RESEARCH PAPER

Reference Centile Curves for Body Fat Percentage, Fat-free Mass, Muscle Mass and Bone Mass Measured by Bioelectrical Impedance in Asian Indian Children and Adolescents

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Objectives: To create gender-specific percentile curves for percent body fat (%BF) by Bio electrical Impedance Analysis (BIA) for screening adiposity and risk of hypertension in Indian children and generate reference curves for percent fat-free mass (%FFM), muscle mass (%LM) and bone mineral content (BMC) by using bioelectrical impedance.

Design: Secondary analysis of data from previous multicenter cross-sectional studies.

Setting: Private schools from five regions of India.

Participants: A random sample of 3850 healthy school children (2067 boys) (5-17 yr) from private schools in five major Indian cities.

Methods: Anthropometry, blood pressure (BP) and body composition were measured by bioelectrical impedance. Reference curves were generated by the LMS method.

Main outcome measures: %BF, %FFM, %LM, BMC and BP

Results: Median %BF increased by 6% from 5 to 13 years of age and declined (around 2%) up to 17 years in boys. In girls, %BF increased by 8% from 5 to 14 years and thereafter declined by 3%. Based upon the risk of hypertension, the new cut-offs of 75th and 85th percentile of %BF were proposed for detecting over fatness and excess fatness in children. Median %FFM was 90% at 5 yrs and decreased till 12 years, and then showed a slight increase to 84% at 17 yrs in boys. In girls, it was 86% at 5 yrs and decreased till 15 yrs, and plateaued at 71.8% at 17 yrs.

Conclusions: Reference curves for percent body fat for Indian children would be useful to screen children for health risk in clinical set up.

Key words: *Body composition, Cut-offs, Metabolic syndrome, Reference curves.*

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Obesity has become a major health concern in childhood as it is a marker of cardio-metabolic risk in later life. Recent studies have also demonstrated that not only excess fat mass [1] but lean mass and muscle fitness [2] are also associated with health risk. Assessment of body composition is an appropriate method to judge adiposity and lean mass, and can help in early detection of cardio metabolic risk.

Bioelectrical impedance (BIA) is a valuable alternative to dual energy *X*-ray absorptiometry (DXA) in assessing body composition as it is portable and convenient to use in clinical setting and field surveys. However, a local reference database is essential to assess the body composition status of children [3]. For the DXA, we have generated such a reference database for Indian children and adolescents [4-6]. However, lack of Indian reference values for BIA reduces its utility to evaluate nutritional status, and associated health risk in Indian children.

Thus, the objectives of the present study were: (*i*) to create gender-specific percentile curves for percent body fat (%BF) by BIA for screening adiposity in Indian children, (*ii*) to explore the possible cut-offs of reference curves for risk of hypertension, and (*iii*) to generate reference curves for percent fat free mass (%FFM), muscle mass (%LM) and bone mineral content (BMC) by BIA.

METHODS

Data of 3832 schoolchildren (2054 boys) aged 5-17 years collected in previous cross-sectional studies were

analyzed to generate age- and gender-specific reference percentile curves for total body fat percentage by BIA. It was a multicenter study conducted in 5 major cities (Delhi, Chennai, Pune, Kolkata and Raipur) from 5 states of India [7] along with one center in Gujarat (Ahmadabad) during 2011 to 2014. Detailed methodology has been previously described [7]. From a list of schools catering to children of socio-economically well-off families from each city, six schools were randomly selected and approached for permission to carry out measurements. All 2- to 17-year-old children from participating schools whose parents consented to measurements were included [7]. The studies were approved by the Ethics Committee of the Jehangir Clinical Development Pvt. Ltd., Pune, which is a recognised Institute by Department of Scientific and Industrial research (DSIR), Government of India.

Data were collected by the same team at each site; equipments were calibrated daily. The mean inter- and intra-observer coefficients of variation were <1% for weight, height and body composition measurements.

Height-for-age (HAZ), weight-for-age (WAZ) and BMI-for-age (BAZ) *Z*-scores were computed as deviations from the median [8]. Adult equivalent BMI *Z* scores were also computed using adult equivalent values for Asians [9] as normal weight (BMI at age 18 d-23 kg/m^2), overweight (BMI 23 to 28 kg/m^2) and obese (BMI >28 kg/m^2).

