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Contribution of body composition and activity behaviors to variation of total energy expenditure in pre-school children

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ABSTRACT

Background: Although it is widely accepted that the pre-school age is a critical period in the development of obesity, there are few published data on factors contributing to variation of total energy expenditure in this age group.
To investigate the contribution of body composition, gender and activity on total energy expenditure (TEE) in pre-school children.
Methods: The total energy expenditure of 89 children (2-6 years old) was measured using the doubly labelled water (DLW) technique. Total and regional body composition were also measured using deuterium dilution and dual energy X-ray absorptiometry (DXA) respectively. Physical activity and sleep patterns were evaluated using an accelerometer. Principal components analysis (PCA) were performed on regional fat mass (FM), regional fat free mass (FFM) and behavioural variables (activity, inactivity and sleep patterns), to generate orthogonal predictors for regression analyses.
Results: No significant difference was found between boys and girls for, log weight, height, BMI sds, total FFM and log FM measured by DXA and total FFM and log obtained with deuterium dilution. No significant gender difference was found for regional traits of FFM and FM except for trunk' FFM ($p=0.02$) and legs' FFM ($p=0.04$), which were higher in boys. FFM and FM measured by DXA explained 70.9% of the variation in TEE, and TEE was significantly lower amongst girls after adjustments for body composition were made ($p<0.01$). The first component of FFM (PC1-FFM), FM (PC1-FM) and behavioural variables (PC1-BV) were positively ($p<0.01$) related to TEE and there was a significant negative relationship with PC2-FM ($p<0.05$) and PC4-BV ($p<0.01$).
Conclusion: Most variation in TEE in this group was explained by difference in FFM and FM. Behavioural factors, particularly time spent in high activities could make an additional contribution of 20.9% in variation of free-living TEE. More research is needed to confirm these findings in other population.

Introduction

The prevalence of obesity in children is a major health problem that has increased dramatically during the past few decades in both developed and developing countries [1,2]. Obesity in children arises from a mismatch between energy intake and energy expenditure (EE) resulting in prolonged positive energy balance beyond that required for growth [3].

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This mismatch in energy balance is likely to have arisen as a consequence of changes in both diet and energy expenditure in recent years, but the relative importance of such changes remains unclear.

Previous studies have suggested that low energy expenditure might contribute to the increased prevalence of childhood obesity [4,5]. However, not all studies confirmed this hypothesis [6,7], rather they have suggested that changes in energy intake are the main cause of increased obesity. Total energy expenditure (TEE) is highly variable between and within individuals [8], and there is a need for more information about this variation in pre-school children. Given that there are many studies demonstrating that body composition contributes to the variation of resting metabolic rate (RMR), available data on how physical activity (PA) and body composition affect total energy expenditure in children are limited. Moreover, few studies have used an accurate body composition assessment technique, such as dual energy X-ray absorptiometry (DXA) or an objective measure of PA [9,10]. As the level of physical activity is the most variable component of TEE, it may also play a role in the variation of TEE between individuals. Children's activity varies in type, duration and intensity, and it is therefore possible that the total amount of PA and the fraction of time spent at sedentary, moderate and vigorous activity have different impacts in the variation of TEE. Previous studies in adults [11] and children [12] have indicated that variations in TEE are largely the result of variations in the intensity of physical activity. These two studies suggested that more moderate [11] and light [12] physical activity is required to significantly raise TEE. Montgomery et al [12] suggested that the difference between these two studies is the result of their definition for PA categories. However, due to differences in defining cut-offs for different intensity levels with the use of different accelerometers, further investigations are needed to improve the understanding of relationships between physical activity and TEE. Most studies on physical activity using subjective methods have been unable to categorize the pattern of activity.

The pre-school age is a critical period of physical and mental development, as well as an important time to target the prevention of childhood obesity. It also overlaps with the period of adiposity rebound (between 3 and 7 years of age), which is when BMI begins to increase, after the initial decline from birth, throughout the rest of childhood and adolescence [13]. Although there has been considerable debate over the issue of whether the adiposity rebound exists or not [14], the available evidence suggests that the pre-school age is a critical period for the development of later obesity [15]. The majority of studies on childhood obesity have focused on school age children. However, it is important to conduct studies on pre-school children to detect any significant effects of activity and body composition on variation of total energy expenditure. This may allow us to identify risk factors that relate to childhood obesity. Identification of relevant risk factors may allow early screening and targeting of children at increased risk and providing potential interventions at population and individual levels.

The doubly labelled water (DLW) technique allows us to accurately measure free-living energy expenditure, which in turn allows the relationship between energy expenditure and obesity to be investigated more reliably. Moreover, the combination of DLW and an activity monitor provide important insights into the relationship between TEE and patterns of physical activity in children [16]. The purpose of this study was to assess the effects of body composition and activity behaviors on total energy expenditure in pre-school children.

