Grow first, gain fat in the meantime. Longitudinal study of anthropometric changes around menarche

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Abstract: There is a long dispute among anthropologist over which factor is more important – skeletal maturation or energy accumulation – for menarche occurrence. Here we report results of longitudinal study conducted on the sample of 178 girls followed for the period from 2 years before to 2 years after the age of menarche. Each year during this period anthropometric measures of waist and hip circumference, pelvis breadth, subscapular, triceps and abdominal skinfold thickness were taken to document girls’ physical development. We found that changes in hip circumference and pelvis breadth were the most closely associated with menarche appearance. We also found that changes in anthropometric measures of lower body part preceded changes in anthropometric measures of upper body part and tended to peak one year before menarche occurrence while changes in upper body part tended to peak one year after menarche occurrence. These results suggest that both skeletal maturation and energy accumulation in the form of fat are equally important for menarche to occur. Furthermore, we are proposing a new indicator describing allometric changes of pelvis around menarche: hip circumference to pelvis breadth ratio (HCPBR).

Key words: menarche, body fat, hips circumference, pelvis breadth, HCPBR

Introduction

Menarche, an occurrence of the first menstrual bleeding, is a landmark of woman’s reproductive life. Its onset is proceeded by the complex physiological process of puberty that can be described as a continues transition from childhood to adulthood (Elias 2011). Puberty is initiated with increase in the activity of hypothalamic-pituitary-gonadal (HPG) axis induced by disinhibition of the GnRH pulse generator in the arcuate nucleus of the hypothalamus. High levels of GnRH stimulate secretion of gonadotropins from pituitary, which in turns induces final gonadal maturation, sex steroids production and the maturation of the gametes. Factors and physiological changes behind the disinhibition of GnRH puls
generator remains unclear and a series of different signaling systems have been identified as permissive for puberty onset (Ojeda et al. 2006).

The age of menarche varies widely both within and between populations and the source of this variation is not clear. Several studies reported delayed age of first menstruation in populations and individuals with poor nutrition (Günther et al. 2010, Simondon et al. 1997, Thomas et al. 2001 but see Maclure et al. 1991). In contrast, earlier menarche generally occurs in girls from populations with high-energy intake and increased prevalence of overweight and obesity (Gluckman & Hanson 2006, Thomas et al. 2001). Frisch and Revelle (1970) proposed that menarcheal age depends on a critical amount of fat stored in a female body. Sixteen kilograms of fat typically stored during childhood and puberty could provide additional energy required during the pregnancy and lactation. Thus girls who attain this amount of fat earlier should be ready for reproduction and start to menstruate earlier (Frisch & McArthur 1974). The mechanism behind this phenomenon could include leptin, a hormone produced by fat cells (Maffei et al. 1995, Wauters et al. 2000). This hormone acts in the brain to increase pulsatile rate of GnRH secretion. The increased activity of GnRH neurons turns on the hormonal changes that are necessary to begin reproductive period of life. Matkovic et al. (1997) suggested that to trigger reproductive ability in women, some critical blood leptin level is necessary. Indeed, in girls with low body fat, leptin deficiency and delayed menarche was observed simultaneously (Matejek et al. 1999). Studies show, that leptin levels rises dramatically before the menarche (Garcia-Mayor et al. 1997, Ahmed et al. 1999, Bandini et al. 2008). In a period of 6 month foregoing menarche, 28% increase in leptin levels was observed (Blogowska et al. 2005).

Recently, Lassek and Gaulin (2007) hypothesized that instead of total fat amount, it is fat distribution and in particular the ratio of fat stores in lower vs upper body part that is related to the age of menarche. This hypothesis was based on their own results showing that odds of menarche increases with increasing amounts of fat in the hips and buttocks and declines with increasing waist circumference and triceps skinfolds. They also claim that their hypothesis is supported by studies in which declining adolescent waist-to-hip ratio (Fredriks et al. 2005) and increasing hip circumference (Forbes 1992) during the time of menarche were observed. Additionally, hip circumference has been shown to be a significant predictor of blood leptin levels, while the waist circumference was not. Indeed, gluteofemoral fat produces more leptin and contains more mRNA leptin than abdominal fat (Nielsen et al. 2009, Papaspyrou-Rao et al. 1997).

