COMPARISON OF THREE METHODS OF BODY FAT MEASUREMENT IN INDIAN MEN

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Abstract:

Under the framework of this collaboration, we used stable isotope dilution technique as a reference to calibrate population specific Bio-electrical impedance equation to calculate total body water for Indians, since they differ in body composition from Western population. The methods were performed according to set guidelines and all precautions were taken to minimize the errors and bias during the test. Our data shows that there is no significant difference between the total body water and hence, the fat percent values obtained from standard D_2O and the anthropometric method using skinfolds (p=0.411). TBW calculated by using an equation generated by Borgonha et.al (1997) gave significantly lower values (p=0.000), however, the result given by the software supplied with the bioimpedance machine was not significantly different from the D_2O value (p=0.232). Detailed analysis of the TBW values given by the bioimpedance software compared with TBW from the D_2O method shows that there was a bias of 11% with a SD of 1.86. The range of values of TBW determined by prediction equations may yield erroneous results. Thus, prediction formulas for body composition estimates should be chosen from properly sampled population based study for a given sex and age and the guidelines should be followed with great care. We propose our new prediction equation for BIA will help to estimate body composition more precisely than the western equations.

1. INTRODUCTION:

India is experiencing an epidemic of type 2 diabetes and coronary heart disease (CHD), insuline resistant disease (IRS) among its young adult and middle-aged population. It is postulated that by the year 2015 India will have the highest number of diabetic patients anywhere in the world [1,2] and that CHD will be the leading cause of premature death in India [3] Obesity is a major risk factor for diabetes and cardiovascular disease. Body Mass Index (BMI, kg/m^2) is used to define obesity, but it is only a surrogate for body fat. Indians have different body composition at birth [4], as well as in later life compared to the Western population. They have higher fat percent for a given level of BMI compared to the white Caucasians and black Africans [5-7]. This fact is recently recognised and led to a recent change in the BMI criteria for diagnosing obesity in Asians, however there is only sparse data on body fat measurements in Indians by reference methods.

Conventionally, human body is divided into two compartments; fat and fat free mass (FFM). These compartments are measured directly or indirectly by predicting total body water (TBW) [8]. Hydrodensitomentry is the best available method for measurement of body density and hence the fat content, but is difficult to perform, requires high co-operation from the subjects and cannot be performed in epidemiological studies. Stable isotope dilution methods, on the other hand, in good technical hands, require less co-operation from the subjects, are safe and non-invasive and therefore, may be used in a variety of situations, including those potentially at risk, such as pregnant women and children [9]. However, cost, and methodological and analytical constraints preclude its use from large-scale epidemiological studies. Skinfold measurements [10] are simple, relatively accurate and therefore used routinely in epidemiological studies [11-13]. However, regional distribution of subcutaneous fat and proportion of fat situated subcutaneously vary in different populations, necessitating population specific equations. Bioelectrical Impedance Analysis (BIA) is simple, non-invasive and uses portable equipment; therefore, it may be used in large epidemiological studies [14-16]. This method uses population specific equation.

Currently available BIA machines are usually calibrated in western populations, and therefore, may give misleading results in Indians. There are only a few comparative studies in Indians for body fat measurement by BIA and a reference method, based on a small number of volunteers [17-19]. We undertook measurements of body fat by anthropometry (using Durnin's equation) and BIA and compared them with the reference method of deuterated water.

2. MATERIALS AND METHODS:

We aimed at studying 150 men (30 – 50 years of age) from 3 geographical locations: rural, urban slums and urban middle class, selected by multi stage stratified random sampling (figure 9).

3. POPULATION SAMPLING:

We randomly selected two villages approx 50 kilometres from Pune in an area where KEM Hospital and Research Centre offers primary health care, and two middle class and two slum wards from the Pune city.

A total of 1222 men were listed by house -to-house survey from the three locations. Those with diagnosed diabetes, hypertension and heart disease were excluded. Excluding those who declined to participate we measured height and weight to give a representative distribution of BMI in these locations. One hundred and fifty men from each group (total 450) were randomly selected for further study. These subjects underwent extensive clinical, biochemical and radiological investigations for measurements of cardiovascular risk factors.

For the purpose of calibrating anthropometry and BIA we used deuterated water dilution method. We had to restrict the number because of the expense of the test. We randomly selected 50 men from each of the three tertiles of BMI for the whole group irrespective of their place of residence. Thus, rural men are over represented in the lowest tertile while middle class are over represented in the highest tertile. These 150 men underwent deuterated water studies in addition to other measurements.

4. METHODS:

Subjects were studied in the Diabetes Research Centre, KEM Hospital, Pune. They reported to the Unit at 7.00 pm and were fed a standard dinner and then rested.

4.1. Deuterium Dilution:

Five hours after dinner subjects emptied their bladder completely to provide the basal urine sample. Then they drank 75mg of deuterium oxide (D_2O, 99.9%, Europa Scientific, Crewe, UK) per kilogram of body weight from a sterile plastic container with the aid of a straw. This was followed by drinking 3gm / kg body weight of plain water, using the same container and the straw to ensure all deuterium is taken. After this the subjects rested again.

Urine samples were collected every hour between 4 h and 7h after D_2O drink. Samples were immediately frozen at –70°C in a screw capped plastic vial, sealed with Para film. They were transported on ice to St. John's National Academy of Health Sciences, Bangalore where they were stored at –70°C till further analysis.

