

Eszter Völgyi

# Bone, Fat and Muscle Gain in Pubertal Girls

Effects of Physical Activity



STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH 160

Eszter Völgyi

Bone, Fat and Muscle Gain in Pubertal Girls  
Effects of Physical Activity

Esitetään Jyväskylän yliopiston liikunta- ja terveystieteiden tiedekunnan suostumuksella  
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2010

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*“Determine the thing that can and shall be done, and then we shall find the way.”*

Abraham Lincoln

To my Family for their love and support  
Családomnak, folyamatos szeretetükért és támogatásukért

## ABSTRACT

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Finnish summary

Diss.

Obesity and osteoporosis are two disorders of body composition that are growing in prevalence. Increasing fatness and obesity can be observed both in adolescents and in adults worldwide and in Finland. An overweight child is highly likely to become an overweight adult and consequently to be at risk of numerous associated health problems later in life. Osteoporosis is another major global health problem and, whilst it mainly affects the elderly, its origins may lie in childhood. Optimizing peak bone mass through proper diet and sufficient physical activity during growth is a key strategy for preventing fractures in later life. This study aimed to determine the growth patterns of body segments, and of bone-, fat-, and muscle mass, and to investigate the influence of leisure-time physical activity on bone mass, fat mass and muscle mass accrual during puberty. Estimation of fat mass and fat free mass was also compared between bioimpedance (BIA) and dual energy X-ray absorptiometry (DXA) devices. The study subjects were 396 girls aged 10 to 13 years at baseline, 255 mothers, 159 grandmothers and 82 males. Physical activity was assessed by questionnaire. Body composition was assessed by DXA and two BIA devices. Bone mineral density was measured by DXA and peripheral quantitative computed tomography. The results showed that whilst the growth of body segments length had ceased by 18 years of age, body weight and segment widths continued to increase, but more slowly. From 11 to 18 years, most of the girls stayed in the same tertiles of bone mass, lean mass, and fat mass. Bone mass linearly increased until the age of 15 followed by a slower rate of increase into adulthood. Lean mass increased in a curvilinear fashion from pre-puberty until 16 years of age, followed by a plateau. Fat mass continuously increased from age 11 years until 18 years of age, but with a slower rate at the end of the period. A consistently high level of leisure-time physical activity between age 11 and 18 was beneficial in terms of bone mass gain at multiple bone sites, lean mass accrual and lower rate of fat mass accumulation. In addition, girls who increased their activity level from low to high during this period also benefited from these positive changes. Systematic differences were observed between DXA and BIA devices and these were dependent on gender, BMI and recreational physical activity level. In conclusion this study suggests that during puberty it is important to encourage girls to exercise regularly even after menarche. The study provides new insights into the complex and dynamic patterns of physical development during and after menarche, and underscores the importance of appropriate exercise during this period.

Keywords: Bone, fat, muscle, growth, leisure-time physical activity

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## LIST OF ORIGINAL ARTICLES

This thesis is based on the following original papers, referred to as studies I-V in the text.

- I Völgyi E, Tylavsky FA, Xu L, Lu J, Wang Q, Alén M, Cheng S. Bone and body segment lengthening and widening: a 7-year follow-up study in pubertal girls. *Bone*. 2010;4:773-82.
- II Cheng S, Völgyi E, Tylavsky FA, Lyytikäinen A, Törmäkangas T, Xu L, Cheng SM, Kröger H, Alén M, Kujala UM. Trait-specific tracking and determinants of body composition: a 7-year follow-up study of pubertal growth in girls. *BMC Med* 2009;7:5.
- III Völgyi E, Lyytikäinen A, Tylavsky FA, Nicholson PH, Suominen H, Alén M, Cheng S. Long-term leisure-time physical activity has a positive effect on bone mass gain in girls. *J Bone Miner Res* 2010;25:1034-41.
- IV Völgyi E, Alén M, Xu L, Lyytikäinen L, Wang Q, Munukka M, Wiklund P, Tylavsky FA, Cheng S. Long term leisure time physical activity effect on lean mass and fat mass in girls during adolescence. Submitted for publication.
- V Völgyi E, Tylavsky FA, Lyytikäinen A, Suominen H, Alén M, Cheng S. Assessing body composition with DXA and bioimpedance: effects of obesity, physical activity, and age. *Obesity (Silver Spring)* 2008;16:700-5.

The thesis also contains unpublished data

## ABBREVIATIONS

ADP	Air displacement plethysmography
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
BA	Bone area
BIA	Bioimpedance analysis
BM	Bone mass
BMC	Bone mineral content
cBMC	Cortical bone mineral content
BMI	Body mass index
BMD	Bone mineral density
cvBMD	Cortical volumetric bone mineral density
vBMD	Volumetric bone mineral density
BUA	Broadband ultrasound attenuation
CSA	Cross-sectional area
cCSA	Cortical cross-sectional area
mCSA	Muscle cross-sectional area
CT	Computed tomography
Cth	Cortical thickness
CV	Coefficient of variation (expressed as %)
DXA	Dual energy X-ray absorptiometry
DZ	Dizygotic
ECF	Extracellular fluid
FFM	Fat free mass
FM	Fat mass
FM%	Fat mass percent
FN	Femur neck
G <sub>HH</sub>	Group with consistent high level leisure time physical activity
G <sub>HL</sub>	Group who changed their leisure time physical activity from high to low
G <sub>LH</sub>	Group who changed their leisure time physical activity from low to high
G <sub>LL</sub>	Group with consistent low level leisure time physical activity
GH	Growth hormone
HD	Hydrodensitometry
HPA	High physical activity group
ICF	Intracellular fluid
LBM	Lean body mass
LM	Lean mass
LPA	Low physical activity group
LTM	Lean tissue mass
LTPA	Leisure time physical activity
L2-L4	Lumbar spine 2-4
MET	Metabolic equivalent of physical activity

MRI	Magnetic resonance imaging
MVC	Maximal voluntary contraction
MZ	Monozygotic
NAA	Neutron activation analysis
PA	Physical activity
PBM	Peak bone mass
PET	Positron emission tomography
PHV	Peak height velocity
pQCT	Peripheral quantitative computed tomography
SAT	Subcutaneous adipose tissue
SD	Standard deviation
SES	Socioeconomic status
SKF	Skinfold
STM	Soft tissue mass
TB	Total body
TF	Total femur
TRM	Time relative to menarche
VAT	Visceral adipose tissue
WHR	Waist-hip ratio
4C	Four-compartment model

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## 1 INTRODUCTION

Obesity and osteoporosis are two complex diseases of body composition that are growing in prevalence (1, 2). Both have multi-component etiologies such as genetic and environmental factors. A fatness and obesity epidemic can be observed both in growing and adult populations worldwide (3-6) and also in Finland in adolescents (7-9), and in adults (10). The Finnish National 2007 Health Report (1) showed that the proportion of overweight people is increasing, with 68% of men and 52% of women aged 25-64-years old overweight, and more than 20% obese. Secular trends can be observed also in the younger population. Overweight and obesity increased remarkably among Finnish adolescents from 1977 to 2005 (2, 7). According to international reference values, the age-standardized prevalence of overweight increased from 4.0 to 9.8% in girls, between 1977 and 1999. The prevalence of obesity in girls was 0.4% in 1977 and 1.4% in 1999 (7). An overweight child is highly likely to become an overweight adult and to be at risk of numerous associated health problems (11). In addition, many social and psychological problems in teenagers are associated with their body weight (12). In short, obesity is a major problem with significant health, social and economic costs.

In most European countries height has been increasing on average by 10 to 30 mm per decade, but a secular trend is not apparent before the age of 2 years (13). This suggests that birth weight and length may not be increasing. Indeed, an Australian study found no changes in birth length, but did report an increase in birth weight between 1988 and 2005 (14). Also age at menarche is now stable at approximately 13 years in many European countries (13).

Osteoporosis is another major public health problem, characterized by excessive skeletal fragility and susceptibility to low-trauma fracture among the elderly (15, 16). Among 57-65 year olds Finns, 40% of men and 56% of women have had osteopenia and 2% men and 14% women have had osteoporosis in the proximal femur or lumbar spine (1). The incidence of hip fractures is also expected to increase more than could be explained merely by population aging (15). Musculoskeletal disorders have become the most common cause of pain and the second most common cause of work disability in the Finnish

population (1). The same trend is observed worldwide in developed countries and the situation is expected to be even worse in developing countries (17).

Studying the growth patterns of the body segments, and of bone, fat and muscle, may provide valuable insights into the factors that drive development and maturation, and can help us understand the origins of obesity and osteoporosis.

The potential role of physical activity (PA) in preventing obesity and osteoporosis has been increasingly recognized. However, there is still no clear agreement on the type, duration and intensity of exercise required to prevent unwanted fat accumulation. The timing of PA seems to be one key factor affecting the peak bone mass (PBM). Leisure-time physical activity (LTPA) refers to exercises, sports, and physically-active hobbies that are performed in one's leisure time (18). It is important to study LTPA in children because, among the different forms of PA (occupational, commuting, leisure-time), children are in a position to alter their participation in this category alone. Occupational PA in their case means the physical education classes during school time which is determined by the educational program. In Finland, whilst the prevalence of leisure-time PA has increased, the prevalence of occupational and commuting PA has decreased (19).

This study aimed to determine the growth patterns of body segments, and bone-, fat-, and muscle mass, and to investigate the influence of leisure-time physical activity on bone mass, fat mass and muscle mass accrual during puberty.

## 2 REVIEW OF THE LITERATURE

### 2.1 Growth, development and aging of the human body

#### 2.1.1 Growth and development during childhood and adolescence

Growth is the dominant biological activity for the first 2 decades of human life (20). It is widely accepted that genetic background accounts for most of the variance in body development. In a twin cohort study of eight countries Silventoinen and colleagues reported that heritability of body height ranged from 0.68 to 0.93 (21). The general pattern of growth is similar from one individual to another, but environmental factors cause individual variability in size and rate at different ages. The most important environmental factors that can affect growth are nutrition, disease and social status, while season and climate are also thought to affect rates of growth and maturity (22).

From birth to early adulthood, both stature and weight follow a four phase growth pattern: rapid gain during infancy and early childhood, steady gain during middle childhood, rapid gain during the adolescent spurt, and then a slow increase until growth ceases (20). However, body weight usually continues to increase with age. The BMI declines from infancy through early childhood, reaching its lowest value at around age 5 to 6, and then increases linearly until adulthood. Children typically gain more in stature during the spring and summer than during the fall and winter (20).

Due to secular trends in height, weight, BMI and age at menarche, child growth reference data must be updated periodically. In a recent publication, Saari and colleagues (23) described new Finnish growth reference data for children and adolescents. They found that in girls, the mean birth length in the reference 1983-2008 population was 50.3 cm compared to 50.2 cm in the 1959-1971 population (**Figure 1**) (23). Girls at the age of 18 years were 1.4 cm taller, while adults were 1.9 cm taller compared to the old population (**Figure 1**) (23). This indicates that secular change is still an ongoing phenomenon in Finnish females. The new Finnish weight-for-length/height percentile curves for girls from the same study are shown in **Figure 2**.

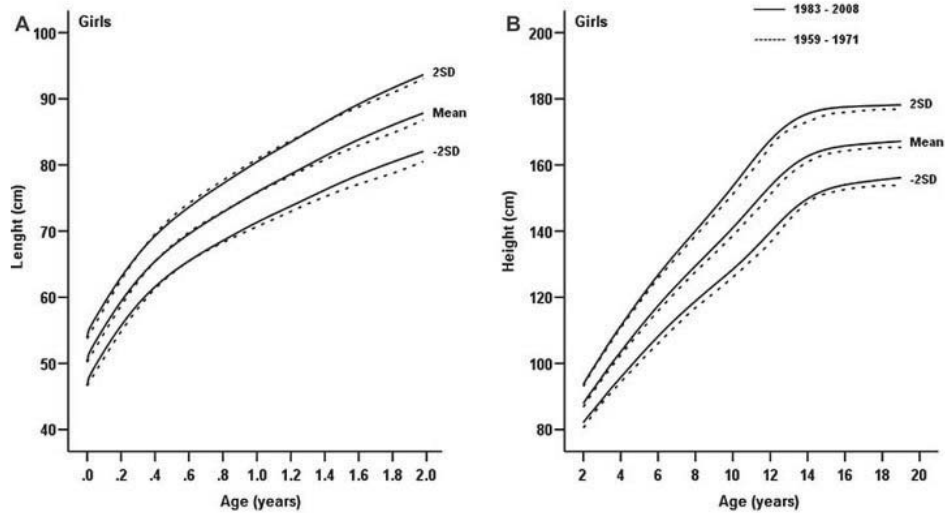


FIGURE 1 The new Finnish length/height-for-age reference (mean  $\pm$  2SD, solid lines) for girls. Curves based on 181,785 measurements from 26,636 full-term healthy subjects born between 1983 and 2008 compared to the current Finnish growth reference based on subjects born between 1959 and 1971 (mean  $\pm$  2SD, dashed lines). A: aged 0-2 years; B: aged 2-19 years). From Saari et al. (23).

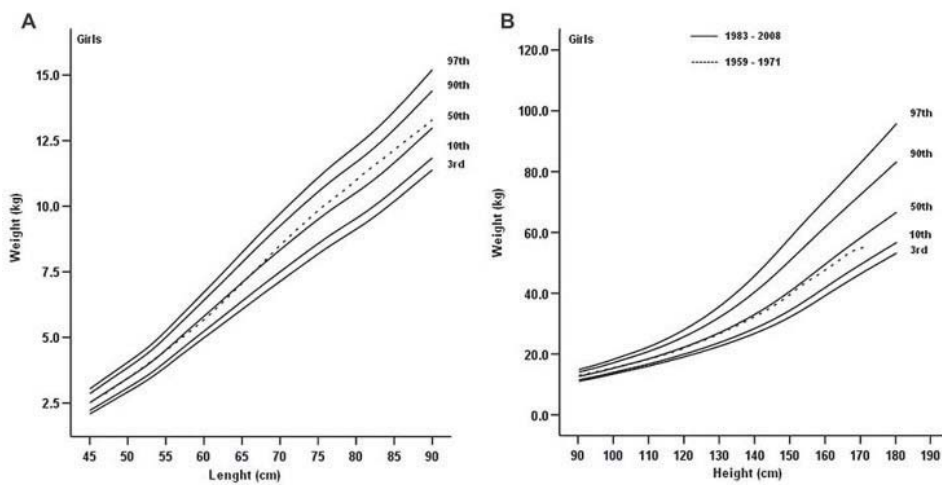


FIGURE 2 The new Finnish weight-for-length/height percentile (3<sup>rd</sup>, 10<sup>th</sup>, 50<sup>th</sup>, 90<sup>th</sup>, 97<sup>th</sup>) curves for girls. Curves based on 428,526 measurements from 73,659 healthy subjects born between 1983 and 2008 (solid lines) compared to the median weight-for-length/height curve of the reference 1959-1971 population. (dashed lines). A: 45-90 cm; B: 90-180 cm). From Saari et al. (23).

Maturation is the process of becoming mature, or of progressing towards the mature state (20). It occurs in all tissues and organs, affecting enzymes, chemical composition and physical organisation. Maturation of the neuroendocrine system is a major factor in sexual, skeletal, and somatic maturation during late childhood and adolescence (20). Sexual maturity is fully

functional reproductive capability. Maturation also refers to the tempo and timing of the progress which varies among individuals. Onset of menarche refers to the first menstrual period, and the age at which menarche occurs is the most commonly reported maturity indicator of female adolescence (20). The development of the secondary sex characteristics is often summarized in five or six stages. The most common used criteria for pubic hair, breast and genital maturation is that of Tanner (24). The development of secondary sex characteristics is result of the circulating concentrations of the hormones that stimulate the maturation process during puberty.