Methods

Subjects

Eighty-nine healthy Caucasian children aged 2-6 years (42 boys and 47 girls) participated in the study. Subjects were from the Rowett Assessment of Childhood Appetite and Metabolism (RASCAL) study and recruited from around Aberdeen, NE Scotland, UK using advertisements in local nurseries and medical health centers. Parents and children were informed about the purpose of the study and the methods that would be used before obtaining written consent from them. Verbal consent was obtained from the children before any measurements were taken. Approval for the study was obtained from the Grampian Research Ethics Committee, and the data reported here are based on measurements taken by trained persons at the Human Nutrition Unit, Rowett Research Institute, Scotland, UK.

Body composition

Body composition was determined by DXA (Norland XR-26, Mark II high-speed pencil beam scanner Norland Corporation, Fort Atkinson, WI) after an overnight fast. Subjects were scanned from head to foot for around 15 minutes whilst lying flat and wearing light clothing. The DXA machine was calibrated on each day that it was used and all calibrations and scan measurements were carried out by a trained coordinator using the procedures provided by the manufacturer. The coefficients of variation (CV) for repeated measurements by this machine in a total-body scan have been reported previously [17]. The CVs of variation for the assessment of body mass content and FM were 1.52 and 2.6 respectively. Subjects were weighed to the nearest 50g whilst in the fasting state using a high precision digital scale (OHAUS Corporation, Pine Brook, USA, Model: CD11). Their height was measured to the nearest 0.1cm using a standard stadiometer (Holtain Ltd, Crymych, Dyfed, Wales). Their Body Mass Index (BMI) was calculated by dividing their weight (in kg) by the square of their height (in m). Data on weight, height and BMI were converted into a standard deviation score (SDS) normalized for age and sex, using UK reference data [14,18].

Total energy expenditure

Total energy expenditure was measured using the DLW method, which has been described in detail elsewhere [19, 20]. A baseline urine sample was collected from each child prior to them being given an oral dose of ^2H_2O and $H_2{}^{18}O$ was individually based on the weight of the child, giving 0.15g/kg ^2H_2O (99% enriched) and 1.5g/kg $H_2{}^{18}O$ (10% enriched: normalized). The first three urine samples were collected at approximately 2, 4 and 6 hours post-dosing; further urine samples were collected at daily intervals for a total of 14 days. Isotope enrichments were measured in duplicate using isotope ratio mass spectrometry at the stable isotope facility of the Rowett Research Institute, Scotland, UK. The techniques used have been described previously by Scrimgeour et al., 1993 [21]. The rate of carbon dioxide production was obtained from the differential disappearance of the 2 isotopes. The multipoint method was used to evaluate the elimination constants of deuterium and oxygen-18 [20]. An average food quotient (FQ) of 0.85 was assumed and the Weir equation was used for calculation of TEE [22].

Accelerometry

The Actiwatch-L (Cambridge Neurotechnology Ltd, Cambridge, UK) was used to estimate free-living physical activity and monitor sleep pattern of the children for 7 consecutive days. This small, uniaxial device is specifically designed to measure acceleration over 0.05g, converting these accelerations to activity counts whilst suppressing high accelerations to eliminate readings outside the 3-11Hz ranges. The Actiwatch was programmed to register activity counts and light in 1-min epochs prior to the commencement of data collection. Children were instructed to wear the Actiwatch on the non-dominant wrist for 7 consecutive days, except during bathing, showering and water-based activities. Actiwatch data was downloaded using a reader unit and was analyzed using the Actiwatch analysis software (Cambridge Neurotechnology Ltd, Cambridge, UK). The proportion of time spent on a certain intensity level was calculated using an equation produced in a validation study of the Actiwatch compared to direct observations [23]. Sleep parameters, such as the time of wake in and up (actual sleep time) and get in and up (assumed sleep time), were derived from recorded light exposure and movement using the manufacturers' software. The data were evaluated over different time periods week, weekday, weekend, woken time, and time of day (morning 8-12, afternoon 12-16, evening 16-20, night 20-24) using a macro written in Microsoft Excel.

Statistical analyses

All data were analyzed using version 14 of MINITAB statistical software package (Minitab Inc., State College, PA). Means and standard deviations were calculated for the descriptive statistics, and frequency distributions were computed for categorical data. Distributions of continuous variables were examined for skewness and non-normally distributed variables were log transformed prior to further analysis. One-way analysis of variance was used to examine differences between boys and girls in relation to physical activity, inactivity characteristics, total energy expenditure, and body composition. Simple and multiple regression analysis were used to assess the relations between the independent variables (age, FM, and FFM) and TEE. To remove the effect of body composition, energy expenditure was adjusted for the effects of fat mass and fat free mass by using multiple regression analyses and calculation of residuals.

