

## Original Article

# The development of a whole-body potassium counter for the measurement of body cell mass in adult humans

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**Background and Objectives:** Total body potassium (TBK), has a natural radioactive isotope, which can be measured to derive body cell mass (BCM), making it useful in clinical conditions, early growth and pregnancy. The objective was to build a whole-body potassium counter (WBKC), to accurately measure TBK in the body. **Methods and Study Design:** A WBKC was designed and constructed using a shadow shield. A cellular four compartment (4C) model of fat free mass (FFM), using estimates of TBK along with total body water (TBW), was compared with a molecular 4C model of the body in twenty healthy adults (10 men and 10 women). The molecular 4C model used measurements of TBW, bone mineral content (BMC), and body volume from deuterium dilution (DD), dual energy x-ray absorptiometry (DXA) and air displacement plethysmography (ADP) respectively. **Results:** The accuracy and precision of the WBKC were 2.8% and 1.9% with TBK phantoms. The mean estimate of FFM by the molecular 4C model was 40.4±6.8 kg, while it was 41.2±7.3 kg using the cellular 4C model. **Conclusions:** A WBKC constructed from base principles, was relatively low cost, efficient, safe and non-invasive, but requires some design considerations. Its measurement of FFM compared well with the molecular 4C model. Once constructed, it offers a relatively costless, accurate and repeatable method to measure body composition in conditions with uncertain hydration status, at all life stages.

**Key Words:** whole-body potassium count, body composition, body cell mass, potassium, fat free mass

## INTRODUCTION

The measurement of body composition provides an objective means of nutritional assessment. The most common approach to measuring body composition is to define a two-compartment model (2C) of the body, consisting of fat mass (FM) and fat free mass (FFM). A more precise cellular four compartment (4C) model divides the body into fat, body cell mass (BCM), extra cellular fluid (ECF) and extra cellular solids (ECS).<sup>1</sup>

The BCM is defined as a “component of body composition containing the oxygen-exchanging, potassium-rich, glucose-oxidizing, work-performing tissue”,<sup>2</sup> in effect, consisting of all the cellular portions of the tissues, excluding non-essential fat.<sup>3</sup> In undernutrition, when the BCM shrinks, it can be accompanied by an expansion of the ECF compartment.<sup>4,6</sup> As a result, FFM as measured by a 2C model can appear to change little, while changes in the hydration can give erroneous FFM results. A safe, accurate and non-invasive method that is unaffected by changes in FFM hydration status,<sup>7</sup> is required to measure body composition in such conditions. Furthermore, in early life, when FFM hydration is elevated by an increased proportion of ECF, total body potassium (TBK)

measurement provides an unbiased estimate of BCM.

Since 98% of TBK<sup>7,8</sup> is held in tight homeostasis in the BCM, the measurement of TBK can accurately measure BCM, which is not affected by changes in the ECF volume or the hydration status of the body. The TBK can be measured by exploiting its isotopic composition, since it contains a very small amount of radioactive <sup>40</sup>K (natural abundance 0.012%), which importantly, exists in a constant proportion to the major stable isotopes of potassium, <sup>39</sup>K (natural abundance 97.3%) and <sup>41</sup>K (natural abundance 6.7%). This allows for the measurement of TBK by measuring the natural radioactivity of <sup>40</sup>K within the body, which can be done safely and non-invasively using a whole-body gamma counter, which is an array of sodium-iodide (NaI) detectors positioned above or around the

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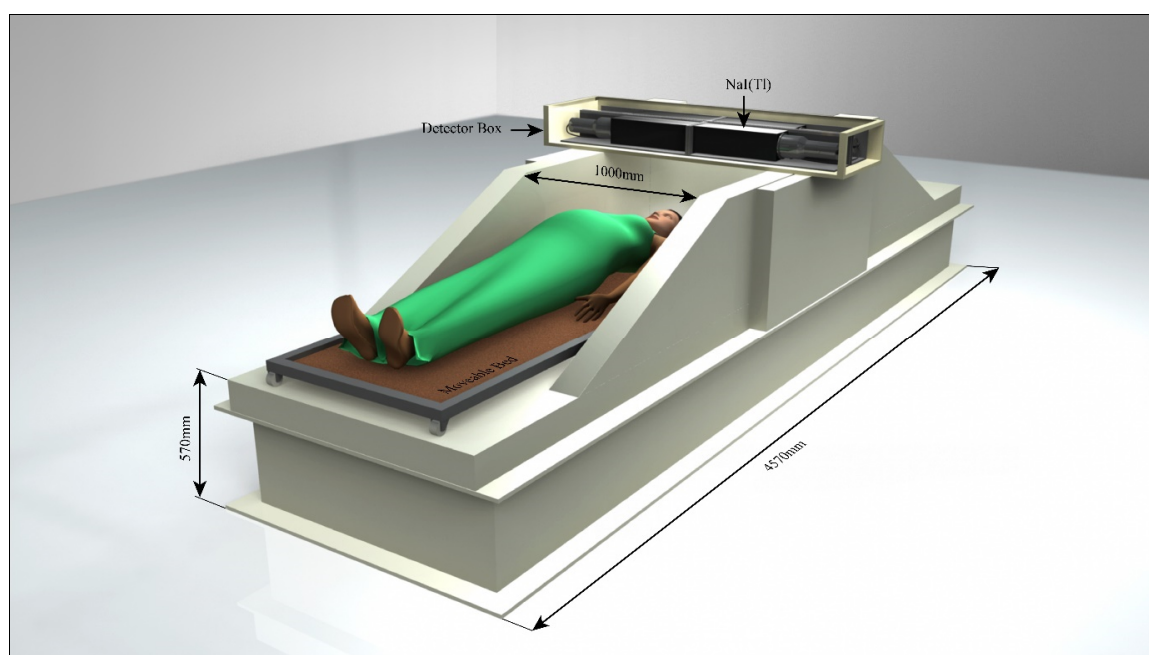
body, housed inside a room or shadow shield structure to reduce the background interference. Calculation of the BCM uses the near constant ratio of TBK to BCM, 108.7 mmol K/kg BCM invariant across age and gender.<sup>3</sup>