### 2.1.2 Changing of bone mass, density and length during growth

Bone tissue accounts for approximately 98% of stature (20, 25). It also accounts for about 15% of body weight in the infant and about 17% in adults under age 50 years (20). Growth during the first year of life may influence later bone mass and therefore skeletal fragility later in life (26, 27). Peak bone mass attained during skeletal growth is an important determinant of the risk of osteoporosis and osteoporotic fractures (28, 29). In a cross-sectional study, average total body (TB) bone mineral content (BMC) increased by 389%, and TB bone mineral density (BMD) increased by 157% during infancy, and the dominant predictor was the body weight (30). The skeleton accounts for about 15% of the body weight in a newborn infant and about 17% in adults. This proportion is even lower for the dry, fat-free skeleton, which represents approximately 3% of body weight in newborns and about 6 to 7% in adults (20, 25). It has been shown that one influencing factor is maternal vitamin D status during pregnancy, which can affect bone mineral accrual during the intrauterine life and which influences bone size (31).

After birth, the dry total bone BMD of long bones decreases by about 30% during the first few months, which is partly caused by the decrease of the relative cortical area (32) and the rapid increase of the bone marrow cavity (33). This is followed by a rapid increase until about 2 years of age, and a slower, linear increase thereafter (32). The relative ash content of the skeleton is constant; about 63% to 66% of the dry, fat-free skeletal weight among infants, children, adolescents, and adults (20). In early and middle childhood, TB BMC and BMD increase steadily with age and then a rapid rate of increase occurs during adolescence (20). The data of Boot and colleagues show the increase in bone mass with age (**Figure 3**) (34). Girls gain approximately 40% of their adult bone mass (900-1000 g) between age 12 and 18 (35-37). The peak velocity for bone mineral accumulation occurs between ages 12 and 14 years (38).

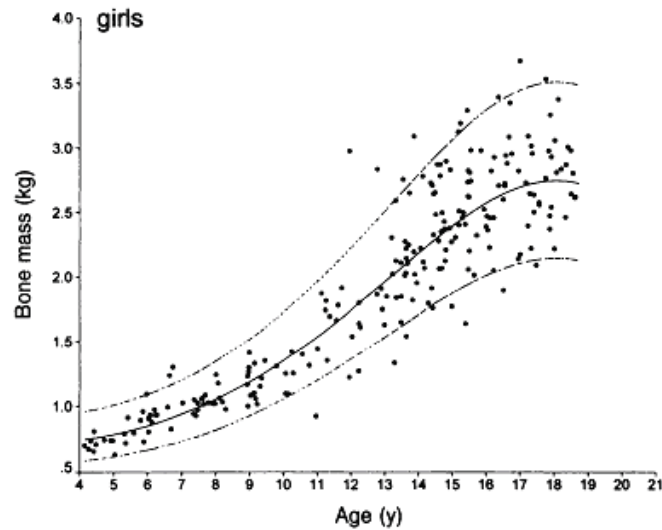


FIGURE 3 Relation between age and bone mass derived from dual-energy X-ray absorptiometry in girls. The lines show the curve with best fit. The dotted lines represent the 5% and 95% reference centiles. Adapted with permission from Boot et al. (34).

The growth curve for TB BMC appears to reach a plateau at about age 15 and 16 in girls with only a slight increase in late adolescence (36). Bachrach and colleagues (39) found in their study that TB BMD reached a plateau at 16.4 years. According to Bailey, 35% of TB BMC was laid down during the 4-year adolescent period surrounding peak linear growth velocity (40). Some large studies using DXA have reported reference data for normative BMD (39, 41) for children and have calculated childrens' BMC Z-scores for age, size, sex and ethnicity (42).

The tracking of BMC and BMD throughout growth has been of recent interest among researchers seeking to understand the normal physiological pattern of bone mineral accrual and changes in bone mineral density. Of children (aged 6 to 15 years) who were categorized as having low bone mass (BMC and BMD Z-score  $< -1.5$ ), 65-77% still had low bone Z-score after 1 year, and 44-62% remained low after 3 years at various bone sites (43). Among those who were classified as having high bone mass (BMC and BMD Z-score  $> 1.5$ ) at baseline, 55-77% still were in the same category after 1 year, and 50-62% remained there after 3 years at various bone sites (43). The degree of tracking varied according to age at baseline: it was less for those who had begun puberty compared to those who were either prepubertal or sexually mature (43) and was influenced by race (43). After adjusting the results for height and weight, initial BMC/BMD was still highly predictive of BMC/BMD 3 years later (43). Foley and colleagues described a high level of tracking for total body, spine and total hip BMC and BMD from 8 to 16 years of age (44). The majority of children

who were in the lowest or highest tertile of bone mass at 8-years old remained in the lowest or highest tertile, respectively, as adolescents (44).

Rates of growth in length and width of bones vary among individuals and with age. Earlier data on longitudinal limb growth were dependent on X-ray films (45-51), and only few studies used longitudinal observation. Dual-energy X-ray absorptiometry provided a new opportunity to estimate both the length and width of selected skeletal sites (52). In early puberty, the long bones grow first, in a synchronous way, to attain the individual's optimal height. It has been found that, while individual values for tibia length increase from age 10 to 13 in girls, group means and individuals' values track within their quartiles of origin (53).

### 2.1.3 Changes of fat mass and distribution during growth

Fat mass (FM) is the most variable component of human body composition. Between-individual variability ranges from about 6 to 60% of total body weight (54). At birth, infants' fat mass percent (FM%) is about 15% on average for girls, increasing to about 26% by 6 months of age (55), and then starts to decline during early childhood (55, 56). According to a recent study the greatest increase in FM% occurred between birth and 6 weeks of age in Australian infants, with FM% doubling over this period (57). The term "adiposity rebound" refers to the point where the BMI values of children start to increase (58). According to the literature this happens between about 5 and 7 years of age (58, 59). In Finnish girls, Saari and colleagues (23) reported that adiposity rebound happens at 6 years of age. Before age 5.5 yr an early rebound is followed by a significantly higher adiposity level than a later rebound after age 7 (58). The data of Boot and colleagues illustrate the trends for both FM and FM% to increase with age in children and adolescents (**Figure 4**) (34). Fat mass accrual continues to increase during puberty and adolescents with an estimated rate of 1.4 kg/year in girls (60). The same study found that FM% increases in girls from about 20 to 26% between age 9 and 20, and that tracking of FM and FM% persists from childhood to adulthood over 5- and 10-year time periods (60). In a recent study Wright and colleagues found that of those children who were obese at age 7, 75% were still obese at age 11, while of those who were overweight 16% had become obese and 20% had normal BMI (61). Vink and colleagues in a Dutch study also demonstrated that girls (aged 9-12 years at baseline) in the lowest and highest quartile of FM had a 77% and 55% chance respectively of staying in the same quartile after 10 years (62). They also found that FM increased from 7 kg at age 9 to 19 kg at age 18 with a significant increase in fat mass from 12 kg at the moment of menarche to 15 kg 1 year after menarche. In the Amsterdam Growth and Health Longitudinal Study, Nooyens and co-workers (63) showed that those women, who were in the highest tertile at the mean age of 13 years in BMI or sum of four skinfolds had a higher risk of becoming an adult with high FM%. These findings support the hypothesis that overweight and obese adolescents are at risk of becoming overweight and obese later in adulthood.

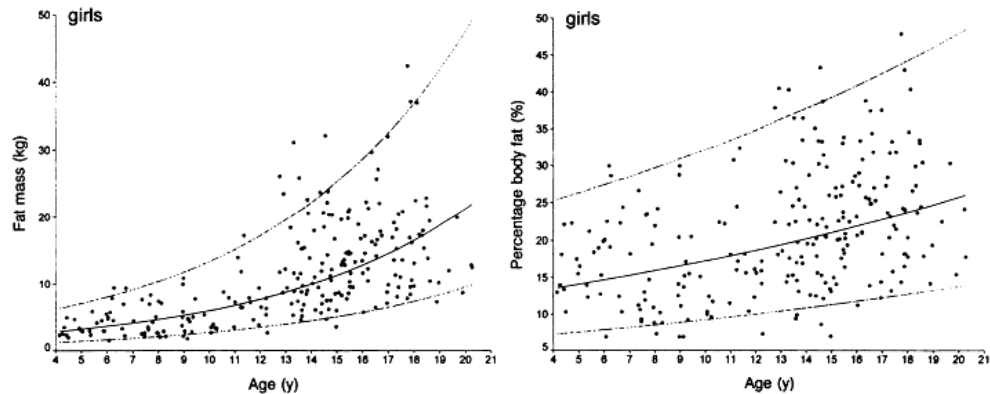


FIGURE 4 Relation between age and fat mass and fat mass percentage derived from dual-energy X-ray absorptiometry in girls. The lines show the curve with best fit. The dotted lines represent the 5% and 95% reference centiles. Adapted with permission from Boot et al. (34).

Variation of the distribution of both subcutaneous and visceral fat is a risk factor for several diseases such as non-insulin dependent diabetes mellitus and cardiovascular diseases (20). Girls have more subcutaneous fat than boys at all ages from early infancy to 18 years of age. The major changes occur during childhood and adolescence, and therefore this age group is of major interest for clinical research. Subcutaneous fat follows the general trends in fat mass growth; it rapidly increases during the first 6 months of life, then there is a reduction thorough 6 and 7 years of age. After this girls show a linear increase during puberty until young adulthood (20). Visceral fat refers to the fat tissue that is located around the viscera in the abdominal cavity (20). MRI, CT and ultrasound are widely accepted, precise methods to quantify the visceral fat in children and infants. Preterm and term-born infants have been measured using whole-body MRI to identify the differences in total, subcutaneous, and intra-abdominal adipose tissue, and it has been shown that preterm infants have significantly less subcutaneous adipose tissue and significantly more intra-abdominal adipose tissue than that of the term-born ones (64). It has been found that those adults who had experienced rapid infant weight gain had significantly greater total and abdominal adipose tissue levels than those who had experienced slower infant weight gain (65). Velde and colleagues (66) reported that lower birth weight was related to higher subcutaneous fat in adulthood especially in the trunk. Abdominal fat area does not increase much during childhood, and the mean area is around 30 cm<sup>2</sup> for normal weight children (20). The mean values for normal weight adolescents are about 40 to 50 cm<sup>2</sup>, while during adulthood, and this value goes up to 70 cm<sup>2</sup> and 94 cm<sup>2</sup> for normal weight women and men (age 25 to 40 years), respectively (20).



### 2.1.4 Changing of FFM/muscle mass during growth

Muscle is the largest and main energy-consuming tissue mass in the human body and provides the forces required for movement and physical activity. Childhood and adolescence are periods of rapid skeletal muscle accrual, followed by a relatively stable phase up to middle adulthood (54). There are different techniques available to assess muscle mass of the total body. Creatinin excretion measurement has been used extensively, as well as the new imaging methods such as DXA, CT, pQCT and MRI or ADP. In recent studies it has been shown that after a decrease in fat-free mass (FFM) during the first 5 days in newborns (67), rapid growth (155 g/wk) occurs until the first 6 weeks of life. Subsequently FFM accrual slows down to a rate of 94 g/wk between wks 6 and 13, and to 79 g/wk between wks 13 and 19 in female infants (57). These results were consistent with Eriksson and colleagues' findings in terms of FFM gain (105 g/wk) during the first 12 wks in female infants (68). Earlier reports from radiographic studies showed no clear evidence of an adolescent muscle growth spurt in females (69). The growth velocity of muscle width in the arm shows first a decrease between age 3 and 5, followed by a plateau until age 15 with an approximate growth rate of about 1-2 mm/yr (69). The velocity of the calf muscle width expansion slightly decreases from age 3 to age 10 (3 to 2 mm/yr), then a rapid increase can be observed until age 12, where the annual growth stays at about 3 mm/yr rate until age 15 (69). By about age 17 years, girls begin to reach a plateau in lean tissue mass (Figure 5) (34).

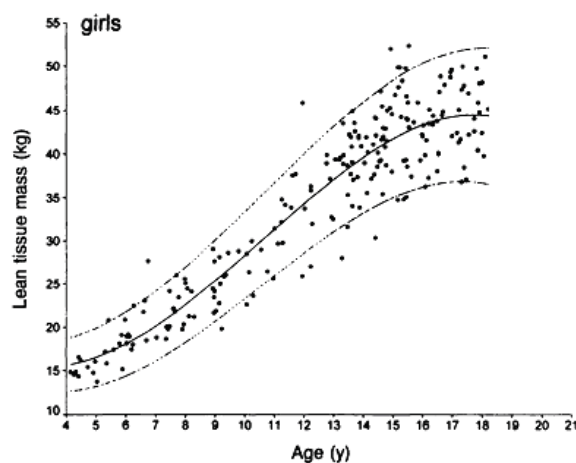


FIGURE 5 Relation between age and lean mass derived from dual-energy X-ray absorptiometry in girls. The lines show the curve with best fit. The dotted lines represent the 5% and 95% reference centiles. Adapted with permission from Boot et al. (34).

### 2.1.5 Aging of the human body

Advancing adult age is associated with significant changes in body composition that affect the overall health status of the elderly. Bone mineral density

decreases with age after reaching its peak in young adulthood. The rate of decrease accelerates in women during and after menopause (70), with a possible acceleration in old age (71). Low bone mass and microarchitectural deterioration of bone tissue define osteoporosis as a systemic skeletal disease (72). Currently, about 10 million Americans suffer from osteoporosis, and the disease is responsible for about 1.5 to 2 million fractures annually in the US (73). This leads to annual medical costs in excess of \$19 billion associated with osteoporosis (73). Peak bone mass might be an important determinant of the risk of osteoporosis in later life (74, 75).

During adulthood fat mass increases slowly with age, but the rate of increase varies according to race, sex and assessment method (54). From the Fels-study, it has been reported that as women became older they gained about 0.55 kg body weight annually and significantly increased their FM by an average of 0.41 kg/year (76). In the same study Siervogel and colleagues reported increases of 0.44 kg/year in FM and 0.41% in fat mass percent (FM%) in women between ages 18 to 45 years, and increases of 0.52 kg/year in FM and 0.47%/year in FM% between ages 45 to 66 years (77). Mott and colleagues found that the relationship between age and body fat was curvilinear indicating a peak amount of body fat in late middle age (53 to 61 years for FM and 55 to 71 years for FM% in the various ethnic groups) and lower amounts of body fat at younger and older ages (Figure 6) (78).

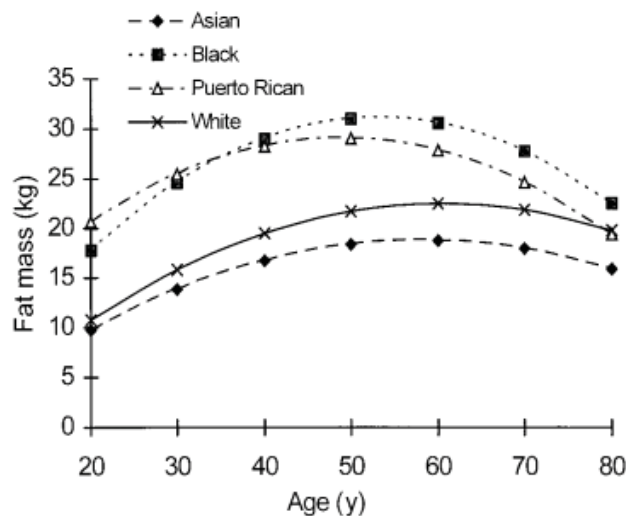


FIGURE 6 Patterns of change in fat mass from 20 to 80 yrs of age. (Plot of regression models for fat mass as a function of age in women.) Adapted with permission from Mott et al. (78).