For the exploratory factor analysis, four principal component analyses (PCA) were undertaken on regional body fat mass (FM), regional fat free mass (FFM) and behavioral variables (BV) to avoid the pitfalls of multiple comparisons, collinearity and redundancy of information. PCA is a statistical technique, which entails a mathematical procedure that uses the linear relationships among variables to reduce related variables into a smaller number of summary factors to facilitate subsequent analyses. In addition, it is a suitable projection method to score a large number of correlated variables into a smaller number of orthogonal variables.

DXA can be used to provide estimates of segmental body composition (head, trunk, abdomen, arms, legs, and whole body), thereby providing estimates of FFM, FM and bone mineral content (BMC). The four principal components (PC's) are: regional fat mass (PC – FM), b: regional fat free mass (PC-FFM), c: combination of FFM and FM and d: behavioral variables (PC-BV), which reflecting variation of fat mass, fat free mass, FFM plus FM (body mass minus BMC) and behavioral factors (activity, inactivity and sleep patterns) respectively, were used in further analyses. Statistical significance was accepted at P < 0.05 for all analyses.

Results

Descriptive statistics of children

Subject characteristics are shown in Table 1. 18.8% of children were overweight (above the 85th centile), and 11.8% obese (above the 95th

Table 1. Physical characteristics of pre-school children involved in the study.

	Boys		Girls		Total	
	Mean	**SD (n)**	**Mean**	**SD (n)**	**Mean**	**SD (n)**
Age (years)	4.02	1.48 (42)	4.1	1.20 (47)	4.06	1.33 (89)
Weight (kg)	18	5.14 (42)	17.66	5.01 (46)	17.82	5.04 (88)
Height (cm)	1.05	0.11 (40)	1.04	0.10 (45)	1.04	0.11 (85)
BMI sds	0.15	1.36 (40)	0.14	1.25 (45)	0.15	1.30 (85)
FM (DXA)(kg)	6.72	3.06 (25)	7.47	3.13 (32)	7.14	3.10 (57)
FFM (DXA) (kg)	11.84	2.72 (25)	10.68	2.80 (32)	11.19	2.80(57)
FM (Deut)(kg)	4.86	2.38 (33)	5.14	2.76 (34)	5.00	2.57 (67)
FFM (Deut) (kg)	13.88	3.52 (33)	12.52	2.84 (34)	13.19	3.24 (67)
TV viewing (h/d)	1.75	1.00 (41)	1.97	0.98 (46)	1.87	0.99 (87)

Deut = Deuterium, For comparison between boys and girls, TV viewing, FFM and FM have been corrected for age effect.

Table 2. Regional values of body composition in 57 children (Boys=25, Girls=32).

	Boys		Girls		Total	
Fat Free Mass (kg)	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Head	1.9	0.51 (25)	1.9	0.47 (32)	1.88	0.48 (57)
Trunk	5.30*	1.23 (25)	4.70*	1.17 (32)	4.95	1.23 (57)
Abdomen	2.27	0.48 (25)	2.11	0.64 (32)	2.18	0.58 (57)
Arms	1.29	0.38 (25)	1.09	0.49 (32)	1.18	0.45 (57)
Legs	3.27*	0.24 (25)	3.07*	1.12 (32)	3.16	1.14 (57)
Fat Mass (kg)						
Head	0.89	0.34 (25)	0.84	0.46 (32)	0.87	0.41(57)
Trunk	2.95	1.60 (25)	3.42	1.59 (32)	3.21	1.59 (57)
Abdomen	1.13	0.62 (25)	1.33	0.63 (32)	1.24	0.62 (57)
Arms	0.79	0.51 (25)	0.84	0.59 (32)	0.82	0.55 (57)
Legs	2.21	1.01 (25)	2.4	1.00 (32)	2.32	1.00 (57)

** p<0.05, For comparison between boys and girls, each trait of FFM and FM was corrected for the rest of other traits and age effects.*

centile). There was no gender differences for age (F=0.08, p=0.78), log weight (F=0.45, p=0.51), height (F=0.19, p=0.67), BMI sds (F=0.01, p=0.97). No significant gender differences were found with respect to total FFM (F=2.62, p=0.11) and log FM (F=2.07, p=0.16) measured by DXA and total FFM (F=1.77, p=0.19) and log FM (F=0.48, p=0.49) obtained with deuterium dilution after correction for age effect.

The regional body composition values of children are shown in Table 2. There were significant correlation between regional traits of FFM and FM (p<0.01). To analyze the gender effect, we corrected each trait of FFM and FM for the rest of other traits and ages. After these corrections, no significant difference was found between boys and girls for regional traits of FFM and FM except for trunk' FFM (F=6.32, p=0.015) and legs' FFM (F=4.56, p=0.04), which were higher in boys.

