

1 Bioimpedance index for measurement of total body water in severely malnourished children:
2 assessing the effect of nutritional oedema

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19 **Introduction**

20 Restoration of body composition indicates successful management of severe acute malnutrition
21 (SAM), but no easy and accurate method is available (1–3). Bioimpedance method (BIM),
22 whole-body (4) or segmental (5), is a safe, rapid and easy technique often used to predict total
23 body water (TBW) and lean mass can in healthy individuals. However, its conventional
24 application, commonly referred to as bioimpedance analysis (BIA), requires population-specific
25 equations (6,7), and its accuracy is limited in general (8). This is due in part to inter-individual
26 variability in body proportions (e.g. limb lengths), as narrow cylinders such as limbs contribute
27 disproportionately to total body impedance (9). In healthy children, age or body size-to-age
28 variation in impedance (Z in Ohm) could affect accuracy of TBW prediction (10). Stunted
29 children with some degree of wasting produce higher R compared with anthropometrically
30 normal children (11) and thus reflects the influence of abnormal body composition and/or body
31 proportion. The poorer the ability of BIA to predict TBW, the less suitable it will be for clinical
32 monitoring of body composition.

33 In most four-electrode (tetrapolar) measurements, Z is measured with $800\mu\text{A}$ alternating current
34 at 50 kHz passing through the body, between the wrist and ankle (12). Two relationships
35 between the Z of the body and its volume (V) are central to this method (4). First, derived from
36 Ohm's Law, V is inversely related to Z and directly to conductive distance, approximated by
37 height or length (H): $V = \rho H^2/Z$. Tissue specific resistivity, ρ , is a frequency-dependent
38 constant inversely related to the number of free ions per V (13). Theoretically it is independent
39 of body size, shape and age but could be affected by abnormal tissue hydration and/or
40 osmolality (10–12). Second, at low frequencies electric current flows around the cell without
41 penetrating into the cell, whereas at high frequencies the membrane capacitance is no
42 impediment to the current and it flows indiscriminately through both intracellular and
43 extracellular space, and thus assumed to reflect TBW better (4).

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45 Decreased total body potassium, increased total body sodium and increased TBW are well
46 recognized and common features of SAM, and often indicate diminished body cell mass and
47 expanded extracellular fluid (1,2,16). Yet, how these abnormalities, particularly oedema, affect
48 the performance of BIA is little studied. This study explores the performance of BIA in
49 estimating TBW in children with SAM and the influence of oedema, using deuterium dilution
50 method as a reference.

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55 **Subjects and Methods**

56 *Study setting and subjects*

57 Children 0.5-14 years of age with SAM (MUAC <11.0 cm or weight-for-height <70 % of the
58 median of the NCHS growth reference and/or nutritional oedema) admitted to Jimma University
59 Hospital were included after informed consent. Children with life threatening conditions such as
60 shock were excluded.

61

62 *Data collection*

63 Weight was measured to the nearest 10 g using a digital scale (Tanita BD 815 MA, Tokyo,
64 Japan) and length to nearest 0.1 cm using a length board (SECA 416, Hamburg, Germany) for
65 children less than 2 years of age. For older children, height was measured using stadiometer
66 (SECA 214, Hamburg, Germany) to nearest 0.1 cm. Pitting oedema was checked by gentle
67 pressure with the thumb on the feet for 3-5 seconds.

68 TBW was determined by deuterium dilution at a dose of 0.5g of $^2\text{H}_2\text{O}$ (Sercon, Crewe, UK) per
69 kg body weight diluted in 5 ml of sterile water. Older children drank the deuterium whereas for
70 younger children it was dripped into the mouth using a plastic tube attached to a syringe. Any
71 spillage was collected in a tissue, weighed and subtracted from the dose. Pre-dose, and 3-hour
72 post-dose samples of saliva were collected in all children. An additional 4-hour post-dose sample
73 was collected in 15 children. In two children (1 with oedema), samples were collected hourly till
74 8-hour post-dose. Children were not given feeds 30 minutes before and 15 minutes after
75 deuterium dosing. Saliva samples were kept at -20°C before shipment to the UK for analysis.
76 Though the dose used was based on Fourier transform infrared (FTIR) protocol, it was difficult
77 to get the minimum (2 ml) saliva volume required for this method(17) and analysis was therefore
78 undertaken at Institute of Child Health, UK using isotope-ratio mass spectrometry (Delta Plus
79 XP; Thermofisher Scientific, Bremen, Germany). Samples were analysed in duplicate, with all
80 enrichments normalized to values for international standard water samples, and the average value
81 used in subsequent calculations. The mean precision of ^2H analyses was, 9.4 deltas, inducing
82 imprecision on TBW of 0.8%. For calculating TBW, it was assumed that ^2H dilution space
83 overestimated TBW by a factor of 1.044(ref. 16).