Due to changing hydration status,<sup>9,10</sup> measurement of body composition is difficult in pregnant women and children in early life using traditional methods, as well as in severe acute malnutrition, where oedema alters the body hydration status.<sup>4-7</sup> Measurement of BCM offers an important body compositional method in these conditions. The estimation of TBK and BCM has also been observed to be useful in monitoring sarcopenia, the age-related loss of skeletal muscle mass<sup>11,12</sup> and the age-related decline in FFM-adjusted resting energy expenditure.<sup>13</sup> This study aimed to construct a whole-body potassium counter (WBKC) in India, capable of measuring TBK and BCM in humans of all ages, including pregnant women.

### MATERIALS AND METHODS

The construction of the WBKC included considerations of shielding from extra-corporeal origin gamma rays and detection of the corporeal origin gamma rays by sodium iodide detectors. For shielding, a shadow shield was constructed from a mixture of mild steel and lead (Figure 1), based on previous experience of shadow shield WBKC designs.<sup>14,15</sup> This measured 4570 mm x 1400 mm x 1200 mm and weighed 14 tons. A strong 200 mm deep concrete plinth was constructed using low potassium aggregate and topped with a 12 mm steel plate measuring 4595 mm x 1430 mm. The steel had been screened to demonstrate that it contained negligible <sup>60</sup>Co activity. Above this, a platform measuring 4570 mm x 1400 mm x 400 mm was constructed with I-beams, around which a water tank with a capacity of 3647 litres was constructed for additional

shielding from the ground. Subsequent tests however showed that filling the tank with water yielded little reduction in the potassium background, so this volume no longer contains water. Seven additional steel plates of 20 mm thickness each, alternating with 3 layers of lead sheets of 3mm each, were placed on top of the water tank to act as the base of the WBKC. Eight steel sheets of 20 mm thickness each (Akruthi Fabs, Bengaluru, India), formed the 800mm high lateral walls of the shadow shield. On top of the lateral walls, a box made from 6 layers of 20 mm steel alternating with 4 layers of 3mm lead sheets was constructed to house the detectors. This detector box measured 1632 mm x 730 mm x 385 mm, and rested on a 20 mm steel plate, measuring 1600 mm x 730 mm, which had two apertures of 150 mm each cut into it on either side, with a 50 mm gap in between. Four thallium doped sodium iodide (NaI(Tl), Saint-Gobain Crystal and Detectors, Hiram, USA) detectors measuring 406.4 mm x 101.6 mm x 101.6 mm were placed within this box, on top of the apertures, to have the desired line of sight below to enable a count only of gamma rays emanating from the subject lying beneath on the moveable bed, and not from other sources. Each detector was fitted with a single, low potassium photomultiplier. A low power high voltage supply, preamplifier, amplifier and multi-channel analyser unit was fitted to each photomultiplier tube and connected via USB cable to the control computer (usbBase plug on MCA, Bridgeport Instruments, USA). A moveable bed for the subjects was positioned at bed height, to facilitate the measurement of subjects with reduced mobility and rolled into the aperture of the WBKC which is 1000 mm wide, 400 mm high with a 4570 mm bed length. The open design of an aperture in the WBKC allows for an infant up to 2 years of age to be counted in a static



**Figure 1.** Four sodium iodide (NaI(Tl)) detectors, placed in a shielded detector box (shown without the top layer of shielding, for visualization purposes) have the desired line of sight to enable counting of gamma rays emanating from the subject lying beneath. The subject lying on the moveable trolley bed was measured for 10 minutes each in three different positions for a total of 30 minutes (from superior to inferior for the body) with respect to the detectors. In the first position the crown of the head was positioned directly under the detectors. In the second position the bed was moved 70 cm such that the approximate centre of the body would be located under the detectors. In the third position the bed was moved another 70 cm in the same direction.

geometry, at maximum sensitivity, positioned perpendicular to the moving bed, and at all the while within direct eyesight of a parent or trained operator.

Radioactive  $^{40}\text{K}$  emits a characteristic gamma ray of 1461 kiloelectronvolt (keV) with a nearly constant emission rate of 200.4 gamma rays/minute/gram of potassium.<sup>16</sup> These were collected by four NaI(Tl) detectors, which were used as they produce a large scintillation light output, and in large crystals, have high gamma-ray detection efficiency. The gamma-ray spectroscopy system used in this study thus included four NaI(Tl) detectors coupled to their respective photomultiplier tubes, a high-voltage unit consisting of a preamplifier and shaping amplifier, a multichannel analyser and a data readout device.

To store and analyse the signal output from the detectors, a software package was developed in R. The software package aggregated data for pre-assigned time interval and produced a spectral curve of counts vs channels for each detector. A peak associated with the gamma rays produced by  $^{40}\text{K}$  was identified in a specific region of interest (channels 800-2000), using the CERN ROOT package.<sup>17</sup> The 800-2000 channel range was chosen to avoid any intersection with  $^{214}\text{Bi}$  (Bismuth) peak at 609 keV and  $^{208}\text{Tl}$  peak at 2614 keV. The background counts underneath the  $^{40}\text{K}$  peak, were estimated using a linear fit function in the area surrounding the peak. The peak was then fit to a Gaussian curve. The area under the Gaussian curve (200 channels on either side of peak channel), obtained after the subtraction of background gave the true value of counts for each detector. The counts were scaled by the length of the time interval, taken in seconds to give an average number of counts per second (CPS).

