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Evidence for energy conservation during pubertal growth

A 10-year longitudinal study (EarlyBird 71)

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Abstract

Background Diabetes is closely linked to obesity, and obesity rates climb during adolescence for reasons that are not clear. Energy efficiency is important to obesity, and we describe a temporary but substantial fall in absolute energy expenditure, compatible with improved energy efficiency, during the rapid growth phase of puberty.

Methods In a longitudinal cohort study lasting 10 years, we measured voluntary energy expenditure as physical activity (PA) by accelerometry, involuntary energy expenditure as resting energy expenditure (REE) by oxygen consumption, BMI, and body composition by DEXA annually on 10 occasions from 7-16y in the 347 children of the EarlyBird study. We used mixed effects modelling to analyse the trends in REE and their relationship to BMI, lean mass, fat mass, age, physical activity and pubertal stage.

Results Relative REE and total PA fell during puberty, as previously described, but the longitudinal data and narrow age-range of the cohort (SD±4m) revealed for the first time a substantial fall in absolute REE during the period of maximum growth. The fall became clearer still when adjusted for fat mass and lean mass. The fall could not be explained by fasting insulin, adiponectin, leptin, LH or FSH.

Conclusion There appears to be a temporary but substantial reduction in energy expenditure during puberty which is unrelated to changes in body composition. If it means higher energy efficiency, the fall in REE could be advantageous in an evolutionary context to delivering the extra energy needed for pubertal growth, but unfavourable to weight gain in a contemporary environment.

Introduction

Childhood obesity has become an important issue over recent time because of its association with early metabolic disturbance.[1] Metabolic (type 2) diabetes, which in most cases is directly related to obesity, was rarely described in young people a few decades ago, but is now the fastest growing chronic disorder of childhood.[2] The factors responsible for childhood obesity are still unclear.

Body mass is the integral of past energy intake and expenditure. Intake is a single variable that can be controlled voluntarily across its range. Energy expenditure, on the other hand, has two components, only one of which (physical activity, PA) is voluntary. PA makes the smaller contribution to energy expenditure,[3] and declines progressively during adolescence, the more so in girls.[4,5] The larger part of energy expenditure is involuntary, fuelling the metabolic processes that sustain life, and is commonly reported as resting energy expenditure (REE). REE is accounted for mostly by metabolism within the body's fat-free

tissues (chiefly brain, liver, heart and kidneys), though some is attributable to fat cells. Muscle contributes little at rest.

However, not all energy is used efficiently. Some, such as the thermic response to feeding (dietary induced thermogenesis), is lost as heat through a process of energy uncoupling in brown adipose tissue,[6] and constitutes a further and variable component of involuntary energy expenditure. Conversely, tight energy coupling can conserve energy. The amount of brown adipose tissue wanes with age, but appears to rise temporarily during puberty, though there is wide individual variation.[7]

REE can be expressed as total (absolute) REE, or as REE relative to size. Absolute REE is deemed to increase during adolescence alongside the increases in lean mass (LM) and fat mass (FM) that characterise the pubertal growth spurt.[8] An increase in absolute REE with growth is intuitive, because body mass is demonstrably the single best predictor of REE,[9] but the evidence tends be based on cross-sectional studies comparing small numbers of children at different ages,[10] or on correlation. Small numbers carry the risk of unintended selection bias which can confound cross-sectional comparisons, and a positive correlation does not necessarily mean that the correlates are moving in the same direction – a correlation can be positive in cross-section, even though the outcome variable (in this case, REE) is falling over time while the explanatory variable (body mass) is rising. Furthermore, unless the age range is tight, a sample incorporating an age-dependent variable will not be fairly represented by the mean age, and ability to detect age-related change over time (resolution) will be compromised.

There have been few cohort studies of REE in childhood, and what truly longitudinal data there is tends to have focused on REE relative to body composition.[6,11] Relative REE appears to fall during puberty, and one theory to explain the fall cites changing body composition, whereby muscle mass increases proportionally during adolescence at the expense of other, metabolically more active, tissues. Thus, the brain, liver, heart and kidneys, all of which have a high metabolic rate, increase in mass by a factor of ×5-12 from birth to maturity, while skeletal muscle, which has a low metabolic rate at rest, increases in mass by a factor of forty.[12]

Here, we describe the trends in physical activity and REE over the course of childhood in a single cohort of contemporary children, and use new observations to explore an alternative paradigm – that there are programmed reductions in PA and *absolute* REE during adolescence, creating energy savings which may have been important over evolutionary

time in meeting the additional needs of pubertal growth, but which in a time of plenty now compound the risk of adolescent obesity.