Associations between TEE and other variables

TEE was significantly correlated with log weight (r^2= 57.2%, p< 0.001), FFM measured by DXA (r^2= 57.7%, p< 0.001), log ffm measured by deuterium dilution (r^2= 58%, p< 0.001), log FM measured by DXA (r^2= 46.4%, p< 0.001),

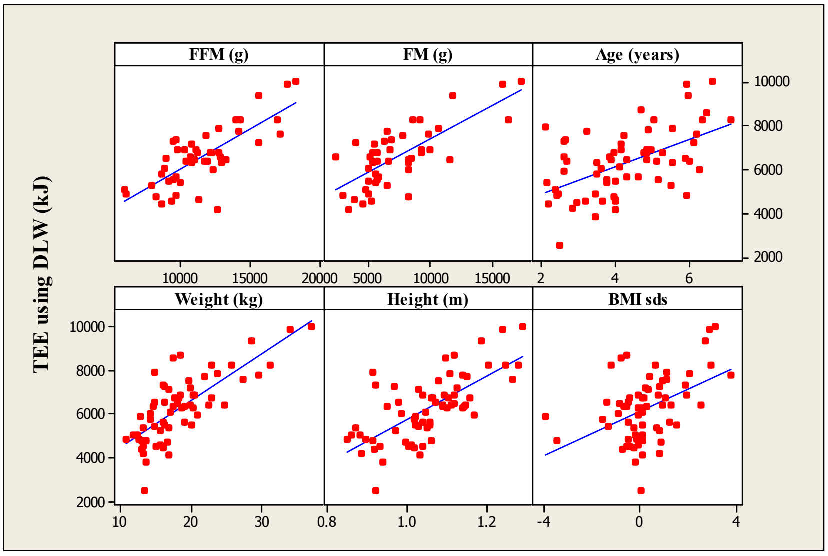

Figure 1. Scatter plot of association between TEE by DLW with FFM measured by DXA (r^2= 57.7%; p< 0.001, n=47), FM measured by DXA (r^2= 46.4%; p< 0.001, n=47), Age (r^2= 29.9%; p< 0.001, n=66), Weight (r^2= 57.2%; p< 0.001, n=66), Height (r^2= 48.9%; p< 0.001, n=66) and BMI sds (r^2= 20.6%; p< 0.001, n=66) in pre-school children.

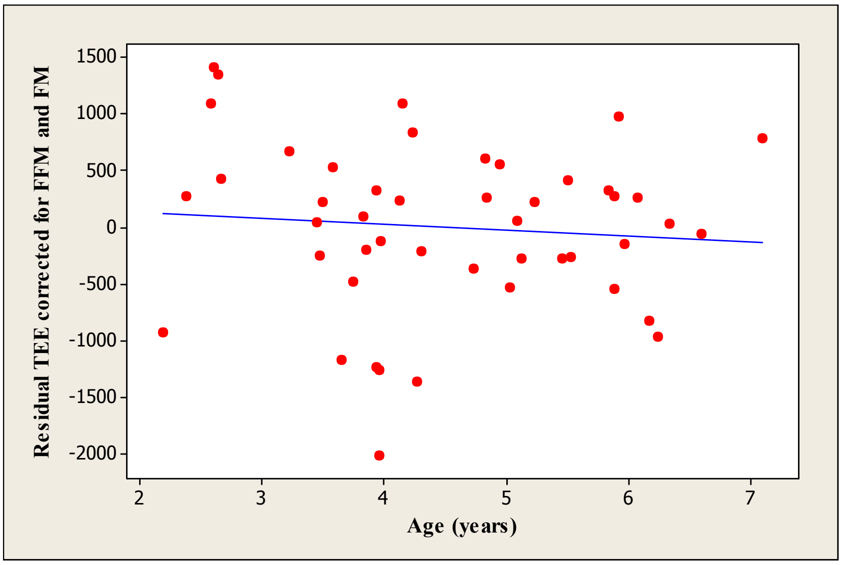

Figure 2. Scatter plot of association between residual of TEE corrected for FFM and FM with age in pre-school children (r^2= 0.8%, p=0.55, n=47).

Table 3. Descriptive summary of total energy expenditure and pattern of activity and sleep of children.

	Boys		Girls		Total	
	Mean	**SD (n)**	**Mean**	**SD (n)**	**Mean**	**SD (n)**
TEE (KJ)	6756*	1371 (32)	5850*	1523 (34)	6289	1511 (66)
APA (7days)	458.1	97.8 (40)	428.5	105.9 (45)	442.4	102.6 (85)
APA (Weekend)	444.6	109.6 (40)	442.3	113.1 (45)	443.4	110.8 (85)
APA (Weekdays)	464.6	106.1 (40)	423.1	112.4 (45)	442.6	110.8 (85)
ATIB (7days)	641.66*	53.70 (40)	615.64*	37.82 (45)	627.5	47.27 (85)
ATIB (Weekend)	643.4*	65.8 (40)	604.40*	52.91 (45)	622.2	61.89 (85)
ATIB (Weekdays)	641.4	64.5 (40)	619.87	42.1 (45)	629.7	54.16 (85)
AST (7days)	526.53	43.48 (40)	527.88	44.87 (45)	527.3	43.96 (85)
AST (Weekend)	533.8	70.9 (40)	515.10	58.86 (45)	523.6	64.88 (85)
AST (Weekdays)	527.6	43.65 (40)	531.32	48.61 (45)	529.6	46.16 (85)