For calibration, the relationship of CPS to the TBK had to be defined. For this, 5 anthropomorphic shaped dummies or phantoms, weighing 19.19, 29.29, 55.77, 85.05 and 123.48 kg, with a constant height of 160 cm were prepared with known quantities of potassium chloride (KCl). Multiple phantoms were used since calibrating against a single-size reference phantom can result in inaccurate TBK values.<sup>18-21</sup> Phantoms were made using a combination of 1L plastic bottles and smaller 300mL plastic bottles to simulate the head, arms, trunk and legs, the relative proportions of which were kept constant across the phantoms. Water phantoms containing deionized water equivalent to the weight and shape of the corresponding phantoms were also constructed and scanned. Detector specific water phantom CPS were subtracted from the KCl phantom CPS to give the net CPS. Each phantom was measured three times to calculate precision and mean counting error. Monte-Carlo calculations were used to simulate the phantoms and human bodies of different shapes and sizes.<sup>18,21,22</sup> Detector responses and efficiencies for different sized phantoms were also measured. The specific accuracy of the WBKC was measured with a phantom of 60 kg weight and 414 g K. The mean counting error (as a percentage, and providing a measure of reproducibility) was also calculated, using the following equation<sup>23</sup>

$$\text{PE} = \left\{ \left[ \frac{N_i}{t_i} + \frac{W_{\text{cps}}}{t_i} + \frac{W_{\text{cps}}}{t_b} \right]^{0.5} \right\} \times \{100/N_i\}$$

where PE is the percent error,  $N_i$  is the net cps for the phantom or subject,  $W_{\text{cps}}$  is the cps for water phantom,  $t_i$  is the counting time for the KCl phantom or the subject,

and  $t_b$  is the counting time for the water phantom.

The measurement of BCM from TBK assumes that the BCM has a fixed K concentration, and uses the formula<sup>3</sup>

$$\text{BCM (kg)} = 0.0092 \times \text{TBK (mmol)}$$

Using a whole-body ratio of potassium to nitrogen of 2.15 mmol K/g N,<sup>24</sup> total body nitrogen (TBN) can be calculated from TBK. If it is assumed that all TBN is incorporated in protein, total body protein (g) can be estimated as  $6.25 \times \text{TBN}$ .<sup>1</sup>

The FFM measurements from the cellular 4C model, using TBK measurements from WBKC and total body water (TBW) using deuterium dilution (DD) were compared to the FFM measurements from a molecular 4C model. The molecular 4C model assumes that the body is composed of 4 compartments; fat, protein, water and mineral.<sup>25</sup> Twenty healthy individuals (10 men and 10 women) aged 24-56 years were recruited for this study, from the staff of the St. John's National Academy of Health Sciences. The study was approved by the Institutional Ethical Review Board. The objective of the study was clearly explained to all the subjects and a written consent was obtained from them. The subjects reported to the WBKC room between 0900 h to 1500 h and their body weight (to the nearest 0.1 kg), height (to the nearest 0.1 cm), abdominal circumference and hip circumference (to the nearest 0.1 cm) were measured utilising standard methods.<sup>26,27</sup>

For the TBK measurement, the subject lay supine on a trolley bed that ran directly under the detectors. The measurement was for 10 minutes each in three different positions (from superior to inferior for the body) with respect to the detectors. In the first position the crown of the head was positioned directly under the detectors. In the second position the bed was moved 70 cm such that the approximate centre of the body would be located under the detectors. In the third position the bed was moved another 70 cm in the same direction. The same measurement protocol was used for the calibration phantoms.

For TBW measurements, which is common to both 4C models, the DD technique was used. After a baseline saliva sample collection, an oral dose of deuterated water ( $\text{D}_2\text{O}$ , 0.1 g/kg body weight; 99.8 atom% purity, Sercon Ltd UK) was administered to each subject, followed by 10 mL of water after the dose. Two post-dose saliva samples were collected at 2.5 and 3 h. Saliva samples were measured for their deuterium enrichment in duplicate by Fourier Transformed Infrared Spectrophotometry (4500t FTIR, Agilent Technologies, CA, USA) and TBW (kg) was calculated from a gravimetric dilution of the  $\text{D}_2\text{O}$  dose compared with an average of the saliva deuterium enrichment at 2.5 and 3 h after receiving a known dose of  $\text{D}_2\text{O}$ , and after correction for the background. To account for the exchange of labile hydrogen that occurs in humans during the equilibration, a correction factor for non-aqueous dilution of  $\text{D}_2\text{O}$  was applied.<sup>28</sup> For other measurements of the molecular 4C model, dual energy x-ray absorptiometry (DXA; Lunar Prodigy Advanced PA+301969, GE Medical Systems, USA) was performed to measure bone mineral content (ash). The air displacement plethysmography (ADP) method (BODPOD 4529, COSMED, Italy) was used to measure the body volume, and therefore body density, after correcting for lung vol-

ume.<sup>29,30</sup> Using the estimates of TBW, BMC (ash), body volume and weight as measured by the techniques described above, fat (kg) was calculated using the following formula<sup>25</sup>

$$\text{Fat (kg)} = 2.747 \text{ BV} - 0.710 \text{ TBW} + 1.460 \text{ A} - 2.050 \text{ Wt}$$

where BV is body volume, TBW is total body water, A is ash and Wt is body weight

The FFM for the molecular 4C model was calculated as the difference between the body weight and fat derived from the equation mentioned above. The estimates of TBW measurements from the FTIR had an internal precision of 1.26%. The precision of the DXA machine for ash content was 2.1%. The precision of ADP method for body volume estimates was 2.3%.