As the body ages, while FM is increasing there is a marked decline in FFM. After the rapid skeletal muscle accrual in childhood and adolescence, a relatively stable phase exists up to middle adulthood (30 to 40 years of age) followed by a decrease which begins at about age 40 which accelerates in later life (54). The loss of FFM is mainly due to the loss of skeletal muscle mass and

bone mineral. Between the ages 29 and 75 years, lean body mass (LBM) decreases by about 11% (79). The Third National Health and Nutrition Examination Survey (NHANES III) showed in a large cohort that FFM increases from adolescence to mid-adulthood, but then declines (80).

Age-related loss in skeletal muscle and strength is referred to as sarcopenia (81). Population studies have reported that sarcopenia affects over 20% of 60 to 70-year-olds, and 50% of those above 75 years of age (82). The health care costs of sarcopenia-related disability in the United States have been estimated to be at least \$12 billion in the year 2004 (83). Loss of muscle mass is related to physical disability (84, 85), and this leads to reduced quality of life. The absolute amount of muscle mass is relatively stable up to 45 years of age, but after there is an accelerated decrease (86). This loss is mainly attributed to skeletal muscle loss in the lower body (86). In a large US population based dataset, Janssen and colleagues found that the observed weight gain between age 18 and 40 yr was predominantly due to increasing fat mass, and that therefore the proportion of skeletal muscle relative to body mass starts decreasing relatively early in life (**Figure 7**) (86). These findings are consistent with others who have reported that muscle cross-sectional area (87) and body cell mass do not change significantly until about 45 years of age (88). The later dramatic decrease in skeletal muscle mass and FFM is associated with declining energy expenditure and heat production (89).

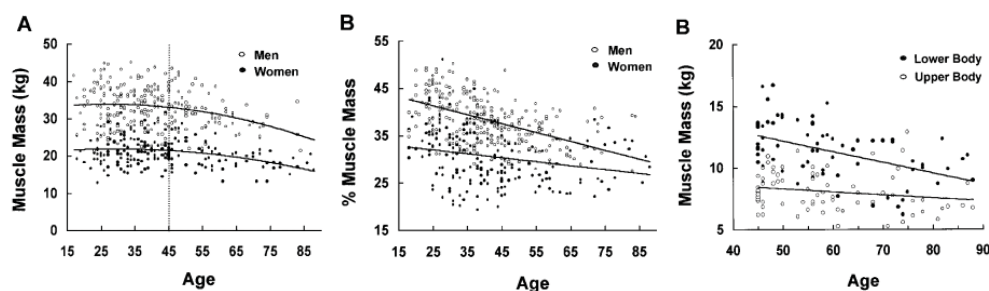


FIGURE 7 A: Relationship between whole body skeletal muscle mass and age. B: Relationship between relative skeletal muscle mass (skeletal muscle mass/body mass) and age. C: Relationship between upper body and lower body skeletal muscle mass in women aged 45+ yr. Solid lines, regression lines. Janssen et al. (86) used with permission.

## 2.2 Factors related to growth and body composition

### 2.2.1 Physical activity

Physical activity occurs in a variety of forms and contexts, including free play, household chores, exercise, physical education, and organized sports (20). It refers to any body movement produced by the skeletal muscles and that results in a substantial increase over the resting energy expenditure (20). Regular

physical activity is an environmental factor which is important in regulating body weight, bone mineral, and lean mass during childhood and adolescence, and therefore it has a long term beneficial influence on health. PA is often characterized by type, intensity, duration and frequency of each activity session. Estimates of PA are usually gathered from questionnaires, interviews, diaries, pedometers, accelerometers or heart rate monitors. However, one validation study found that pedometer, heart rate monitor and PA questionnaire measure different aspects of physical activity (90). Physical activity behavior tends to track during childhood (91), but there is a decline in the amount of PA during adolescence (92), especially in girls (93).

Changes in lifestyles, and therefore in the different types of physical activity, have occurred in the past 2 decades, mainly due to the increasing mechanization of everyday life. In Finland, a longitudinal study by Borodulin and colleagues (19) found that occupational and commuting physical activity has decreased over the past 30 years, whilst LTPA has increased. They suggest that commuting physical activity together with leisure-time physical activity should be emphasized to gain more health benefits.

Regular PA does not have significant effects on body height (94), but it is associated with increases in body weight, fat mass, skinfolds (95, 96), bone mass (97-99) and lean mass (100). Interestingly, school-based or nursery-based exercise or physical activity intervention studies have not found significant improvements in BMI (101, 102).

The influence of regular physical activity on FM and FM% is apparent in studies with overweight and obese children (103, 104). Kriemler and colleagues (96) also found that a one school year physical activity program could decelerate the BMI gain and skinfold thickness in 7- and 11-year old children. In cross-sectional studies it has been reported that the level of PA is negatively associated with fat mass index (105) or FM% (106) and positively associated with FFM (105), percentage of FFM (106), or lean mass (95). However, other studies have failed to see positive effects of physical activity on fat mass in children (107) and female adolescents (108). In a longitudinal study, Baxter-Jones and colleagues (100) found that habitual physical activity during puberty had a significant independent influence on lean body mass accrual in girls when the results were controlled for maturation and growth (100). Parizkova reported that those boys who were regularly training between age 11 and 18 increased their FFM more than controls (107). Kemper et al. (109) observed a relationship between high levels of physical activity and FM, but they did not find such a relationship between PA and BMI.

PA already starts to regulate bone biology in prenatal life. Regular fetal kicks against the uterine wall result in mechanical stimulation and such activity plays an important role in ossification, and bone modeling and remodeling (33). There might be different explanations for the postnatal loss in BMD. One explanation might be that, after birth, the infant can move more freely and that smaller loads are imposed on the skeleton, leading to a decrease in postnatal BMD (33). On the other hand, after birth the infant is no longer surrounded by

fluid, but has to start to support itself against gravitational forces, implying potentially greater skeletal loading. An alternative explanation for this phenomenon could be that the change from placental nutrition to milk could lead to a temporary bone mineral density loss. With passive range-of-motion exercises it is possible to increase BMC and reduce the loss of BMD in preterm babies (110). There is evidence that LTPA has beneficial effects on bone mass accrual during childhood (111-114). The intensity of exercise is the most important factor for increasing the accrual of BMD (115, 116). MacKelvie et al. (111, 117) reported that only weight-bearing exercise interventions in school-program had significant effects on bone mass gain. It has been found in female soccer players (118) that intense exercise after puberty was associated with higher BMD accrual, and that decreased PA intensity in both the short-term and long-term was associated with higher BMD loss compared to controls. Some longitudinal studies have also indicated that moderately intense training was sufficient to increase the accrual of bone mineral in a young population (111, 117, 119). A daily school-based exercise intervention of 40 minutes per school day was beneficial for spinal BMD and BMC in boys aged 7 to 9 years (120). Baxter-Jones and colleagues (99) reported that girls who had been active during adolescence had 9% and 10% more adjusted BMC at the total hip and femoral neck (FN) than their peers at follow up aged 23- to 30-years old. The outcomes of exercise intervention studies vary depending on the pubertal maturity level of the cohort at the beginning of the intervention. Studies with pre- or early pubertal children have found significant increases in lumbar spine and femoral neck BMD (113, 119, 121), but no significant differences in total body and lumbar spine BMD and BMC have been reported in postpubertal girls (122). In an adult population, Nevill and colleagues (123) reported that there was no association between bone measurements (BMD and BMC of lumbar spine and femoral neck) and any component of physical activity in 20 to 25-year-old women. Kemper and colleagues (124) showed that both the metabolic and mechanical components of physical activity during adolescence had a strong relationship with lumbar BMD at age of 32 years. Between the ages of 13 to 16 years, only the metabolic component of the physical activity had a strong relationship with adult lumbar BMD, while during young adulthood (21-27 years) only the mechanical component was related to adult lumbar BMD (124). The same study group also reported that daily physical activity during adolescence and young adulthood was positively related to lumbar and hip BMD measured at the mean age of 28 years (125, 126). Bakker et al. (127) also did not find a relationship between the metabolic and mechanical components of physical activity and lumbar BMD in a 10 year longitudinal study in adult females.

### 2.2.2 Diet

Nutrition is an environmental factor that affects growth. Energy and nutrient intakes and their influences vary during the growing years. Energy and nutrition are needed to maintain the basal metabolic rate, to support maturation,

repair, and maintain the tissues or to conduct PA. Requirements vary by age, sex and FFM mass.

Of the major minerals, calcium and phosphorous are essential for bone growth and metabolism, but researchers have reported no association between calcium intake and BMC in girls (128, 129). The estimated daily increment in the calcium content of the body is 110 mg in females between age 10 and 20, while during the peak of the adolescent growth spurt this goes up to 240 mg (20). In a Finnish study Cheng and colleagues reported that calcium intake from cheese appears to be more beneficial for cortical bone mass accrual than the consumption of tablets containing a similar amount of calcium (130).

Rowlands and colleagues reported that in 8- to 11-yr-old children dietary calcium intake should be 700-800 mg/day and that participation in vigorous activity is needed for a positive impact on bone indices (131). Evidence on the effect of dairy product or calcium supplementation on body weight or FM reduction is contradictory. One review of randomized trials found little evidence of effects (132), while in another review Heaney and colleagues found that increasing calcium intake by 300 mg was associated with substantially decreased weight gain and could reduce the risk of overweight by, perhaps by as much as 70% (133). For the normal growth and maturation other minerals such as magnesium, sodium, potassium and chlorine are also needed.

### 2.2.3 Hormones

Several hormones act to impact growth and development throughout the life cycle. Growth hormone (GH) is a major anabolic hormone which triggers the growth of all body cells, and which is therefore crucial to physical development in early life. GH deficiency in children (134) and in adults (135) is associated with increased body fat. In puberty, sex hormones play a key role in the profound changes in body composition. For example, the increase in muscle mass in boys during puberty is associated with testosterone levels.

Sex steroid hormones of women affect FM distribution during puberty and after menopause (136). Combined treatment with estradiol and testosterone is more effective in increasing BMD at hip and lumbar spine in post menopausal women than is estradiol alone (137). Estradiol has a positive effect on bone geometric and densitometric development in early pubertal girls (138). Estrogen may prevent fat accumulation in the upper-body before menopause (139). Leptin is a hormone which is secreted by adipocytes and circulates in the blood in concentrations related to FM (140). Caprio et al. (141) and Gutin et al. (142) reported that a stronger association exists between leptin and subcutaneous adipose tissue and total FM than between leptin and visceral adipose tissue in children. It has been also shown that leptin concentrations are closely related to FM% and physical activity after adjustment for body fat (143). Leptin is also believed to be an "alarm" hormone in situations of increased energy expenditure or reduced energy intake to help defend the body against weight loss (144).

Adiponectin is an adipocyte-derived hormone, whose circulating concentration negatively correlates with adiposity, namely FM% in children (145), adolescents (146) and adults (147).

#### 2.2.4 Other factors

Biological inheritance represents the effects of the parents and grandparents on the child. Weight at birth is one of the most studied physical characteristic in relation to genetics. It is widely accepted that genetic background accounts for most of the variance in body development. However, it has been shown that fetal genotype accounts for only about 15% to 20% of the variation in birth weight (20). The current knowledge on the genetic regulation of body height and weight has been summarized in three points by Malina and colleagues (20): First, genes associated with length and weight at birth have only a small effect on adult stature and weight. Second, a set of genes are correlated with adult height and weight. Third, another independent group of genes regulate the growth rate of height and weight. The genetic control of adult stature is greater (60%) than that of the body weight (40%). Silventoinen and colleagues (21) found that, in women, heritability for height varied between 68% and 84%, in a twin cohort study spanning eight countries. A genetic study indicated that the variation in tibia length is under genetic control with a heritability of 77–80% (148). In a recent study, Chinappen-Horsley and colleagues (149) found heritability of 65% for spine length, 73% for femur length, 65% for tibia length, 57% for humerus length, and 68% for radius length (measured from DXA scans) in 3751 Caucasian females. Skeletal muscle tissue as the major component of FFM has a significant genetic contribution to it. The heritability estimate of 52% was found in postmenopausal twins (150), and 87% and 83% were reported for adult female twins for FFM (151, 152). The heritability estimates for FM and adiposity vary between 25% and 40% of the age and sex adjusted phenotypic variance (153). Less information is available on the role of genes in maturation. Results indicate that genotype has a major role in the timing and tempo of the maturational events (20). Sexual maturation is also under genetic control. The correlations between family members and age at menarche are highest in monozygotic (MZ) twin pairs, followed by dizygotic (DZ) twin pairs, biological sisters, and mothers (20). In secondary sex characteristics, MZ girls are more concordant than DZ girls (154).

Ethnicity and race can also influence the phenotypic characteristics. Race is a biologically distinct group, while ethnicity implies a culturally distinct group. Height, weight, body proportions, body composition and maturation are different among those of African (Black), White (European), Mexican and Asian ancestry (20).

Social conditions can also influence growth and maturation. The socioeconomic status (SES) of the family is a significant factor. It includes the parents' educational background, family income, nutritional status, head of household, health-care access and place of residence. Children from the high SES families tend to be on average, taller and heavier than those with a low SES

background (20). During late adolescence girls from lower SES tend to be, on average, fatter than those in the upper SES (20). Howe and colleagues in a British study concluded that the difference in height between different socioeconomic groups is primarily present at birth and this difference does not get significantly bigger through childhood (155). However, it is difficult to draw conclusions because the influence of SES on growth and maturation is generally country specific.

Those mothers who smoke regularly during pregnancy have lighter and shorter babies at birth (156). The effect is greater for weight. Children whose parents smoke at home tend to be, on average, shorter than those whose parents do not smoke (157). Ethanol use during pregnancy can cause developmental delay and disturbances (158). Smoking and drinking at four years after menarche, even at low levels, appear to be associated with increased visceral adipose tissue deposition in adolescent females (159). It has been also speculated that alcohol consumption during early adolescence may delay the onset of female puberty through inhibiting the production of IGF-1 in the liver (160).

Heat and cold may have effects on growth and maturity, but it is difficult to separate the specific influence of temperature. There are other factors, like living conditions, nutritional resources, infectious and parasitic diseases and racial/ethnic variations. To study the effect of high altitude is easier. It has been found that the mean birth weight, height-for-age, weight-for-age, and weight-for-height indicators decline significantly with increasing altitude, starting at an elevation >1500 m (161). Growth and development under conditions of chronic hypoxia results in a different pattern of growth in Andean highlanders than in lowlanders. Growth at high altitude results a small delay in linear growth (162), and such a delay is probably established at or soon after birth (163). In highlanders, the median age of menarche is greater by about 0.8 years (162).

### **2.3 Body composition assessments**

Different body composition measurement devices can yield different results, and so conclusions based on a specific device may need careful interpretation. The various methods for estimating body composition in vivo use very different measurement principles, and they therefore vary in applicability, pricing and validity. In addition, body composition methods rely on several assumptions that are often age dependent.