Body weight was recorded after the last urine sample was collected, and used in all calculations.

Samples were analysed in duplicate for deuterium enrichment by zinc reduction followed by duel-inlet mass-spectroscopy (Europa Scientific, Crewe, UK) as recommended by the IDECG [20]. Repeated analysis for natural background samples gave a CV (delta vs. SMOW) equal to 0.02%. With high enrichment samples using IAEA standard no 302, (enrichment equal to 500 vs. SMOW), CV was equal to 0.22%.

Deuterium oxide (99.9% D_2O) provided by the manufacturers (Europa Scientific, Crewe, UK) was analysed to ascertain its enrichment using dilution method. The value obtained was incorporated into the calculation of the deuterium pool size using the equation [21].

$$N \text{ (moles)} = \frac{W \times A}{18.02 \times a} \times \frac{(E_a - E_t)}{(E_s - E_p)}$$

Where 'W' was the weight of water used for diluting the dose to assess its enrichment, 'A' was the amount weighed to dose administered to each of the subjects, 'a' was the weight of dose diluted for analysis of enrichment, 'E_a' was enrichment of the dose, 'E_t' was the enrichment of tap water, 'E_s' was the enrichment of post-dose sample and 'E_p' was the enrichment of pre-dose baseline. All calculations to total body water by D_2O (TBW_D) assumed that the deuterium space was 1.04 times the TBW [21].

4.2. Anthropometry

Height was measured using a wall fixed stadiometer (CMS Instruments, London), and body weight using portable Sohenel scale. Biceps, triceps, subscapular and suprailiac skinfold thickness were measured on the left side of the body using Harpenden skinfold callipers (CMS Instruments). Body fat percent was calculated from four skinfold thicknesses using formula ([4.95/Density]-4.5) x 100, where Density = (1.1599 – (0.0717 x $\log_{10}$ (Sum of four skinfolds)) [10]. Body fat percent was converted to fat in kilograms using the formula ([Body weight x Fat percent]/100). FFM was calculated by subtracting body fat in kilograms from total body weight. Total body water by anthropometry (TBW_A) was calculated from FFM using hydration constant (TBW_A = FFM * 0.7194) [22].

4.3. Bioelectrical impedance

The procedure was carried out according to the recommendations in the NIH Technology Assessment Conference Statement (1994)[23]. All measurements were carried out in the morning (~07:30h) using Multiscan 5000 (Bodystat Ltd, Isle of Man, UK). Subjects had rested for ~12h, fasted ~6h, and emptied there bladder before measurements. Metal ornaments were removed and subjects were rested supine on a wooden couch for 5 min. Their arms and legs are abducted at a 30 – 45 degree angle from the trunk. Electrode sites were cleaned with ether, for proper attachment and to reduce local resistance. The source and the sensor electrodes were applied at the first metacarpophalangeal joint and first tarsophalangeal joint, on the right side using adhesive electrodes supplied by the manufacturer. An alternating current (800 mA), with an increment of 5 KHz between 5 KHz and 500 KHz frequencies was used to measure impedance between the two electrodes, and values recorded for all the above frequencies were downloaded on a PC computer and soft were supplied by the manufacturer is used to calculate TBW in litres and converted to kg (TBW_I) by multiplying with 0.99336 (density of water at body temperature).

Impedance at 50 KHz was used to calculate TBW using the predictive equation given by Borghonha et.al. [17] (TBW_B) with height^2/ Impedance as predictive variables for men. TBW_D, TBW_I and TBW_B are used to calculate FFM with the help of hydration constant, 0.7194 [22] (Siri, 1961). Fat mass was obtained by subtracting FFM from body weight.

4.4. Statistical analysis:

Data is shown as median and inter-quartile range. For variables not normally distributed, we used appropriate transformations for normalization.

Statistical significance of the difference of means between groups was tested by unpaired and paired t-test wherever appropriate. The significance of correlations was tested by Spearman's method. Multivariate analysis was carried out by multiple linear regression technique using stepwise method. All statistical analysis was performed using SPSS for Windows (version 10.0).

5. RESULTS

Three subjects with low impedance value and hence negative fat percent were excluded from the analysis. The analysis was done on 139 subjects on whom body fat measurements were available by all methods. The basic characteristics of the study subjects are shown in Table.1.

Comparison between the TBW's of all the three methods revealed no significant difference Viz D_2O vs Anthropometry, p=0.411 and D_2O vs Bioimpedance by machine, p=0.232. However, there was a significant difference between D_2O and Bioimpedance by Borgonha et al, p=0.000.

Figure 1 shows a strong and significant correlation between TBW_I and TBW_D ($r=0.84$, $p<0.001$) suggesting a linear relationship. However this does not guarantee the agreement between the two methods [25]. Hence, we plotted a Bland and Altman plot (Figures 4 to 6) which shows the limits of agreement for TBW's i.e. D_2O and Anthropometry (3.6, -3.9 litres) these limits are not wide so causing the difference of ~11% in TBW suggesting a good agreement between the methods; similarly there is a good agreement between D_2O and Bioimpedance Machine where the limits of agreements are (3.9,-3.5) . But when we compare D_2O and Bioimpedance by Borgonha et al the limits of agreements are (5.7,-3.2) these limits of agreement are wide enough, causing a difference of ~10 to 17 % in TBW measurement suggesting a rather poor degree of agreement between these two methods.