Body composition was assessed using Bioelectrical Impedance Analyzer (BIA), (Tanita Model BC-420MA) after a minimum of 3 hours of fasting, and voiding before measurements (10 am onwards) [10]. This analyzer measures body composition using a constant current source with a high frequency current (50kHz, 90µA). The 8 electrodes are positioned so that electric current is supplied from the electrodes on the tips of the toes of both feet, and voltage is measured on the heel of both feet. BIA measures body composition as fat%, fat mass, fat free mass, total body water, bone-free lean tissue mass (LTM), bone mineral amount included in the entire bone (bone mass) by measuring bioelectrical impedance in the body in standing position of subject. Measurements were tested for test-retest reliability on pilot sample of ten subjects separately by measuring them on BIA at two different time points. Reliability coefficient was significant for the body fat percent, fat mass, fat-free mass and muscle mass (intra class correlation coefficient = 0.96, P=0.0001). Clinical examinations were carried out by pediatricians to assess health status of children to ensure that only apparently healthy children were included in the study.

Statistical analysis: All statistical analyses were performed using SPSS software (version 16.0. 2007). All results were expressed as mean (SD) for comparability with other studies. Smoothed gender-specific reference plots showing 2nd, 9th, 25th, 50th, 75th, 85th and 95th percentiles of %BF and FFM were derived using LMS method (LMS chart-maker Pro version 2.4, 2008; by Pan and Cole), which constructs reference percentiles adjusted for skewness [11]. Each variable of interest was summarized by three smooth curves plotted against age, representing the median (M), coefficient of variation (S) and skewness (L) of the measurement distribution [12]. Models were checked for goodness of fit using the detrended Q-Q plot, Q Tests and worm plots [13]. The LMS method was found to be appropriate to use for this data as the measure of skewness of the data was 1.1 with a standard error of 0.03. The possible cut-offs of derived %BF percentiles were tested for their efficacy against the BP values by classifying the children into three groups: normal BP (SBP/DBP <90th percentile), pre-hypertension (SBP/DBP 90th-95th percentile), and hypertension (SBP/DBP >95th percentile) [14]. Pearson's correlation coefficient was used to assess relationship of BP and various body composition parameters, *i.e.* BMI, FMI, LMI, FFMI and %BF.

RESULTS

***Table* I** illustrates anthropometric and body composition parameters for both the genders from 5 to 17 years of age. Mean (SD) height-for-age Z-scores in boys [girls] were 0.11 (1.0) [0.10 (1.0)]; weight for age Z-scores were 0.25 (1.0) [0.12 (1.1)] and BMI for age Z-scores were 0.21 (0.98) [0.09 (1.1)]. Majority of the children (95.5%) had normal Z-scores for height, weight and BMI with reference to contemporary Indian growth references [8].

When compared with adult-equivalent cut-offs of BMI for Asians corresponding to 23 and 28 kg/m^2 [9], 65.2% boys (86.9% girls) had normal BMI, 23.3% boys (11.1% girls) had BMI >23 kg/m^2 adult cut-off and 11.5% boys (2% girls) had >28 kg/m^2 adult cut-off.

Mean body fat percent in boys and girls increased gradually till 14 years of age and then showed a decline up to 17 years; though the decline was small and mean fat percent was higher in girls than boys (P<0.05). Mean muscle mass and fat-free mass also increased with age in both boys and girls though boys had a significantly higher muscle mass than girls after 11 years of age (P<0.05). Bone mass of boys and girls increased with age, and after 13 years of age, bone mass of girls showed a plateau while boys showed increase till 17 years.

BMI showed a significant correlation with %BF (r=0.87, P <0.01). Considering the adult equivalent Asian