#### **2.3.1 Bioimpedance based methods**

*Bioimpedance analysis (BIA)* assessments are based on the estimation of the water content of the body. BIA assumes that the body is approximately cylindrical; therefore it allows the calculation of its volume. BIA estimates total body water (TBW), and it gives an estimate of FFM based on previous finding that 73% of FFM is TBW (164-166). The first commercial impedance analyzers and related articles appeared in the mid-1980s (167). Single-frequency impedance analyzers



generally operate at 50 kHz frequency. The hydration constant is not the same among individuals, especially with higher body weight (165). To increase the validity of single-frequency impedance analyzers, it is recommended to use them in combination with other anthropometric data to predict body composition (168). The estimation of body composition from BIA devices requires regression equations validated against a gold standard method. However there are several equations available and their usability is limited to specific gender, race and age groups (167). Since single-frequency impedance analyzers are not able to distinguish between intra- and extra-cellular fluid (ICF, ECF) compartments, multifrequency impedance analyzers have been developed. Impedance values measured at several different frequencies can explain inter-individual variations in body composition more precisely (167). However, in general, multifrequency impedance has not improved the estimate of body composition compared to the single-frequency method (169). A number of factors can affect BIA determinations such as eating or drinking before measurements, menstrual status, dehydration, physical activity, or metal in the body.

### 2.3.2 X-Ray based Methods

*Dual-energy X-ray absorptiometry (DXA)* is a widely accepted method to estimate body composition. It replaced single- and dual-photon absorptiometry in the 1990s and was initially used to estimate BMD for lumbar vertebrae and parts of the femur. DXA is an improved technology compared to the earlier radioisotope methods, and uses an X-ray tube with a filter to create low-energy (40kV) and high-energy (70 or 100kV) photons (170), giving greater precision and more accurate estimates of BMD and soft-tissue composition. DXA provides estimates of bone mineral-free lean tissue mass (LTM), fat mass (FM), soft tissue mass (STM=LTM+FM), and fat-free mass (FFM=LTM+BMC) (170, 171). Different manufacturers use different radiation sources and imaging geometry, such as pencil beam or fan beam. The pencil beam devices are more accurate but slower, and they have higher resolution and lower radiation dose than that of the fan beam devices (172).

Validation studies have been carried out with both pencil beam (173, 174) and fan beam (175) devices. In general, pencil beam DXA shows good results when compared to multicompartment methods. However, Tylavsky and colleagues found that the Hologic QDR 4500A (**Figure 8**) systematically overestimated FFM and underestimated FM in older adults compared to the four compartment (4C) model (175).

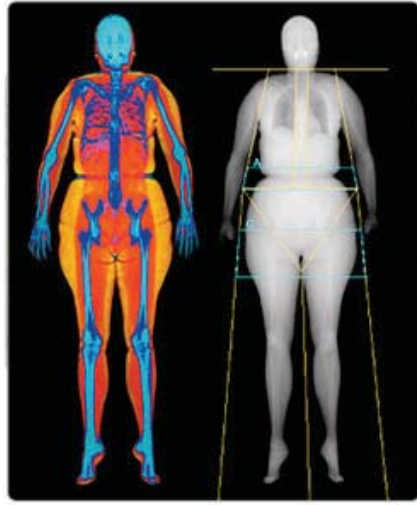


FIGURE 8 Total body picture from Hologic's Advanced Body Composition™ assessment.

Norcross and Loan found that the Lunar Prodigy fan beam DXA instrument is accurate and valid compared with 2C and 3C models in young adults (176). Tylavsky and colleagues described in their study that fan-beam calibration provides higher estimates of lean soft tissue mass and lower estimates of FM than the pencil beam approach (177). Factors that influence the accuracy of the measurement include subject thickness, size, calibration procedures, software version, and instrument, company and model (171). Limitations of DXA measurements include the radiation exposure and the high cost.

### 2.1.3 Other Methods

Early body composition methods divided the human body into 2 components, namely fat mass and fat free mass (178). Later the three-compartment method was introduced with three measurable quantities: body mass, body volume and TBW (179-181). Adding bone mineral as the fourth compartment to the previous method, four quantities of the FFM can be measured which allows for the calculation of the density of the FFM (168, 171). Hydrodensitometry is often considered as the “gold standard” method for body composition validation assessments (182), but the four-compartment (4C) model has been recommended as the “new gold standard” in which the four compartments of body mass, bone mineral, body volume, and total body water are measured, respectively, by scales, dual-energy X-ray absorptiometry, underwater weighing, and deuterium dilution method, allowing the determination of the density of the FFM (180, 183).

*Hydrodensitometry (HD)* is often considered to be the reference method for body composition validation assessments (182). HD requires an uncomfortable test, involving the measurement of residual volume in the lungs, as well as complete submersion in water to estimate the body volume. With the corrections for residual volume and gastrointestinal tract gas volume, body

density can be calculated. Due to its impractical nature, this method has not been widely used.

*Air displacement plethysmography (ADP)* uses the pressure-volume relationship to estimate volume and density of the human body. It is suitable for elderly, children, disabled, and other special populations. A new system called the Bod Pod (Life Measurement Instruments, Inc., Concord, CA, USA) has been developed to increase the precision and accuracy compared to hydrodensitometry (184). This new device applies Poisson's law to determine the body volume. An air circulation system is used to mix the air between the reference chamber and the chamber where the subject is seated. Validation in the general population suggests that ADP is an accurate, reliable and widely-applicable method for assessing body composition, but problems can occur during calibration in children because of the smaller body size (185). Another system, the PEA POD (PEA POD; Life Measurement Inc, Concord, CA) has been found to provide a reliable and accurate assessment of FM% in infants (186).

*Skinfold (SKF)* measurement is suitable for all populations, but it can be difficult to measure severely obese persons because of the maximum jaw opening of the calipers. The most common sites for skinfold thickness are over biceps and triceps, subscapular, suprailiac, anterior thigh and medial calf. The correlation of skinfold thickness with FM% is high (0.7-0.9) (187, 188). Another limitation of this method is the missing estimation of the visceral adipose tissue (VAT), since it takes into account only the subcutaneous adipose tissue (SAT). When skinfold thickness is measured, the pressure by the calipers displaces some ECF, or may force some lobules of adipose tissue to slide into areas of lesser pressure (189). Accuracy of the result depends on the measurer and the equation used in the calculation. The SKF method has low correlations with FFM (189), and FM% (190) compared to DXA, and SKF is inaccurate when compared to the 4C method (191).

*Ultrasound* instruments can measure subcutaneous adipose tissue and muscle thickness. A linear-array transducer is generally usually used to measure the tissue thickness. This is, in theory, a simple method, but in practice the interpretation of the result requires experience (189). In one recent study, ultrasound was used to estimate visceral fat in children and showed good agreement with CT (192).

Imaging methods such as *magnetic resonance imaging (MRI)* and *computed tomography (CT)* are considered to be the most accurate in vivo three-dimensional methods for quantification of body composition at the tissue-organ level (193). However, their high cost, radiation exposure (for CT) and operator skill requirements are factors limiting their wider use in this context (194). In general, CT provides slightly more reliable and repeatable data than MRI, but MRI does not involve ionising radiation. *Positron emission tomography (PET)* with tracers is a technique that can provide information about specific metabolic processes in certain organs and tissues. Visualization of brown fat or energy expenditure in specific organs and tissues is possible with this new

technique (195). When mass is known, it is also possible to calculate whole-body volume simply and quickly by means of segmentation of PET transmission data (196).

The *Dilution* principle can be used to measure TBW. The volume of water in the body can be measured by isotope dilution, using tritium, deuterium, or  $^{18}\text{O}$ -labeled water. The precision and accuracy of this method is between 1 to 2%, and it is considered to be the golden standard to measure TBW (197). However, careful attention is needed to design the measurement protocol (198).

*Neutron activation analysis (NAA)* allows for direct in vivo chemical analysis at the atomic level. The 6C model used this technology as a reference method. Body elements such as calcium, sodium, chlorine, phosphorus, nitrogen, hydrogen, oxygen, and carbon can be determined with NAA. It is one of the most sophisticated methods available, but the technology and skills needed, together with high expense and radiation dose, tend to severely limit its use in practice (170).

*Total body potassium (TBK)* is often used as a reference measure of body cell mass. The measurement requires a gamma ray detector, a shielded room to reduce the radiation, and a computer-based data acquisition system that can identify the unique gamma rays from  $^{40}\text{K}$  (170). High cost and lack of availability makes this method impractical for routine use.

### 3 PURPOSE OF THE STUDY

Understanding the effects of physical activity and diet on growth of the different body components such as tissues, organs and bones is important in promoting healthy body composition and peak bone mass in children as well as preventing obesity and osteoporosis in later life. Therefore the main purpose of this observational study was to determine the growth patterns of body segments, and bone, fat, and muscle mass, and to investigate the influence of physical activity on bone, fat and muscle mass accrual during puberty. The study was also designed to compare DXA and BIA body composition assessments in estimating body fat mass.

More specifically the objectives were the following:

- 1) To determine the growth patterns for various body segments and relate these to mothers' and grandmothers' values (I).
- 2) To track body composition components through childhood and adolescence (II).
- 3) To examine whether the level and consistency of leisure-time physical activity during adolescence affect the quantity and distribution of lean mass and fat mass and the bone mineral content and bone mineral density attained at early adulthood (III, IV).
- 4) To verify to what extent the DXA, InBody (720) and Tanita BC 418 MA devices yield similar results for body fat mass and fat free mass in men and women with different levels of BMI and physical activity (V).

## 4 MATERIAL AND METHODS

### 4.1 Subjects and study design

This study was part of the Calex and Calex-family study (Effects of physical activity and vitamin D on musculoskeletal properties and fitness in three generations). A detailed description of the Calex study is given elsewhere (130). In brief, the subjects were first contacted via class teachers teaching grades 4 to 6 (age 9-13 years old) in 61 schools in the city of Jyväskylä and its surroundings in Central Finland (96% of all the schools in these areas). The flow chart of the participants has been described in our earlier reports (130) (II) and can be seen in **Figure 9**. Of those eligible, 396 girls participated in the laboratory tests 1-8 times over a maximum period of 8 years (mean duration of total follow-up was 7.5 years and mean age at last follow-up was 18.3 years). Of the 396 girls, 258 participated in the first 2-year intervention study, and 235 participated in the 7-year follow-up assessments (II). From the intervention group, 101 girls had bone assessments at baseline and at the 7-year follow-up, and 134 from the eligible sample at the follow-up. Due to missing physical activity questionnaires, 33 subjects (15 from the intervention group and 18 from the non-intervention group) were excluded also from the final analysis; therefore 202 girls were included in the final analysis (III). No intervention effect on body composition and bone was found, thus data were pooled in the present analysis (I-IV).

In addition, 255 mothers (age 35 to 60 years) and 159 grandmothers (age 52 to 92 years) participated in the same study procedures as the girls. The results are presented separately for 141 premenopausal mothers (PrM; mean age 45, range 35 to 55 years) and for 107 postmenopausal mothers (PoM; mean age 49.3, range 36.9 to 60.4) for comparison with their daughters at age 18 (I and II). In addition, 72 male volunteers (age 37 to 81 years) participated in the study (V).

Background information including the health status was collected via a self-administrated questionnaire. Persons with serious metabolic, cardiovascular, or endocrine diseases were excluded from the study. Written informed consent was obtained prior to the laboratory examinations. The study protocol was approved by the ethical committee of the University of Jyväskylä,

the Central Finland health care district, and the Finnish National Agency of Medicines. Informed consent was given by all subjects and their parents prior to the assessments. All data were handled confidentially.

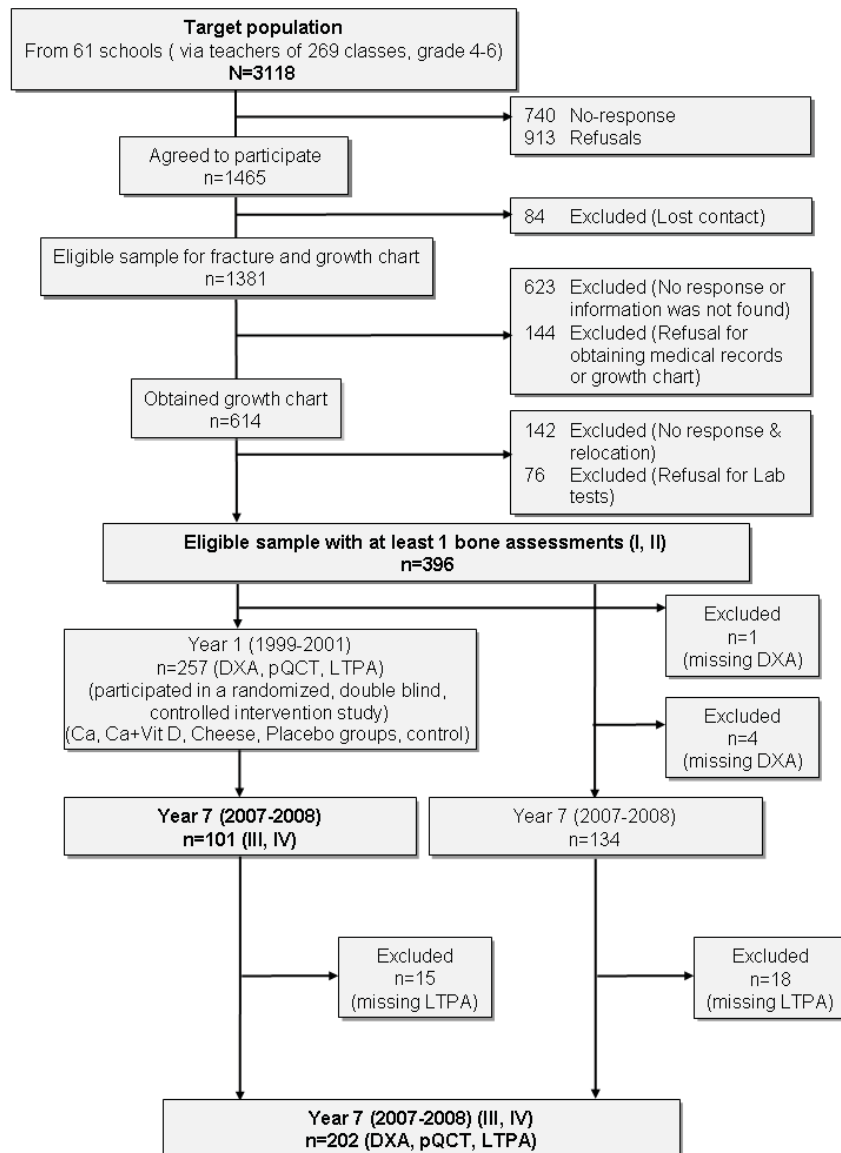


FIGURE 9 Study population flow of the Calex girls.

## 4.2 Measurements

### 4.2.1 Anthropometric measurements

All measurements were performed after an overnight fasting (12h). Participants were weighed with light clothes and without shoes. Height was determined using a fixed wall-scale and weight was determined using an electronic scale, calibrated before each measurement session. BMI was calculated as weight (kg) per height (m)<sup>2</sup>.

Different body segment widths and lengths were measured from dual-energy X-ray absorptiometry (DXA Prodigy; GE Lunar Corp., Madison, WI USA) scan images using bones as landmarks. Details of the novel method have been described in the original article (I).

For the purpose #1 of this study, we classified the subjects into three groups on the basis of clinical cut points of BMI for overweight and obese (V). The normal group was defined as BMI < 25 kg/m<sup>2</sup> (31 men, 44 women), overweight as BMI = 25-30 kg/m<sup>2</sup> (40 men, 27 women), and obese as BMI > 30 kg/m<sup>2</sup> (11 men, 15 women).