Methods

EarlyBird is a non-intervention longitudinal study of healthy school children reviewed every six months as a single cohort from 5-16y, and has been described in detail elsewhere.[13] It was conducted in the city of Plymouth, UK, from 2000-2013, and addressed the question: which children become insulin resistant, and why? All 67 Plymouth primary schools were identified and their head teachers asked for agreement to participate in the study. Fifty-four schools agreed, and were stratified into quartiles according to the proportion of children entitled to free school meals, a socio-economic proxy. A random selection was made from each quartile and registration for the study invited during school induction meetings, where parents expressing interest were given a full explanation. With parents' written consent and children's assent, a total of 307 children (137 girls, 170 boys) who started school between January 2000 and January 2001 became the EarlyBird cohort. A further 40 children were added at age 9y to redress a gender imbalance. Importantly for the resolution of age-related change, the variance in age of the cohort did not exceed SD±4m throughout, and 80% of the children were retained for the 12 years of the study. Ethical approval was given in the summer of 1999.

Anthropometry: Height was measured every six months to the nearest 1mm (Leicester Height Measure, Child Growth Foundation, London), weight to the nearest 200g in light clothing (Tanita Solar 1632W electronic scales, West Drayton, Middx). A minimum of three 'blind' repeats were made of each anthropometric measure at each visit. BMI was defined as mass(Kg)/height^2(m)

Physical activity (PA) PA was measured objectively each year using Actigraph accelerometers (Model: 7164 - formerly MTI/CSA - Fort Walton Beach, FL). Actigraph accelerometers are of good technical reproducibility,[14] and correlate well with criterion measures of free-living activity-related energy expenditure.[15] The accelerometers were worn on an adjustable elastic belt around the child's waist, and were set to run continuously for seven days (five school days and a weekend) at each annual time-point. Only recordings that captured at least four days monitoring (each of at least nine hours wear time) were included in the analyses, as this has been shown to be the minimum required to achieve >70% reliability.[16] The Actigraph records the intensity of movement every 1/10th of a second,

and for this study the counts were collected into epochs of one minute and stored against clock time. Periods of noncompliance reported by the parents, and periods recording 0 cpm for ≥17 consecutive minutes (assumed to be unreported noncompliance) were replaced with the mean accelerometer counts recorded at the same clock time on the remaining days of the recording week. The sensitivity of each accelerometer was measured under controlled conditions by a motorized turntable.[17]

Resting energy expenditure (REE) REE was measured annually, during the week prior to PA and on the same day as anthropometry, by indirect calorimetry using a ventilated flow-through hood technique (Gas Exchange Measurement; Nutren Technology Ltd, Manchester, UK). Performance tests report a mean error of 0.3% ± 2.0% in the measurement of oxygen consumption and 1.8% ± 1% in that of carbon dioxide production.[18] The recording was rejected if the calibration test lay outwith the range 20.90-20.99% O_2. The children were fasted overnight, and measured at around 9 am in all cases. They were given a "settling in" period of up to 10 minutes under the hood before data collection over a minimum of 10, usually 15, minutes, once the minute-long readings had stabilised. Any one-minute interval during which the recorded REE lay two SD or more above the child's overall mean was ignored. We have referred throughout the text to absolute REE and to relative REE, which is absolute REE adjusted for tissue mass.