The FFM for the cellular 4C model was calculated as a sum of BCM, ECF and ECS. The BCM was obtained from WBKC. The extra cellular water (ECW) was calculated as the difference between TBW and intracellular water (ICW). The ICW was assumed to be 70% of the BCM, assuming that hydration of BCM is tightly regulated.<sup>1</sup> The ECF was calculated assuming that ECW consistently comprised 98% of ECF. The ECS was calculated as 14% of TBW.<sup>10</sup> Using the ratio of TBW (derived as sum of ECW and ICW) and FFM, the hydration factor of FFM was calculated. FM was calculated as the difference of body weight and FFM.

The data are summarised as mean  $\pm$  standard deviation (SD). FFM from the cellular 4C model was compared with the FFM from the molecular 4C model. The difference in FFM between the two 4C models was computed and the mean difference is reported with the 95% confidence interval (95% CI).

Estimate of the overall measurement precision of FFM from the two 4C models were calculated using the propagation of error method.<sup>31</sup> The reason for calculating this error was that each primary measurement's precision propagated into the final FFM estimate from the two 4C models. The precision estimates were based on a 66 kg person with 45% TBW and 40% BCM. The error on TBW measurement due to the DD was 1.26%, for TBK it was 1.9%, for body weight it was 0.1 kg and for body fat

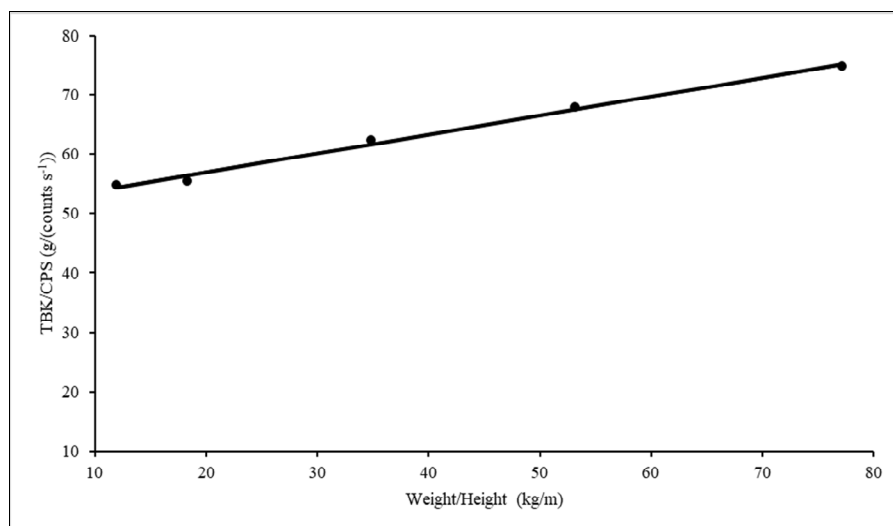
from the molecular 4C model the error was 0.42%. The precision of estimated amount of FFM using the molecular and cellular 4C model, obtained from the propagation of error method for a 66 kg person were  $\pm 0.29$  and  $\pm 0.46$  kg respectively.

## RESULTS

There was a strong linear relationship of the ratio of the TBK to CPS to the ratio of Weight to Height<sup>32-34</sup> (Figure 2), confirming observations that different bodies would result in different detector efficiencies determined by their body geometry.<sup>18-21</sup> The geometry correction factor derived from Monte Carlo simulations ranged from 1.5 to 1.8 as compared to point source measurements. The accuracy of the WBKC was calculated using a phantom that was not part of the calibration series, which contained 414.4 g K. With a measured TBK of 402.9 g, it gave an accuracy of 2.8%. The mean precision of the instrument was 1.9% of TBK. The mean counting error ranged from 0.81 to 2.66% for the phantoms.

The mean age of the study subjects was  $31.8 \pm 7.1$  years, while the mean body weight, height and BMI were  $66.3 \pm 14.0$  kg,  $161.5 \pm 6.8$  cm and  $25.3 \pm 4.8$  kg/m<sup>2</sup>, respectively. Based on their TBK, the subjects had an estimated BCM of  $25.9 \pm 5.9$  kg, which was  $39.6 \pm 7.4\%$  of their body weight. The body composition measurements for the subjects are summarized in Table 1. The TBW as calculated by DD, and BCM as calculated by WBKC, were used to estimate ECW, ECF, ECS, ECW to ICW ratio (E:I), and the hydration of FFM. In Table 2, the measurement of FFM and FM, calculated by the molecular and cellular 4C models, are presented. The mean estimate of FFM by the molecular 4C model was  $40.4 \pm 6.8$  kg, in comparison to  $41.2 \pm 7.3$  kg as measured by the cellular 4C model. The mean difference was  $-0.8 \pm 1.3$  (95% CI: -1.4, -0.2 kg).