84 A tetrapolar portable bioimpedance (BI) analyser (BODYSTAT QuadScan 4000, British Isles,
85 England), emitting 200 μA root mean square alternating current at 5, 50, 100 and 200 kHzs, was
86 used to measure resistance (R), reactance (X_c) and Z. Self-adhesive disposable electrodes were
87 attached at the right hand and foot, injecting leads were connected to the electrodes just behind
88 the finger and toe and the measuring leads were then connected to the electrodes on the right

89 wrist and right ankle. Measurement was done after deuterium dosing and in triplicate, 5 minutes
90 apart, while children were calm and supine on stretcher with limbs abducted from the body.
91 Triplicate values were averaged for each subject.

92 Among 7 oedematous children with TBW data at 3 and 4 hours, there was an average increase in
93 isotopic enrichment, which indicated a delayed deuterium equilibration time. The calculated
94 TBW values therefore decreased during this period by 3.5% (95% CI: -10.6, 3.4). Among 7 non-
95 oedematous children, there was no average change in TBW calculated from 3- and 4-hour post
96 dose samples (average difference -0.2%, 95% CI -5.4, 5.0). Data on deuterium enrichment up to 8
97 hours in two children are shown in **Figure 1**. In the non-oedematous child, enrichment declined
98 from 3 hours, indicating equilibration by 3 hours and subsequent dilution of body water by fluid
99 intakes. In the oedematous child, enrichment increased between 3 and 4 hours, and then declined.
100 This suggests that equilibration was complete by 4 hours in this child. On this basis, we assumed
101 that all oedematous children were equilibrated by 4 hours. Therefore, 3-hour TBW values were
102 reduced by 3.5% in all children with oedema, but no adjustment was made to the 3-hour TBW
103 values in the non-oedematous children.

104 The study was approved by the Research Ethical Review Committee, College of Public Health
105 and Medical Sciences, Jimma University. Before giving consent, caretakers were given verbal
106 and written information. All the data were collected by two research nurses. The study was
107 conducted from December 2009 to October 2011.

108 *Statistics and data handling*

109 Data were double entered into EpiData version 3.1 (EpiData Association, Odense, Denmark) and
110 analysed with Stata/IC 12.1 (StataCorp, Texas, USA). Anthropometric z-scores, based on WHO
111 child growth standard, were calculated in Stata and WHO Anthro Plus v 1.0.3 (WHO, Geneva,
112 Switzerland)(19). BI index H^2/Z (cm^2/ohm) was calculated, where H is height or length. TBW
113 from deuterium was regressed on BI index for SAM children as a single group, and separately
114 for oedematous and non-oedematous. Then their regression coefficients and slopes were
115 compared. Data were also expressed graphically, using the bioimpedance vector analysis (BIVA)
116 approach of Piccoli (20). This approach, through RXc plot, allows axes of variability in the
117 magnitude and hydration of lean tissue to be visualized. Xc and R were height-indexed (Xc/H ,
118 ohm/m and R/H , ohm/m), and Xc/H plotted against R/H .

119 **Results**

120 The study comprised 16 non-oedematous and 19 oedematous children with SAM and having
121 median (interquartile range) age of 42 (26-54) months 48 (26-60) and 36 (30-48) months,
122 respectively (**Table 1**). The minimum and maximum ages of the children were 10 month and 144
123 months, respectively. Both non-oedematous and oedematous children were severely stunted ($p=$

124 0.70), but the latter had higher BMI-for-age ($p < 0.001$). As shown in **table 2**, the primary BI
125 parameters (Z, R and Xc) were lower among oedematous children compared with non-
126 oedematous, even when adjusted for height ($p < 0.001$) whereas the BI indices at the two
127 frequencies were not different between the two groups ($p > 0.086$).