In contrast, to the fat/leptin hypothesis, stands the view that the age of menarche is closely correlated to bone age (Marshall & De Limongi 1976). According to this hypothesis the reproduction in women should begin only when the appropriate stature and bone maturation is attained. In particular it is the bone pelvis size that is critical for the successful childbearing, thus the time of the initiation of reproductive period should be closely correlated with the age when the proper pelvis breadth is attained (Ellison 1982). However, as Ellison (1982) pointed out, due to several reasons it is difficult to discriminate between those two hypotheses. First, there is a signifi-
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Material and Methods

Study sample

Girls from the study sample participated in the longitudinal Wroclaw Growth Study conducted between years 1961 and 1972. They were included in the study at the age of 7 and further followed for the next 11 years. All girls were inhabitants of the city of Wroclaw in southwestern Poland (Bielicki & Waliszko 1975). The project started with 435 girls, however at the end of the examination period, the number of participants was reduced to 212 due to random dropout. In addition, only girls with completed data were included in analysis, thus total number of subjects dropped to 178.

Anthropometric measurements and age at menarche recording

The subjects underwent the anthropometric measurements in the spring (April–May) annually. Each year the following anthropometric measurements were taken by the trained study assistant: biilliac breadth (ic-ic) measured by caliper to the nearest mm, waist and hip circumferences measured by elastic steel tape to the nearest cm, triceps, subscapular and abdominal skinfold thickness measured by Lange skinfold caliper to the nearest mm. Waist and hip circumference were used to calculate waist to hip ratio (WHR). Hip circumference and biilliac breadth were used to calculate hip circumference to pelvis breadth ratio (HCPBR). HCPBR index reflects changes of hip circumference in relation to pelvis breadth. Slight, non-significant fluctuations of HCPBR with age around menarche should thus indicate parallel in-
crease in both traits with the same rate. In contrast, significant HCPBR increase should indicate faster growth of the hip circumference in relation to the pelvis breadth increase.

During each examination girls were asked if their first menstruation had already occurred. The girls had been instructed to register the exact date of their first menstruation thus, during the following examinations majority of them were able to report this date with the accuracy within one day (Koniarek 1971).

Statistical analysis

Changes of all features around menarche were analyzed in five one year age classes, two years before (–2, –1) and two years after menarche (1, 2). Zero was assigned to the year in which menarche occurred. Total and relative percent changes for anthropometrical parameters were calculated. Total percent change were calculated to depict absolute parameter change during the whole observation period (–2 to 2) and change from year –1 to 0 while relative percent changes were calculated to depict parameter changes in consecutive years related to the change during the whole observation period.

Natural logarithms of skinfold thickness and sum of skinfolds were included in the analysis due to highly rightly skewed distribution in all age groups as indicated by the results of Kolmogorow-Smirnow test. Univariate analysis of variance with repeated measurements was used to assess changes of anthropometric measurements around menarche. Post-hoc Tukey test was used to assess significance of anthropometric parameter changes between the consecutive years around menarche. Size effects were expressed by partial eta squared ($\eta^2_p$).

T-Student test for dependent samples was used to assess changes between age -1 and 0. All calculations were carried out in the STATISTICA 10.0 (Statsoft, 2011).

Results

Mean values of all analyzed traits in the five years around menarche (from 2 years before to 2 years after menarche) are shown in Table 1. All anthropometric characteristics except for WHR, which gradually decreased during the period around menarche, increased during this period and these increases were highly statistically significant. In particular, as indicated by the results of post-hoc Tukey test significant differences in the analyzed anthropometric parameters were observed in each age classes except for the differences between classes –2 and –1. Differences between these age classes were non-significant for abdominal and triceps skinfolds and for HCPBR. Differences in hip circumference, pelvis breadth and waist circumference had the highest effect size as revealed by ANOVA tests (Table 1). In addition, changes in these characteristics in the year preceding menarche (–1 to 0) had the highest significant difference as indicated by results of t-test (Table 1).

Furthermore, the difference between two Pearson’s correlation coefficients (from –1 to +1 years range) calculated for relationship between metrical age of menarche and hip circumference, and metrical age of menarche and pelvis breadth proved to be significant ($r=0.78$, $p<0.01$ for age and hip circumference, and $r=0.72$, $p<0.01$ for age and pelvis breadth, $z=-3.43$, $p<0.01$ for the coefficients difference). It means that significant changes in hip circumference and pelvis breadth are both strongly associat-
Table 1. Mean and standard deviation (in parenthesis) of 9 analyzed anthropometric features analyzed in one year age classes, two years before and two years after menarche in 178 girls (menarche appeared in 0 age class)

