performed all five measurements at the same occasion, over a period of 3.5 hours. The subjects attended the laboratory in the morning, after an overnight fast. Body weight was measured with the subjects in their underwear to the nearest 0.1kg using an electronic balance. Height was measured with a stadiometer to the nearest 0.1cm.
Dilution Techniques. A baseline urine sample, 5-10 ml, was collected, whereupon the subject drank a solution containing 1ml 3.7MBq/ml $^3$H, radiation dose 0.07mSv and 45ml 5% sodium bromide (NaBr). A postdose blood sample, 9ml, was collected 3 hours after the oral dose. The baseline urine sample was used to subtract possible natural occurring $^3$H and Br from the postdose blood sample.

The serum was centrifuged. Approximately 2 ml serum was sublimated to separate H$_2$O and $^3$H from other molecules (proteins, ions etc.). 0.5 ml sublimate + 0.5 ml standard solution (plasma, $^3$H 3.7MBq/ml, 1:40 000 diluted) were analysed in a Tri-Carb liquid scintillation counter. TBW was calculated as:

$$\text{TBW}_{^3\text{H}} (\text{L}) = \frac{\text{Dose} \times 40,000 \times (\text{Std. count} - \text{blank count})}{1000 \times (\text{Serum count} - \text{Urine count})}$$  \hspace{1cm} (1)

where Dose = Amount (in ml) of test solution administered, 40 000 = Standard solution dilution factor, Std. count = Activity in standard solution, Blank count = Background activity, 1000 = Conversion from ml to litres, Serum count = Activity in serum water, Urine count = Activity in zero urine water.

Methanol was used for protein deposit in the bromide analysis whereupon the supernatant was extracted. The bromide concentration (mmol/L) was determined using a high performance liquid chromatography (HPLC). ECW, estimated as corrected bromide space (CBS) was calculated as (55):

$$\text{CBS}_{\text{Br}} (\text{L}) = \frac{\text{Br dose}}{[\text{Br serum}]} \times 0.90 \times 0.95 \times 0.94$$ \hspace{1cm} (2)
where \( \text{Br dose} = \) Amount of bromide (in mmol) in test solution administered, \([\text{Br serum}] = \) Concentration (in mmol/L) bromide in serum, 0.90 = correction factor for bromide distribution in intracellular spaces, 0.95 = correction for the Gibbs-Donnan effect, 0.94 = correction factor for the concentration of water in serum.

ICW was defined as (31):

\[
\text{ICW}_{(\text{Dilution})} (\text{L}) = \text{TBW}_{3\text{H}} - \text{ECW}_{\text{Br}}
\]  

\((3)\)

**DXA.** The DXA measurement was performed using a GE Lunar iDXA (enCORE, USA) whole body scanner, radiation dose 0.006mSv, measuring FM, lean soft tissue mass (LSTM) and bone mineral content (BMC).

FFM was defined as (35):

\[
\text{FFM}_{\text{DXA}} (\text{kg}) = \text{LSTM}_{\text{DXA}} + \text{BMC}_{\text{DXA}}
\]  

\((4)\)

TBSMM was calculated using a predictive model based on ALST (36):

\[
\text{TBSMM}_{\text{DXA}} (\text{kg}) = 1.19 \times \text{ALST}_{\text{DXA}} - 1.65
\]  

\((5)\)

**SFB7.** The measurement was performed right after the DXA measurement giving the subject a natural rest in supine position for 5-10 minutes. Four electrodes were positioned on the subject’s right side, two on the hand/wrist and two on the
foot/ankle. Values for TBW, ECW, ICW, FM and FFM were received using the proprietary software and equations of SFB7.

TBSMM was calculated using a predictive model, developed in 75 year olds and derived from equation 5 above, according to equation 2 in Tengvall (40):

\[
TBSMM_{SFB7} (kg) = -23.953 + (0.333 \times Ht) + (-0.004 \times Ri) + (-0.010 \times Re) + (-1.72 \times 7 \times \text{gender}) + (0.042 \times BW)
\]

where \(Ri\) = intracellular fluid resistance, \(Re\) = extracellular fluid resistance, \(Ht\) = Height in cm, \(BW\) = Body Weight in kg, gender (women = 1; men = 0)

*Whole body counting for TBK*. The laboratory is a room partly underground, shielded by iron ore concrete walls, floor and ceiling to reduce background radiation. A chamber made of plate armour and lead plates further surrounds the detector system and a bunk intended for the subject. The subjects were dressed in their underwear and a special coat, intended to avoid radiation (e.g. radon) from their own clothes. The subjects lay in the chamber for 300 s. The counts per second (CPS) from \(^{40}\text{K}\), a radioactive isotope of natural body potassium were measured. TBK was calculated as:

\[
TBK (\text{mmol}) = \frac{\text{CPS} (^{40}\text{K}) \times \text{CF}}{1.195}
\]

where \(\text{CF} = \text{Calibration Factor} = 138 \times \sqrt{\text{BW}/\text{Ht}} + 66.5\), where \(\text{BW} = \text{Body Weight in kg}, \text{Ht} = \text{Height in cm}\). The Calibration Factor was derived from measurements on plastic phantoms of various sizes, containing known amounts of \(^{40}\text{K}\).

BCM was calculated as (56):
BCM\textsubscript{TBK} (kg) = 0.00833 \times TBK \quad (8)

ICW was calculated as (38, 57):

\[ \text{ICW}\textsubscript{TBK} (L) = 0.70 \times \text{BCM}/0.99371 \quad (9) \]

where 0.70 is the assumed proportion of water in BCM and 0.99371 is the density of water at 36°C.