128 Results of the regression of TBW on BI indices for SAM children as a single group and
129 separately for oedematous and non-oedematous are shown in **Table 3**. In the single group, there
130 were marginal differences in the estimates between the two frequencies. In each separate group,
131 all the regression estimates were similar for the two frequencies, and the intercepts were also
132 comparable. This indicates that little is gained by using 200 kHz, and further analyses described
133 below were therefore undertaken using Z50 only. The non-oedematous children had about 60%
134 higher coefficient of determination (R^2) and 20% lower standard error of estimate (SEE) than
135 oedematous children, indicating a much tighter association between BI indices and TBW than in
136 oedematous children.

137 Although the difference in slopes between oedematous and non-oedematous group was not
138 significant (**table 3**), for a given amount of TBW, oedematous children had a lower Z value and
139 hence higher index (**Figure 2**). Additionally, in Figure 3 it is evident that the contrast in the
140 slopes between oedema and non-oedema declines slightly at Z200 compared to Z50. So, there is
141 a weak indication that at higher frequencies, where the current passes through both extracellular
142 and intracellular space, the impedance-TBW association is not quite so different as when the
143 current mainly passes through ECW. Using BIVA approach, the oedematous and non-
144 oedematous children showed contrasting association between height-adjusted Xc and R (**Figure**
145 **3**).

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149 **Discussion**

150 In this study the poor agreement between TBW and the BI index showed the complexity of
151 assessing TBW in SAM patients, particularly among oedematous children. Fluid and electrolyte
152 abnormalities in SAM might alter tissue electrical properties and thus make prediction of TBW
153 using the BI index invalid.

154 Deuterium equilibration was delayed in oedematous (4hr) but not in non-oedematous children
155 (3hr). This reflects and confirms the hemodynamic abnormality in children with SAM;
156 analogous to hypothyroidism, they are characterized by significant prolongation of circulation
157 time and expanded extracellular water(21). The longer equilibration duration among oedematous
158 children could be explained by their excess ECW, which is clinically evident as oedema. This is
159 a methodological issue which can thus be resolved by adapting the protocol; a separate issue is
160 whether the association between the BI index and TBW is also affected by oedema.

161 In this study, oedematous children were found to produce lower impedance per unit of height,
162 although they were as severely stunted as the non-oedematous children. Walker et al (11)
163 showed that stunted Jamaican children have higher R than the non-stunted despite having the
164 same TBW%. However, in the current study stunted children with oedema had lower BI
165 parameters. The lower ρ , as estimated from the regression coefficients, may explain this
166 difference. Theoretically ρ varies with frequency but not the size and shape of individuals (13).
167 Alterations in the amount and composition of extracellular fluids, expected to be extreme in
168 oedematous children, influence tissue-specific resistivity (22). The variations in the RXc plot
169 between oedematous and non-oedematous may further support difference in their lean tissue
170 hydration.

171
172 Regressing of TBW on H^2/Z appears to give weak predictive power generally for SAM.
173 However, when the oedematous and non-oedematous children are analysed separately, the poor
174 overall accuracy can be attributed primarily to a much looser fit between TBW and H^2/Z in the
175 oedematous group (lower R^2 and higher SEE). The predictive power was similar between 200
176 kHzs and 50kHzs. The criteria for deciding on equations however is somehow empiric and also
177 relies on the assumption that impedance has been measured equally well at each frequency(23).
178 Our study suggests that BIA might help monitor TBW of non-oedematous children through
179 treatment, but would be of less use in oedematous children. However, the hydration of lean tissue
180 in non-oedematous SAM children is unknown (24) hence at present there is insufficient
181 information to convert TBW to lean mass.