TABLE I ANTHROPOMETRY AND BODY COMPOSITION MEASUREMENTS BY AGE AND R IN INDIAN CHILDREN AND ADOLESCENTS

Age (yr)	*n*	*Height (cm)*	*Weight (kg)*	*Fat Mass (kg)*	*Fat-free mass (kg)*	*Muscle mass (kg)*	*Bone mineral content (kg)*	*Fat Percent*
Boys								
5	36	114.6 (5.2)	20.5 (5.1)	2.9 (3.2)	17.5 (2.2)	16.8 (2.0)	0.7 (0.2)	12.2 (8.1)
6	145	118.6 (5.7)	22.3 (4.7)	3.6 (3.2)	18.9 (2.2)	18.1 (2.1)	0.8 (0.1)	14.3 (8.7)
7	120	124.5 (5.5)	25.2 (5.4)	4.4 (4.1)	21.0 (2.3)	20.1 (2.2)	0.9 (0.1)	15.5 (10.5)
8	144	129.0 (6.4)	27.4 (5.4)	4.6 (4.7)	22.8 (2.6)	21.7 (2.5)	1.0 (0.2)	14.7 (10.4)
9	177	135.4 (6.7)	32.3 (7.6)	6.7 (5.8)	25.5 (2.9)	24.3 (2.7)	1.2 (0.2)	18.4 (11.3)
10	176	140.7 (7.0)	36.4 (8.4)	8.4 (6.7)	28.0 (3.4)	26.6 (3.2)	1.4 (0.2)	20.5 (12.3)
11	232	145.8 (8.2)	40.2 (11.7)	9.2 (9.4)	30.8 (4.1)	29.2 (3.9)	1.5 (0.2)	19.8 (12.7)
12	268	151.4 (8.0)	44.1 (11.6)	10.2 (9.3)	34.3 (4.9)	32.6 (4.6)	1.7 (0.3)	19.9 (12.8)
13	234	156.9 (8.5)	48.1 (11.2)	10.4 (8.9)	37.8 (5.5)	35.9 (5.2)	2.0 (0.3)	19.9 (11.9)
14	175	164.4 (7.5)	56.2 (13.0)	13.2 (10.8)	43.3 (5.3)	41.0 (5.0)	2.3 (0.3)	20.9 (12.3)
15	146	167.7 (6.8)	58.4 (13.4)	11.5 (9.4)	47.2 (6.1)	44.7 (5.7)	2.5 (0.3)	17.5 (10.5)
16	131	169.2 (6.7)	60.1 (12.2)	10.2 (6.9)	50.5 (7.1)	47.8 (6.8)	2.6 (0.3)	15.7 (7.5)
17	70	170.3 (6.4)	63.8 (12.8)	11.6 (7.5)	52.5 (6.8)	49.8 (6.7)	2.7 (0.3)	16.9 (7.6)
Girls								
5	40	114.1 (5.6)	20.3 (4.0)	3.3 (2.2)	16.8 (2.2)	16.0 (2.1)	0.7 (0.1)	15.4 (6.7)
6	126	117.6 (5.8)	21.7 (5.3)	3.7 (2.7)	17.9 (2.8)	17.09 (2.6)	0.8 (0.1)	15.5 (7.3)
7	95	123.8 (5.9)	24.5 (5.6)	4.6 (3.4)	19.9 (2.6)	19.0 (2.4)	0.9 (0.2)	17.2 (8.2)
8	135	129.6 (6.6)	28.5 (8.2)	6.2 (4.9)	22.4 (3.8)	21.3 (3.5)	1.1 (0.3)	19.3 (9.1)
9	122	133.8 (6.7)	30.7 (8.1)	6.6 (5)	23.9 (3.9)	22.7 (3.6)	1.23 (0.3)	19.3 (9.4)
10	181	139.6 (8.0)	33.9 (9.0)	7.7 (5.3)	26.3 (4.6)	24.9 (4.2)	1.4 (0.3)	20.4 (9.2)
11	205	146.6 (7.7)	38.5 (9.6)	8.9 (5.8)	29.6 (4.9)	27.9 (4.5)	1.7 (0.4)	21.1 (9.0)
12	244	150.1(7.0)	41.8 (9.7)	10.4 (6.3)	31.5 (4.4)	29.7 (4.1)	1.8 (0.3)	23.2 (8.9)
13	215	153.8 (6.7)	46.7 (10.8)	13.1 (7.3)	33.7 (4.6)	31.7 (4.2)	2 (0.3)	26.0 (9.2)
14	147	155.4 (6.0)	50.7 (10.9)	16.1 (8.5)	34.6 (4.1)	32.6 (3.8)	2.0 (0.3)	30.1 (8.1)
15	117	156.6 (5.6)	52.3 (9.1)	16.1 (6.7)	36.2 (3.7)	34.1 (3.4)	2.1 (0.3)	29.8 (6.8)
16	103	157.0 (6.1)	51.1 (8.3)	14.6 (5.1)	36.5 (4.1)	34.5 (3.8)	2.0 (0.4)	27.8 (5.7)
17	48	158.4 (7.4)	55.1 (11.6)	16.5 (7.6)	38.7 (6.1)	36.5 (5.7)	2.2 (0.4)	28.9 (6.6)

Values are Mean (SD).