\textit{ADP}. The subjects entered the ADP-device (BodPod, Cosmed, Italy), an ovoid chamber, measuring body volume, for 2 × 40 s. To reduce body surface area they wore only their underwear. A swim cap was used to reduce hair volume. An electronic balance determined their weight. By the density (mass/unit volume), the Siri Equation was used to calculate percent fat (58):

\[ \text{Percent Fat}\textsubscript{ADP} = \frac{4.95}{\text{density}} - 4.50 \quad (10) \]

FM was derived from percent fat:

\[ \text{FM}\textsubscript{ADP} (kg) = \frac{\text{Percent fat}}{100} \times \text{BW} \quad (11) \]

where \text{BW} = \text{Body Weight in kg}

\textbf{Statistical methods and variable analyses}

Statistical analyses were performed using IBM\textsuperscript{®} SPSS 24.0 for MAC (SPSS Inc., Chicago, IL, USA). Data were analysed in three parts: as a whole group (\(n=50\)) and divided by gender (male, \(n=25\); female, \(n=25\)).
The number of subjects was equal to study populations used in previous similar research (43), however limited by a late approval from the regional ethical review board in Gothenburg.

Estimates from reference methods and SFB7 were compared using linear regression from which Pearson correlation (rP) was received. Paired-Samples T-Test’s were used to compare means between a reference method and SFB7 within each group. Independent-Samples T-Test’s were used to compare estimates between the female group and the male group. Bland Altman plots examined the difference in a variable measured with a reference method and SFB7 as a function of their mean. By using linear regression in the Bland Altman plots, systematic bias could be found. Statistical significance were equated with p-values < 0.05.

Comparisons were made between:

- TBSMM estimates by SFB7 and DXA
- FM estimates by SFB7 and DXA
- FM estimates by SFB7 and ADP
- FM estimates by ADP and DXA
- FFM estimates by SFB7 and DXA
- TBW estimates by SFB7 and $^3$H-dilution
- ECW estimates by SFB7 and Br-dilution
- ICW estimates by SFB7 and Dilution Techniques
- ICW estimates by SFB7 and TBK
Data analysis

A systematic inspection of manually entered data (Height, Body Weight, gender etc.) for each method was made ensuring correct measurements. Deviating values were identified by the minimum and maximum values in descriptive statistics (table 1) and by outliers and extremes in boxplots.

Outliers were defined as values deviating > 1.5 interquartile ranges (IQR) from the end of the boxes. Extremes were defined as values deviating > 3 IQR from the end of the boxes. Variables used to identify outliers and extremes are presented in textbox 1.

Textbox 1: Variables used to identify outliers and extremes.

TBSMM measured by SFB7 and DXA
FM measured by SFB7, DXA and ADP
FFM measured by SFB7 and DXA
TBW measured by SFB7 and $^3$H-dilution
ECW measured by SFB7 and Br-dilution
ICW measured SFB7 Dilution Techniques and TBK
Difference in TBSMM (DXA-SFB7)
Difference in FM (DXA-SFB7, ADP-SFB7)
Difference in FFM (DXA-SFB7)
Difference in TBW ($^3$H-dilution - SFB7)
Difference in ECW (Br-dilution - SFB7)
Difference in ICW (Dilution Techniques-SFB7, TBK-SFB7)

Outliers and extremes were examined in the whole group, male group and the female group.
Ethics

The study contributed to a greater insight for each subject regarding body composition. There were examples where the subject’s test results were not consistent with their own appreciation, e.g. regarding body fat. This could possibly result in negative alterations in self-image, eating habits and exercise. Furthermore, there was a risk to detect abnormal nutritional status or an illness. One subject had to recur for further investigations because of a tendency to low bone mineral density.

The radiation dosage from the DXA (0.006mSv) and $^3$H-dilution (0.07mSv) measurement was less than the dosage from a dental X-ray, however not to be considered negligible. A few subjects found the blood and urine specimen collection unpleasant. The author underwent all the measurements.

Data could be linked to the subjects only in connection with the measurements. Before data processing all names were replaced with a key. Informed consent was obtained from all participants.

A medical doctor with specialized expertise in clinical nutrition reviewed all the measured values. Adequate follow-up was available in case of deviation.

The regional ethical review board in Gothenburg (application number: 773-16) and the Radiation Protection Committee (application number: 16-43) in the Region of Västra Götaland approved this study.
Results

There was no significant difference in age between the genders. The male group was taller, weighed more and had a larger BMI. For full descriptive statistic, see table 1.

All 50 participants took part in all measurements. Due to technical errors 9 subjects repeated the TBK measurement and 2 subjects repeated the SFB7 measurement within a week from the original occasion. These were included. The systematic inspection of manually entered data and descriptive statistics (table 1) required no exclusion. 31 outliers and 6 extremes in 12 subjects were found. One female, who was an outlier in the “difference in ICW (TBK-SFB7)” variable and who repeated the TBK measurement at a different occasion was excluded in a recast of statistics regarding TBK. The remaining outliers and extremes were included.

SFB7 measurements for each variable (TBSMM, FM, TBW, ECW and ICW) are further validated under separate subheadings.

TBSMM estimates, a comparison between DXA and SFB7

SFB7 underestimates mean TBSMM in comparison with DXA in all three groups (table 2). The underestimation was more pronounced in the male group than in the female group (p = 0.012).

The correlation between DXA_{TBSMM} and SFB7_{TBSMM} was high in the whole group (r_P = 0.943, p < 0.001) and the male group (r_P = 0.914, p < 0.001) but only moderate in the female group (r_P = 0.572, p = 0.003) (table 2, figure 1).

A systematic bias was found in both the whole group and the male group according to
the linear regression in Bland Altman plot. The correlation was positive in both the whole group \((r_P = 0.450, p = 0.001)\) and the male group \((r_P = 0.779, p < 0.001)\), reflecting an increasing underestimation by SFB7 as mean TBSMM increases. No systematic bias was found in the female group (table 2, figure 2).