### 4.2.2 Health status, medical information, socio-economic status, diet and physical activity

All the information was collected and laboratory tests performed within a two week period during the same month of the year throughout the 7-year follow-up to avoid seasonal effects.

Lifestyle and behavioral characteristics as well as medical history were collected via a self-administered questionnaire. Girls under age 15 filled in the questionnaire with their parents' assistance, and all the questionnaires were checked by a study nurse. Breast feeding was expressed in months. The socio-economic factors included the highest level of parental education, how wealthy the family is, and whether the girl was living with both parents or with a single parent. Birth crown-heel length and birth weight were obtained from the girl's growth chart.

Dietary information was obtained from a food-intake diary kept for three days (two ordinary school days and one weekend day) as has been described in detail elsewhere (199) and earlier (II). Dietary intakes were analyzed using Micro-Nutrica software (version 2.5), Finland. The nutrients related to body composition and bone accrual, including intake of carbohydrate (g/day), fat (g/day), protein (g/day), calcium (Ca) (mg/day), potassium (K) (mg/day), phosphorus (P) (mg/day) and magnesium (Mg) (mg/day), as well as total energy intake (kcal/day) were chosen as background variables.

Leisure time physical activity (LTPA) level was evaluated using a self-administrated physical activity questionnaire which was a modified version of a validated questionnaire used in a previous WHO study (200, 201). The modification has been described earlier (III and IV).



For the purposes #4 and #5 (III and IV) of this study, girls were divided first into two groups according to the median values of their LTPA scores at screening and at the 7 year follow-up visit. Four activity groups were then formed as follows: consistently high ( $G_{HH}=50$ ), consistently low ( $G_{LL}=53$ ), changed from high to low ( $G_{HL}=48$ ), or low to high ( $G_{LH}=51$ ).

Physical inactivity (PIA) was calculated as the sum of sitting and lying hours per day.

#### 4.2.3 Bone and body composition measurements

##### *DXA assessment*

Dual-energy X-ray absorptiometry (Prodigy, GE Lunar Corp., Madison, WI USA with software version 9.3) was used to estimate bone mineral content (BMC), bone mineral density (BMD) and bone area (BA) of the total body (TB), total femur (TF), and lumbar spine (L2-L4) (**Table 1**) (III). The whole body fat mass (FM), percentage of FM (FM%), fat free mass (FFM), bone mass (BM) and lean tissue mass (LM) were also determined (**Table 2**) (II, IV and V). Details of the measurement procedures are presented in the original reports (III, IV and V). Precision of the repeated measurements expressed as percent coefficients of variation (CV%) ranged from 0.6% to 1.2% for BMC, from 0.9% to 1.3% for BMD, and from 0.6% to 1.2% for BA of the whole body, total femur, and L2-L4. Precision was 2.2% for FM%.

##### *pQCT assessment*

A pQCT device (XCT-2000, Stratec Medizintechnik, GmbH, Pforzheim, Germany) was used to scan left tibia. Details of the measurement procedures are presented elsewhere (202, 203). Muscle cross-sectional area (mCSA), total cross-sectional area (CSA, mm<sup>2</sup>), bone mineral content (BMC, mg/mm), volumetric bone mineral density (vBMD, mg×cm<sup>-3</sup>) and cortical CSA (cCSA), BMC (cBMC), vBMD (cvBMD), and thickness (CTh, mm) (**Table 1**) of the left calf were analyzed using the manufacturer's software package (version 5.40) and Geanie 2.1 (Commit Ltd, Espoo, Finland) (III). The CV% was 1% for the mCSA and CSA, and was less than 1% for BMC, vBMD, and CTh.

##### *QUS assessment*

Calcaneal Broadband Ultrasound Attenuation (BUA) of the left calcaneus was measured using a gel-coupled scanning quantitative ultrasonometer QUS-2 (Quidel Corporation, San Diego, CA, USA). Details of the measurement procedure have been presented earlier (III) (204). The short-term reproducibility with repositioning (determined on the same day by a single technician) expressed as CV% was <1.2%.

TABLE 1 Scanned sites and obtained variables of the bone measurements.

Device	Scanned sites	Analyzed sites	Variables
DXA	Lumbar Spine	L2-L4	BMC, BMD, BA
	Left Femur	FN and TF	BMC, BMD, BA
	Total Body	TB	BMC, BMD, BA
pQCT	Distal Radius	Total Bone	BMC, CSA, vBMD
	Middle Radius	Total Bone	BMC, CSA, vBMD
		Cortical Bone	BMC, CSA, vBMD
	Tibial Shaft	Total Bone	BMC, CSA, vBMD
		Cortical Bone	BMC, CSA, vBMD, Cth
QUS	Left Heel	Heel	BUA

#### *Bioimpedance assessments*

InBody (720) (Biospace Co., Ltd, Korea) and Tanita BC 418 MA Segmental Body Composition Analyzer (Tanita Corp., Japan) devices were used to estimate FM%, intra cellular fluid (ICF) and extra cellular fluid (ECF) (Table 2) (V). FFM was estimated by multiplying TBW by 0.73. Details of the measurement procedure have been presented earlier (IV and V). Precision of the repeated measurements expressed as CV was, on average, 0.6% (InBody) and 0.3% (Tanita) for FM%.

TABLE 2 Scanned sites and obtained variables of the body composition measurements.

Device	Scanned sites	Analyzed sites	Variables
DXA	Total Body	TB	FM, LM, BM, FM%, FFM
		Arms	FM, LM, BM, FM%
		Legs	FM, LM, BM, FM%
		Trunk	FM, LM, BM, FM%
		Android	FM, FM%
		Gynoid	FM, FM%
		ROI1	FM, FM%
		ROI2	FM, FM%
		ROI3	FM, FM%
		InBody	Total Body
Tanita	Total Body	TB	FFM, FM%

### 4.3 Statistical analysis

Data were checked for normality by Shapiro-Wilk's W test and for homogeneity by Levene's test before each analysis. A p-value of less than 0.05 with 2-tails was considered statistically significant. The correlation among FM% values measured by the different devices was estimated using Kendall Tau's correlation (V). Longitudinal correlations were used to analyze the associations between body height as the dependent variable and the body segment's width and length as the independent variable (I). The agreement in FM% between the two BIA devices and DXA were checked by Bland and Altman analysis (205) (V). Using regression analyses differences were compared between the 3 devices with regard to age and the influence of age in differences obtained

between Tanita and InBody with BMI and physical activity levels (V). One-way ANOVA with Tukey test or Kruskal-Wallis ANOVA (when data was not normally distributed) with Mann-Whitney test were used to test the differences in FFM and FM% (V), anthropometric variables (III), bone variables (III) among the groups. Analysis of covariance (ANCOVA) with height as covariate at 7-year follow-up and Tukey post-hoc tests were used to test the differences in bone (III) and body composition (IV) variables among the LTPA groups. Mann-Whitney U test (when data was not normally distributed), and independent t-test were used to compare gender differences and two groups by physical activity. A hierarchical linear model with random effects was employed to explore the growth patterns of height, weight, BM, LM, and FM (II), and of width and length of different body segments (I). Detailed description of the model has been given in the original articles (I and II). Statistica for Windows (StatSoft Inc., Tulsa, OK), SPSS and MLwiN (Multiple Project, Institute of Education, University of London, UK) software packages were used to perform the statistical analyses.

## 5 RESULTS

### 5.1 General characteristics

**Table 3** presents the basic physical characteristics of the girls. Those in the intervention group (n=101) were younger ( $17.9\pm 1.0$  vs.  $18.6\pm 1.0$  years) and their mean age at menarche was greater ( $13.4\pm 1.0$  vs.  $12.6\pm 1.3$  years) than those in the non-intervention group at the end of the study (n=134) ( $p<0.001$ ). There was no significant difference in body height, mass and BMI between the intervention and non-intervention groups. According to the criteria of Cole and colleagues (206), 12.4% of the girls were overweight and obese.

TABLE 3 Basic characteristics of the girls throughout the study

	Girls scr-84m		Girls 0-84m	
	scr	84m (Year 7)	0m	84m (Year 7)
N	1381	235	257	101
Age (yrs)	11.1 (0.9)	18.3 (1.1)	11.2 (0.8)	17.9 (1.0)
Height (cm)	146 (9.0)	166 (5.6)	146 (8.0)	165 (6.0)
Mass (kg)	39.4 (9.4)	60.3 (9.9)	39.2 (8.7)	58.9 (10.3)
BMI (kg/m <sup>2</sup> )	18.0 (3.0)	21.9 (3.1)	18.3 (2.9)	21.5 (3.3)
Menarche age (yrs)*		13.0 (9.0-17.1)	13.3 (10.6-17.1)	13.2 (10.6-17.1)

Scr: screening phase of the study, 0m: baseline, 84m: 7-year follow-up.

\* Reported as median and range.

**Table 4** presents the physical characteristics of the adult study populations. Men weighed more, were taller and had higher BMI than women ( $p<0.05$ , respectively). Grandmothers were older, shorter and had higher values of BMI than both the pre- and post-menopausal mothers ( $p<0.001$ ) (I).

## 5.2 Tracking of body composition

Most of the girls remained in the same tertile of BM, LM and FM at follow-up as they had been in as 11-yr olds at baseline (**Figure 10**).

TABLE 4 Basic characteristics of the adult subjects

	Study V		Studies I-II		
	Men	Women	PreM	PostM	GM
n	82	86	141	107	149
Age (yrs)	54.2 (11.0)	56.1 (11.7)	45.1 (4.1)	49.3 (4.9)	70.1 (6.6)‡
Height (cm)	176 (6.5)*	163 (6.1)	165 (5.8)	165 (6.0)	160 (5.2)‡
Mass (kg)	82.3 (11.0)*	68.2 (12.5)	69.7 (14.0)	70.3 (11.5)	72.4 (12.8)
BMI (kg/m <sup>2</sup> )	26.5 (3.2)*	25.5 (4.6)	25.6 (4.7)	25.8 (4.4)	28.4 (4.8)‡

\* Differences between men and women  $p < 0.05$  (V); † differences between grandmothers and mothers  $p < 0.001$  (I and II).

PreM: pre-menopausal mothers, PostM: post-menopausal mothers, GM: grandmothers.

For BM, 24% of the girls were in the highest, 21% in the middle, and 23% in the lowest tertiles both at baseline and follow-up. Two percent of the girls changed their tertiles from high to low and 3% from low to high during the 7 years. For LM, 19% of the girls were in the highest, 18% in the middle, and 23% in the lowest tertiles both at baseline and follow-up. Six percent of the girls changed their tertiles from high to low and 1% from low to high during the 7 years. The proportion of girls who changed by only one tertile varied from 4 to 13%. In the case of FM, 20% of the girls were in the highest, 14% in the middle, and 18% in the lowest tertiles both at baseline and follow-up. Four percent of the girls changed their tertiles from high to low and 3% from low to high during 7 years. For BMI, 19% of the girls were in the highest, 15% in the middle, and 20% in the lowest tertiles both at baseline and follow-up. Four percent of the girls changed their tertiles from high to low and 3% from low to high during 7 years. In FM%, 19% of the girls were in the highest, 14% in the middle, and 17% in the lowest tertiles both at baseline and follow-up. Four percent of the girls changed their tertiles from high to low and 5% from low to high during 7 years.

Girls at the age of 18 years were similar in height to their mothers but they were 9kg lighter and their BMI was 3.7 units lower than that of their mothers ( $p < 0.001$ , respectively). In body composition girls had 6% less BM, 9% less LM, and 21% less FM than that of their mothers' ( $p < 0.001$ , respectively) (II).

### 5.2.1 Bone growth

Timing of peak growth velocities of bone and body segment lengths and widths is summarized in **Figure 11**. Femur length peaked the earliest at 21 months before menarche, followed by the shoulder width, humerus, tibia and radius length at around 18 months before menarche. The growth velocity of lesser pelvis and greater pelvis width peaked at 13.5 and 11.5 months pre-menarche, respectively, followed by the trunk length at 12 months pre-menarche. These results indicate that the peripheral limb bones peaked about 5 months earlier than central trunk ones (I).

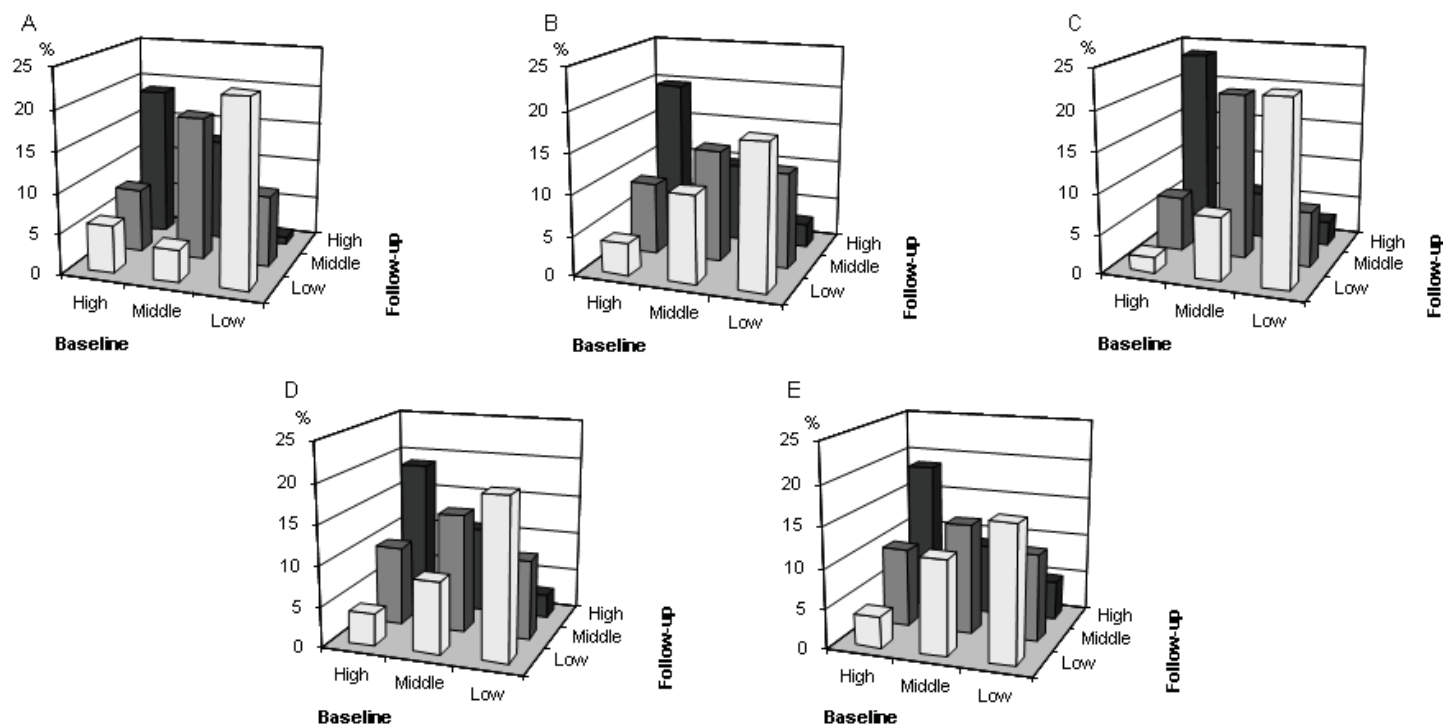


FIGURE 10 The proportion of subjects that were in the lowest, middle and highest tertile of DXA measurements for (A) LM, (B) FM, (C) BM, (D) BMI and (E) FM% as 11-year-olds that were in the lowest, middle and third highest tertile of DXA measurements as 18-yr olds. The majority of children, who were in the lowest and highest tertiles as 11-year-olds, remained in the lowest and highest DXA tertiles as 18-year-olds.