182
183 In conclusion, this study demonstrated that BI parameters were lower in oedematous compared
184 with non-oedematous. Prediction of TBW using the BI index was unsatisfactory mainly among

185 oedematous SAM, but performed better in non-oedematous patients. Predictions of TBW at 200
186 kHz and 50 kHz didn't differ. The study also showed that isotope equilibration in children with
187 oedematous SAM is delayed. Larger sample size and narrower age range could have
188 demonstrated the variation in BIA prediction better. The potential utility of the BI index for
189 monitoring changes in body composition in SAM patients therefore varies substantially between
190 oedematous and non-oedematous children. Further work is required to develop BIA technology
191 for clinical monitoring of these patients; in the meantime our equation for non-oedematous
192 children may be valuable for research studies, but should not be applied in clinical practice.
193

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203 for write up of the paper while all co-authors reviewed the draft manuscript and accepted the
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208 **References**

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276 Fig 1. Patterns of deuterium enrichment (A) and corresponding calculated total body water (B) in
277 two children with severe acute malnutrition. In Fig 1A, declines in enrichment can be attributed
278 to post-equilibration fluid intake, whereas increases in enrichment indicate continuing isotopic
279 equilibration. The data therefore indicate that the non-oedematous child was equilibrated by 3
280 hours, and the oedematous child by 4 hours.

281 Fig 2. Linear regressions of total body water using deuterium dilution on BI index (H^2/Z ,
282 cm^2/Ohm) of children with severe acute malnutrition, where H is height or length and Z
283 impedance. The contrast in the slopes between oedema and non-oedema declines slightly at Z200
284 compared to Z50. The solid (non-oedematous) and broken (oedematous) lines represent the fitted
285 values.

286 Fig 3. RXc plot of reactance (X_c) on resistance (R) of children with severe acute malnutrition by
287 oedema. Height or length (H).

288

Table 1. Selected characteristics of children with severe acute malnutrition admitted to Jimma University

Hospital by nutritional oedema ^a

	Non-oedematous (n=16)	Oedematous (n=19)	p-value
Age, month	48 (26 - 60)	36 (30 - 48)	0.01 ^b
Sex, female	10 (62.5)	8 (42.1)	0.23 ^c
Z- score ^d			
Height-for-age	-3.9 ± 2.8	-3.6 ± 1.7	0.70
Body mass index-for age	-4.3 ± 1.4	-1.5 ± 1.4	<0.001
Weight-for-age	-5.3 ± 1.5	-3.3 ± 1.6	<0.001

^a mean ± SD or median (interquartile range) or n(%), ^b Kruskal-Wallis rank, ^c Chi-square and independent *t-test*

Table 2. Bioimpedance parameters and total body water of children with severe acute malnutrition by nutritional oedema ^a

	Non-oedematous n = 16	Oedematous n = 19	P - value
Impedance at 50kHz, Z50 (Ohm)	1128.9 ± 222.8	792.9 ± 197.8	<0.001
Impedance at 200khz, Z200 (Ohm)	1040.4 ± 199.2	752.1 ± 185.1	<0.001
Resistance, R50 (Ohm)	1117.5 ± 218.4	738.5 ± 215.2	<0.001
Reactance, Xc50 (Ohm)	56.0 ± 17.4	33.6 ± 14.9	<0.001
R50/H (Ohm/m)	1354.6 ± 374.6	872.6 ± 233.9	<0.001
Xc50/H (Ohm/m)	66.6 ± 22.1	40.0 ± 18.1	<0.001
H ² /Z50 (cm ² / Ohm) ^b	7.4 ± 4.5	9.6 ± 2.6	0.09
H ² /Z200 (cm ² / Ohm)	7.9 ± 5.0	10.0 ± 2.6	0.11
Total body water (L)	7.9 ± 3.2	8.2 ± 1.5	0.75

^a Mean ± SD, total body water (TBW) using deuterium dilution and ^b height or length (H)

Table 3. Regressions of total body water using deuterium dilution on bioimpedance index (H^2/Z) by nutritional oedema of children (n=35) with severe acute malnutrition ^a

	Intercept	β (95%CI)	SEE	R^2
$H^2/Z50$ (cm ² /ohm)				
Both groups	2.70	0.50 (0.35 - 0.64)	0.07	0.59
Oedematous	3.72	0.35 (0.11- 0.60)	0.12	0.37
Non-oedematous	2.33	0.60 (0.39 - 0.81)	0.10	0.73
$H^2/Z200$ (cm ² /ohm)				
Both groups	2.50	0.48 (0.35 - 0.61)	0.06	0.65
Oedematous	3.43	0.36 (0.14 - 0.60)	0.11	0.38
Non-oedematous	2.27	0.55 (0.37 - 0.74)	0.09	0.76

^a Height or length (H) / impedance (Z at 50 or 200 kHz), standard error of estimate (SEE) and coefficient of estimate (R^2). Intercepts and β (slopes) for oedematous and non-oedematous are not different between 50 kHz and 200 kHz, $p > 0.05$.