Body composition Whole body dual energy x-ray absorptiometry (DEXA) scans were performed with the Lunar Prodigy Advance fan beam densitometer on the same day as anthropometry and REE, and analysed using EnCore 2004 software version 8.10.027 GE (Lunar Corporation, Madison WI, USA). We were particularly concerned to record fat mass (FM) and fat-free (lean) mass (LM). CVs for body composition analysis using this system have been reported to range from 0.18-1.97% among paediatric subjects.[19]

Tanner stage From 9y, each child (and their parent in the early years) was shown line drawings representing genital development for boys, breast development for girls, and pubic hair development for both, and asked to choose the picture for each that most closely matched their own development. The drawings have been validated,[20] and agree, to within one Tanner stage, by 76% with clinical assessment of genital development (kappa 0.48), and by 88% with pubic hair development (kappa 0.68). A mean score for both Tanner measures (genital/breast and pubic hair development) was calculated at each age. TS1 represents pre-puberty (no phenotypic change), TS2 early puberty (first phenotypic change), TS3 mid puberty, TS4 late puberty, TS5 the end of puberty (adult phenotype).

Height velocity Annual height velocity was calculated from serial overlapping six-month height measurements in order to establish the growth pattern of the cohort, and age at peak height velocity (APHV). Although we incorporated Tanner Stage in the models, APHV is arguably a more objective and metabolic measure of adolescent development, and was included for these reasons.

Statistics

All children age 7-16y were considered for analysis, and all analyses were carried out in statistical software package Stata version 14.1 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP). Three separate linear mixed effect models were developed. The first (M-1) considered PA (cpm/day) as the outcome variable, and BMI, gender and age as explanatory variables. A random coefficient model was developed that permitted each child random intercepts from repeated measurements, and age-related random slopes for PA. A log likelihood ratio test suggested that the random coefficient model was significantly better than a random intercept model (χ^2 75.48, p<0.001). The strength of maximum likelihood (ML) based algorithms for mixed effect models lies in their ability to accommodate missing data points, as the best parameter estimate is derived when the likelihood for a probabilistic distribution of the data is at maximum.[21] Thus, unlike list-wise deletions applied to conventional regression estimators, ML tolerates (within limits) cases where outcomes are missing for some points. Accordingly, 322 out of the 347 children contributed to the analysis of M-1. M-2 (n=323) modelled REE (kCal/day) as outcome. The number of minute-long measurements used for REE was included as a predictor in the random intercept model, along with age and gender, to adjust for any variance associated with duration of measurement. There was no evidence statistically of random age-related slopes for REE, but a random coefficient model with slopes related to minutes measured fitted the model significantly better than the random intercept model (χ2 18.11, p<0.001). M-3 (n=320) was similar to M-2 except for further adjustments to lean mass (Kg), fat mass (Kg) and APHV, allowing examination of the impact of lean/fat mass on the relationship between age and REE. The random slope related variance exhibited in the previous model (M-2) disappeared when the model was adjusted for lean and fat mass. Accordingly, a random intercept model was fitted which was significantly a better fit than a single level model (χ^2 64.11, p<0.001). Interaction effects between gender, age and other variables were included in the model where appropriate. The relationships between age and PA/REE were not linear, so that age was fitted to the 2nd degree higher order polynomial for PA (M-1), and 4th degree polynomial for REE (M-2 and M-3). In order to obtain consistent age-related estimates for the linear and higher order polynomials, age was centred to its overall mean (11.32 y), regardless of gender, to include 'zero' in its range. Centring age irrespective of

gender did not introduce bias, as the age difference between the genders was negligible (mean 0.12y, SD±0.04y). M-3 was further adjusted for APHV and Tanner stage (both as a categorical, and as a continuous predictor), but the effect of Tanner stage became insignificant (Tanner stage: p=0.23) once the model was adjusted for lean/fat mass. Outcomes and their residuals at both levels were normally distributed. Results for all three models are presented with their coefficients and 95% confidence intervals (CI). Outcomes are predicted and plotted separately in this report in relation to their explanatory variables.

RESULTS

POPULATION STUDIED

The basic cohort characteristics at ages 7y, 11y and 16y are shown in Table 1, with the proportions deemed obese at each age.

Table 1 Basic characteristics of the cohort during the course of the study

Importantly, the age of the cohort was uniform at each visit, with very little variance, and the boy's ages closely matched those of the girls. The girls were the same height as the boys at 11y, but significantly shorter by 16y. They were fatter than the boys throughout.

GROWTH VELOCITY (Fig 1a/1b)

The velocity curves (Fig 1a/1b) show the annualised six-monthly rates of height and weight gain in boys and girls. The period of growth acceleration (growth spurt) spanned the interval 11-15y. Peak height velocity was achieved by 14y in both genders, and peak weight velocity by 13y in the girls and by 16y in the boys. Weight appeared to accelerate faster in the boys, and height in the girls.