Figure 3 shows a sex wise comparison of FFM as obtained from the cellular 4C model and the molecular 4C model. Since the FFM is strongly dominated by BCM and TBW, the relationship between the latter two estimates may provide insight on the relative sizes of intracellular and extracellular compartments within FFM. The estimates of BCM given by the WBKC correlated well with



**Figure 2.** The equation describing the relationship is  $y = 0.316x + 50.681$  ( $r^2=0.99$ ;  $p<0.01$ ;  $n=5$ ).

**Table 1.** Body composition measurements of the subjects

Variable	Combined (n=20)	Men (n=10)	Women (n=10)
TBK (g)	110.1±25.2	124.3±20.1	95.9±22.2*
BCM (kg)	25.9±5.9	29.2±4.7	22.6±5.2*
% BCM	39.6±7.4	44.0±6.8	35.2±5.2*
TBW by DD (kg)	29.2±5.0	32.1±4.9	26.2±3.1*
% TBW	44.7±5.7	48.1±5.1	41.3±4.2*
ICW (kg)	18.1±4.2	20.5±3.3	15.8±3.6*
% ICW	61.9±7.6	64.0±6.4	59.7±8.4
ECW (kg)	11.0±2.5	11.6±3.0	10.4±1.8
% ECW	38.1±7.6	36.0±6.4	40.3±8.4
ECF (kg)	11.2±2.5	11.9±3.1	10.6±1.8
ECS (kg)	4.1±0.7	4.5±0.7	3.7±0.4*
E: I	0.6±0.2	0.6±0.2	0.7±0.3
Hydration of FFM	0.71±0.02	0.70±0.01	0.71±0.02

TBK: Total Body Potassium; BCM: Body Cell Mass; % BCM: BCM as percentage of body weight; TBW: Total Body Water; DD: Deuterium dilution method; % TBW: TBW as percentage of body weight; ICW: Intra Cellular Water; % ICW: ICW as percentage of total body water; ECW: Extra Cellular Water; % ECW: ECW as percentage of total body water; ECF: Extra Cellular Fluid; ECS: Extra Cellular Solids; E: I- Ratio of Extra Cellular Water to Intra Cellular Water; FFM: Fat Free Mass.

Values are mean±SD.

\*Significance at  $p < 0.01$ ; independent t-test.

the estimates of TBW (Figure 4).

## DISCUSSION

In this study, the design of a WBKC to measure the TBK and hence the BCM is described. The measurement of TBK makes a good and accurate metric for the measurement of BCM, because of the tight regulation of the intracellular concentration of potassium.<sup>1</sup> Total body potassium can be a valuable and accurate way of estimating the ICW and other aspects of body composition. For instance, muscle cells are potassium rich and skeletal muscle is a major component of FFM. Total body potassium has formed the basis of methods to estimate skeletal muscle mass in adults and in children.<sup>35,36</sup> The WBKC measures potassium's natural radioactivity, by measuring the small amount of radiation that emits naturally from body, and by shielding this from the background radiation coming from surrounding bodies. The estimates of FFM from the WBKC were compared to the estimates of FFM from a molecular 4C model.

Body geometry is a major variable in counting gamma rays emanating from the body. When a point source of gamma rays is measured, one would expect the ratio of the total potassium in the point source to the CPS to be a

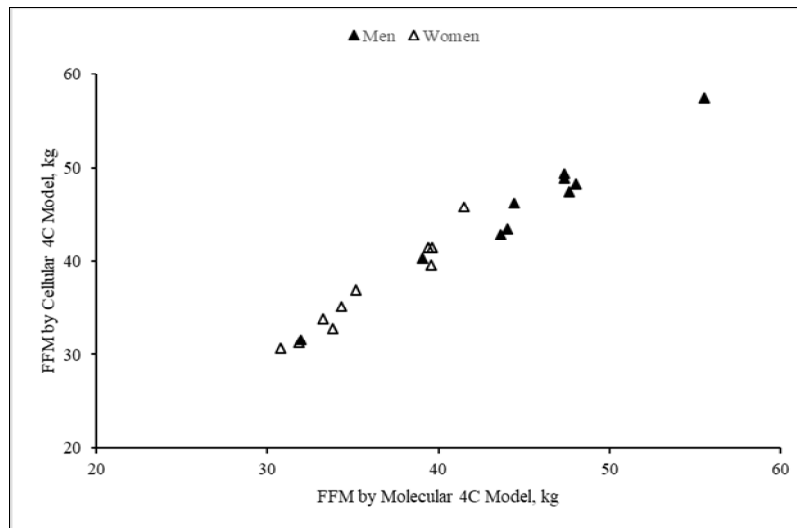
constant value. However, owing to the distribution of the potassium over a large volume with an irregular geometry (like the human body), this ratio should vary. The point of origin of radiation is not fixed, and the self-absorption of gamma rays by overlying body tissue would influence the amount of radiation that is detected from the body. As the scanning of the body was performed directionally along the subject's axis the efficiency of the detector in measuring gamma rays should decrease in a linear fashion against the weight to height ratio as the potassium is distributed over a greater volume per unit height as observed by Bhati et al<sup>18</sup> in their study on measurements of high energy photon emitters for radiation workers of different physiques. The TBK is proportionate to the expected number of gamma ray events, and consequently the number of counts expected to hit the detector in the absence of large and irregular geometry. The problem of irregular geometry can be accounted for using Monte Carlo simulations which can be based on minimal anthropometric measurements.<sup>20</sup> The Monte Carlo simulations account for the different parts of the body, with different dimensions, and can be modified for body shapes that do not conform to a standard set of proportions.<sup>21</sup>