We further attempted to improve the agreement between the two methods (D_2O and Bioimpedance Machine) by mathematically controlling factors which affect these measurements and over which we have no control.

We used stepwise regression method to predict the TBW. The predictor variables tried in the model were, Height^2 / Impedance (at 50 KHz) [26,27], age of the subject and body weight (kg). Removal of age from the equation resulted in a significant gain in R^2 (to 88.8% from the previous 85.0%) and reduction in standard error (to 1.52 from 1.72) of regression estimates. The new model is as follows.

$$\text{TBW (LITRE)} = 0.38\ (\text{HEIGHT}^2/\ \text{IMPEDANCE}) + 0.13\ \text{WEIGHT} + 5.75$$

This equation was internally validated by dividing the whole group randomly into two equal sized groups and by inspecting the distribution of residuals (Mean =0.033, SD=1.52) (Figure 8), which is a normal distribution suggesting the validity of the equation obtained by regression analysis.

6. DISCUSSION:

The rising prevalence of diabetes and cardiovascular heart disease in India is emerging as a major public health problem. Obesity has been identified as one of the major risk factors.

Gain or loss of total body water and fat percent during recovery of the patient reflects the health condition, which will help the clinicians to decide the treatment. The disturbance in the ratios of fat and water content may help to predict future health problems. Hence there is a need to measure the body composition by very accurate methods. In India, anthropomenty and BIA are usually used to predict body composition. Indians have different body composition than western population, and hence there is need to calibrate these methods for local population.

This is the first properly sampled population based study in India to compare these two methods against the standard isotope dilution method.

We have taken all precautions while performing the methods. A specific Bodystat calibrator was used daily to confirm the reproducibility of the machine. No water retaining drugs, fasting for minimum 5 hours, physical activity prior to measurement, empty bladder, resting for at least 5 min. to settle hydrodynamics, not to rest on / near conducting material which may affect the impedance values, proper cleaning of the electrode sites and placement of electrodes. Displacement of electrode by one cm. can result in 2% change in resistance

Anthropomentric parameters such as height and weight must be taken with great care. Over or under estimation of height by 2.5cms has been shown to cause an error of 1.0 litres of TBW. Over or under estimation of weight by 1.0 kg can cause an error of 0.2 litres of TBW [23].(NIH).

Many health clubs, gymnasium centres and general clinics use this method to measure the body fat, but they do not follow the guidelines.

The types of machine usually used are small, portable, hold and stand or stand on the machine. These machines do not require proper electrode attachments and are not calibrated for local population. Thus they also need to have proper machines and require predictive equations.

Our results show that there is a good agreement of TBW_D with TBW_A. TBW_B is lower compared with TBW_D hence fat percent calculated by BIA method is higher as compared to D_2O.

Our results support the need to calibrate the predictive equations for Indians. The predictive equation we derived will give better results than the equations that were developed before on small and restricted samples, and equations that originate from western populations.

TABLE-1: BASIC INFORMATION ON SUBJECTS STUDIED (N=139)

	Median	IQR
Age (yrs)	39.0	(34.0-45.0)
Height (cm)	165.2	(161.7-170.0)
Weight (kg)	59.3	(52.5-67.4)
BMI (kg/m^2)	21.5	(19.9-25.3)
TBW_D (Kg)	32.6	(29.9-35.5)
TBW_A (Kg)	32.9	(30.4-35.4)
TBW_I (Kg)	32.4	(30.3-34.9)
TBW_B (Kg)	31.6	(29.8-32.7)
TBW Difference ($TBW_D - TBW_A$)	-0.06	(-1.09-0.89)
TBW Difference ($TBW_D - TBW_B$)	1.32	(-0.18-2.67)
TBW Difference ($TBW_D - TBW_I$)	0.15	(-1.04-1.07)
TBW Difference ($TBW_A - TBW_B$)	1.49	(-0.09-2.82)
TBW Difference ($TBW_A - TBW_I$)	0.07	(-0.97-1.20)
Fat % by D_2O	24.2	(16.9-29.0)
Fat % by Anthropometry	23.3	(17.8-27.7)
Fat% by BIA (Machine)	24.2	(19.6-27.9)
Fat% by BIA (Borgonha et al)	26.1	(19.2-32.2)

TBW_D= total body water by D_2O method, TBW_A= total body water by anthropometry, TBW_I = total body water by software from the manufacturer, TBW_B total body water by Borgonha et al formula.

TABLE-2: COMPARISON OF MEAN BODY FAT% (N=139)

Methods	p-value (Paired t-test)	Anthropometry	Bioimpedance (Machine)	Bioimpedance (Borgonha et al)
D_2O		p=0.411	p=0.232	p=0.000
Anthropometry		--	p=0.116	p=0.000

p-value by paired t-test

ABBREVIATIONS:

SD: Standard deviation
TBW: Total body water
TBW_A:Total body water by Anthropometry
TBW_D:Total body water by Deuterated water
TBW_I:Total body water by Impedance machine
TBW_B:Total body water by Borgonha etal
BIA: Bio-electrical Impedance
FFM: Fat free mass
BMI: Body mass index
IQR: Inter quartile range
SPSS: Statistical package for social sciences