** $p<0.05$, Average Physical Activity (APA) (Count/min), Average Time in bed (ATIB) (Min), Average Actual Sleep Time (AST) (Min)*

log FM measured by deuterium dilution (r^2= 28%, p< 0.001), height (r^2= 48.9%, p< 0.001), BMI sds (r^2= 20.6%, p< 0.001), and age (r^2= 29.9%, p< 0.001) (Figure 1). FFM and log FM measured by DXA explained most of variation on TEE (r^2= 70.9%). When TEE was adjusted for these two components, the residual of TEE was not significantly influenced by age (r^2= 0.8%, p=0.55), log weight (r^2= 0%, p= 0.94) and height (r^2= 1.8%, p= 0.42). When data was adjusted for log FFM measured by deuterium dilution, no significant association was found between TEE with log FM (r^2= 0.7%, p= 0.50), age (r^2= 0%, p=0.97), log weight (r^2= 0.3%, p= 0.659) and height (r^2= 0%, p= 0.99).

TEE was significantly higher in boys (F=6.43, p=0.014) than in girls (Table 3). After adjustment for log FM and FFM (r^2= 70.9%, p< 0.001), the residual of TEE was still significantly higher in the boys (F=7.77, p=0.008). There were no significant differences between girls and boys for PA measured by Actiwatch during weekend (F=0.01, p=0.92), weekdays (F=3.04, P=0.09), 7 days (F=1.78, p= 0.19), afternoon (F= 0.87, p= 0.35), evening (F= 0.82, p= 0.37), and night (F= 3.46, p= 0.07). However boys had significantly higher PA during morning (F=5.31, p=0.02). There was no significant gender differences with time spent in different PA intensities (percentage of moderate F=0.96, p= 0.33, sedentary F= 0.96, p= 0.33) during the week. Sleep pattern data collected objectively by Actiwatch showed no significant difference between boys and girls for actual sleep time during weekend (F=1.64; P=0.20), weekdays (F=0.13, p=0.72) and 7 days of week (F=0.02, p=0.89). But, time in bed was significantly higher in boys during weekend (F=8.54, p=0.005) and 7 days of week (F=6.35, p=0.014). No significant difference was found between boys and girls in case of the time in bed during weekdays (F=3.18, p=0.08).

Associations between TEE and regional body composition

Correlation between most cluster of regional body FM, regional body FFM, and behavioral factors were significant (data not shown). Due to observed collinearity between each cluster of variables, PCA was applied to remove these multi-collinearities for further analyses. Tables

Table 4. Principal components of regional fat free mass in pre-school children (n=56). Values show eigenvector loadings of traits on each PC.

Variable	**PC1**	**PC2**	PC3
FFM (Head)	0.260	-0.849	-0.359
FFM (Trunk)	0.524	-0.043	0.263
FFM (Abdomen)	0.480	0.106	0.564
FFM (Arms)	0.395	0.515	-0.690
FFM (Legs)	0.521	0.021	-0.082
PVE (%)	64	20	9

Table 5. Principal components of regional fat mass in pre-school children (n=54). Values show eigenvector loadings of traits on each PC.

Variable	**PC1**	**PC2**	**PC3**
FM (Head)	0.013	-0.990	-0.125
FM (Trunk)	0.512	0.019	-0.298
FM (Abdomen)	0.504	0.047	-0.446
FM (Arms)	0.472	-0.114	0.834
FM (Legs)	0.511	0.065	-0.029
PVE (%)	72	21	5

Table 6. Principal components of regional FFM and FM in pre-school children (n=54). Values show eigenvector loadings of traits on each PC.

Variable	PC1	PC2	PC3
FFM (Head)	0.248	0.372	0.476
FFM (Trunk)	0.346	-0.266	0.309
FFM (Abdomen)	0.300	-0.308	0.272
FFM (Arms)	0.245	-0.407	0.070
FFM (Legs)	0.378	-0.248	-0.569
FM (Head)	0.024	-0.494	-0.569
FM (Trunk)	0.363	0.264	-0.284
FM (Abdomen)	0.338	0.047	-0.446
FM (Arms)	0.472	0.024	-0.152
FM (Legs)	0.362	0.252	-0.245
PVE (%)	55	20	15