FAT MASS AND LEAN MASS (Fig 1c/1d)

Fat mass (FM) was systematically higher in the girls, and continued to rise linearly throughout puberty (Fig 1c). In contrast, the early rise of FM in boys tended to level off in puberty. Lean mass (LM) followed much the same upward trajectory in boys and girls until early puberty, when it accelerated in the boys but increased little further in the girls (Fig 1d).

Figure 1 Height (1a) and weight (1b) velocity curves of the cohort; Trends in fat mass (1c) and lean mass (1d) with age in boys and girls 7-16y.

MIXED EFFECTS MODELS (Table 2)

The analyses here are based on the three models established in Table 2.

Voluntary energy expenditure (PA) The behaviour of PA predicted by M-1 is plotted in Fig 2. There was a progressive decline in PA from 7y to 16y in both genders, which accelerated with age. Girls were systematically less active than boys and their activity declined more rapidly with age.

Table 2 Mixed effect models (PA/REE/REE further adjusted for lean mass, fat mass and APHV)

Fig 2 Trends in physical activity in boys and girls (adjusted for BMI) with 95% confidence limits

Involuntary energy expenditure (REE)

Fig 3, the key figure in this report, models the age-related trends in REE before (3a) and after (3b) adjustment for lean and fat mass. The unadjusted model shows the rise in REE expected of growing children,[22] but only up to the age of 11y, after which REE fell unexpectedly in both genders to the age of 15y, when it started to recover. Yet the interval 11y-15y was the period of maximum growth (Fig 1 c/d), during which the girls tended to gain fat rather than lean, and boys lean rather than fat. Fig 3b (M-3) models the same age-related REE after removing the variation in REE attributable to metabolically active tissues (LM and FM). Predictably, the rise in REE observed pre-puberty in Fig 3a is lost, as its rise was explained by growth. Paradoxically, however, the decline in REE during puberty is now more, rather than less pronounced, and could not be ascribed to changing body composition. Indeed, none of the variables introduced into the model could explain the fall in REE during the period of rapid adolescent growth.

Figure 3 Trends in age-related REE for boys and girls, before (3a) and after (3b) adjustment for fat and lean mass (both models adjusted for Tanner stage, APHV and minutes of REE recorded).

INTERACTIONS

The association between LM and REE weakened with age (Fig 4a and b). Although the correlation between them remained positive throughout at each point in time, REE was falling over time while LM was rising. Thus, an increase in mean LM in boys of 18kg (girls 10kg) from 11y to 15y was accompanied by a fall of REE in the age-related model

amounting to 114 kCal/day (girls 284), p<0.001. The adolescent decline in REE was greatest among those of highest lean mass (Fig 4c and d), though the interaction with age had practically disappeared by 16y. Interaction between age and APHV was significant, suggesting a positive association between APHV and REE over the period of time.

Figure 4 Interaction of age with the relationship between lean mass and REE (4a and b), and of lean mass with the relationship between age and REE (4c and d).

ENERGY CONSERVED DURING PUBERTY

Voluntary energy expenditure, expressed as the mean of total PA adjusted for BMI, fell by ~120,000 Actigraph counts/day from age 10-16y in the boys, and by ~130,000 in the girls. An equation has been proposed in the past to convert Actigraph counts into calories,[15] but only in girls of a single age group, so that we have not attempted to use it here.

Involuntary energy expenditure, expressed as the unadjusted mean of REE, fell by ~110 kCal/day from age 10-15y in the boys, and by ~190kCal/day in the girls. The unadjusted figures are drawn from the raw data, so that any fall is offset by the increase in REE associated with the cumulative rise in body mass over the same period and the metabolic energy expended in driving it. Even then, the net result is a decline in REE. Fig 3b, however, models the fall in REE independently of changes in body mass (both lean and fat), and reveals the real reduction in intrinsic REE – up to 450 kCal/day over the end of the five year period in both genders.