The mean TBK values from the present study were 124.3±20.1 g and 95.9±22.2 g for men and women respectively. The observed BCM values were 44.0±6.8% and 35.2±5.2% of the body weight for men and women respectively. These values of TBK<sup>11</sup> and BCM were similar to earlier observations in other populations.<sup>37,38</sup> The study also produced data on the hydration of FFM, the BCM to FFM ratio and the E:I ratio in Indian adults. The BCM to FFM ratio and the E:I ratio can vary from individual to individual. As observed by Wang et al<sup>39</sup> the expected range for these ratios are 0.4 to 0.7 (for BCM:FFM) and 0.5 to 1.4 (for E:I). In the present study the range of the BCM to FFM ratio was 0.5 to 0.7 and the calculated E:I ratio varied from 0.32 to 1.22. While the BCM to FFM ratio was within the expected range, the E:I ratio was much lower than has been observed elsewhere, and is likely a result of the higher body fat percentage observed

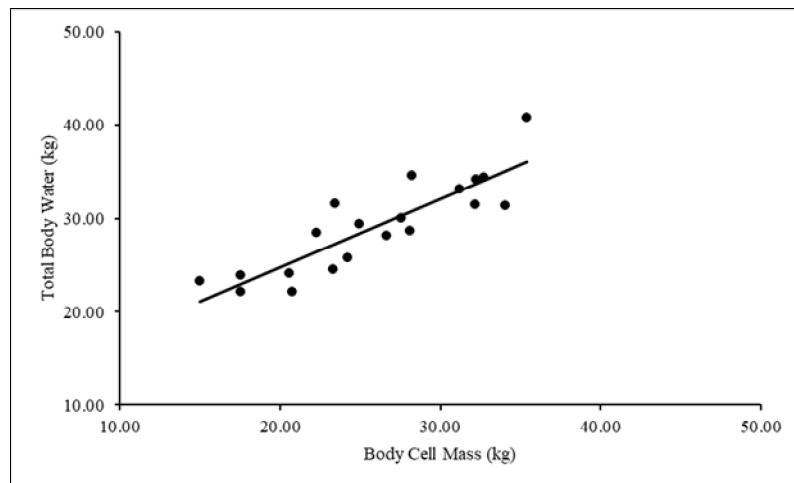
**Table 2.** Fat and Fat-Free Mass by the two different four compartment (4C) models (n=20)

Variable	Mean±SD
Cellular 4C Model	
Fat (kg)	25.1±9.0
FFM (kg)	41.2±7.3
% Fat	36.8±8.4
% FFM	63.2±8.4
Molecular 4C Model	
Fat (kg)	25.9±9.8
FFM (kg)	40.4±6.8
% Fat	37.9±9.1
% FFM	62.1±9.1

FFM: Fat Free Mass; % Fat: Fat as percentage of body weight; % FFM: Fat Free Mass as percentage of body weight.



**Figure 3.** The equation describing the relationship for men is  $y = 1.081x - 2.945$  ( $r^2=0.98$ ;  $p<0.01$ ;  $n=10$ ); The equation describing the relationship for women is  $y = 1.289x - 9.404$  ( $r^2=0.95$ ;  $p<0.01$ ;  $n=10$ ).



**Figure 4.** As FFM is strongly dominated by the BCM and the TBW, the relationship between the two estimates provide insight on the relative sizes of these compartments within FFM. The figure shows that the estimates of BCM given by the whole-body potassium counter have a linear relationship with the estimates of TBW measured by deuterium dilution. The equation describing the relationship is  $y = 0.732x + 10.198$  ( $r^2=0.756$ ;  $p<0.01$ ;  $n=20$ ).

in Indians.<sup>40-42</sup> Similarly, the estimates of TBW in Indian adults, as a percentage of total body weight remained fixed in a very narrow range for both men and women at  $48.1\pm 5.1\%$  and  $41.3\pm 4.2\%$  respectively, consistent with the literature.<sup>43</sup> The hydration of FFM was  $0.71 \pm 0.02$ , (range 0.68-0.74). Except for one outlier with the value of 0.68, this was also consistent with the literature, which states that the value of 0.73 is quite constant across all human beings.<sup>10</sup>

FFM hydration is tightly regulated: ECW and ECF can be derived from TBW and ICW, the latter derived directly from BCM; ECS can be estimated from DXA or predicted, as it is the smallest component of FFM. It is thus implied that there is a relationship between TBW and BCM. Indeed, there was a reasonable linear relationship between TBW and BCM (Figure 4), which formed the basis of the use of TBK to estimate FFM in the early years of its use.<sup>44,45</sup>

The comparison in the estimates of FFM as calculated by the molecular 4C model agreed with the cellular 4C model based estimate, to within 5%. This is consistent

with an earlier study which also found that estimates of BCM from a DXA and TBK based 4C model correlated well with estimates of BCM derived from a DXA and bromide dilution based cellular 4C model.<sup>46</sup>

The strength of this study is the innovative design of a shadow shielded WBKC, which allows for detection of emanating gamma rays from the body without enclosing the subject in a shielded room, allowing for greater subject comfort. In addition, Monte Carlo modelling was used to allow for variable body geometries. The accuracy and precision of the WBKC were also acceptable. One limitation of the design of the WBKC was that due to the high cost of the NaI(Tl) detectors, only four were used. This increases the counting time, and for smaller body sizes with less potassium, a longer counting time will be required. Another limitation is related to the small sample size of the present study, due to the intensive nature of the 4C model measurements.

In conclusion, the cellular 4C model that used WBKC estimates of BCM to estimate the FFM strongly correlated with the FFM measured by the molecular 4C model.