4-6 contain the component-loading matrix of regional body FFM measures, regional body FM and regional body FFM plus FM, respectively. The first and second principal component (PC1-FFM and PC2-FFM) contained 64% and 20%, respectively, of the total variation in FFM. All regional body FFM had positive loadings on component 1 varying from 0.26 (Head) to 0.52 (Leg and Trunk). Also, PC1-FM and PC2-FM were the new variables of regional body FM that together contained 93% of the total variance in FM. Fat mass of all regional body except head showed high loading on the first component, whereas head fat mass had high negative correlation in second component (Table 5). Because the masses of regional FFM and regional FM are not independent of each other, thus we combined all traits in one PCA run (Table 6). All regional measures loaded positively in PC1-FFM plus FM, which explained 55% of variation in FFM and FM. In the second components of FFM plus FM, which explained extra 20% in variation of FFM plus FM, except for head, all measures of FFM loaded negatively and all measures of FM loaded positively.

TEE was significantly positively related to PC1-FFM (r^2= 56.9%, p< 0.001), PC1-FM (r^2= 51.4%, p< 0.001) and negatively related to PC2-FM (r^2= 8.9%, P < 0.05). TEE was adjusted for these components (r^2= 70.9%, p< 0.001) and residual of TEE was used in further analysis. Figure 1 shows the associations between TEE with PC1-FFM, PC1-FM and PC2-FM. When the combination of regional body FFM and FM was analyzed in one PCA run, association between TEE with PC1-FFM+FM, which loaded highly by all traits except head's FM was significant (r^2= 69.7%, p< 0.001). This association was increased by adding the PC2-FFM+FM with high loading for all traits except abdomen and arms' FM in to the model (r^2= 73.7%, p< 0.001). No association was found between TEE and PC3-FFM+FM, which loaded by all traits except arms' FFM (r^2=2%, p=0.36).

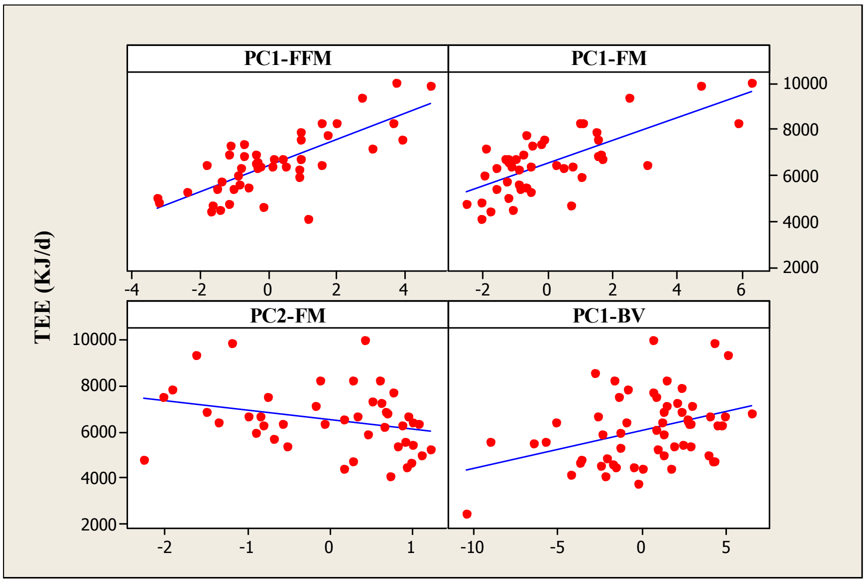

Figure 3. Scatter plot of association between TEE with PC1-FFM (r^2= 56.9%, p< 0.001, n=46), PC1-FM (r^2= 51.4%, p< 0.001, n=44), PC2-FM (r^2= 8.9%, p< 0.05, n=44) and PC1-BV (r^2= 14%, p= 0.004, n=58) in pre-school children.

Table 7. Principle components of behavioural factors in pre-school children (n=77). Values show eigenvector loadings of the original traits on the observed PC's.

Variable	PC1	PC2	PC3	PC4
Average PA (7days)	0.278	0.039	-0.071	-0.064
Average weekend PA	0.243	0.054	-0.025	0.276
Average weekday PA	0.264	0.027	-0.082	-0.188
Average morning PA	0.225	0.012	0.039	-0.112
Average afternoon PA	0.246	-0.047	-0.048	-0.126
Average evening PA	0.257	0.008	-0.061	0.041
Average night PA	0.046	0.185	-0.129	0.049
Average TIB (7 days)	-0.013	-0.439	-0.061	0.033
Average TIB (weekend)	-0.015	-0.328	-0.193	-0.409
Average TIB (weekdays)	-0.009	-0.389	0.010	0.226
Average AST(7day)	-0.060	-0.417	-0.029	0.128
Average AST (weekend)	-0.007	-0.317	-0.187	-0.366
Average AST (weekdays)	-0.068	-0.358	0.037	0.327
Average of woken PA (7days)	0.274	-0.023	-0.055	-0.093
Average of woken PA (weekend)	0.245	-0.032	-0.018	0.240
Average of woken PA (weekdays)	0.257	-0.018	-0.063	-0.211
Average of no activity (7 days)	-0.134	0.119	-0.624	0.145
Average of no activity (weekend)	-0.070	0.153	-0.325	0.004
Average of no activity (weekdays)	-0.126	0.066	-0.578	0.161
Average of sedentary activity (7 days)	-0.242	0.141	0.124	-0.074
Average of sedentary activity (weekend)	-0.241	0.086	0.099	-0.204
Average of sedentary activity (weekdays)	-0.221	0.150	0.115	-0.019
Average of moderate activity (7 days)	0.276	0.062	-0.020	0.006
Average of moderate activity (weekend)	0.234	0.036	0.088	0.390
Average of moderate activity (weekdays)	0.266	0.068	-0.046	-0.134
PVE (%)	50	17	7	6