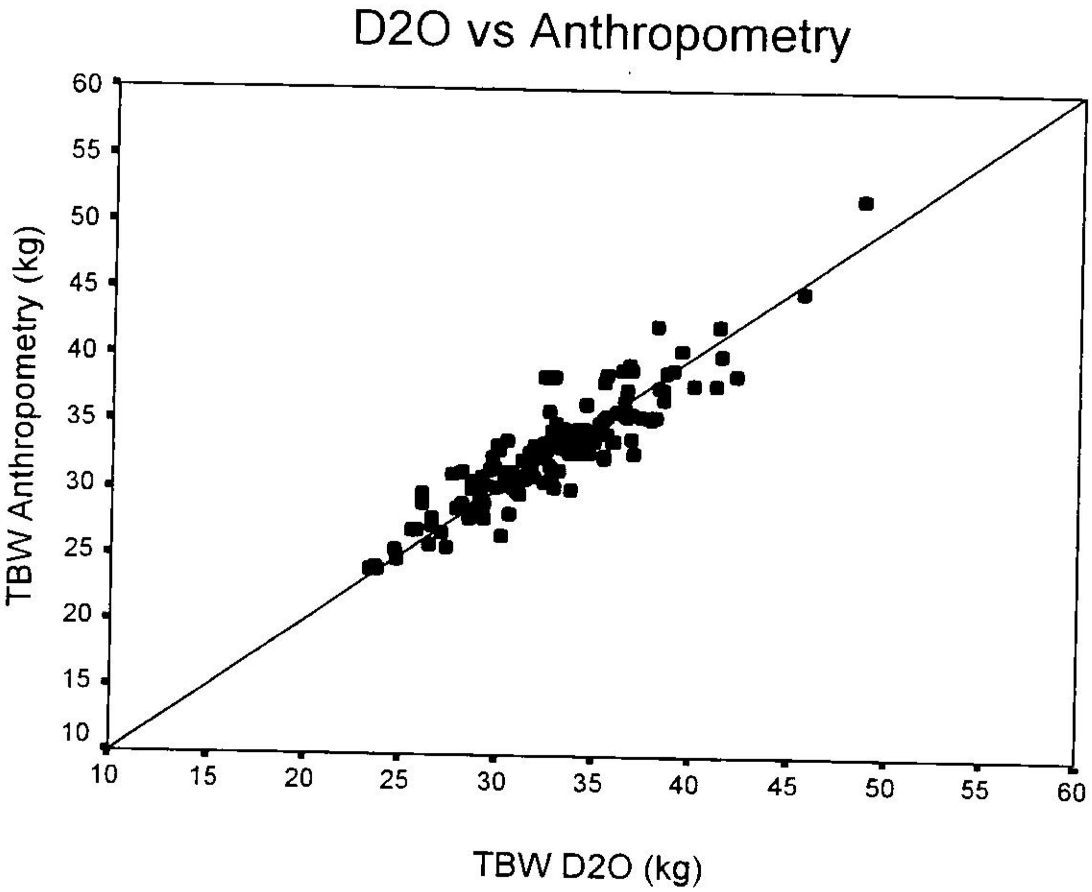


Figure-1: Shows Scatter plot of TBW_D vs TBW_A.