The cellular 4C model is advantageous as it allows for splitting the FFM compartment into three further separate compartments, BCM, ECF and ECS.<sup>1</sup> The measurement of BCM is free of assumptions of body hydration status and in subjects with uncertain hydration status such as pregnant woman, children in early stages of life and people with acute malnutrition its use may be more reliable. The newly built WBKC that was designed at St. John's Research Institute, Bangalore, India can be used in clinical and research settings in future to study the body composition of children undergoing nutritional rehabilitation, where repeated measures of BCM can help in the planning of nutritional interventions. Additionally, this method would be useful in children with cancer undergoing treatment, children with chronic kidney diseases and in assessing growth of children, as each measurement is almost costless, and can be repeated any number of times. Since it is not affected by movement, it is comfortable for the subject. Important considerations are the initial cost and the great weight required to adequately shield background radiation, and these considerations limit its use in the field. However, the subsequent low running cost and high accuracy give good measurements of BCM and protein accretion.

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#### AUTHOR DISCLOSURES

All authors report no conflict of interest.

#### REFERENCES

- Wang Z, Shen W, Kotler DP, Heshka S, Wielopolski L, Aloia JF, Nelson ME, Pierson Jr RN, Heymsfield SB. Total body protein: a new cellular level mass and distribution prediction model. *Am J Clin Nutr.* 2003;78:979-84.
- Moore FD. The body cell mass and its supporting environment: body composition in health and disease. Philadelphia: WB Saunders Co.; 1963.
- Wang Z, St-Onge MP, Lecumberri B, Pi-Sunyer FX, Heshka S, Wang J et al. Body cell mass: model development and validation at the cellular level of body composition. *Am J Physiol Endocrinol Metab.* 2004;286:E123-8.
- Garrow JS. Total body-potassium in kwashiorkor and marasmus. *Lancet.* 1965;2:455-8.
- Garrow JS, Fletcher K, Halliday D. Body composition in severe infantile malnutrition. *J Clin Invest.* 1965;44:417-25.
- Nichols BL, Alleyne GA, Barnes DJ, Hazlewood CF. Relationship between muscle potassium and total body potassium in infants with malnutrition. *J Pediatr.* 1969;74:49-57.
- Murphy AJ, Ellis KJ, Kurpad AV, Preston T, Slater C. Total body potassium revisited. *Eur J Clin Nutr.* 2014;68:153-4. doi: 10.1038/ejcn.2013.262.
- Murphy AJ, Davies PS. Body cell mass index in children: interpretation of total body potassium results. *Br J Nutr.* 2008;100:666-8. doi: 10.1017/S0007114507901269.
- Rasmussen KM, Yaktine AL. Composition and components of gestational weight gain: physiology and metabolism. In: Weight gain during pregnancy reexamining the guidelines. Washington (DC): National Academies Press (US); 2009. pp. 71-110.
- Wang Z, Deurenberg P, Wang W, Pietrobelli A, Baumgartner RN, Heymsfield SB. Hydration of fat-free body mass: new physiological modeling approach. *Am J Physiol Endocrinol Metab.* 1999;276:E995-1003.
- Kehayias JJ, Fiatarone MA, Zhuang H, Roubenoff R. Total body potassium and body fat: relevance to aging. *Am J Clin Nutr.* 1997;66:904-10.
- Ribeiro SML, Kehayias JJ. Sarcopenia and the analysis of body composition. *Adv Nutr.* 2014;5:260-7.
- Roubenoff R, Hughes VA, Dallal GE, Nelson ME, Morganti C, Kehayias JJ, Singh MA, Roberts S. The effect of gender and body composition method on the apparent decline in lean mass-adjusted resting metabolic rate with age. *J Gerontol A Biol Sci Med Sci.* 2000;55:M757-60.
- Boddy KE, Elliott AL, Robertson I, Mahaffy ME, Holloway I. A high sensitivity dual-detector shadow-shield whole-body counter with an 'invariant' response for total body in vivo neutron activation analysis. *Phys Med Biol.* 1975;20:296-04.
- Boddy K, King PC, Henderson JT, Shearman DJ, Finlayson ND, Simpson JD, Tothill P. A direct intercomparison of two whole-body monitors in the measurement of the absorption of an oral dose of vitamin B12 labelled with <sup>57</sup>Co and <sup>58</sup>Co. *Phys Med Biol.* 1972;17:374-80.
- Ellis KJ, Shypailo RJ, Abrams SA, Wong WW. The reference child and adolescent models of body composition: a contemporary comparison. *Ann N Y Acad Sci.* 2000;904:374-82.
- ROOT Data Analysis Framework. [cited 2017/10/15]; Available from: <https://root.cern.ch/>.
- Bhati S, Patni HK, Ghare VP, Singh IS, Nadar MY. Monte Carlo calculations for efficiency calibration of a whole-body monitor using BOMAB phantoms of different sizes. *Radiat Prot Dosimetry.* 2012;148:414-9. doi: 10.1093/rpd/ncr203.
- Shypailo RJ, Ellis KJ. Monte Carlo efficiency calibration of a neutron generator-based total-body irradiator. *J Radioanal Nucl Chem.* 2009;282:247-53.
- Shypailo RJ, Ellis KJ. Whole body counter calibration using Monte Carlo modeling with an array of phantom sizes based on national anthropometric reference data. *Phys. Med. Biol.* 2011;56:2979-97.
- Kramer GH, Burns LC, Guerriere S. Monte Carlo simulation of a scanning detector whole body counter and the effect of BOMAB phantom size on the calibration. *Health Phys.* 2002;83:526-33.
- Briesmeister JF. MCNP: a general Monte Carlo code for neutron and photon transport. Version 3A. Revision 2. Springfield, VA: National Technical Information Service, US Department of Commerce, U.S. Government Printing Office; 1986.
- Ellis KJ, Shypailo RJ. Total body potassium in the infant. *J Radioanal Nucl Chem.* 1992;161:61-9.
- King JC, Calloway DH, Margen S. Nitrogen retention, total body 40K and weight gain in teenage pregnant girls. *J Nutr.* 1973;103:772-85.
- Fuller NJ, Jebb SA, Laskey MA, Coward WA, Elia M. Four-component model for the assessment of body composition in humans: comparison with alternative methods, and evaluation of the density and hydration of fat-free mass. *Clin Sci (Lond).* 1992;82:687-93.
- Gordon CC, Chumlea WC, Roche AF. Stature, Recumbent Length, and Weight. In: Lohman TG, Roche AF, Martorell R, editors. *Anthropometric Standardization Reference*