BMI cut-offs of obesity and adiposity, 90.8% of boys and 91.8% of girls with high adiposity were correctly identified by BMI (sensitivity or true-positive rate), and 91.7% of boys and 82.2% of girls without high adiposity were also correctly classified (specificity or true-negative rate). Among those adolescents considered as overweight or obese by BMI cut-offs, only 69.3% of girls and 85.0% of boys had excess adiposity (the predictive value).

With advancing age, SBP and DBP increased slowly; the mean blood pressure was within reference range [14] in 87% boys and 90% girls. BMI showed a significant correlation with SBP (r= 0.67, $P<0.01$) and DBP (r= 0.54, $P<0.01$). A positive significant correlation was observed between percent body fat with SBP (r=0.53, $P<0.01$) and DBP (r=0.44, $P<0.01$). According to hypertension cut-offs, 41.1% overweight or obese boys and 30.6% girls showed high blood pressure, whereas with excess fatness 45% boys and 36.4% girls were having hypertension. Around 4% to 6% children and adolescents were misclassified as hypertensive with BMI cut-offs than the BIA cut-offs as also with the proposed body fatness cut-offs.

To examine the relative fatness with height, indices of fat mass, muscle mass and body mass were computed (***Table* II**). In boys with increasing age, average increase in FMI and BMI was around 3% whereas increase in

TABLE II FAT MASS INDEX, MUSCLE MASS INDEX AND BODY MASS INDEX BY AGE IN BOYS AND GIRLS

Age (yr)	*Boys*				*Girls*			
	FMI	*MMI*	*FFMI*	*BMI*	*FMI*	*MMI*	*FFMI*	*BMI*
5	2.06 (2.0)	12.56 (0.7)	13.26 (0.7)	15.41 (2.6)	2.50 (1.5)	12.26 (0.8)	12.82 (0.9)	15.52 (2.2)
6	2.45 (2.0)	12.79 (0.5)	13.36 (0.6)	15.76 (2.3)	2.56 (1.7)	12.27 (0.9)	12.87 (1.0)	15.50 (2.6)
7	2.81 (1.8)	12.80 (0.6)	13.51 (0.5)	16.15 (2.8)	2.90 (2.0)	12.30 (0.8)	12.94 (0.9)	15.84 (2.8)
8	2.67 (2.5)	12.91(0.7)	13.65 (0.7)	16.3 4(2.8)	3.55 (2.5)	12.62 (1.1)	13.28 (1.2)	16.73 (3.5)
9	3.56 (2.9)	13.22 (0.6)	13.87 (0.5)	17.49 (3.0)	3.56 (2.5)	12.59 (1.0)	13.27 (1.1)	16.89 (3.4)
10	4.13 (3.2)	13.41 (0.7)	14.08 (0.7)	18.21 (3.2)	3.79 (2.5)	12.87 (1.0)	13.61 (1.1)	17.19 (3.3)
11	4.27 (3.8)	13.74 (0.8)	14.54 (0.8)	18.71 (3.9)	4.06 (2.6)	13.04 (1.2)	13.72 (1.3)	17.74 (3.6)
12	4.21 (3.9)	14.07 (0.9)	14.84 (0.9)	19.04 (4.1)	4.57 (2.8)	13.40 (1.0)	14.22 (1.1)	18.42 (3.5)
13	4.17 (3.7)	14.45 (0.9)	15.37 (0.9)	19.48 (3.8)	5.42 (2.9)	13.54 (1.1)	14.38 (1.2)	19.62 (3.7)
14	4.74 (3.9)	15.05 (1.0)	16.0 (1.0)	20.58 (4.3)	6.67 (3.5)	13.47 (1.1)	14.41(1.3)	21.02 (4.1)
15	4.11 (3.4)	15.86 (1.4)	16.75 (1.5)	20.72 (4.6)	6.57 (2.6)	13.88 (1.0)	14.72 (1.0)	21.31 (3.3)
16	3.58 (2.4)	16.65 (2.0)	17.58 (2.0)	20.95 (3.9)	5.94 (2.1)	13.98 (1.1)	14.81 (1.2)	20.74 (3.1)
17	3.85 (2.5)	16.95 (1.7)	18.0 (1.8)	22.01 (4.1)	6.57 (2.6)	14.50 (1.6)	15.37 (1.7)	21.91 (4.0)