POSSIBLE MECHANISMS

The following hormones were measured alongside REE and body composition, and each was incorporated in turn into the final model shown in Figure 4b: insulin (Diagnostic Products Corporation, Los Angeles, CA**),** IGF1 (University of Surrey, Guildford, UK), LH and FSH (Bayer Diagnostics, Newbury, Berkshire, UK), leptin and adiponectin (to 14y, University of Glasgow, UK).

While each showed distinct trends over time, none was able to explain the fall in REE independently of body composition, age, gender or maturity (IGF-1, p=0.28; LH, p=0.56; FSH, p=0.43; adiponectin, p=0.52). Only fasting insulin (p=0.01) and leptin (p=0.02) appeared to have a statistically significant impact on the fall in REE in the final model, but their effect size was small, and not sufficient to explain the fall. Thus, a one-unit increase in fasting insulin was associated with a 3kCal/day increase in REE (15kCal/day for one

standard deviation). Similarly, a one-unit increase in leptin was associated with a 1kCal decline in REE. Neither altered the age-related decline in REE shown in Fig 3b.

Discussion

The data presented here suggest that both voluntary and involuntary energy expenditure decline systematically during the pubertal years of rapid growth. Where it has been noted before, the decline in REE has been attributed to relative REE (ie REE adjusted for body mass), and ascribed to the changes in body composition which characterise puberty.[8,11,23] The decline in absolute REE at a time of rapid growth reported here is counterintuitive, because body mass is deemed the single most important determinant of REE. A decline in absolute REE implies an intrinsic reduction in energy expenditure of metabolically active tissues, one that would conserve energy. Story and Stang estimate that puberty imposes an additional 20-30% in energy needs.[24] However speculative, we believe that the decline in intrinsic energy expenditure shown here may represent a period of programmed energy conservation ('efficiency savings') which has evolved to assure the extra energy needed for adolescent growth.

Whatever the interpretation given to the behaviour of absolute REE, we believe that the body composition explanation given in the past to the perceived fall in relative REE may not be consistent with observation. First, the fall in REE from 10y to 15y was not just relative, but absolute. While there may be changes in body composition associated with pubertal growth, such that the proportion of energy-spending tissues falls, there is no evidence from any source that metabolically active tissue is actually lost, whether lean or fat. Any increase in muscle mass relative to other tissues might attenuate the rise in absolute REE associated with growth, but could arguably not reduce it. Second, whereas the boys gained substantially more lean mass than the girls, their REE declined less. While the rise in lean mass from 12y onwards in the boys was likely to have been attributable in large part to muscle, there is little corresponding change in lean mass among the girls, whose decline in REE was nevertheless greater than the boys'. The behaviour of REE in boys, compared with that in the girls, appears to be the reverse of what is predicted by the body composition hypothesis. Most importantly, perhaps, the decline in REE over time remains – indeed, becomes clearer – after adjustment for lean mass and fat mass, and is therefore unlikely to be explained by either. Rather than reflect changing body composition, we believe these data may reveal how the human body adapts to puberty by conserving the energy it needs for rapid growth.

We did not expect the decline in REE, because absolute REE is usually reckoned to increase with body mass – and body mass does not merely increase during puberty, it accelerates. However, as we have shown in Fig 4, there is nothing incompatible with a correlation that remains positive while the mean of the outcome variable (in this case REE) declines. The measures suggest that REE is declining intrinsically, and the small within-cohort variance in REE compared with the large decline over time that the change is systematic. Systematic change in biological systems implies control, and controlled change infers that there is survival advantage to be gained.

We do not know what mechanisms are responsible for reducing voluntary activity, or for switching down the energy expenditure of metabolically active cells, but the falls in PA and REE were profound. We tested a number of candidate hormones that were measured alongside REE and body composition, but none was able to explain the fall in REE. We did not measure oestrogen levels in EarlyBird because of their random fluctuations in adolescent girls, and measurements of testosterone were incomplete. In any event, neither appears to be implicated in the control of REE, at least in adults.[25]

Growth hormone, and in consequence IGF-1, levels rise from early puberty, but the rise is associated with an increase, rather than a fall, in REE which reportedly occurs prior to the changes in body composition which might confound it.[26] Adiponectin levels are reported to be inversely related to REE in adults,[27,28] though we were not able in an earlier study to show the same relationship in pre-pubertal children.[29] Neither of these two studies analysed trends. The study by Ruige and colleagues was cross-sectional and applied to adults who were either overweight or obese. That of Pannacciulli was also cross-sectional, and confined to Pima Indians. In the present study, adiponectin interacted with age only to the extent that it was inversely related to body fat. When body fat was included in the model, the coefficient for adiponectin fell and lost its statistical significance.