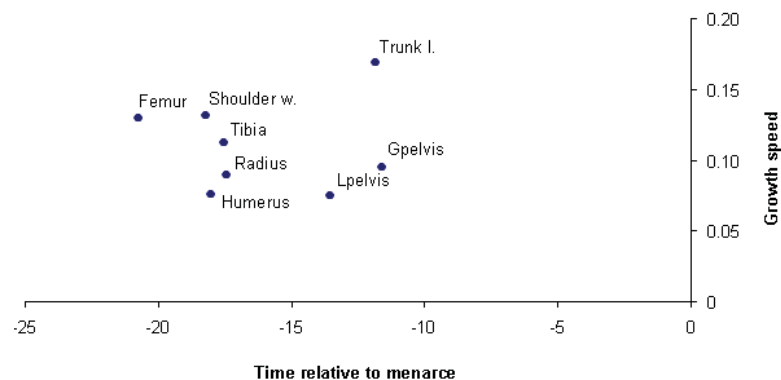


FIGURE 11 Timing of peak growth velocities of bone lengths and widths. The median of age at menarche was 13.0 (SD: 1.11 yr) years-of-age.

Girls had similar values at age 18 for shoulder width, and femur, tibia, humerus and radius length compared to their pre-menopausal mothers. They had 5% narrower greater pelvis, 7% narrower lesser pelvis and 2% longer trunk length than their pre-menopausal mother ( $p < 0.001$ ). There were no differences in any of the measured bone and segment length and width variables between the pre- and post-menopausal mothers. Grandmothers had 3% wider greater pelvis, and 5% shorter trunk length, 3% shorter tibia and radius length compared to the post-menopausal mothers ( $p < 0.01$ ) (I).

### 5.2.2 Muscle and fat mass accrual

The growth patterns of LM, BM and FM as a function of time relative to menarche in the girls are presented in **Figure 12**.

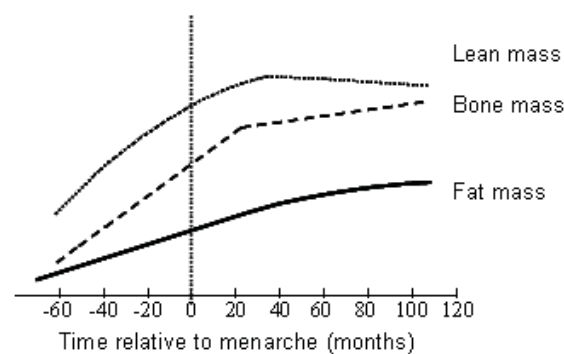


FIGURE 12 Growth curves for whole body lean mass, bone mass, and fat mass in girls. The X-axis is the time relative to menarche (months). The lines indicate the best fitting line estimated by a hierarchical linear model with random effects. (Lean-, bone, and fat mass in arbitrary units (not plotted to same scale).

Lean mass increased in a curvilinear way from pre-puberty until 3 years after the onset of menarche, followed by a plateau until adulthood. Bone mass

increased linearly until 2 years after the onset of menarche, followed by a slower rate of increase into adulthood, while FM increased continuously from pre-puberty until adulthood, but with a slower rate at the end of the period.

The body weight of the girls was 13% (9 kg) less than that of their mothers owing to 6% (0.16 kg) less BM, 9% (3.7 kg) less LM, and 21% (5.2 kg) less FM (II).

### 5.3 Effect of physical activity

#### 5.3.1 Bone

The differences in bone mineral content of total body, lumbar spine, and total femur among the LTPA groups are shown in **Figure 13**. In BMC, girls in the  $G_{LL}$  group had significantly lower values than  $G_{HH}$  and  $G_{LH}$  at TB (8%), at L2-L4 (8%), and at TF (10% and 9%, respectively).  $G_{LL}$  was found to have significantly lower values of BMC (7%) than that of  $G_{HL}$  only at L2-L4.

The differences in bone mineral density of total body, lumbar spine, and total femur among the LTPA groups are shown in **Figure 14**. For BMD,  $G_{LL}$  girls had significantly lower values than  $G_{HH}$  and  $G_{LH}$  at TB (4% and 5%, respectively), at L2-L4 (6% and 4%, respectively), and at TF (9% and 8%, respectively).  $G_{LL}$  was found to have significantly lower values of BMC than that of  $G_{HL}$  at TB (4%) and L2-L4 (5%).

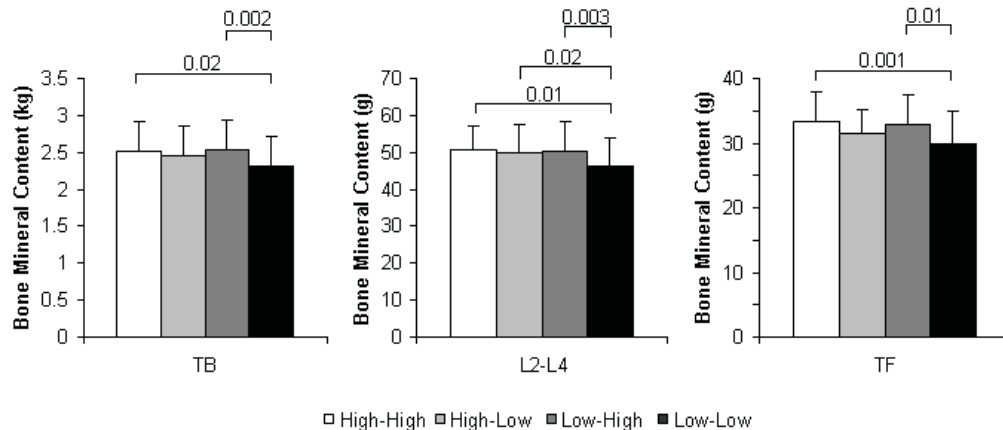


FIGURE 13 Comparison of BMC of total body (TB), lumbar spine 2-4 (L2-L4) and total femur (TF) among LTPA groups with body height as covariate at the age of 18 years. Error bars show SDs.

Of the pQCT measurements, the  $G_{LL}$  group had significantly lower values of BMC (7%) and CSA (9%) of distal radius, BMC, cBMC, and cCSA of middle radius (7%), and BMC (7%), cBMC (8%), cCSA (8%) and Cth (8%) of tibial shaft than the  $G_{LH}$  group (III).



In addition, a significant difference was found in BUA ( $p=0.013$ ) among the LTPA groups, but no significant differences were localized between any two specific groups (III).

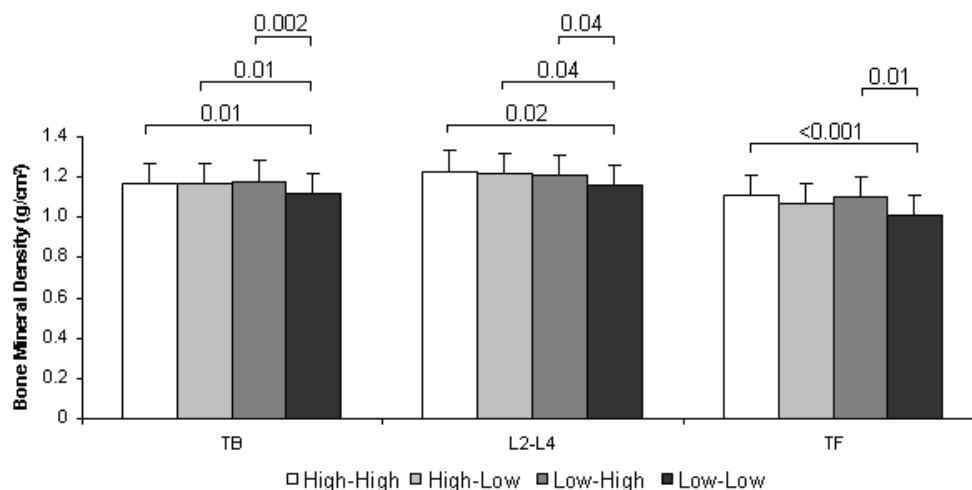


FIGURE 14 Comparison of BMD of total body (TB), lumbar spine 2-4 (L2-L4) and total femur (TF) among LTPA groups with body height as covariate at the age of 18 years. Error bars show SDs.

### 5.3.2 Lean mass and fat mass

The differences in lean mass and fat mass of total body, arms, legs and trunk among the LTPA groups are shown in **Figure 15**. In LM, girls in the  $G_{LL}$  had significantly lower values than  $G_{HH}$  and  $G_{LH}$  at TB (5% and 6%), arms (7% and 10%), legs (7%, respectively), and at trunk (4%, and 5%). Girls in  $G_{HL}$  also had significantly lower values than  $G_{HH}$  and  $G_{LH}$  at TB (5%, respectively), arms (4% and 7%), legs (6% and 7%), and at trunk (4% and 5%). There were no significant differences in FM at any site, however F-test revealed a significant difference in FM% of total body among the LTPA groups ( $p=0.04$ ); but no significant differences were localized between any two specific groups (IV).

There was a significant difference in mCSA ( $p=0.001$ ) among the LTPA groups, but no significant differences were localized between any two specific groups (IV).

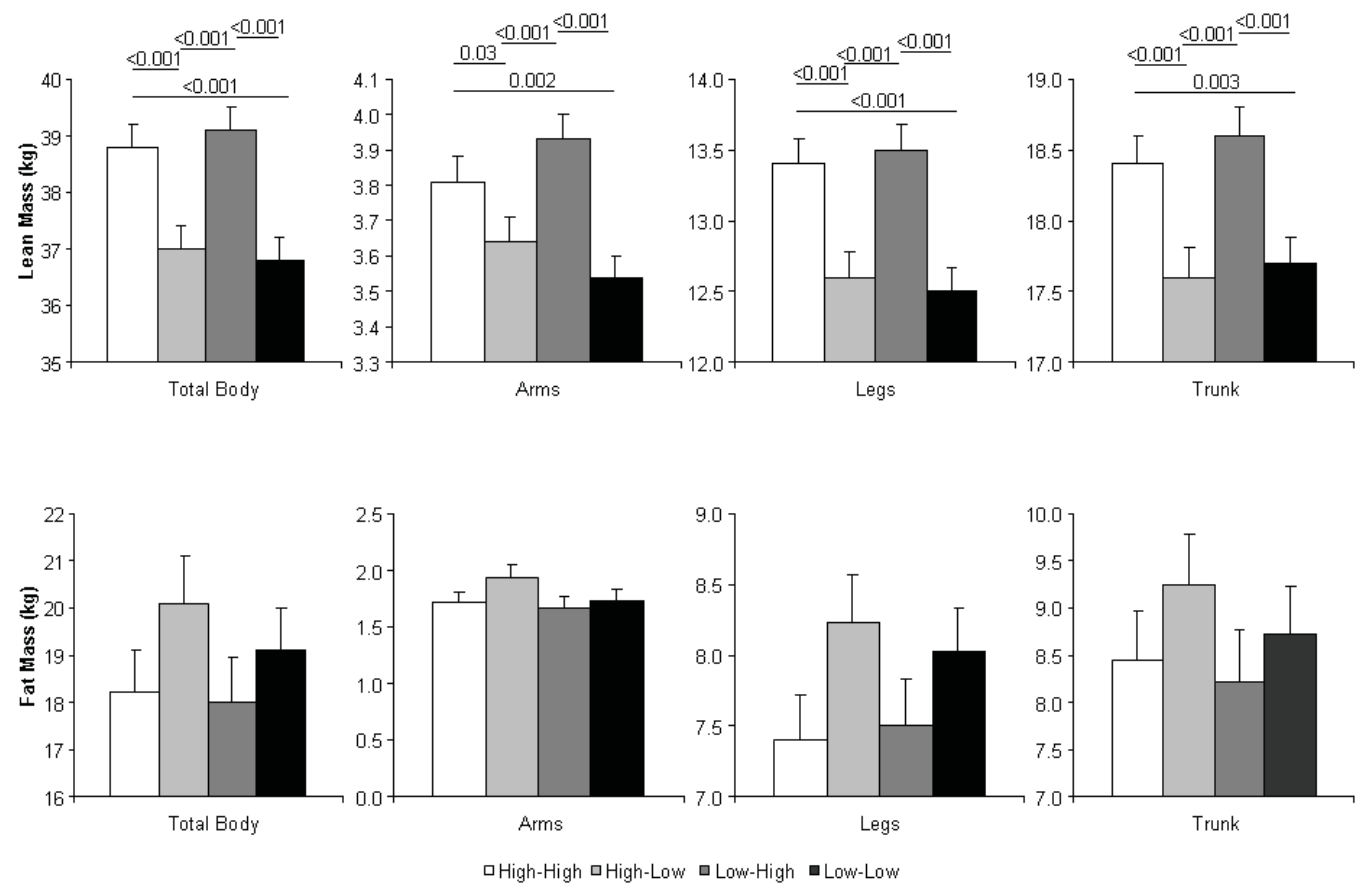
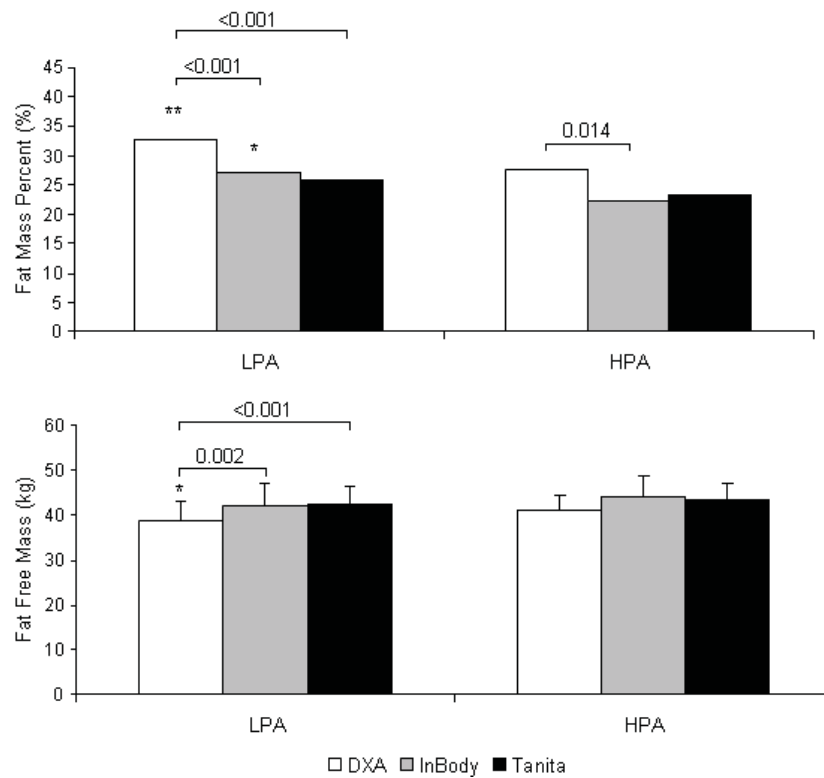


FIGURE 15 Comparison of LM and FM of total body, arms, legs and trunk among LTPA groups with body height as covariate at the age of 18 years. Error bars show SDs.