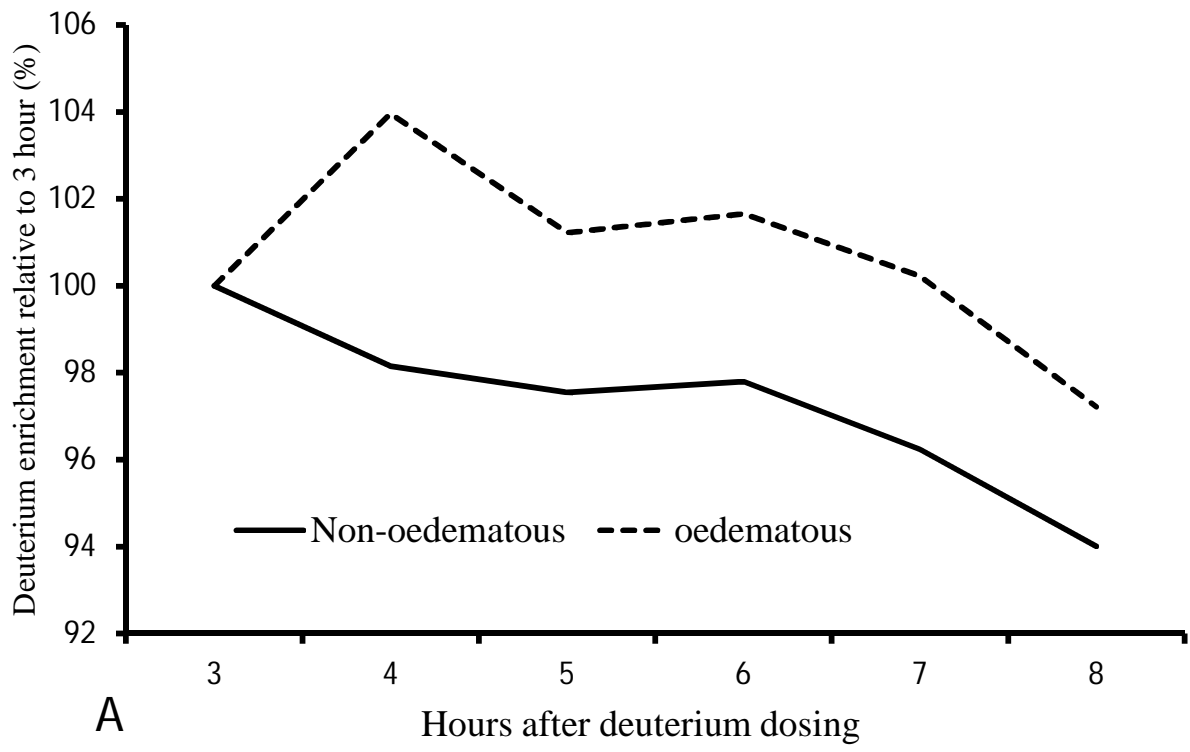


Fig 1A