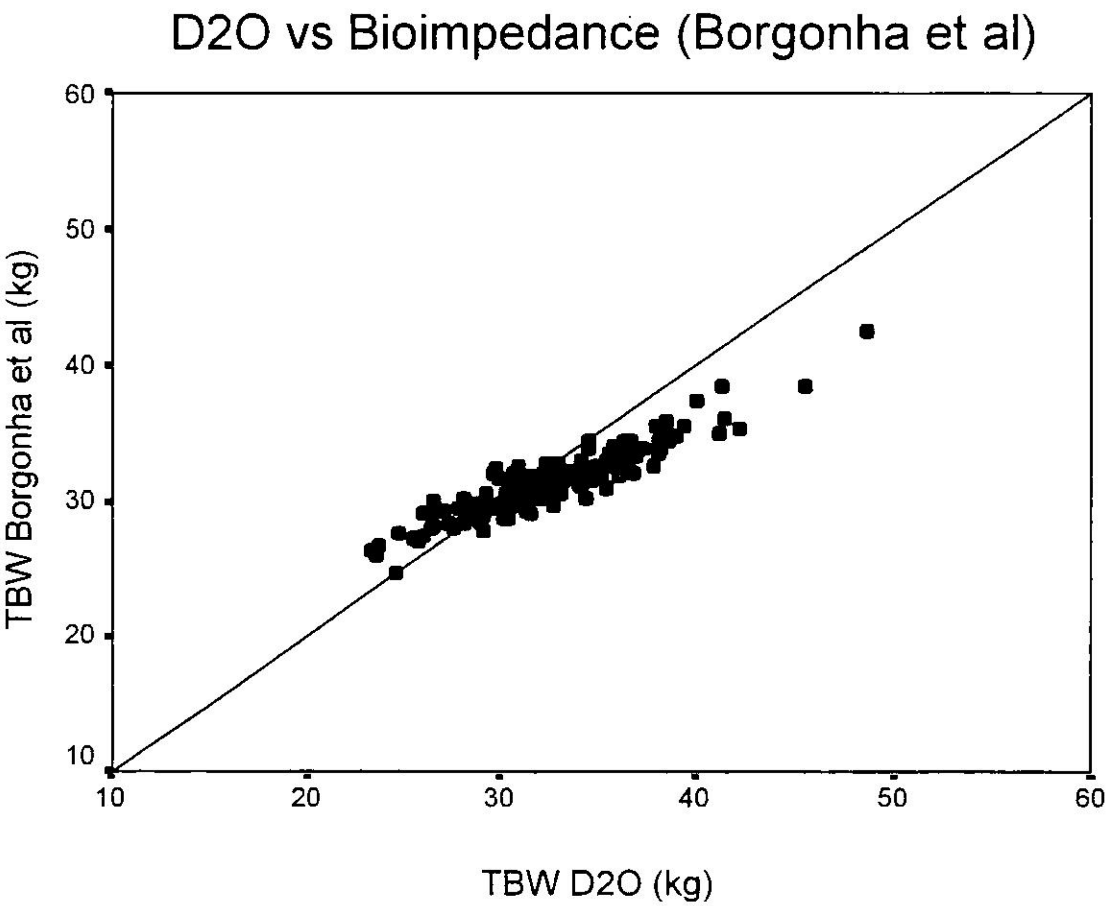


Figure-2: Shows Scatter plot of TBW_D vs TBW_B.

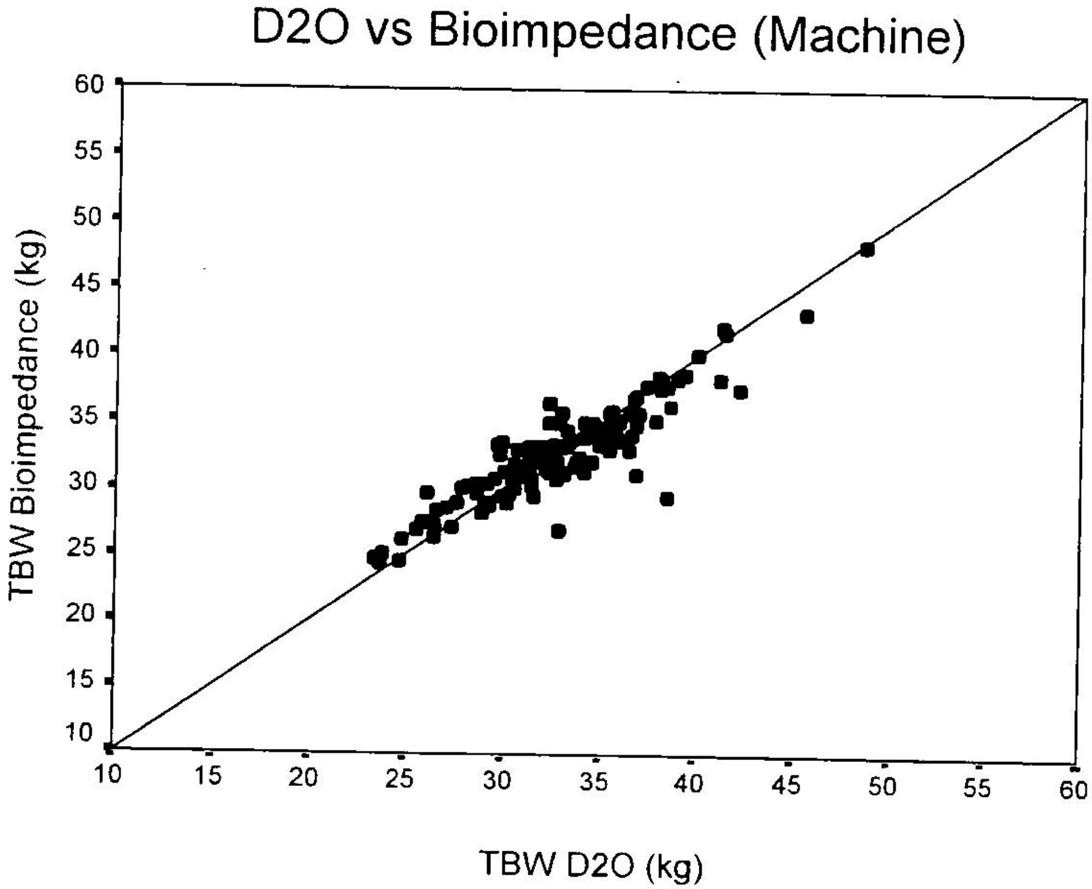


Figure-3: Shows Scatter plot of TBW_D vs TBW_I.