## 5.4 Comparison of different body composition assessment methods

Fat mass percent and fat free mass of the girls at age 18 ( $n=74$ , age:  $17.8\pm 1.1$  yrs, body height:  $164\pm 6$  cm, body weight:  $57.9\pm 8.7$  kg, BMI:  $21.4\pm 2.7$   $\text{kg}\cdot\text{m}^{-2}$ , menarche age:  $13.4\pm 1.0$  yrs), estimated using three devices according to PA categories are shown in **Figure 16**. In girls, the InBody device gave significantly lower estimates of FM% than DXA in both LPA (17%) and HPA groups (19%). The Tanita device also yielded significantly lower values of FM% than DXA (20%), but only in the LPA group. Both BIA devices gave higher estimates of FFM than DXA (InBody: +9%, Tanita: +10%), but only in the LPA group. Based on the BMI groups, both InBody and Tanita devices gave significantly lower estimates of FM% (-18% and -19%, respectively) and significantly higher estimates of FFM (+8%, respectively) than DXA in the normal group (**Figure 17**).



**FIGURE 16** Comparison of three methods in estimating fat mass percent (FM%) of total body (upper figure) and fat free mass (FFM) of total body (lower figure) among PA groups in girls. Error bars show SDs. \* Significantly different from HPA group  $p<0.05$ ; \*\* Significantly different from HPA group  $p<0.01$ .  $n=48$  for LPA and  $n=24$  for HPA.

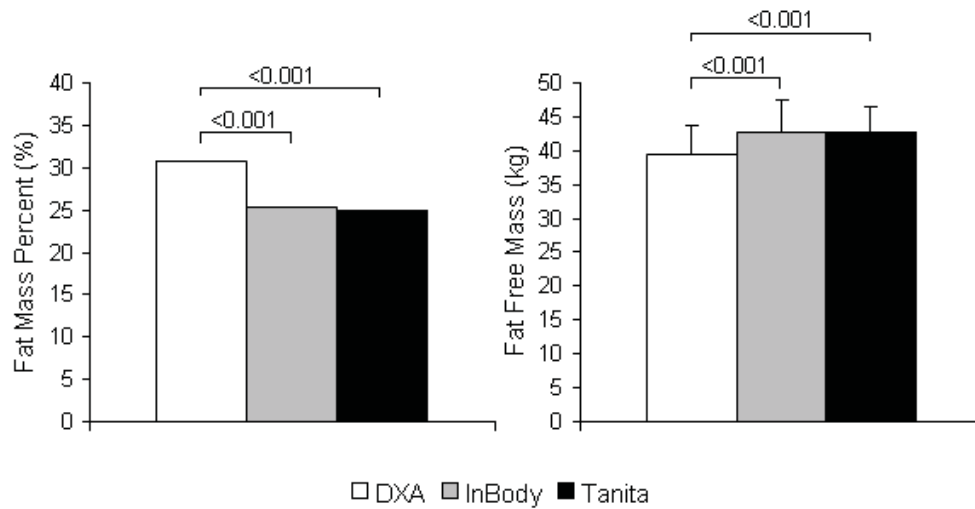


FIGURE 17 Comparison of three methods for estimating fat mass percent (FM%) of total body (left figure) and fat free mass (FFM) of total body (right figure) in girls with normal BMI ( $<25 \text{ kg} \times \text{m}^{-2}$ ). Error bars show SDs.

Girls' results at age 18 were comparable to those of the older women (from 38 to 81 years), except that there was no difference in the HPA group and only InBody gave higher estimate of FFM than DXA in women.

The results of Bland-Altman analysis with respect to the PA groups are shown in **Figure 18**. We found that the absolute difference in FFM between Tanita and InBody was zero, but a negative trend was found. In those who have less FFM, Tanita gives a higher estimate for FFM than InBody, while in those who have a bigger proportion of FFM, InBody gives higher estimates of FFM. In FM%, we did not find such phenomenon between Tanita and InBody estimations.

In both men and women, InBody gave significantly higher estimates of FFM than did DXA in normal group (+5% for men, and +8% for women) and in low physical activity group (+5% for men and +8% for women). Tanita provided significantly higher estimates for FFM than DXA only in the LPA group in both genders (+5% for men and +6% for women). Further, in women, both InBody (+8%) and Tanita (+7%) gave higher estimates for FFM than did DXA in the overweight group (V).

In both men and women, both InBody and Tanita gave significantly lower estimates of FM% than DXA in the normal (men: -26% InBody, -19% Tanita; women: -18% InBody, -12% Tanita), overweight (men: -16% InBody, -15% Tanita; women: -12% InBody, -11% Tanita) and LPA groups (men: -17% InBody, -16% Tanita; women: -13% InBody, -11% Tanita). Further, in the obese women both BIA methods yielded significantly lower estimates of FM% than DXA (-7% for both InBody and Tanita) (V).

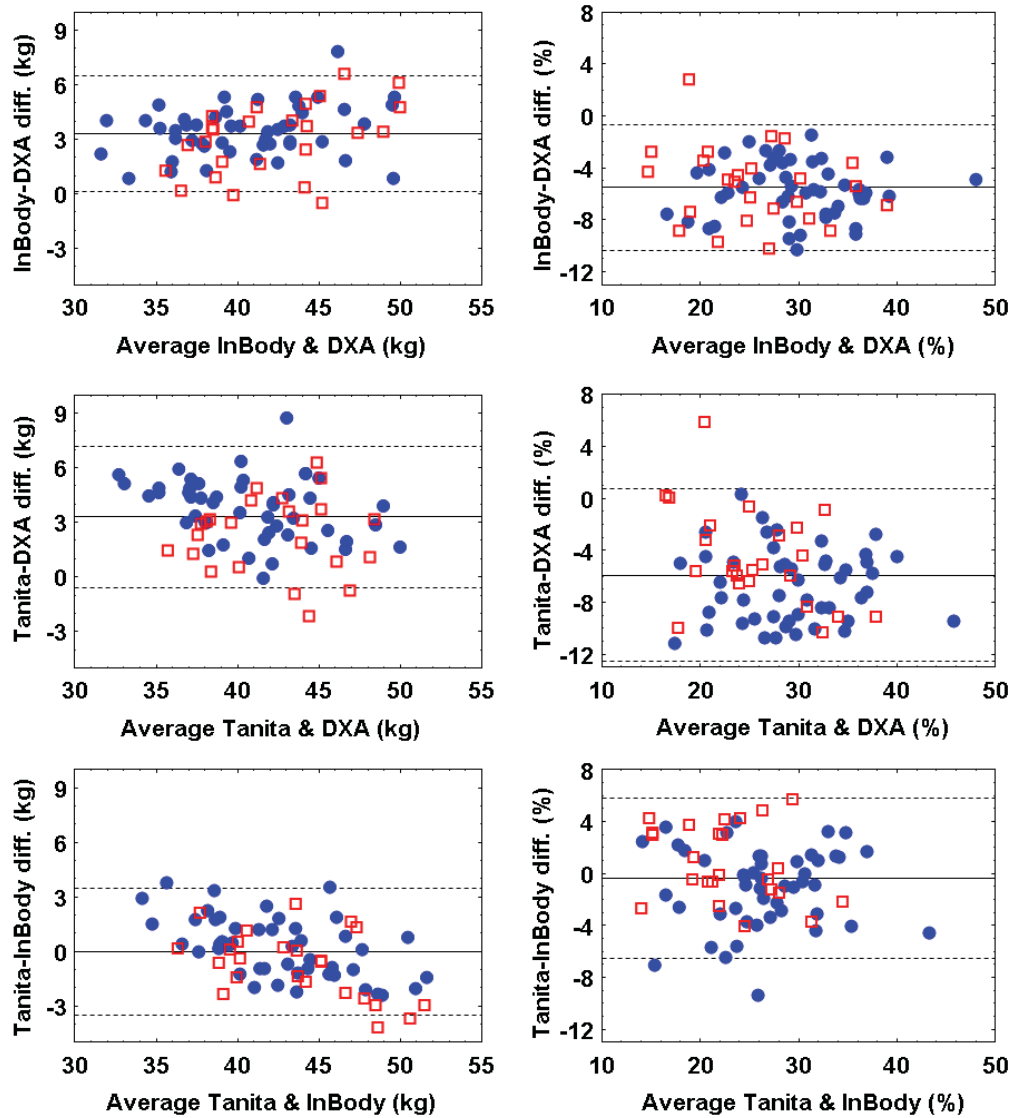


FIGURE 18 Bland-Altman analysis plotted for FFM (left panel) and FM% (right panel) among all methods and distinguished by PA group. The solid lines represent the mean and the broken lines represent  $\pm 2SD$ , and each dot represents an individual girl. Filled circles represent the low PA group (less than 4 times or 4 hrs/week), while open squares represent the high PA (at least 4 times and 4 hrs/week) group.

## 6 DISCUSSION

This longitudinal investigation of pubertal girls had three components. Firstly, the tracking and growth patterns of body composition and body segments were studied over a period from 11 to 18 years of age. Secondly, the influence of leisure-time physical activity on body composition was explored. Finally, methodological issues regarding the body composition devices, namely the DXA (Prodigy, GE Lunar) and BIA (InBody 720 and Tanita BC 418) machines, were investigated.

### 6.1 Tracking of body composition

The individual differences in BM, FM and LM were established before puberty. Tracking was stronger in BM than LM and FM during the 7-year follow-up. After 7 years, 78% of the girls remained in the same tertile in BM, 60% in LM, and 52% in FM. Our finding was consistent with previous reports in which 75% of those children who were obese at the age of 7 were also obese after 4 years (61) and girls had a 77% and 55% chance of staying in the highest and lowest quartile, respectively, of FM after 10 years (62). Guo and colleagues also reported that total FM, FM%, and LM track from childhood to young adulthood and from post-puberty to young adulthood (207). Low, but significant tracking was also reported for total FM and LM from early childhood to young adulthood (208, 209). These data together with the results of earlier studies (210, 211) suggest that it is possible to identify children who are prone to develop low or high BM, FM and LM. This finding supports the hypothesis that those children who are obese and overweight are at risk of being obese and overweight in adulthood (212).

Tracking is influenced by both heritable and non-heritable factors. Diet is a non-heritable but important contributing factor to body composition, especially to bone mass. The positive association between calcium and bone health has been widely described (130, 213, 214). High LTPA during puberty is also an independent predictor for low gain in FM (215, 216) or higher gain in

BM (99, 217) and LM (100). The bone trait variance in the population is mainly the result of the individual differences in genetics (211). Nguyen and colleagues (218) found that the estimated heritability of lumbar spine, FN, and total body BMD was 78%, 76%, and 79%, respectively. Heritability of FM and LM was 65% and 84% respectively. In the same study 72% of the intersubject variance of BMI was attributed to genetic factors (218). Our results suggested that BM (69%) was subject to the greatest genetic influence, followed by FM (57%) and LM (50%) (219).

The increase in BM was greatest near menarche while high LM and FM accumulation occurred much earlier before menarche. The girls did not show increased LM after age 16, but they had reached only 91% of their mother's LM values. The inter-relationship between the BM, FM and LM suggests that having a higher proportion of LM during growth can affect BM and the proportion of FM.

### 6.1.1 Bone growth

When longitudinal growth of long bones and trunk length slows down, the width of pelvis and shoulder continues to increase. Peripheral limb bones growth peaked about 5 months earlier than central trunk bones. Compared to Smith and colleagues (220), in our study the peak growth velocity of the non-weight-bearing long bones of the humerus and radius occurred 7 months later. This could be due to methodological differences in measuring limb bone length (radiographs vs. DXA scans) or to race/ethnicity differences (USA vs. Finland), or to factors such as climate, nutrition and physical activity. During the first phase of puberty, the limb bones grow first to define the individual's optimal height. The delay in central body growth could be related to sexual development and therefore to the preparation for reproduction. However, the widening of the great pelvis apparently continues steadily with age, as shown by the fact that the grandmothers had a wider pelvic girdle than the mothers, and the mothers had a wider great pelvis than girls at age 18. Weight gain and mass distribution may also be related to central body growth. It is also possible that the timing of bone lengthening and body segment widening are controlled by the same genes that control body size (203). In genetic studies of Caucasian females, heritability for height has been reported to be 68-84% (21), for spine length 65% (149), for tibia length 65-80% (148, 149), for femur length 73%, for humerus length 57%, and for radius length 68% (149).

Since osteoporosis is a major health concern and the increasing prevalence of this disease puts serious extra costs burdens upon the health systems in each country (73, 221, 222), it is crucial to maximize bone accrual during childhood and adolescence. Optimizing peak bone mass is critical to prevent fragility fractures in later life (74). Bone strength, and therefore fracture risk, depends on bone size, volumetric bone density, microarchitecture, and intrinsic bone tissue properties (72). Bone mineral content and bone mineral density are two of the most frequently assessed bone properties. In this study we found that BMC linearly increased until 2 years after the onset of menarche, followed by a

slower rate of increase into adulthood. A recent report (203) based on the same study population found that volumetric cortical BMD kept increasing throughout the whole follow-up period, but at a lower velocity 2 years after menarche (**Figure 19**). Peak growth velocity of the volumetric cortical BMD occurred exactly at the onset of menarche (203). These results are in line with other studies which have estimated that about 26% of adult whole-body bone mineral is accrued during the 2 years around peak growth and that about 35% of total-body and lumbar spine bone mineral content and over 27% of femoral neck BMC are laid down during the 4 years surrounding peak height velocity (PHV) (37, 40). In our study PHV occurred 13.5 months before menarche, while the fast growth period of BMC and BMD ended 24 months after the onset of menarche and for BMD it started 24 months before menarche. This suggests that the right side of the above mentioned window is 3 years after PHV and the left side is 1 year before PHV. Therefore, it is important to optimize nutrition and physical activity from early puberty through to 3 years after PHV.

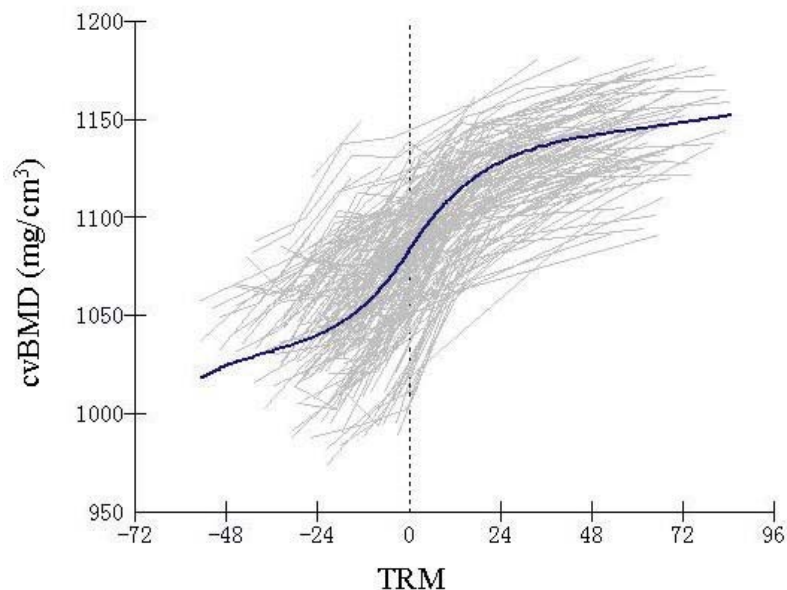


FIGURE 19 Growth pattern of volumetric cortical BMD in girls. Gray lines represent longitudinal change of each individual's values and the black line is the best fitting line derived from a hierarchical statistical model. The y-axis represents cortical volumetric bone mineral density and the x-axis represents time relative to menarche. Reproduced with permission from John Wiley and Sons, Xu et al. (203).