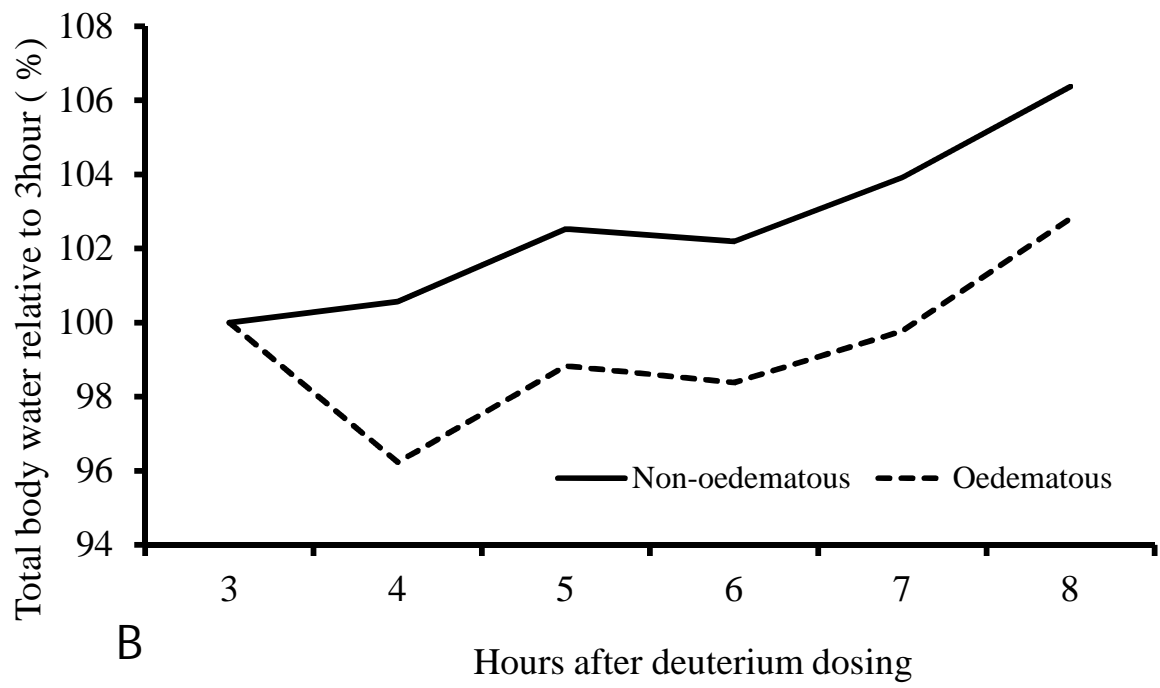


Fig 1B

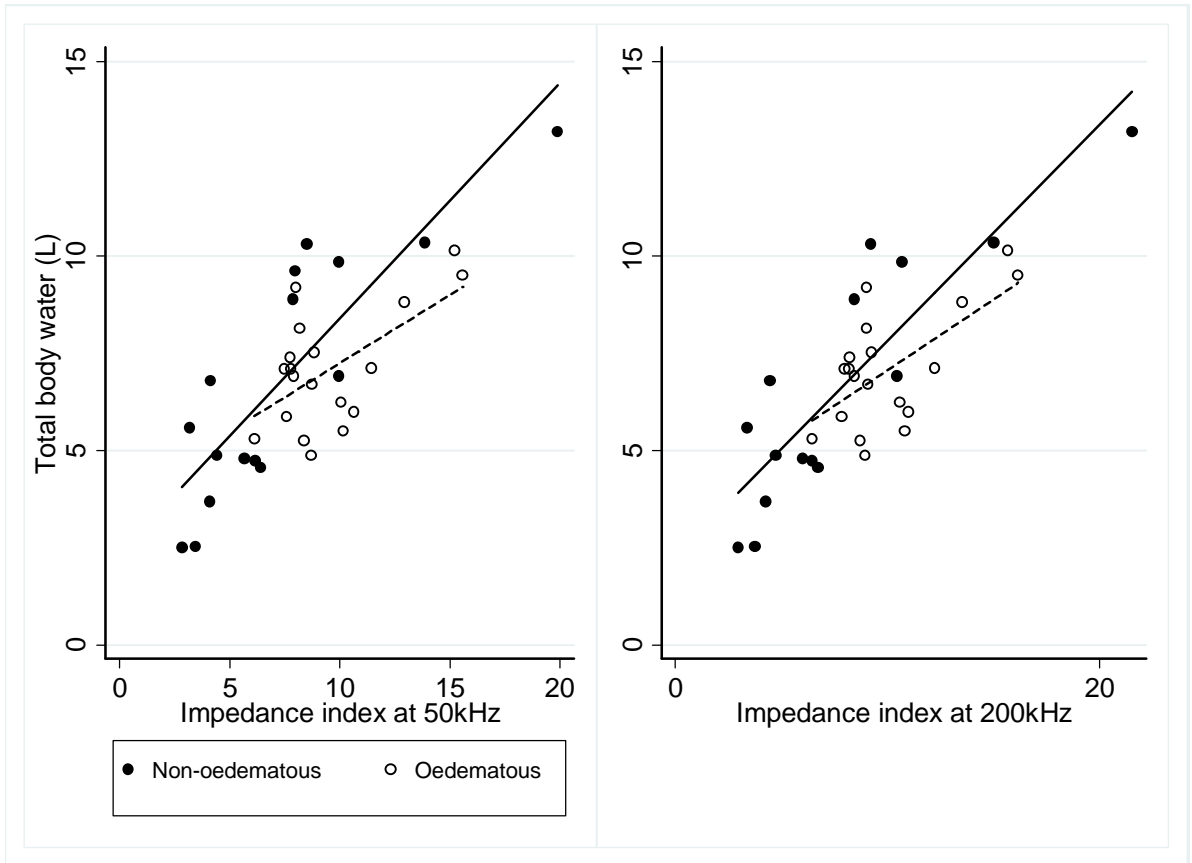