**FM estimates, a comparison between DXA, ADP and SFB7**

SFB7 underestimates mean FM in comparison with DXA in all three groups (table 3). There was no significant difference in mean FM between the genders \((p = 0.444)\).

The correlation between \(\text{DXA}_{FM}\) and \(\text{SFB7}_{FM}\) was high in the female group \((r_P = 0.898, p < 0.001)\), somewhat lower in the whole group \((r_P = 0.816, p < 0.001)\) and moderate in the male group \((r_P = 0.636, p = 0.001)\) (table 3, figure 3a-c).

SFB7’s underestimation of FM in comparison with DXA was balanced by an overestimation of FFM (table 3).

SFB7 underestimates mean FM in comparison with ADP in the whole group and the female group. There was no significant difference in mean FM between ADP and SFB7 in the male group. The correlation between \(\text{ADP}_{FM}\) and \(\text{SFB7}_{FM}\) was high in the female group \((r_P = 0.890, p < 0.001)\), moderate in the whole group \((r_P = 0.745, p < 0.001)\) and low in the male group \((0.408, p < 0.043)\) (table 3, figure 3d-f).

No systematic bias was found in any of the groups, neither when comparing SFB7 to DXA or SFB7 to ADP, according to the linear regression in Bland Altman plot. However, a wide spread along the y-axis and a wide spread in the same vertical line is prominent in the plots, especially in the whole group and the male group (table 3,
The wide spread in the same vertical line reflects how SFB7 both underestimates and overestimates FM by several kg among subjects with the approximate same amount of FM.

The correlation between FM measured by the reference methods (ADP and DXA) was high in all three groups. However, ADP tends to provide lower estimates for FM than DXA. No systematic bias was found, according to the linear regression in Bland Altman plot (table 3).

**TBW estimates, a comparison between $^3$H-dilution and SFB7**

SFB7 slightly overestimates mean TBW in comparison with $^3$H-dilution in the whole group. In both the male group and the female group SFB7 mean difference for TBW were consistent with Dilution Techniques. The correlation between $3\text{H-dilution}_{\text{TBW}}$ and SFB7$_{\text{TBW}}$ was high in the whole group ($r_P = 0.958, p < 0.001$), rather high in the male group ($r_P = 0.880, p < 0.001$) but moderate in the female group ($r_P = 0.763, p < 0.001$) (table 4, figure 5).

There was no systematic bias in any of the groups, according to the linear regression in Bland Altman plot. However, a wide spread along the y-axis and in the same vertical line is prominent in the plots (table 4, figure 6).

**ECW estimates, a comparison between Br-dilution and SFB7**

SFB7 overestimates mean ECW in comparison with Br-dilution in all three groups (table 5). The overestimation is more pronounced in the male group than in the female group ($p < 0.001$).
The correlation between Br-dilution$_{ECW}$ and SFB7$_{ECW}$ was high in all three groups (table 5, figure 7).

A systematic bias was found in the whole group ($r_P = -0.790, p < 0.001$) and the male group ($r_P = -0.487, p < 0.014$), according to the linear regression in Bland Altman plot. The correlation was negative in the two groups, reflecting an increasing overestimation of ECW by SFB7 as mean ECW increases. No systematic bias was found in the female group (table 5, figure 8).

**ICW estimates, a comparison between Dilution Techniques and SFB7 and between TBK and SFB7**

*Dilution-SFB7*: SFB7 underestimates mean ICW in comparison with Dilution Techniques in the whole group and the male group. There was no significant difference in mean ICW between SFB7 and Dilution Techniques in the female group. The correlation was rather high in the whole group ($r_P = 0.884, p < 0.001$) but moderate in the male group ($r_P = 0.667, p < 0.001$) and the female group ($r_P = 0.548, p = 0.005$) (table 6, figure 9a-c).

A systematic bias was found in the whole group according to the linear regression in Bland Altman plot. The correlation was positive in the whole group ($r_P = 0.360, p = 0.010$), reflecting an increasing underestimation by SFB7 as mean ICW increases. No systematic bias was found in the male group and the female group. A wide spread along the y-axis and in the same vertical line is prominent in the plots in all three groups (table 6, fig. 10a-c).
**Discussion**

**Findings**

We have validated SFB7’s ability to predict TBSMM, FM, TBW, ECW and ICW in a healthy study population. Unfortunately, this study shows a lack in SFB7’s ability to predict all these values in men, women and when combining the genders.
Adjacent studies

The TBSMM equation for bioimpedance was derived from DXA-estimates. The equation was developed in 75 year olds from Sweden (n=98; men, n=50; women, n=48; medication use in 87/98). In comparison with our whole group (n=50) a similar correlation was found between TBSMM measured by DXA and bioimpedance (rP_{n=98} = 0.96, rP_{n=50} = 0.943), however no systematic bias was found in the other study. Apart from the age difference, the latter could be explained by their use of a different bioimpedance analyser (Xitron Hydra 4200) and DXA-scanner (Lunar Prodigy) (40).

Bioimpedance underestimating FM in comparison with DXA has been shown in a previous study with a slightly different population (Norwegian; n=93; men, n=57; women, n=36; age 51 ± 11.5 years; BMI 30.9 ± 4.5 kg/m^2), using the same DXA but a different bioimpedance analyser (BodyScout). The difference in mean FM between DXA and bioimpedance was more pronounced in that study (Δmean FM_{n=93} = 4.1 ± 3.6kg, Δmean FM_{n=50} = 2.8 ± 3.4kg) but they presented a higher correlation (rP_{n=93} = 0.95, rP_{n=50} = 0.816). In contrast to our study, their difference in mean FM was significantly higher in the male group than in the female group and a systematic bias was found in the whole group. The study concluded that the proprietary software of the bioimpedance device should not be used in estimation of body FM on an individual level (59).