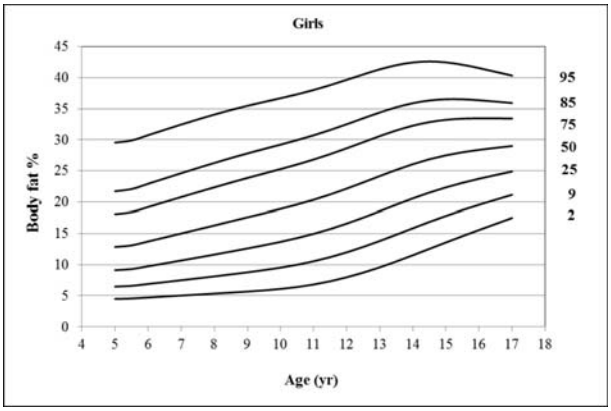
**Values are Mean ± SD; FMI = Fat Mass Index (Fat mass (kg)/ Height^2 (m)); MMI = Muscle Mass Index (Muscle Mass (kg)/Height^2 (m); FFMI = Fat Free Mass Index (Fat Free Mass (kg)/ Height^2 (m)); BMI = Body Mass Index (weight (kg)/Height^2 (m)).*

Muscle mass index (MMI) was 7%. In girls, BMI showed a similar rate of increase of 3% with age but average increase in FMI was 9% and in MMI 1.4%. The correlations of SBP with FMI, MMI, and BMI (r=0.57-0.69, *P*<0.01) even after adjusting for age in both boys and girls.

The reference percentile curves generated for %BF by BIA are illustrated in ***Fig.* 1a** (boys) and ***Fig*. 1b** (girls). A significant gender difference was seen in the shape of fat percentile curves. Lower percentiles of boys were flatter than girls and around 10 years of age, body fat% showed higher increase in girls than boys. Median fat% percentile of boys was also lower than the median percentile of girls. Median fat% of boys declined after 13 years of age while for girls there was a steady increase with age. Median fat percent in boys showed an average increase of 6% from 5 to 13 years of age, and then a decline of around 2% up to 17 years of age. However, median fat% in girls increased by 8% from 5 to 14 yrs, and by 3% thereafter up to 17 years of age.

To explore the association of over fatness and obesity with the risk of hypertension, the percentages of children in the hypertensive or pre-hypertensive range or with BP <90th percentile wereclassified in successive fat percentile categories. It was observed that %BF percentile groups; with <50th, 50th-75th, 75th-85th, and >85th reference percentile, exhibited a significant difference in prevalence of hypertension in various percentile groups (*P*<0.01). Up to the 75th fat percentile,

(a)



(b)

FIG.1 *Smoothed reference percentile curves for percent body fat for Indian boys (a); and girls (b).*

the percent children with hypertension was relatively small which increased in the later %BF percentile groups (P<0.05). Percentage of children with pre-hypertension also increased from the 75th percentile (P<0.05). Thus, the75th and 85th reference percentiles may reveal the risk of hypertension (***Fig.* 2**).

Reference percentiles for %FFM by age and gender are provided in ***Fig.* 3a** and **3b**, respectively. Median percentile of %FFM was 90% at 5 years of age, and it decreased gradually to 82.5% by 12 years of age in boys, after which it increased gradually to 84% at the age of 17 years. In girls, the 50th percentile of %FFM was lower (86% at 5 yr of age) than boys and it decreased to 71% by 15 years of age and showed a plateau till 17 years of age.

WebFig.* 1*a and **1*b*** represent reference curves for percent muscle mass in boys and girls, respectively. In boys, the lower percentiles (2nd and 9th) exhibit a dip between 7 to 14 years of age and then show a small increase till 17 years; while, higher percentiles are flat and almost parallel to the horizontal axis. In girls, average decrease of 0.8% was seen in median %lean mass with increasing age.