Time in bed (TIB), Actual sleep time (AST), PVE= Proportion of variance explained.

Associations between TEE and behavioral factors

Variation in pattern of activity and sleep can be explained by the four extracted components (Table 7). PC1-BV loaded greatly by higher levels of activity and this component explained around 50% of total variation in all activity and sleep patterns. Time in bed and sleeping loaded highly in second component of behavioral factors, which explained an extra 17% of the total variation. The third component, which explained only 7% of the total sleep and activity variation loaded highly by average time spend in inactivity. Among the four extracted components, only PC1-BV (r^2= 14%, p=0.004) positively and PC4-BV (r^2= 11.6%, p=0.009) negatively were associated with TEE. Only, the relationship between the first principal components of behavioral factors (r^2= 20.9%, p=0.005) remained significant with TEE after adjusting for PC1-FM, PC2-FM, PC1-FFM and sex. Similar association was found between PC1-BV and corrected TEE for PC1-FFM+FM, PC2-FFM+FM and sex (r^2= 18.2%, p= 0.008). The percentage of the variance in behavioral factors explained by this component was 50% among the set of all variables and represented a more specific aspect of higher levels of activity pattern. However, no significant association was seen between first component of behavioral factors and PC1-FM (r^2= 0.04%; p=0.82), PC2-FM (r^2 = 0.5%, p=0.63) and PC1-FFM (r^2 = 0%, P=0.98). Although age had no relation with PC1-BV (r^2= 0.4%, p= 57) and residual of TEE corrected for PC1-FFM, PC1-FM and PC2-FM, it was positively correlated to PC1- FFM (r^2= 37.6%, p< 0.001) and PC1-FM (r^2= 19.9%, p< 0.001).

Discussion

There are limited data on the effects of body composition on TEE in pre-school children, thus the aim of this study was to investigate how TEE varies in relation to body composition and daily activity. Most studies on children, teenagers and adults have shown that TEE is highly influenced by FFM and FM [9, 24, 25]. For example, in a study by Goran et al., 1993 [24], where the energy expenditure of 4-6 year old children was measured over 14 days under free-living conditions by DLW, FFM explained most of the variation in TEE (r^2= 0.76%). In another study of 4-10 year old children, Goran et al [25] found that FFM explained 66% of variation in TEE, whilst FM had an independent influence on the variation of TEE. Our findings on the effects of body composition (70.9%) are in agreement with these results. Taken together with previous studies it can be concluded that body composition is the main contributor in variation of TEE between individuals and other factors such as PA, sex, age, diseases and ethnicity that might explain the remaining variance.

The majority of energy expenditure studies in humans have suggested that TEE is higher in males than in females [26-29]. However, the results of these studies are conflicting after normalizing TEE for either body mass or FFM. For example, in a longitudinal study of children aged 3 - 24 months carried out by Butte et al, 2000 [26], the significant influence of sex disappeared when TEE was adjusted for body weight. Similar findings have been reported in studies on 4-6 year old Caucasian children [24], 4-10 year old children [25] and pre-pubertal children [29]. Conversely, the results of a study in adults indicated that, after adjusting for FFM, TEE was higher in males than in females [28]. Another study observed that normalized TEE for body composition was significantly influenced by sex in 9-year-old children and it was higher in boys [30]. Our study suggested that boys had higher TEE than girls and sex was a significant determinant of TEE even after controlling for FFM and FM. An explanation that has been suggested previously was the effects of sex through RMR [25]. The lower TEE in girls may suggest that they are more susceptible to energy imbalance, therefore, weight gain and obesity.