## 6.2 Effect of physical activity on body composition

### 6.2.1 Effect of physical activity on bone mass

Based on our results, long-term LTPA had a positive effect on bone mass gain at multiple bone sites in girls during puberty. In addition, girls who increased their physical activity during adolescence also benefited from bone mass gain. This result is in line another longitudinal study (99) which reported that active adolescent females had 9% and 10% more adjusted BMC at the total hip and FN, respectively, than their peers when they were adults. We found that those girls who were in the consistent LTPA group and in the increased LTPA group had 12% and 11% more BMC, respectively, at the TF than that of the consistently low LTPA group. A study based on a short-term high-impact school exercise intervention program in early childhood (223) showed that the intervention was still beneficial on hip BMC after 8 years. Another research group found an association between MET (metabolic equivalent of physical activity) scores and BMD at the FN (224). The osteogenetic effect of exercise is region-specific and load-dependent, but it is evident that higher levels of physical activity can increase bone health (225). The findings of this current study emphasize that not only can those girls who consistently maintain a high level of leisure-time physical activity benefit from bone gain but also those who increase their level of LTPA from an initially low level. We found that these latter girls started to increase their level of physical activity around menarche, in the middle of the fast growth window. This result supports the idea that the onset of menarche is a critical time when girls tend to change their behavior patterns (20). Studies have reported that physical activity has the greatest impact on bone during pre- and early pubertal periods (113, 226), and this is consistent with our earlier finding that prepuberty (Tanner stage I) is likely to be a more sensitive period for PA to exert or exhibit its beneficial effects on bone than peripuberty (Tanner stage II) (217). However, in postmenarchal adolescent girls, despite significant strength gains, there were no significant changes in total body and lumbar spine BMC after 26 weeks of resistance training (122). The effect of physical activity on bone mineral density might be site specific. It is also possible that exercise affect lumbar bone mineral density via FFM as it was shown in a Dutch study (227).

The type and duration of physical activity, in addition to its timing, can have important effects on bone development (217). In this study the most common types of LTPAs (the activities listed in the questionnaire in first place) were swimming (38%), cycling (26%), and horseback riding (13%) at age 11, and cycling (35%), walking (21%) and jogging (11%) at age 18 (IV). We also found that 68% of the total sample participated in a nonweight-bearing PA at age 11, but only 36% at age 18 (IV). The amount of weight-bearing exercise increased in all LTPA groups from baseline until the end of the study, but nonloading exercises still had a preponderance among those girls whose level of LTPA stayed at a low level throughout the study (IV). These results are in line with

others who found that impact PA was more efficient than non-impact PA for BMC accrual (217), and that high bone strains from weight-bearing exercises are the most influential on BMD accrual (37, 115, 116).

### **6.2.2 Effect of physical activity on lean mass and fat mass**

In the present study we found that girls with consistently high LTPA during puberty had significantly higher lean mass at various sites, including arms, legs, trunk and total body than their inactive peers. Girls who changed the level of LTPA from low to high between the ages of 11 and 18 years could also benefit from lean mass accrual by the age of 18 comparing to those who stayed at low level or dramatically decreased their LTPA during the study.

In a longitudinal study design, a Canadian research group (100) reported that habitual physical activity had a significant, independent effect on lean mass accrual during adolescence. In an earlier study (107), Parizkova found that regular training between age 11 and 18 increased FFM in boys. Tobias and colleagues (95) found, in a cross-sectional study design, that lean mass was positively related to the level of physical activity in 11 year old children. Goulding and colleagues showed that despite the fact that lean mass was continuously increasing throughout the Tanner stages, the distribution remained fairly constant (228). These results are consistent with our finding that girls who participated at least for 5 times/week and 5 hours/week in LTPA during puberty could benefit more from lean mass accrual.

Skeletal muscle, which is the largest tissue in the human body, plays an important role in locomotion, energy balance and in prevention and management of various diseases (229). Therefore, maintaining, increasing or slowing down the decline in lean mass with age thorough regular physical activity throughout life is a undoubtedly of key importance in remaining healthy.

We also found that high level leisure time physical activity during puberty has an effect on the amount and distribution of fat mass by age 18. This finding is important because obesity is strongly associated with a variety of chronic diseases, especially type 2 diabetes (230, 231), cardiovascular disease (230) and hypertension (232), certain cancers (232) and increased mortality (232). Furthermore in children, obesity is also linked to low self-esteem (233) and a higher level of anxiety (234). The influence of regular physical activity on FM and FM% is apparent in studies of overweight and obese children (103, 104), but the results from the normal population are not consistent as to whether physical activity or exercise can affect fat mass. Kriemler and colleagues (96) reported that a one academic year PA program could slow down the gain in BMI and skinfold thickness in 7- and 11 year old children. However, others (107, 108), (235) did not find positive effects of PA on FM in children. In cross-sectional studies it has also been reported that the level of PA was negatively associated with fat mass index (105) or FM% (106). Our overall analysis showed that there was a significant difference in FM and FM% among the LTPA groups, but we could not localize the differences between any two

specific groups. The lack of significant differences could be due to increased error in measurement or sample size limitations.

While school-based or nursery-based exercise or physical activity intervention studies have not revealed a significant effect on BMI (101, 102), studies with adults have showed that those females who were physically active at age of 16 gained BMI slower between 16 and 45 years and between 23 and 45 years than their less active peers (236). This result suggests that activity at around the age of 16 may have a prolonged benefit on weight maintenance. Guo and colleagues (76) found that the effect of physical activity on body composition was more profound in post-menopausal than in pre-menopausal women.

The cause and effect relationship between fatness and physical activity may not only be one-directional. It has been speculated that exercise and overweight may have a two-way causal pathway. It may well be that overweight people find it difficult to take part in higher intensity activities that would consume more energy (237).

A recent study has shown that the combined prevalence of overweight and obesity has decreased significantly in 2-year-old Finnish children and it remains stable in 15-year-old girls (9). However, the obesity epidemic still exists in other age groups. Higher education and level of physical activity are related to a lower prevalence of overweight (237), but between 1978 and 2002 the prevalence was increasing in all educational and PA groups (237).

### **6.3 Methodological considerations**

When assessing overweight or obesity, use of the body mass index (BMI) and waist-hip ratio (WHR) can give misleading results. There are many different methods and devices available to estimate body composition, but each one has its own specific advantages and limitations. Therefore objective body composition assessments and comparisons are needed for the research and healthcare fields.

In this study, we compared two types of BIA devices and DXA in their ability to assess FM% and FFM in subjects with different levels of PA. We found that DXA provided higher estimations of FM% and lower estimations of FFM compared to both BIA devices in all comparisons. The two BIA devices yielded similar group means, but we found a non-systematic bias between the InBody and Tanita devices for estimates of FFM in girls and estimates for FM% in men and women (V). A validation study using the same DXA device as used in this current study reported no significant bias of FM% in nonobese girls compared to the 4C method (238). This implies that both BIA devices used in this study may provide a true underestimate of FM% for girls. Other studies have also reported that BIA tends to overestimate FFM, thereby underestimating FM% in obese subjects and overestimating FM% in athletes (239-241). However, there

are studies that report that BIA is a valid estimator for body composition in healthy individuals (239, 242).

The Bland-Altman plots revealed discrepancies between the BIA devices and DXA for both FFM (3 kg) and FM% (5.5-6%) in absolute amounts, and with decreasing FFM for the Tanita-DXA comparison. Interestingly, the absolute difference between Tanita and InBody devices was highly dependent on the amount of FFM: Tanita tends to overestimate compared to InBody at lower levels of FFM, while InBody tends to overestimate compared to Tanita at higher levels of FFM. In studies that compared InBody (720) to DXA devices other than the Lunar Prodigy, Salmi (243) found that DXA overestimated FM% by 4.7% in overweight and obese subjects, while Demura et al. (244) reported only a 0.1% difference in a Japanese sample using a Lunar DP-X DXA. When the Tanita system was compared to the Prodigy, the BIA device underestimated total fatness by 5.0% in overweight and obese subjects (245), and by 1.5% in normal and obese subjects (246) compared to the Lunar DP-X.

The Tanita and InBody devices use different algorithms for determining FM%. Tanita incorporates the level of physical activity age, height, gender and impedance into the equation. In contrast, the InBody uses only the electrical properties obtained from the BIA device. We found in our study (V) that both age and gender contributed to the difference in FM% between InBody and Tanita. Further reasons for the discrepancy between the BIA devices could be that the hydration levels differ between very active and relatively inactive persons (26) and they may have different distribution of FFM and FM. The arms and legs provide 85% of the total body impedance but only 35% of total body volume (27). Thus, PA levels could conceivably affect estimation of FFM and, therefore, FM%.

Those under rigorous PA training may have a different distribution of FFM and both DXA and InBody could detect significant differences in FM% between the LPA and HPA groups, while the Tanita could not. Therefore the question arises of whether the selection criterion for the athletic mode in Tanita is accurate or whether it might in fact be a source of additional error.

## 6.4 Limitations of the study

This study is a prospective longitudinal study using a sample from an intervention study (the Calex study). Pooling the intervention and non-intervention subjects will not have influenced the outcome of this current investigation, since there were no intervention effects on bone growth and body composition, as has been reported earlier (130) (I-IV).

Although this study was rather wide ranging, it has a number of limitations. It is difficult to establish the precise physiological mechanisms that drive the developmental adaptation of human body composition from puberty to early adulthood on account of the array of potentially confounding factors (247). The high drop-out rate in the follow-up assessment was mainly due to

relocation. However, by using hierarchical longitudinal models we were able to cope with this since it allows inclusion of data from every subject regardless of missing data. A limitation of the hierarchical modeling approach was that we were unable to statistically compare the peak growth velocity times.

Another limitation of our study is the use of a questionnaire to assess LTPA. While questionnaires have been reported to be the most practical method to estimate PA in large populations (248), subjects may tend to over report their PA (249) and therefore it may not reflect the actual level of PA. However, Kurtze and colleagues have shown that a PA questionnaire can be reproducible and provide a useful measure of LTPA (250). Accelerometers or heart rate monitors may provide more accurate estimates of physical activity, but these methods were not feasible because of the longitudinal study design. Even though we have information about the girls LTPA from more measurement time points, we were unable to analyze such data because of the large number of participants who dropped out.

In paper I, our use DXA as a reference measurement is a limitation because it is not generally considered as a “gold standard” for body composition assessments. Difference between manufacturers, beam configurations and technology in DXA devices are all potential sources of measurement variations, and therefore our results apply only to the specific devices used in the study.

In this study population, 12.4% of the participants were classified as overweight/obese using the system of Cole and colleagues (206). This was similar to the prevalence of overweight/obesity (11%) published by the Finnish national report for female students (15 to 24 years of age) (251) but was less than reported by Saari and colleagues (23) in the latest growth reference data (17.9%). The reason for this is the different method used in the recent study (percentile vs. BMI cut-offs). Only one of our participants met the requirements for the obese category (1%), which is comparable to the report from Saari et al. (23) (1.8%). Thus the results of our study are applicable to females who are normal or overweight and representing Finnish general teenage population.

## 7 MAIN FINDINGS AND CONCLUSIONS

On the basis of this observational study, we conclude that:

- 1) Most of the girls remained in the same tertile in BM, LM, and FM at age 18 as they were in when they were 11-year-olds. Lean mass increased in a curvilinear way from pre-puberty until 3 years after the onset of menarche, followed by a plateau until adulthood. Bone mass increased linearly until 2 years after the onset of menarche, followed by a slower rate of increase into adulthood, while FM continuously increased from pre-puberty until adulthood, but at a slower rate at the end of the period. This indicates that it is possible to identify early those children who are prone to develop low or high BM, FM and LM later in life.
- 2) After the growth of body height and length of various body segments had ceased by age 18, body weight and segment widths continued to increase, although at a slower speed into adulthood. These results underscore the importance of early pubertal changes and give more information about peripheral limb- and the central trunk bones growth during the 4-year rapid growth window of puberty.
- 3) Girls whose leisure-time physical activity remained at a consistent high level or increased from low to high levels between age 11 and 18 benefited in terms of bone mass gain in multiple bone sites, lean mass accrual and less fat mass accumulation. Therefore it is important to encourage girls to exercise regularly, especially in weight bearing activity, even after menarche.
- 4) BIA devices (InBody720 and Tanita BC 418 MA) yielded systematically higher values of FFM than DXA (Prodigy, GE Lunar). These differences depended on gender, BMI and habitual recreational PA level which highlights the importance of taking these factors into account when evaluating body composition.

## TIIVISTELMÄ

Lihavuus ja osteoporoosi ovat kehon koostumuksen häiriöitä, joiden esiintyvyys on lisääntymässä. Lihavuus on lisääntynyt maailmanlaajuisesti sekä nuorilla että aikuisilla. Ylipainoisesta lapsesta tulee todennäköisesti ylipainoinen aikuinen, jolla on myöhemmin elämässä lukuisia lihavuuteen liittyviä terveysongelmia. Osteoporoosi on toinen merkittävä vanhemman iän terveysongelma, joka voi saada alkunsa jo lapsuudessa. Luun huippumassan optimointi kasvuiässä asianmukaisen ruokavalion ja riittävän fyysisen aktiivisuuden avulla on keskeinen strategia osteoporoottisten murtumien ehkäisyssä.

Tämän tutkimuksen tavoitteena oli määrittää kehon segmenttien sekä luiden, rasvan ja lihasten massan kasvumalleja ja selvittää vapaa-ajan fyysisen aktiivisuuden vaikutusta luu-, rasva- ja lihasmassan kertymiseen murrosiässä. Tutkimuksessa verrattiin myös bioimpedanssia (BIA) ja kaksiennergistä röntgenabsorptiota (DXA) rasvan ja rasvattoman massan arviointimenetelminä.

Tutkittavina oli 396 alun perin 10-13-vuotiasta tyttöä, 255 äitiä, 159 isoäitiä ja 82 miestä. Fyysinen aktiivisuus määritettiin kyselylomakkeen avulla ja kehon koostumus BIA- ja DXA-laitteilla. Luun mineraalitiheys mitattiin DXA:n ja perifeeristen luiden kvantitatiivisen tietokonetomografian (pQCT) avulla.

Tulokset osoittivat, että kehon segmenttien pituuskasvu oli loppunut 18 vuoden ikään mennessä, mutta kehon paino ja segmenttien leveydet lisääntyivät edelleen, joskin hitaammin. Useimmat tytöt pysyivät 11. ja 18. ikävuoden välillä samoissa luun, rasvan ja rasvattoman massan tertiileissä. Luumassa lisääntyi lineaarisesti 15 vuoden ikään asti, jonka jälkeen kasvunopeus hidastui. Rasvaton massa lisääntyi käyräviivaisesti 16 vuoden ikään asti, mitä seurasi tasannevaihe. Rasvan massa lisääntyi jatkuvasti 11. ja 18. ikävuoden välillä, mutta hitaammin seurantajakson loppupuolella. Pysyvä aktiivinen liikunnanharrastus kyseisellä aikavälillä oli yhteydessä useiden luukohtien ja rasvattoman massan kasvuun ja vähäisempään rasvan kertymiseen. Vastaavanlaisia myönteisiä tuloksia todettiin tytöillä, joiden aktiivisuus lisääntyi seurantajakson aikana. DXA- ja BIA-laitteiden välillä havaittiin systemaattisia, sukupuolesta, painoindeksistä ja fyysisestä aktiivisuudesta riippuvia eroja.

Tutkimuksen tulokset viittaavat siihen, että on tärkeää rohkaista murrosiässä olevia tyttöjä liikkumaan säännöllisesti. Tutkimus tuo esille uusia näkemyksiä murrosiän fyysisen kasvun monitahoisista ja dynaamisista malleista ja korostaa asianmukaisen liikunnan ja ravinnon merkitystä kyseisenä aikana.

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