Reference curves for bone mineral content by BIA for Indian boys and girls showed increasing trend with age till 17 year in boys **(*Web Fig.* 2*a*)** and till 15 year in girls **(*Web Fig.* 2*b*)**. In boys, 50th percentile of BMC increased rapidly up to 13 year and then gradually till 17 year of age. In girls, median percentile of BMC showed an increase up to 11 year of age after which the curve was flatter till 17 year of age.

DISCUSSION

The present study describes age-and gender-specific reference curves for body fat percentage measured by BIA (BC-420MA) for children and adolescents using a large sample representing various regions of India. The possible cut-offs of 75th and 85th percentile have been suggested based on the risk of hypertension for defining over fat and excess fat, respectively. Our study also provides reference percentiles of %FFM, %LM and BMC by age and gender.

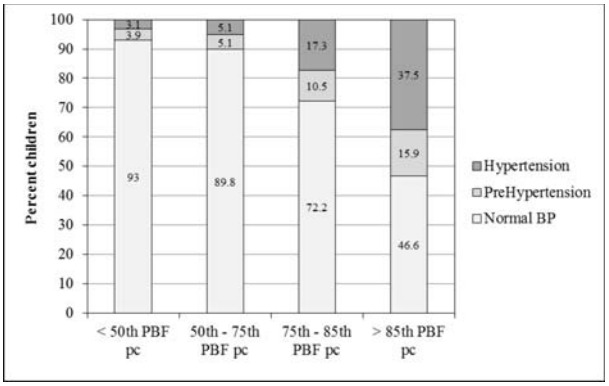
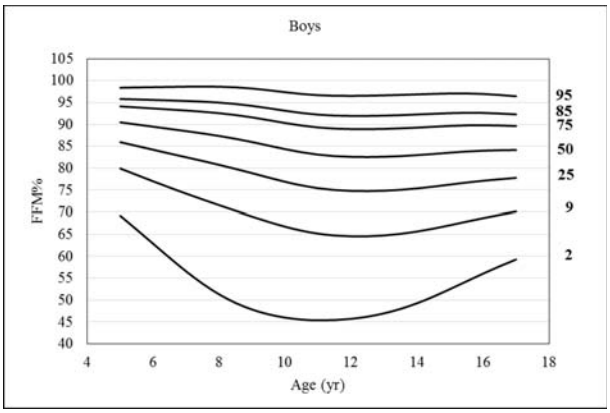
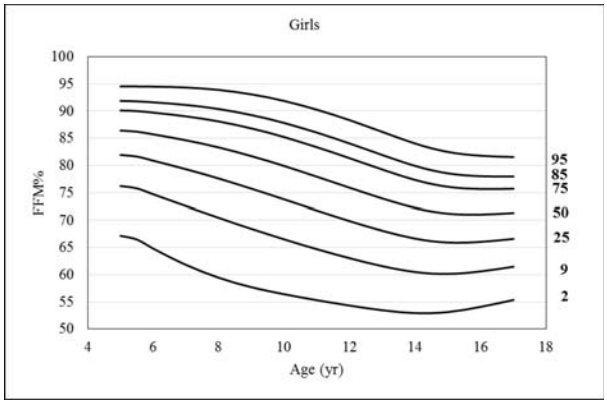


FIG. 2 *Association of hypertension risk with degree of fatness by Indian body fat percentiles.*



(a)



(b)

FIG. 3 *Smoothed Reference percentile curves for percent FFM for Indian boys (a); and girls (b).*

The present fat mass percentiles are device- and country-specific, and may not be applicable to other BIA devices. Another limitation of the study is that the cut-offs for %BF and various body composition parameters could not be assessed with metabolic markers. Unlike what is known about BMI and Waist circumference [15], there are no meaningful cut-off values established to indicate cardiovascular and metabolic risk. We used hypertension as a marker for health risk and proposed the 'excess fat' cut-offs. Our results showed a better correlation of BMI with blood pressure than %BF. This may be due to use of adult equivalent Asian BMI cut-offs over IOTF or other cut-offs. However, misclassification of hypertension risk was more with BMI cut-offs than proposed excess fatness cut-offs. These results suggest the utility of body fat assessment in evaluating possible health risk in youth. Results also indicate the need for future research to

WHAT IS ALREADY KNOWN?