A previous study in healthy subjects (n=73, 36.2 ± 10.0 years) presented a similar correlation between ICW measured by TBK and bioimpedance (rP_{n=73} = 0.85; rP_{n=50} = 0.895). However, their difference in mean ICW was more appealing (0.08 litres). The differences could be explained by their use of a different bioimpedance analyser.
(Xitron 4000B), different gender distribution (63 men, 10 women), nationality (Italian) and probable differences in whole-Body Counting constructions and calibrations (44).

A previous Dietary Thesis at the University of Gothenburg in Sweden (n=42, equal gender distribution, healthy subjects, age 47.4 ± 18.6 years) presented a similar correlation between TBW measured by $^3$H-dilution and bioimpedance ($r^2_{n=42} = 0.94$; $r^2_{n=50} = 0.917$; where $r^2 = rP$ squared) and ECW measured by Br-dilution and bioimpedance ($r^2_{n=42} = 0.93$; $r^2_{n=50} = 0.914$) but a higher correlation between ICW measured by Dilution Techniques and bioimpedance ($r^2_{n=42} = 0.90$; $r^2_{n=50} = 0.781$). They used the same Dilution Techniques as in our study. Bioimpedance overestimated FFM in comparison with DXA in our study, while no significant difference in mean FFM was found in their study. The correlation between DXA$_{FFM}$ and bioimpedance$_{FFM}$ was similar ($r^2_{n=42} = 0.90$; $r^2_{n=50} = 0.925$). Differences could be explained by their use of a different bioimpedance analyser (Xitron Hydra 4200 devices (Xitron Technologies, San Diego, USA) and DXA-scanner (Lunar progidy, GE Lunar Corp, Madison, USA) (43).

**Methodological considerations**

In reference to a high frequency of malnutrition (2-14) and fluid imbalance (22, 23) among hospitalized patients, this study is a relevant contribution to the pursuit of simpler methods to estimate body composition. The research questions were concise and designed to be answered with a simple yes or no. The accuracy in choice of reference methods and statistical analyses provides a high credibility to the answers. The study was performed in a controlled environment. Competent personnel with many years of experience in the field performed a key part of the measurements.
Strengths and Weaknesses

One of the greatest strengths of this study was the usage of several reference methods and statistical analyses. Since the study population was evenly divided by gender, we were able to make proper comparisons in SFB7’s ability to determine body composition estimates between men and women.

Considering weaknesses, the study population was very homogenous. A large part of the group had a great common interest in fitness, among them elite athletes on a national level. Higher correlation was often found in the whole group (n = 50) than in the gender groups (n = 25), indicating the need of a larger study population to validate SFB7’s ability to determine body composition estimates in men and women separately. Also, several subjects repeated the TBK and SFB7 measurement on a different occasion, during which time normal variations in body composition may have occurred, possibly resulting in inaccurate values.

To further elucidate the value of bioimpedance in clinical settings it would have been of interest to compare devices from different brands and to use more accurate reference methods such as MRI-scans.

Clinical significance and improvements
Whether SFB7’s inability to predict body composition estimates lies within the software used to determine raw data (resistance, reactance) or the equations further used to predict body composition estimates or within both is for us impossible to say. Since we do not know the equations used to predict these values, speculations for improvements are difficult. A possibility is to use more individualized values for body density. Bioimpedance equations rely on an appreciated value for body density.
The ADP in this study provided values for body density extending from 1.01 – 1.08 g/ml, indicating room for improvement.

An attempt to develop bioimpedance equations for TBW and ECW specific for elite athletes was made recently (60). Even though our study population should not be considered elite athletes, they sure differed from the general population regarding physical fit. Perhaps data collected in this study could underpin a cross-validation study focusing on developing bioimpedance equations specific for moderate fitness performers and athletes.

The equation used to predict TBSMM (40) needs improvement to fit a wider population. Among its weaknesses is the simplification were a differentiating between men and women is made using only a constant (−1.727).

Since SFB7 provides unsatisfying values in our healthy study population we cannot expect it to provide proper values in patients with additionally medication and diagnoses affecting fluid balance.

Perhaps, as suggested by a recent study in peritoneal dialysis patients, we should apply caution, and not let bioimpedance replace our subjective assessment (61).

Transferability to the ill
The results from this study are not applicable to the ill, nor were they intended to. If SFB7 had provided more satisfying body composition estimates in our healthy study population, further research in the ill would have been of interest. Our homogenous,
young and healthy study population, including several lean and muscular subjects, is quite the opposite of the typical elderly patient.

As known from previous research, muscle mass tend to decrease with age (62). When comparing TBSMM determined by equation 2 in Tengvall and SFB7 with TBSMM determined by DXA, a systematic bias was found in our whole group and male group reflecting a lack of precision in SBF7’s ability to determine TBSMM dependent on the subjects’ amount of muscle mass.

TBW tends to decrease with age as well. Whether it is due to the loss of ICW and BCM, or ECW is controversial (63). However it may have an impact on the bioimpedance estimates.

The elderly has an increased amount of Visceral Adipose Tissue (VAT) in comparison with the young (64), which may have an impact on the bioimpedance estimates for FM.

Intermuscular Adipose Tissue (IMAT) increases in the elderly (65) which may affect the bioimpedance ability to estimate both TBSMM and FM.

**Conclusions and Implications**

SFB7’s proprietary software and equations does not provide values for FM, TBW, ECW and ICW consistent with reference methods in healthy subjects. Equation 2 in Tengvall applied on SFB7 does not correctly estimate TBSMM.
We are brought further away from the vision of a manageable method to detect deviant nutrition and fluid imbalance among hospitalized patients. Improvements of SFB7’s equations and equation 2 in Tengvall in general and/or development of more individualized equations are needed. The SFB7’s software should be reviewed.