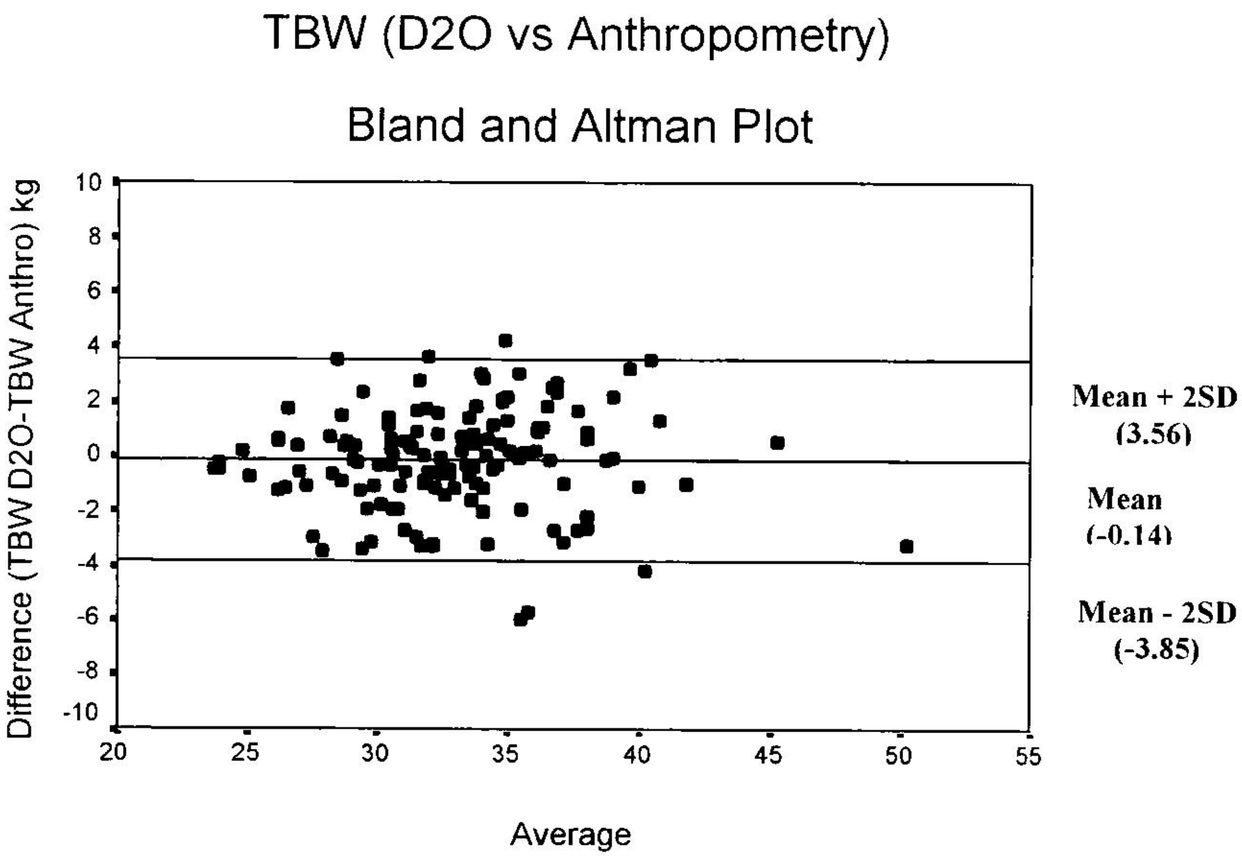


Figure-4: Bland and Altman plot showing the limits of agreements (TBW_D vs TBW_A), $r=0.04$, $p=0.649$

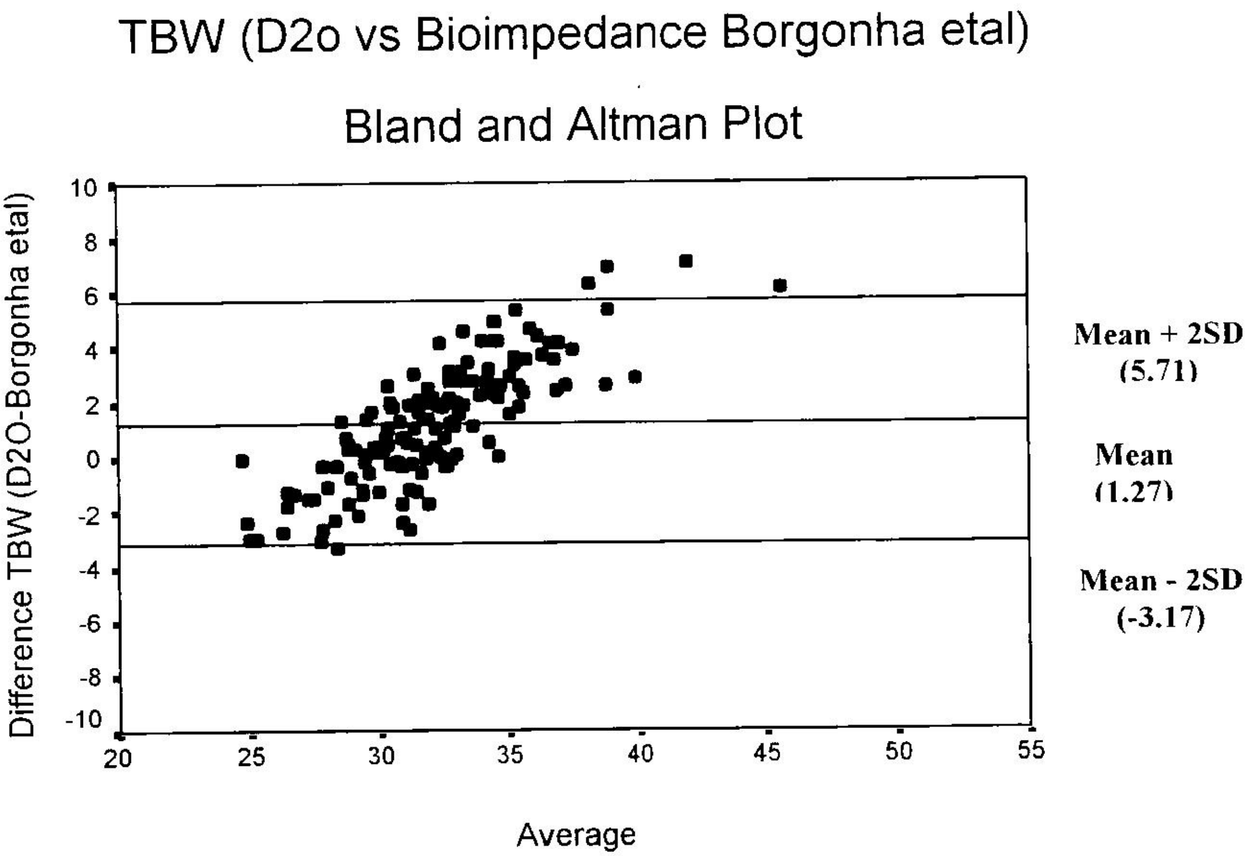


Figure-5: Bland and Altman plot showing the limits of agreements (TBW_D vs TBW_B), $r=0.81$, $p=0.000$