- Body fat percentage for a given BMI is higher in Asian Indian children in comparison to their Caucasian counterparts.

WHAT THIS STUDY ADDS?

- Reference centile curves for body fat percentage, fat-free mass and muscle mass for Asian Indian children and adolescents are provided.
- Based on the risk of hypertension, cut offs of 75th and 85th percentile of body fat percentage have been suggested for correctly classifying excess fatness in clinical and community settings.

establish Indian population-specific prediction equations for BIA estimates of total body water and fat mass.

When compared with other BIA fat percentile data, median fat percentage of Indian boys was lower than the UK, Turkish, German and Chinese boys till the age of 12 years, and then it overlapped with the UK and Turkish boys but remained lower than that of Chinese and German boys [16-19] (***Web Fig.* 3a**). Indian and Chinese girls' median fat percentage was similar with both showing a steady rise with age. Till the age of 13 years, Indian and Chinese median curves were lower than UK, German and Turkish girls and were higher thereafter (***Web Fig.* 3b**). The 85th and 95th %BF percentiles of Indian boys and girls were higher than that of the UK, German and Turkish children **(*Web Fig.* 3c** and **3d)**. Thus, the shape of the Indian fat percentile curves was different, especially during pubertal years, than the UK standards and other population based studies. Therefore, these western reference standards may not assess fatness uniformly over the entire childhood age-range for children of Asian Indian origin. Though some part of these variations may be attributable to the differences in the model and make of BIA instruments [19], the reference curves derived in this study from Indian data may be more appropriate for assessing fatness in Asian Indian children and adolescents.

Percentiles for fat free mass by BIA in adults have been reported [20]. Though %FFM percentiles in pediatric age range have been recently reported in UK population [21], such data are not available for Asian populations. The 50th percentile of UK boys was flat across the age range which is in agreement with our data. For UK girls, the 50th percentile of %FFM was lower compared with boys and declined with age until around 11 years of age (the mean age at which puberty commences); it then continued to decrease at a slower rate up to age 17 years. Indian girls in the present study also showed a similar decline in % FFM with age; however, the age of decline and magnitude of %FFM are lower in our data than in the UK girls and boys.

In summary, suggested reference curves for percent body fat, fat free mass, muscle mass and bone mineral content by BIA for Asian Indian children may be useful to assess body composition in children in clinical and community set up. A cut off of 75th and 85th percentile of %BF may further be beneficial to detect over fatness and excess fatness in Asian Indian children.

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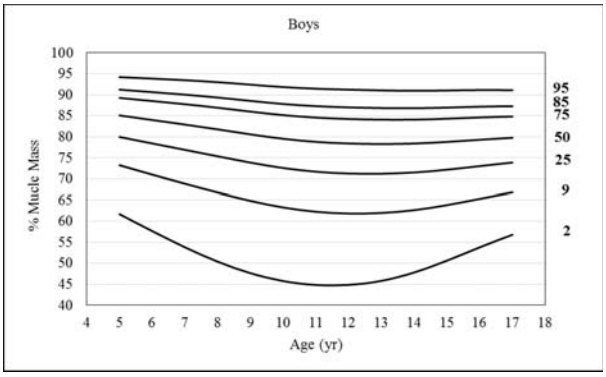
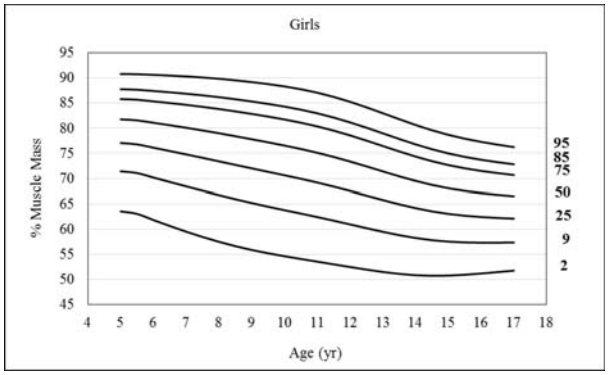
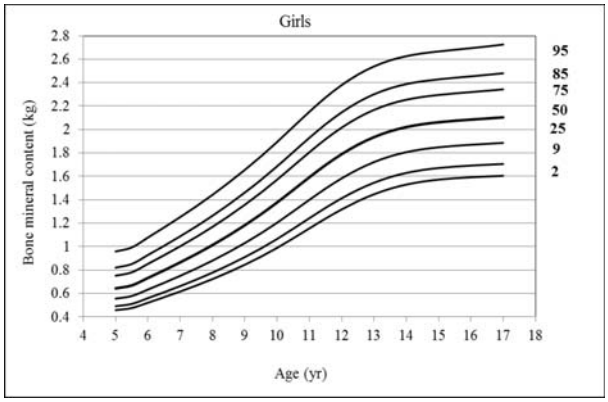