This underlines the need to scrutinize proprietary equation if possible, as these have a profound impact on the performance of the bioimpedance’s output. Thus, bioimpedance methods are not just population specific, but also device- and equation specific.
Populärvetenskaplig sammanfattning – (på svenska)

Bestämning av muskelmassa, fettmassa och kroppsvatten. Är bioimpedans med Impedimed SFB7 överensstämmande med referensmetoder?

Det är vanligt att patienter på sjukhus lider av undernäring och rubbad vätskebalans. Detta leder till längre vårdtider, sjukare patienter, högre vårdkostnader och i värsta fall döden.


Innan bioimpedans kan börja användas som rutin inom sjukvården krävs dock fler studier som utvärderar metoden gentemot referensmetoder. I vår studie lät vi 50 friska forskningspersoner, hälften män och hälften kvinnor, mäta sig med en bioimpedansapparat av modellen Impedimed SFB7 (härefter hänvisad till som SFB7) och 4 referensmetoder under en och samma förmiddag. SFB7 ger värden för de olika vattenrummen samt fettmassa. För att få bioimpedans-värden även för muskelmassa använde vi en extern ekvation. Referensmetoderna bestod av följande:
• Dual-energy X-ray Absorptiometry (DXA), en röntgenundersökning som bland annat mäter fettmassa och muskelmassa.
• Air Displacement Pletysmography (ADP), en kammare som med hjälp av tryckförändringar beräknar kroppens densitet och därför härleder värden för fettmassa.
• Helkroppsräknare för totalkalium, en äldre teknik där kroppens radioaktivitet från kalium mäts med strålningsdetektorer i en kammare. Utifrån kroppens kaliuminnehåll kan vattnet inuti cellerna beräknas.
• En utspädningsteknik där forskningspersonerna fick dricka tritium (som fördelar sig i totalt vatten) och bromid (som fördelar sig i vattnet utanför cellerna). Ämnena koncentrationer analyserades därefter i ett blodprov varpå beräkningar för de olika vattenrummens volymer kunde göras.

SFB7’s värden för fettmassa och de olika vattenrummen jämfördes med referensmetodernas motsvarande värden. Detsamma gällde för muskelmassan som SFB7 beräknade med hjälp av den externa ekvationen. Hur väl mätningarna stämde överens med varandra undersöcktes med medelvärdesjämförelser och korrelation (sambandet mellan SFB7’s värden för en kroppskomponent och en referensmetods värden för samma komponent). Vidare undersöcktes om SFB7 gjorde några systematiska fel beroende på hur mycket av en kroppskomponent forskningspersonerna hade.

Resultaten var dock nedslående. SFB7 gav värden för både fettmassa och de olika vattenrummen, som med en eller flera statistiska metoder skiljde sig från
referensmetoderna på ett betydande sätt. Detsamma gällde de värden för muskelmassa som SFB7 tog fram med hjälp av den externa ekvationen.

Förbättringar av SFB7’s mjuva och/eller dess ekvationer, samt den externa ekvationen som användes för att beräkna muskelmassa krävs innan mätningar på både friska individer och patienter inom sjukvården är lämpligt.

Acknowledgements

The author acknowledges the contributions of the participants, V. Malmros for the H3- and Br-dilution assay, V. Malmros and M. Hoppe for the bioimpedance and DXA measurements, M. Isaksson, N. Dilan and M. Hjellström for the TBK measurements, E. Järvinen for providing BodPod manual and instructions and L. Ellegård and I. Bosaeus for supervision.

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### Table 1: Descriptive statistic in 50 healthy subjects, presented as mean, standard deviation, min and max.

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Male group (n=25)</th>
<th>Female group (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>25.1 ± 3.5</td>
<td>27.8 ± 7.8</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>167.2 ± 6.0</td>
<td>177.0 ± 7.0</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>61.1 ± 7.0</td>
<td>79.0 ± 9.0</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>24.2 ± 2.7</td>
<td>29.7 ± 2.7</td>
</tr>
<tr>
<td><strong>SFB7 TBSMM (kg)</strong></td>
<td>24.4 ± 6.1</td>
<td>29.7 ± 2.7</td>
</tr>
<tr>
<td><strong>SFB7 FM (kg)</strong></td>
<td>12.8 ± 5.8</td>
<td>11.2 ± 4.7</td>
</tr>
<tr>
<td><strong>SFB7 FFM (kg)</strong></td>
<td>57.3 ± 12.6</td>
<td>67.8 ± 8.6</td>
</tr>
<tr>
<td><strong>SFB7 TBW (l)</strong></td>
<td>41.9 ± 9.2</td>
<td>49.6 ± 6.3</td>
</tr>
<tr>
<td><strong>SFB7 ECW (l)</strong></td>
<td>17.4 ± 4.0</td>
<td>20.8 ± 2.7</td>
</tr>
<tr>
<td><strong>SFB7 ICW (l)</strong></td>
<td>24.5 ± 5.4</td>
<td>28.8 ± 3.9</td>
</tr>
<tr>
<td><strong>DXA TBSMM (kg)</strong></td>
<td>27.3 ± 7.2</td>
<td>33.5 ± 4.4</td>
</tr>
<tr>
<td><strong>DXA FM (kg)</strong></td>
<td>15.6 ± 5.4</td>
<td>14.4 ± 4.5</td>
</tr>
<tr>
<td><strong>DXA FFM (kg)</strong></td>
<td>55.2 ± 12.3</td>
<td>65.6 ± 8.4</td>
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<tr>
<td><strong>ADP FM (kg)</strong></td>
<td>13.9 ± 5.5</td>
<td>12.3 ± 4.5</td>
</tr>
<tr>
<td><strong>ADP BD (kg/l)</strong></td>
<td>1.05 ± 0.02</td>
<td>1.04 ± 0.02</td>
</tr>
<tr>
<td><strong>ADP TBW (l)</strong></td>
<td>41.1 ± 9.0</td>
<td>48.6 ± 6.2</td>
</tr>
<tr>
<td><strong>ADP ECW (l)</strong></td>
<td>15.4 ± 2.8</td>
<td>17.5 ± 2.2</td>
</tr>
<tr>
<td><strong>ADP ICW (l)</strong></td>
<td>25.6 ± 6.5</td>
<td>31.1 ± 4.3</td>
</tr>
<tr>
<td><strong>TBK ICW (l)</strong></td>
<td>23.0 ± 5.8</td>
<td>27.8 ± 4.1</td>
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</table>

* p = by Independent-Samples T-Test, comparing means between the male group and the female group.
Table 2: Difference in TBSMM estimates between DXA and SFB7, analysed with Paired-Samples T-Test, linear regression and Bland Altman plot with linear regression.