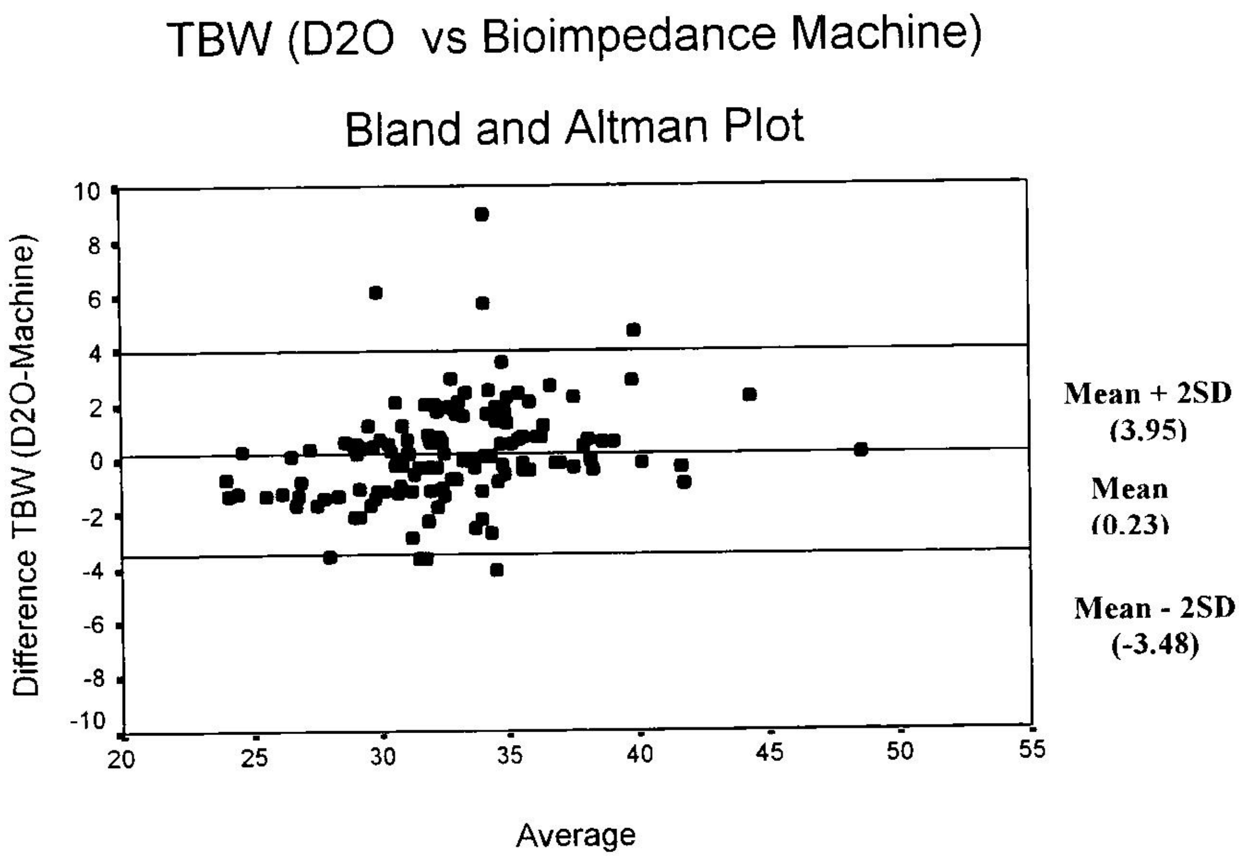
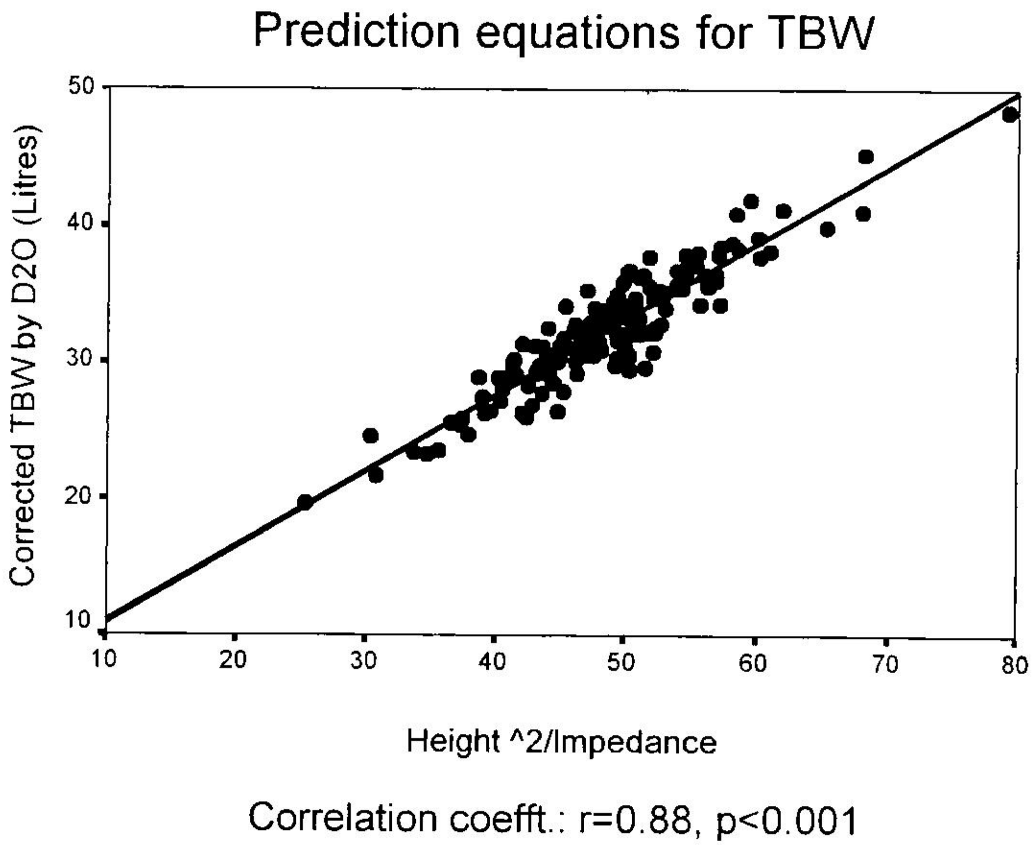