Although, several studies have shown that mass of internal organs contribute more to RMR variation than peripheral FFM (i.e. legs and arms) because of their greater metabolic activity [31,32], there is limited information regarding the effect of these organs and tissues on TEE. The important point is that none of these studies have removed collinearity between regional body composition such as what we found in our study. Principal components analysis allows clustering of logically related variables, hence this method of analysis was used to verify associations among regional body composition to clarify their relation to TEE. The first principal component of FFM, which was almost determined by all proportions of body lean mass can be interpreted as a dimension of the children' FFM and only this component was related to TEE. In addition, PC3-FFM that loaded highly by central body FFM (trunk and abdomen) was not related to TEE. Therefore, the results of present study indicated that distribution of FFM did not explain variability of TEE more than gross FFM. Given the frequency of studies investigating the effect of regional FFM on variation of RMR, further studies need to reveal how the distribution of FFM influences TEE in children and adults.

It has been reported that the body fat has an independent influence on TEE [25] and also RMR [17], although this influence was relatively small compared with the effect of FFM. A possible explanation for the effect of FM on TEE might be that the increase in body mass [either by FM or FFM) influences the energy cost of activity, and consequently TEE [33]. Our findings indicate that a great proportion of the variability in TEE can explain by PC1-FM. This component explained most variation of regional FM and loaded heavily by all regional parts except head. This result can be accepted in agreement with above reports. But, negative association of TEE with PC2-FM which loaded highly by head FM is a new finding and also difficult to interpret. Due to linear correlation between FFM and FM, we combined the regional measures of body FFM and FM in one run of PCA. Compared to total FFM and FM, the first and second components of regional FFM plus FM (together) explained only an extra 3% of the variability in TEE. Given the lack of published data to compare this finding, we thought it was important to provide additional information related to the effects of regional FM and FFM on variation of TEE.

There are substantial data suggesting that physical activity is the most variable and, after RMR, the second largest component of TEE, and that it plays an important role in the regulation of energy balance [24, 30]. However, due to the difficulties of assessing TEE and PA under free-living conditions, the specific nature of this role remains unclear, particularly in children [12]. Activity levels can have a major impact on total energy expenditure among individuals as well as from day to day, but in general PA is responsible for 15-30% of variation in the TEE of sedentary people [34]. Our data demonstrated a positive association between PC1-BV and TEE after adjusting for other factors, which is consistent with results from previous studies [34]. This component loaded heavily for high level of physical activity index such as moderate to vigorous activity. On the other hand, in the second component of behavioral factors, sleep and slightly sedentary activity pattern had higher scores of loading. Relationship between PC1-BV and TEE and lack of significant association between PC2-BV and TEE suggests that intensity of PA appears to be more important in determining TEE rather than total PA. A previous study in adults has shown that physical activity level (PAL) was not influenced by engagement in high-intensity activity but the fraction of time spent on activities of moderate intensity was a significant predictor of PAL [11]. Another study in children has observed that the fraction of the day spent in activities of sedentary and light-intensity were significantly associated with PAL, whereas no association was found between PAL or TEE and the time spent in moderate- and vigorous-intensity physical activity [12]. To our knowledge, although, such a cumulative scale has not been used in prior studies to describe activity pattern, our result in this part of study is consistent with previous studies that found the contribution of physical activity in variation of free living TEE [25,34].

Although, several studies have found a negative association between FM and physical activity [35-37], the relation between time spent in different PA levels and body composition is unclear. Maffeis et al [38], showed that FM in children was directly associated to sedentary activity time, but there was no relation between activity related energy expenditure (AEE) and percentage of FM. In contrast, Roemmich et al., 2000 [39] found that FM was negatively associated to AEE and time spent in very hard level of PA. They also found that the total and sedentary activity times were not related to FM. Another study in obese children showed an inverse relation between FM with total activity time but not with AEE [33]. The results obtained in current study showed no association between behavioral components and the components of FM and FFM. An explanation for these discrepancies in results might be the real differences between populations and the application of different methods for measuring PA (recall, questionnaire, and heart rate, DLW) and body composition (skinfold thickness, DXA). For example, when DLW is used for calculating AEE, it combines both weight-bearing and non-weight-bearing physical activity and therefore, influences the relationship between body composition and PA [33]. Also, another study found that physical activity level (PAL), which is often used for indicating activity level, was not independent of body size [37]. There are two other possible explanations for observing lack of association between FM and PA in our study. First, insufficient variation in PA levels of our predominantly sedentary children. Second, the age effect as PA may need longer time to act on body composition. However, compared to the age of children in previous studies [35-37], our subjects were younger.

It should be noted that like other cross-sectional studies, it is difficult to determine the direct cause and effect between variables of current study. Therefore, future prospective design studies are needed to confirm our finding and to delineate their cause and effect relationships.

In conclusion, body composition especially FFM, was the major determinant of TEE, which contributes 57.7% of variation in TEE, whereas FM had an independent effect and increased the variation (70.8 %). More than a half of the remaining variation was explained by behavioral factors. After detecting the collinearity between regional body composition, our data indicated that central FFM did not contribute more in variation of TEE than peripheral FFM. Increased television watching was also associated significantly with increase in percentage of FM and a decrease in time spent on PA, but not TEE.

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