Fig 2

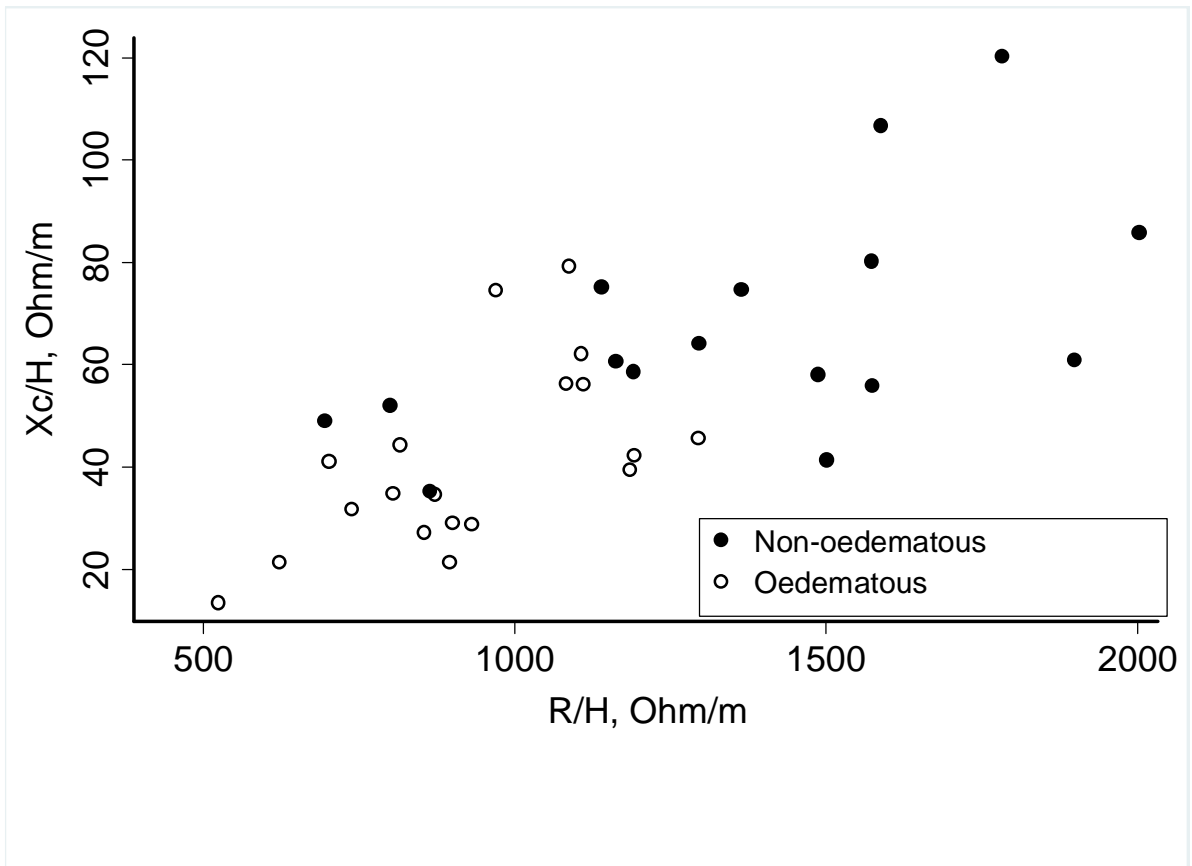


Fig 3