Figure-6: Bland and Altman plot showing the limits of agreements (TBW_D vs TBW_I), r=0.28, p=0.001



The regression equation is:

TBW (Litre) = 0.38 (Height^2/ Impedance) + 0.13 Weight + 5.75

Figure-7: Relationship between height^2 / Impedance and TBW (Litre, as measured by deuterium dilution): $r=0.88$, $p<0.001$.

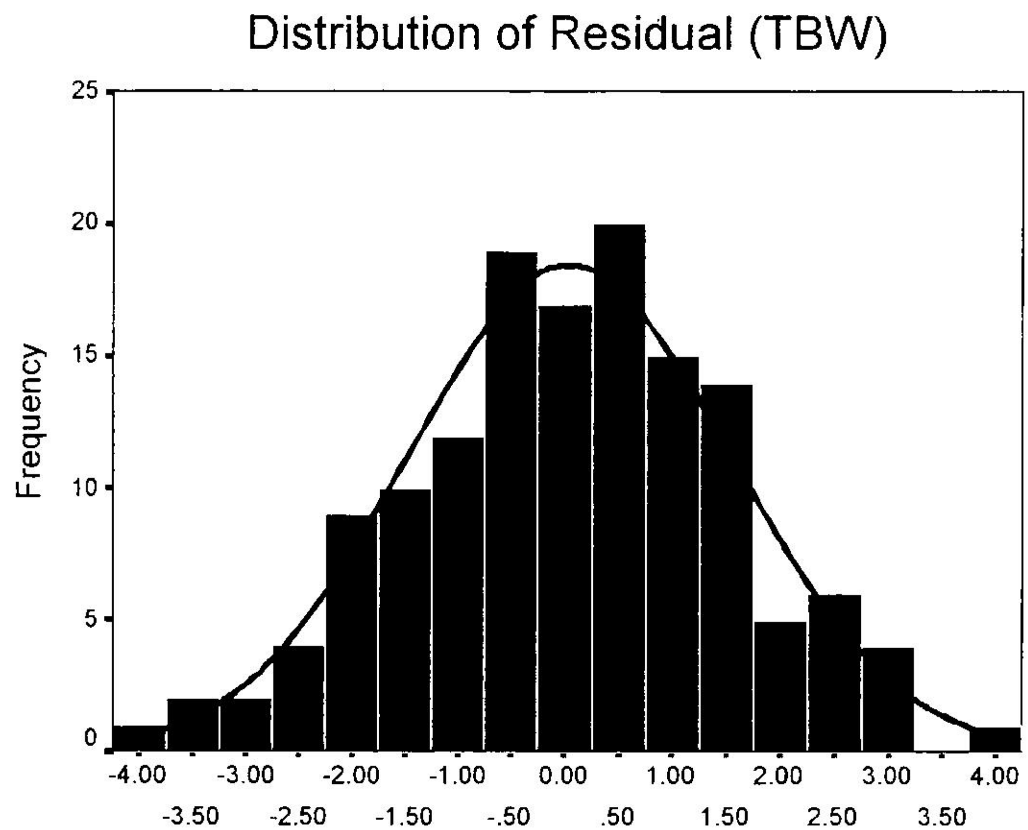


Figure-8: Histogram showing distribution of residual of TBW (Regression analysis).

Population Sampling

	Urban		Rural
	Municipal Wards (4 / 124)		Villages (2 / 6)
	Slums (2)	Middle Class (2)	Hamlets (10 / 36)
Households	1200	373	436
Men (30-50y)	425	443	354
Known DM/ HT/ IHD	15	21	3
Random Selection	200	200	200
Disease	6	2	2
Studied	142	150	149
Core	51	49	47

Figure-9: Shows population sampling

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