<table>
<thead>
<tr>
<th></th>
<th>Difference in mean</th>
<th>Linear regression in BA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>kg</td>
</tr>
<tr>
<td>TBSMM DXA-SFB7 (whole group)</td>
<td>50</td>
<td>2.9 ± 2.5</td>
</tr>
<tr>
<td>TBSMM DXA-SFB7 (male)</td>
<td>25</td>
<td>3.8 ± 2.2</td>
</tr>
<tr>
<td>TBSMM DXA-SFB7 (female)</td>
<td>25</td>
<td>2.1 ± 2.5</td>
</tr>
</tbody>
</table>

Abbreviations; TBSMM, Total Body Skeletal Muscle Mass; DXA, Dual-energy X-ray Absorptiometry; SFB7, Impedimed SFB7 bioimpedance device; BA, Bland Altman plot.

Data presented as difference in mean in kg ± 1 Standard Deviation (SD), ± 1.96 SD Limits of Agreement (LoA) and Pearson correlation (rP).

p$^1$ = by Paired-Samples T-Test. p$^2$ = by linear regression. p$^3$ = by linear regression in Bland Altman plot.

Figure 1: Correlation between TBSMM in kg measured by DXA (y-axis) and SFB7 (x-axis). a = whole group, b = male group, c = female group. Black line, regression line.

Figure 2: Bland Altman Plots with linear regression displaying the relationship between difference in TBSMM (TBSMM$\Delta$) and mean TBSMM (TBSMM$\bar{x}$), where TBSMM$\Delta$ = DXATBSMM-SFB7TBSMM, TBSMM$\bar{x}$ = (DXATBSMM + SFB7TBSMM)/2. a = whole group, b = male group, c = female group. Continuous horizontal line, difference in mean; crosshatched lines, difference in mean ± 1.96 SD, Limits of Agreement (LoA); grey line, regression line.

Abbreviations; TBSMM, Total Body Skeletal Muscle Mass; SFB7, Impedimed SFB7 bioimpedance device; DXA, Dual-energy X-ray Absorptiometry.
Figure 3: Correlation between FM measured by DXA (y-axis) and SFB7 (x-axis) (a-c) and by ADP (y-axis) and SFB7 (x-axis) (d-f). a,d = whole group, b,e = male group, c,f = female group. Black line, regression line.
Abbreviations; FM; Fat Mass, SFB7, Impedimed SFB7 bioimpedance device; DXA, Dual-energy X-ray Absorptiometry; ADP, Air Displacement Plethysmography

Figure 4: Bland Altman Plots with linear regression displaying the relationship between the difference in FM (FM₄₃) and mean FM (FMₓ), where FM₄₃ (a-c) = DXAₐₓ-SFB7ₐₓ, FM₄₃ (d-f) ADPₐₓ-SFB7ₐₓ, FMₓ (a-c) = (DXAₐₓ+SFB7ₐₓ)/2, FMₓ (d-f) = (ADPₐₓ+SFB7ₐₓ)/2. a,d = whole group, b,e = male group, c,f = female group. Continuous horizontal line, difference in mean; crosshatched lines, difference in mean ± 1.96 SD, Limits of Agreement (LoA); grey line, regression line.
Abbreviations; FM; Fat Mass, SFB7, Impedimed SFB7 bioimpedance device; DXA, Dual-Energy X-ray Absorptiometry; ADP, Air Displacement Plethysmography
Table 3: Difference in FM and FMM estimates between SFB7 and reference methods, analysed with Paired-Samples T-Test, linear regression and Bland Altman plot with linear regression.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Difference in mean kg</th>
<th>p&lt;sup&gt;1&lt;/sup&gt;</th>
<th>rP</th>
<th>p&lt;sup&gt;2&lt;/sup&gt;</th>
<th>LoA</th>
<th>Linear regression in BA rP</th>
<th>p&lt;sup&gt;3&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>FM DXA-SFB7</td>
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<tr>
<td>(whole group)</td>
<td>50</td>
<td>2.8 ± 3.4</td>
<td>&lt;0.001</td>
<td>0.816</td>
<td>&lt;0.001</td>
<td>-3.9 – 9.5</td>
<td>-0.126</td>
<td>0.385</td>
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<td>FM DXA-SFB7</td>
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<tr>
<td>(male group)</td>
<td>25</td>
<td>3.2 ± 4.0</td>
<td>0.001</td>
<td>0.636</td>
<td>0.001</td>
<td>-4.6 – 10.9</td>
<td>-0.047</td>
<td>0.824</td>
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<tr>
<td>(female group)</td>
<td>25</td>
<td>2.4 ± 2.9</td>
<td>&lt;0.001</td>
<td>0.898</td>
<td>&lt;0.001</td>
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<tr>
<td>(whole group)</td>
<td>50</td>
<td>1.2 ± 4.1</td>
<td>0.049</td>
<td>0.745</td>
<td>&lt;0.001</td>
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<tr>
<td>(male group)</td>
<td>25</td>
<td>1.1 ± 5.0</td>
<td>0.289</td>
<td>0.408</td>
<td>0.043</td>
<td>-8.7 – 10.9</td>
<td>-0.059</td>
<td>0.779</td>
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<tr>
<td>FM ADP-SFB7</td>
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<td></td>
<td></td>
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<tr>
<td>(female group)</td>
<td>25</td>
<td>1.2 ± 3.0</td>
<td>0.047</td>
<td>0.890</td>
<td>&lt;0.001</td>
<td>-4.6 – 7.1</td>
<td>-0.150</td>
<td>0.475</td>
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<tr>
<td>(whole group)</td>
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<td>-1.6 ± 1.7</td>
<td>&lt;0.001</td>
<td>0.953</td>
<td>&lt;0.001</td>
<td>-4.9 – 1.7</td>
<td>0.061</td>
<td>0.675</td>
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<tr>
<td>(male group)</td>
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<td>-2.1 ± 1.9</td>
<td>&lt;0.001</td>
<td>0.907</td>
<td>&lt;0.001</td>
<td>-5.9 – 1.7</td>
<td>-0.042</td>
<td>0.841</td>
</tr>
<tr>
<td>FM ADP-DXA</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(female group)</td>
<td>25</td>
<td>-1.2 ± 1.3</td>
<td>&lt;0.001</td>
<td>0.979</td>
<td>&lt;0.001</td>
<td>-3.6 – 1.3</td>
<td>0.021</td>
<td>0.921</td>
</tr>
<tr>
<td>FFM DXA-SFB7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(whole group)</td>
<td>50</td>
<td>-2.0 ± 3.5</td>
<td>&lt;0.001</td>
<td>0.962</td>
<td>&lt;0.001</td>
<td>-8.8 – 4.8</td>
<td>-0.090</td>
<td>0.535</td>
</tr>
<tr>
<td>FFM DXA-SFB7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(male group)</td>
<td>25</td>
<td>-2.2 ± 4.1</td>
<td>0.011</td>
<td>0.887</td>
<td>&lt;0.001</td>
<td>-10.2 – 5.7</td>
<td>-0.035</td>
<td>0.868</td>
</tr>
<tr>
<td>FFM DXA-SFB7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(female group)</td>
<td>25</td>
<td>-1.8 ± 2.8</td>
<td>0.004</td>
<td>0.786</td>
<td>&lt;0.001</td>
<td>-7.4 – 3.8</td>
<td>-0.194</td>
<td>0.354</td>
</tr>
</tbody>
</table>