<table>
<thead>
<tr>
<th>Age class according to menarche</th>
<th>F</th>
<th>p</th>
<th>Partial $\eta^2$</th>
<th>t (-1 to 0)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-2</td>
<td>-1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pelvis breadth (ic-ic) (cm)</td>
<td>23.2 (1.65)</td>
<td>24.5 (1.49)</td>
<td>25.7 (1.43)</td>
<td>26.6 (1.40)</td>
<td>27.2 (1.40)</td>
</tr>
<tr>
<td></td>
<td>1361.59</td>
<td>&lt;0.001</td>
<td>0.885</td>
<td>20.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>57.7 (4.96)</td>
<td>59.8 (4.98)</td>
<td>62.2 (4.74)</td>
<td>64.3 (4.77)</td>
<td>66.1 (4.76)</td>
</tr>
<tr>
<td></td>
<td>538.11</td>
<td>&lt;0.001</td>
<td>0.751</td>
<td>15.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>73.6 (5.70)</td>
<td>78.0 (5.56)</td>
<td>83.3 (5.78)</td>
<td>87.5 (5.66)</td>
<td>90.8 (5.19)</td>
</tr>
<tr>
<td></td>
<td>1735.18</td>
<td>&lt;0.001</td>
<td>0.907</td>
<td>30.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.78 (0.04)</td>
<td>0.77 (0.04)</td>
<td>0.75 (0.04)</td>
<td>0.73 (0.04)</td>
<td>0.73 (0.03)</td>
</tr>
<tr>
<td></td>
<td>177.47</td>
<td>&lt;0.001</td>
<td>0.501</td>
<td>8.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCPBR</td>
<td>3.18 (0.19)</td>
<td>3.19 (0.18)</td>
<td>3.24 (0.19)</td>
<td>3.29 (0.19)</td>
<td>3.34 (0.18)</td>
</tr>
<tr>
<td></td>
<td>81.48</td>
<td>&lt;0.001</td>
<td>0.316</td>
<td>5.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triceps skinfold* (mm)</td>
<td>8.4 (3.81)</td>
<td>8.6 (3.61)</td>
<td>9.9 (4.01)</td>
<td>12.1 (4.34)</td>
<td>13.9 (4.56)</td>
</tr>
<tr>
<td>Median</td>
<td>7.5</td>
<td>7.6</td>
<td>8.8</td>
<td>11.2</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>179.05</td>
<td>&lt;0.001</td>
<td>0.508</td>
<td>6.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Subscapular skinfold* (mm)</td>
<td>6.0 (3.46)</td>
<td>7.0 (3.73)</td>
<td>8.4 (4.09)</td>
<td>10.5 (4.31)</td>
<td>12.2 (4.41)</td>
</tr>
<tr>
<td>Median</td>
<td>5.0</td>
<td>6.0</td>
<td>7.2</td>
<td>9.2</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>278.03</td>
<td>&lt;0.001</td>
<td>0.614</td>
<td>8.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abdominal skinfold* (mm)</td>
<td>10.0 (6.80)</td>
<td>10.3 (6.43)</td>
<td>12.7 (7.84)</td>
<td>16.6 (9.40)</td>
<td>18.8 (8.52)</td>
</tr>
<tr>
<td>Median</td>
<td>8.0</td>
<td>8.0</td>
<td>10.0</td>
<td>14.2</td>
<td>17.1</td>
</tr>
<tr>
<td></td>
<td>148.94</td>
<td>&lt;0.001</td>
<td>0.466</td>
<td>7.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>11.2 (1.07)</td>
<td>12.2 (1.04)</td>
<td>13.2 (1.05)</td>
<td>14.2 (1.01)</td>
<td>15.1 (0.93)</td>
</tr>
<tr>
<td></td>
<td>–</td>
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</tr>
</tbody>
</table>
ed with maturation of girls’ reproductive system. It also means that hip circumference is a better predictor of menarche than pelvis breadth.

Table 2 presents total percent change in anthropometric features in the period of 2 years before, and 2 years after the menarche occurrence (–2 to 2) and between one year before menarche and menarche year (–1 to 0). The highest total percent change during the observation period, was observed for the subscapular and abdominal skinfold thickness (increase by 102% and by 88.8% respectively). Similarly, changes in these anthropometric characteristics were also the highest during the year preceding menarche (19% and 23% for subscapular and abdominal skinfold respectively). In contrast, the lowest percent change during the whole observational period and in the year preceding menarche was noted for WHR and HCPBR (6.4% and 5.0% respectively). Interestingly, when changes in anthropometric features in consecutive years where related to the changes during the whole observational period (relative percent changes) the highest change in the year preceding menarche was observed for WHR and HCPBR (40.0% and 31.3% respectively) (Table 3).

In addition, while rate of change in hips and waist circumference tended to peak at the year preceding menarche occurrence (–1 to 0), rate of change in triceps, subscapular and abdominal skinfold thickness peaked at the year following menarche (0 to 1) (Fig 1).