There are strengths and weaknesses to this report. The study was longitudinal and, in view of its unexpected results, every effort was made to account for confounders. The longitudinal design lent itself to mixed effects modelling which can account for missing data and detect interactions over time. The uniform age of the cohort (SD ±4m) was crucial to resolving age-related changes, which were central to the analysis. Blind duplicate measurements of height and weight assured optimum precision of the anthropometric measures and AHPV, and DEXA provided an objective criterion measure of body composition in both absolute and relative terms. However, DEXA could not resolve the components of lean mass, so that we cannot be certain how the proportion of each, and of muscle in particular, changed during adolescence. The accelerometers we used provided an objective measure of PA, but

recorded only vertical movement, and were unlikely to have recorded fidgeting and movement of the upper body while seated. For this reason, we may have underestimated voluntary energy expenditure, though sedentary PA contributes little to overall volume. Tanner stage was obtained by report, which was inevitable where healthy children are concerned, but we place greater store by APHV as an objective measure of adolescent development, and incorporated it accordingly. The numbers we studied were relatively small by epidemiological standards, but attrition was low (<20% over 10 years), and the multiple time points contributed considerable power to the analysis. The population was 98% white Caucasian, which optimises homogeneity, but arguably limits generalisibility.

IMPLICATIONS

The observations reported here seem reliable, and are novel, but our interpretation of them is inevitably speculative. Sustained growth requires a positive energy balance, and the pubertal dip in REE could represent an evolutionary defence against nutritional pressures during transition from childhood into adulthood – a throwback to an era when nutrition was limited, but maximum fertility at maturity crucial to survival of the species. If so, the same characteristic could have adverse implications for contemporary children, acting unfavourably where calories are widely available. The generalised weight gain which we described earlier in this cohort as they enter adolescence could in part be attributable to this phenomenon.[30] Strategies to prevent obesity in children, at its worst among adolescents, might take note of this particularly vulnerable period.

Conflicts of interest: none declared

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Figure 1 Height (1a) and weight (1b) velocity curves of the cohort; Trends in fat mass (1c) and lean mass (1d) with age in boys and girls 7-16y.

Fig 2 Trends in physical activity in boys and girls (adjusted for BMI) with 95% confidence limits

Figure 3 Trends in age-related REE for boys and girls, before (3a) and after (3b) adjustment for fat and lean mass (both models adjusted for Tanner stage, APHV and minutes of REE recorded).

Figure 4 Interaction of age with the relationship between lean mass and REE (4a and b), and of lean mass with the relationship between age and REE (4c and d).