Abbreviations: FM; Fat Mass, FFM; Fat Free Mass; SFB7, Impedimed SFB7 bioimpedance device; DXA, Dual-energy X-ray Absorptiometry; ADP, Air Displacement Plethysmography; BA, Bland Altman plot
Data presented as difference in mean in kg ± 1 Standard Deviation (SD), ± 1.96 SD Limits of Agreement (LoA) and Pearson correlation (rP)

p<sup>1</sup> = by Paired-Samples T-Test. p<sup>2</sup> = by linear regression. p<sup>3</sup> = by linear regression in Bland Altman plot

Figure 5: Correlation between TBW in litres measured by Tritium dilution (y-axis) and SFB7 (x-axis). a = whole group, b = male group, c = female group. Black line, regression line.
Abbreviations: TBW, Total Body Water; SFB7, Impedimed SFB7 bioimpedance device; ³H, Tritium dilution
Table 4: Difference in TBW estimates between Tritium dilution and SFB7, analysed with Paired-Samples T-Test, linear regression and Bland Altman plot with linear regression.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Difference in mean litres</th>
<th>p₁</th>
<th>rP</th>
<th>p²</th>
<th>LoA</th>
<th>Linear regression in BA rP</th>
<th>p³</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBW(^3)H-SFB7 (whole group)</td>
<td>50</td>
<td>-0.8 ± 2.7</td>
<td>0.032</td>
<td>0.958</td>
<td>&lt;0.001</td>
<td>-6.1 – 4.4</td>
<td>-0.072</td>
<td>0.619</td>
</tr>
<tr>
<td>TBW(^3)H-SFB7 (male group)</td>
<td>25</td>
<td>-1.0 ± 3.0</td>
<td>0.110</td>
<td>0.880</td>
<td>&lt;0.001</td>
<td>-7.0 – 5.0</td>
<td>-0.035</td>
<td>0.867</td>
</tr>
<tr>
<td>TBW(^3)H-SFB7 (female group)</td>
<td>25</td>
<td>-0.7 ± 2.3</td>
<td>0.165</td>
<td>0.763</td>
<td>&lt;0.001</td>
<td>-5.1 – 3.8</td>
<td>-0.009</td>
<td>0.966</td>
</tr>
</tbody>
</table>

Abbreviations; TBW, Total Body Water; SFB7, Impedimed SFB7 bioimpedance device; \(^3\)H, Tritium dilution

Table 5: Difference in ECW estimates between Bromide dilution and SFB7, analysed with Paired-Samples T-Test, linear regression and Bland Altman plot with linear regression.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Difference in mean litres</th>
<th>p₁</th>
<th>rP</th>
<th>p²</th>
<th>LoA</th>
<th>Linear regression in BA rP</th>
<th>p³</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECW Br-SFB7 (whole group)</td>
<td>50</td>
<td>-1.9 ± 1.6</td>
<td>&lt;0.001</td>
<td>0.956</td>
<td>&lt;0.001</td>
<td>-5.1 – 1.2</td>
<td>-0.790</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ECW Br-SFB7 (male group)</td>
<td>25</td>
<td>-3.3 ± 1.0</td>
<td>&lt;0.001</td>
<td>0.931</td>
<td>&lt;0.001</td>
<td>-5.3 – 1.2</td>
<td>-0.487</td>
<td>0.014</td>
</tr>
<tr>
<td>ECW Br-SFB7 (female group)</td>
<td>25</td>
<td>-0.6 ± 0.6</td>
<td>&lt;0.001</td>
<td>0.907</td>
<td>&lt;0.001</td>
<td>-1.8 – 0.6</td>
<td>0.165</td>
<td>0.431</td>
</tr>
</tbody>
</table>