- Manual. Champaign, Illinois: Human Kinetic Books; 1988. pp. 3-8.
27. Callaway CW, Chumlea WC, Bouchard C, Himes JH, Lohman TG, Martin AD et al. Circumferences. In: Lohman TG, Roche AF, Martorell R, editors. Anthropometric standardization reference manual. Champaign, Illinois: Human Kinetic Books; 1988. pp. 39-54.
  28. International Atomic Energy Agency report. Using the deuterium dilution technique with analysis of saliva samples by Fourier Transform Infrared Spectroscopy. IAEA Hum Heal Ser. no. 12. Vienna: International Atomic Energy Agency; 2009.
  29. Fields DA, Goran MI, McCrory MA. Body-composition assessment via air-displacement plethysmography in adults and children: a review. *Am J Clin Nutr.* 2002;75:453-67.
  30. Crapo RO, Morris AH, Clayton PD, Nixon CR. Lung volumes in healthy nonsmoking adults. *Bull Eur Physiopathol Respir.* 1982;18:419-25.
  31. Uncertainties and Error Propagation. [cited 2018/06/26]; Available from: [http://burro.case.edu/Academics/Astr306/Stats/Appendix\\_V\\_Error\\_Prop.pdf](http://burro.case.edu/Academics/Astr306/Stats/Appendix_V_Error_Prop.pdf).
  32. Cohn SH, Dombrowski CS. Absolute measurement of whole-body potassium by gamma-ray spectrometry. *J Nucl Med.* 1970;11:239-46.
  33. Naversten Y, Lenger V. Total body potassium determination using a whole-body counter. *Acta Radiol Oncol.* 1983; 22:167-75.
  34. Cohn SH, Dombrowski CS, Pate HR, Robertson JS. A whole-body counter with an invariant response to radionuclide distribution and body size. *Phys Med Biol.* 1969;14:645-58.
  35. Wang Z, Zhu S, Wang J, Pierson Jr RN, Heymsfield SB. Whole-body skeletal muscle mass: development and validation of total-body potassium prediction models. *Am J Clin Nutr.* 2003;77:76-82.
  36. Wang Z, Heshka S, Pietrobelli A, Chen Z, Silva AM, Sardinha LB, Wang J, Gallagher D, Heymsfield SB. A new total body potassium method to estimate total body skeletal muscle mass in children. *J Nutr.* 2007;137:1988-91.
  37. Kyle UG, Genton L, Hans D, Karsegard L, Slosman DO, Pichard C. Age-related differences in fat-free mass, skeletal muscle, body cell mass and fat mass between 18 and 94 years. *Eur J Clin Nutr.* 2001;55:663-72.
  38. De Lorenzo A, Andreoli A, Battisti P, Candeloro N, Volpe SL, Di Daniele N. Assessment of total body potassium in healthy Italian men. *Ann Hum Biol.* 2004;31:381-8.
  39. Wang Z, Heshka S, Wang J, Gallagher D, Deurenberg P, Chen Z, Heymsfield SB. Metabolically active portion of fat-free mass: a cellular body composition level modeling analysis. *Am J Physiol Endocrinol Metab.* 2007;292:E49-53.
  40. Misra A, Vikram NK, Arya S, Pandey RM, Dhingra V, Chatterjee A et al. High prevalence of insulin resistance in postpubertal Asian Indian children is associated with adverse truncal body fat patterning, abdominal adiposity and excess body fat. *Int J Obes Relat Metab Disord.* 2004;28: 1217-26.
  41. Raji A, Seely EW, Arky RA, Simonson DC. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. *J Clin Endocrinol Metab.* 2001;86:5366-71.
  42. Deurenberg-Yap M, Schmidt G, van Staveren WA, Deurenberg P. The paradox of low body mass index and high body fat percentage among Chinese, Malays and Indians in Singapore. *Int J Obes Relat Metab Disord.* 2000; 24:1011-7.
  43. Kuriyan R, Thomas T, Ashok S, Jayakumar J, Kurpad AV. A 4-compartment model based validation of air displacement plethysmography, dual energy X-ray absorptiometry, skinfold technique & bio-electrical impedance for measuring body fat in Indian adults. *Indian J Med Res.* 2014;139:700-7.
  44. Forbes GB, Gallup J, Hursh JB. Estimation of total body fat from potassium-40 content. *Science.* 1961;133:101-2.
  45. Fomon SJ, Haschke F, Ziegler EE, Nelson SE. Body composition of reference children from birth to age 10 years. *Am J Clin Nutr.* 1982;35:1169-75. doi: 10.1093/ajcn/35.5. 1169.
  46. Shen W, St-Onge MP, Pietrobelli A, Wang J, Wang Z, Heshka S, Heymsfield SB. Four-compartment cellular level body composition model: comparison of two approaches. *Obes Res.* 2005;13:58-65.