Table 1. Basic characteristics of the cohort during the course of the study

Variables	Boys Mean ±SD (N)	Girls Mean ±SD (N)
Age (years)	–	–
7y	6.89 ± 0.25 (158)	6.88 ± 0.27 (122)
11y	10.88 ± 0.27 (151)	10.84 ± 0.29 (144)
15y	14.81 ± 0.27 (141)	14.80 ± 0.31 (137)
16y	15.81 ± 0.26 (141)	15.83 ± 0.31 (137)
Height (cm)	–	–
7y	122.58 ± 5.91 (158)	121.59 ± 5.95 (122)
11y	145.13 ± 7.15 (151)	145.19 ± 7.46 (144)
15y	171.54 ± 8.17 (141)	162.98 ± 6.15 (135)***
16y	175.67 ± 7.13 (141)	163.88 ± 6.33 (136)***
Weight (kg)	–	–
7y	24.34 ± 4.73 (158)	25.35 ± 5.41 (122)
11y	38.56 ± 9.25 (151)	41.28 ± 10.71 (144)*
15y	60.84 ± 12.21 (141)	60.09 ± 12.98 (135)
16y	66.63 ± 13.43 (141)	62.42 ± 13.35 (136)**
BMI (kg/m^2)	–	–
7y	16.09 ± 2.03 (158)	17.00 ± 2.37 (122)***
11y	18.13 ± 3.17 (151)	19.35 ± 3.63 (144)**
15y	20.58 ± 3.40 (141)	22.54 ± 4.21 (135)***
16y	21.64 ± 3.86 (141)	23.32 ± 4.34 (137)***
Lean mass(kg)	–	–
7y	18.86 ± 2.26 (152)	17.83 ± 2.20 (120)***
11y	27.73 ± 3.78 (141)	27.26 ± 4.42 (137)
15y	46.31 ± 7.35 (137)	36.78 ± 4.44 (133)***
16y	50.29 ± 6.58 (133)	37.34 ± 4.11 (125)***
Fat mass(kg)	–	–
7y	4.17 ± 3.13 (152)	6.27 ± 4.03 (120)***
11y	9.42 ± 6.39 (141)	12.90 ± 7.08 (137)***
15y	12.26 ± 8.61 (137)	20.62 ± 9.74 (133)***
16y	14.02 ± 10.48 (133)	22.39 ± 10.20 (125)***
PA(count per day/1000)	–	–
7y	555.81 ± 129.63 (132)	500.90 ± 89.47 (97)***
11y	511.93 ± 152.64 (131)	409.56 ± 110.12 (125)***
15y	430.57 ± 136.99 (108)	321.31 ± 100.71 (98)***
16y	396.16 ± 157.07 (102)	303.08 ± 110.18 (106)***
REE(kCal/day)	–	–
7y	1152.48 ± 140.49 (135)	1103.91 ± 171.10 (99)*
11y	1423.12 ± 243.11 (125)	1368.11 ± 248.06 (120)
15y	11379.32 ± 212.38 (82)	1182.35 ± 194.66 (189)***
16y	1520.73 ± 244.41 (93)	1221.45 ± 190.05 (94)***
%Obese[1]	–	–
7y	7% (11)	12% (14)
11y	10% (15)	13% (18)
15y	8% (11)	12% (16)
16y	11% (16)	14% (19)

*** denotes gender difference significance p<0.05, **p<01, ***p<0.001**

[1]Obesity defined as BMI>98th percentile of 1990 UK reference population

Table 2. Mixed effect models (PA/REE/REE further adjusted for lean mass, fat m

Variables	M-1 (PA)[1]
	Estimates (95% CI)
$Age_{(yrs)}$	-11.72 (-14.79 – -8.66)***
$Age^{\wedge 2}$	-1.27 (-1.82 – -0.73)***
$Age^{\wedge 3}$	–
$Age^{\wedge 4}$	–
Gender (Ref: Boys)	–
Girls	-80.06 (-100.09 – -60.02)***
$BMI_{(wt/ht^2 m)}$	-6.03 (-8.44 – -3.63)***
Minute	–
$Lean\ mass_{(kg)}$	–
$Fat\ mass_{(kg)}$	–
APHV	–
Interactions	–
Girls x Age	-6.27 (-10.23 – -2.31)**
Lean mass x Age	–
Fat mass x Age	–
APHV x Age	–

[1]Model coefficients scaled to thousands

*** p<0.05, **p<0.01, ***p<0.001**

ıass and APHV)

M-2 (REE)	M-3 (REE with LM/FM/APHV)
Estimates (95% CI)	**Estimates (95% CI)**
1.80 (-6.79 – 10.39)	-95.51 (-131.49 – -59.52)***
-24.65 (-28.50 – -20.80)***	-23.42 (-28.09 – -18.74)***
1.86 (1.40 – 2.32)***	2.64 (2.14 – 3.14)***
0.73 (0.55 – 0.90)***	0.86 (0.67 – 1.05)***
–	–
-86.58 (-120.57 – -52.59)***	-91.89 (-126.30 – -57.49)***
–	–
17.26 (12.66 – 21.86)***	14.60 (10.06 – 19.13)***
–	21.60 (18.18 – 25.02)***
–	10.80 (8.78 – 12.82)***
–	-4.62 (-17.14 – 7.91)
–	–
-18.05 (-23.75 – -12.34)***	–
–	-1.81 (-2.66 – -0.96)***
–	-1.05 (-1.60 – -0.50)***
–	5.04 (2.55 – 7.53)***