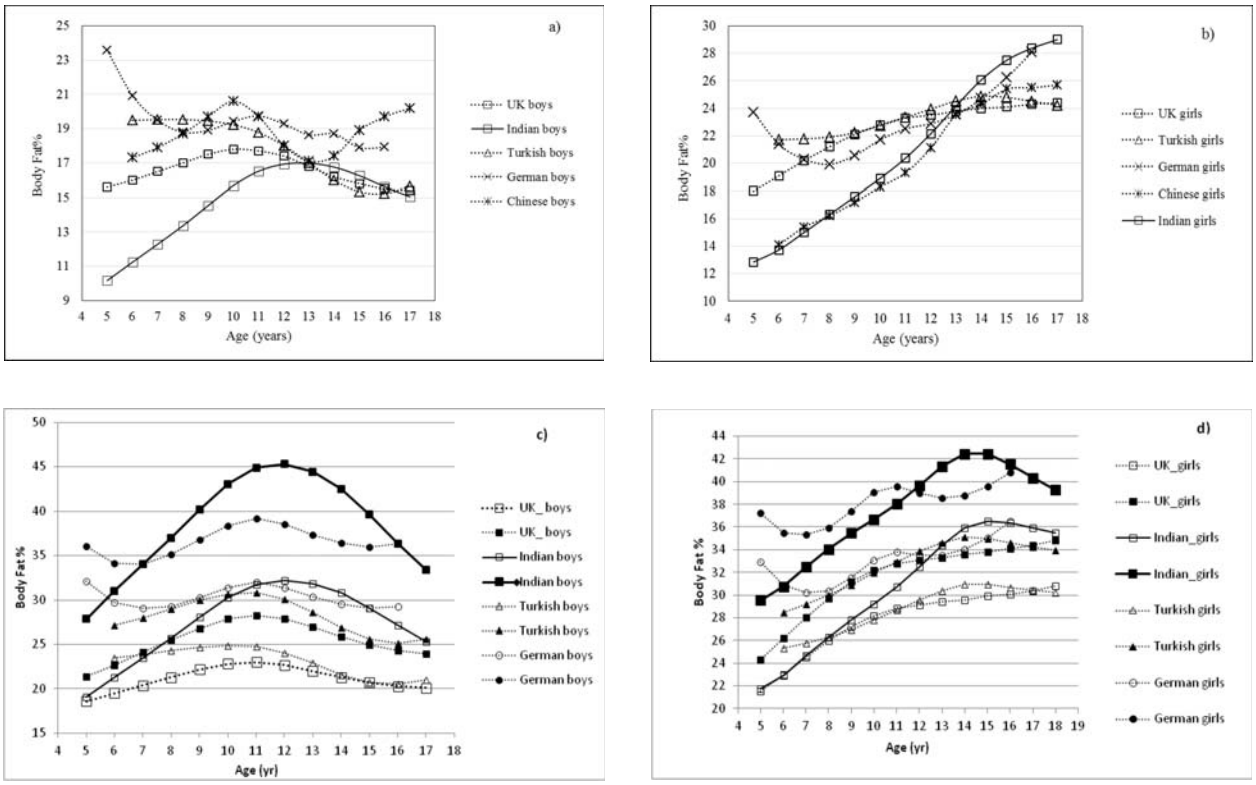
(a) *(b)*

WEB FIG. 1 *Smoothed reference percentile curves for Percent muscle mass for Indian boys (a); and girls (b).*



(a) *(b)*

WEB FIG. 2 *Smoothed percentile curves for bone mineral content for Indian boys (a); and girls (b).*



WEB FIG. 3 *Comparison of Indian percentiles of body fat percentage with those of UK, Turkish, German and Chinese children.*