Abbreviations; ECW, Extracellular Water; SFB7, Impedimed SFB7 bioimpedance device; Br, Bromide dilution; BA, Bland Altman plot

Figure 6: Bland Altman Plots with linear regression displaying the relationship between difference in TBW (TBW\(_Δ\)) and mean TBW (TBW\(_\bar{x}\)), where TBW\(_Δ\) = \(^3\)H\(\text{TBW} - \text{SFB7TBW}\), TBW\(_\bar{x}\) = (\(^3\)H\(\text{TBW} + \text{SFB7TBW}\))/2. a = whole group, b = male group, c = female group. Continuous horizontal line, difference in mean; crosshatched lines, difference in mean ± 1.96 SD, Limits of Agreement (LoA); grey line, regression line.

Abbreviations; TBW, Total Body Water; SFB7, Impedimed SFB7 bioimpedance device; \(^3\)H, Tritium dilution

47
Table 6: Difference in ICW estimates between Dilution Techniques and SFB7 and between TBK and SFB7, analysed with Paired-Samples T-Test, linear regression and Bland Altman plot with linear regression.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Difference in mean litres</th>
<th>p^1</th>
<th>rP</th>
<th>p^2</th>
<th>LoA</th>
<th>Linear regression in BA rP</th>
<th>p^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICW Dill-SFB7</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(whole group)</td>
<td>50</td>
<td>1.1 ± 3.1</td>
<td>0.013</td>
<td>0.884</td>
<td>&lt;0.001</td>
<td>-4.9 – 7.1</td>
<td>0.360</td>
<td>0.010</td>
</tr>
<tr>
<td>ICW Dill-SFB7</td>
<td>25</td>
<td>2.3 ± 3.3</td>
<td>0.002</td>
<td>0.667</td>
<td>&lt;0.001</td>
<td>-4.2 – 8.8</td>
<td>0.131</td>
<td>0.533</td>
</tr>
<tr>
<td>(male group)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICW Dill-SFB7</td>
<td>25</td>
<td>0.0 ± 2.3</td>
<td>0.934</td>
<td>0.548</td>
<td>0.005</td>
<td>-4.6 – 4.5</td>
<td>0.075</td>
<td>0.723</td>
</tr>
<tr>
<td>(female group)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICW TBK-SFB7</td>
<td>50</td>
<td>-1.6 ± 2.6</td>
<td>&lt;0.001</td>
<td>0.895</td>
<td>&lt;0.001</td>
<td>-6.7 – 3.5</td>
<td>0.151</td>
<td>0.295</td>
</tr>
<tr>
<td>(whole group)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICW TBK-SFB7</td>
<td>49*</td>
<td>-1.7 ± 2.5</td>
<td>&lt;0.001</td>
<td>0.901</td>
<td>&lt;0.001</td>
<td>-6.6 – 3.3</td>
<td>0.211</td>
<td>0.146</td>
</tr>
<tr>
<td>(male group)</td>
<td>25</td>
<td>-1.1 ± 2.9</td>
<td>0.074</td>
<td>0.745</td>
<td>&lt;0.001</td>
<td>-6.7 – 4.5</td>
<td>0.073</td>
<td>0.729</td>
</tr>
<tr>
<td>ICW TBK-SFB7</td>
<td>25</td>
<td>-2.1 ± 2.3</td>
<td>&lt;0.001</td>
<td>0.507</td>
<td>0.010</td>
<td>-6.5 – 2.4</td>
<td>-0.268</td>
<td>0.196</td>
</tr>
<tr>
<td>(female group)</td>
<td>24*</td>
<td>-2.3 ± 2.0</td>
<td>&lt;0.001</td>
<td>0.580</td>
<td>0.003</td>
<td>-6.3 – 1.7</td>
<td>-0.180</td>
<td>0.399</td>
</tr>
</tbody>
</table>

Abbreviations; ICW, Intracellular Water; SFB7, Impedimed SFB7 bioimpedance device; Dil, Dilution Techniques; TBK, Total Body Potassium; BA, Bland Altman plot
Data presented as difference in mean in litres ± 1 Standard Deviation (SD), Pearson correlation (rP), ± 1.96 SD Limits of Agreement (LoA)
p^1 = by Paired-Samples T-Test. p^2 = by linear regression. p^3 = by linear regression in Bland Altman plot
*One subject excluded due to repeated TBK measurement at a different occasion.
Figure 9: Correlation between ICW in litres measured by SFB7 (x-axis) and Dilution Techniques (y-axis) (a-c) and by SFB7 (x-axis) and TBK (y-axis) (d-f). a,d = whole group, b,e = male group, c,f = female group. Black line, regression line.
Abbreviations; ICW, Intracellular Water; SFB7, Impedimed SFB7 bioimpedance device; Dil, Dilution Techniques; TBK, Total Body Potassium

Figure 10: Bland Altman plots with linear regression displaying the relationship between difference in ICW (ICWΔ) and mean ICW (ICW̄), where ICWΔ (a-c) = ICW Dil - ICW SFB7, ICW̄ (d-f) ICW TBK - ICW SFB7, ICWΔ (a-c) = (ICW Dil + ICW SFB7)/2, ICW̄ (d-f) = (ICW TBK + ICW SFB7)/2. a,d = whole group, b,e = male group, c,f = female group. Continuous horizontal line, difference in mean; crosshatched lines, difference in mean ± 1.96 SD, Limits of Agreement (LoA); grey line, regression line.
Abbreviations: ICW, Intracellular Water; SFB7, Impedimed SFB7 bioimpedance device; Dil, Dilution Techniques; TBK, Total Body Potassium