Changes of pelvis breadth paralleled changes of the hip circumference until

<table>
<thead>
<tr>
<th>Total % change</th>
<th>–2 to 2</th>
<th>–1 to 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvis breadth (ic-ic)</td>
<td>17.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>14.7</td>
<td>4.0</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>23.3</td>
<td>6.7</td>
</tr>
<tr>
<td>WHR</td>
<td>6.4</td>
<td>2.7</td>
</tr>
<tr>
<td>HCPBR</td>
<td>5.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Abdominal skinfold</td>
<td>88.8</td>
<td>23.5</td>
</tr>
<tr>
<td>Triceps skinfold</td>
<td>65.0</td>
<td>14.5</td>
</tr>
<tr>
<td>Subscapular skinfold</td>
<td>102.0</td>
<td>19.3</td>
</tr>
</tbody>
</table>

Table 3. Relative percent of change in anthropometric features between all consecutive age classes

<table>
<thead>
<tr>
<th>Relative % change</th>
<th>–2 to –1</th>
<th>–1 to 0</th>
<th>0 to 1</th>
<th>1 to 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvis breadth (ic-ic)</td>
<td>322</td>
<td>29.7</td>
<td>22.6</td>
<td>15.5</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>24.8</td>
<td>28.2</td>
<td>25.6</td>
<td>21.5</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>25.3</td>
<td>30.6</td>
<td>24.7</td>
<td>19.3</td>
</tr>
<tr>
<td>WHR</td>
<td>20.0</td>
<td>40.0</td>
<td>40.0</td>
<td>0.0</td>
</tr>
<tr>
<td>HCPBR</td>
<td>6.2</td>
<td>31.3</td>
<td>31.2</td>
<td>31.2</td>
</tr>
<tr>
<td>Triceps skinfold</td>
<td>2.7</td>
<td>22.8</td>
<td>41.7</td>
<td>32.8</td>
</tr>
<tr>
<td>Subscapular skinfold</td>
<td>16.3</td>
<td>22.1</td>
<td>34.0</td>
<td>27.6</td>
</tr>
<tr>
<td>Abdominal skinfold</td>
<td>3.7</td>
<td>27.3</td>
<td>43.8</td>
<td>25.2</td>
</tr>
</tbody>
</table>

WHR – waist to hip ratio
HCPBR – hips circumference to pelvis breadth ratio
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Fig. 1. Relative percentage changes in waist and hip circumference and skinfolds thickness in the period of two years before to two years after menarche (menarche appeared at year 0)

Fig. 2. Pelvis breadth and hip circumference in the period of two years before to two years after menarche (menarche appeared at year 0)

*The range of the two left and right scales are proportionally the same and spread out to 35% of the minimal value of appropriate scale
the age of menarche (Fig 2). After that moment hip circumference increased further at the same rate while the rate of increase in pelvis breadth dropped down. Thus, no change of HCPBR was observed until the year preceding menarche (non-significant post-hoc Tukey test) when sharp increment in hip circumferences in relation to pelvis breadth has occurred. This resulted in sharp and statistically significant increase in HCPBR value (Fig. 3). Changes in HCPBR indicate the gradual arrest of pelvis skeletal maturation and stable increase in fat deposition in hip region occurs around the time of menarche. Simultaneously, faster increment in hip in relation to waist circumference resulted in WHR decrease around menarche (Fig. 3).

**Discussion**

Results of our longitudinal study confirm hypothesis that menarche occurs at the moment when skeletal maturation of pelvis decelerate while fat accumulation accelerate in adolescent girls. They indicate that pelvis bone maturation antecede accumulation of fat in lower body part to the large extent. Although both skeletal maturation and the amount of energy storage in the form of subcutaneous fat tissue are associated with age of menarche, rate of accrual in pelvis breadth decreases while in hips circumference resulted in WHR decrease around menarche (Fig. 3).
increases during the period of two years preceding menarche. These changes are illustrated by decrease in percent change of pelvis breadth and increase in percent change of hips circumference. They are also illustrated by sharp increase in value of HCPBR index in the year preceding menarche. Moreover changes in pelvic maturation and in deposition of fat in lower body part are similarly associated with occurrence of first menstruation, as indicated by similar effect size in repeated measure analysis of variance and results of the correlation analysis.

Furthermore our results indicate that hip circumference to pelvis breadth ratio (HCPBR), as an index proposed in this paper, can be a good predictor of oncoming menarche. This ratio appeared to be better predictor of menarche than widely established WHR. Both indices change around menarche, with WHR decreasing and HCPBR increasing around menarche. However, in contrast to WHR that decreases in relatively constant rate in the two years before and after menarche (Fig 3), HCPBR begins to increase only shortly before menarche and thus seems to better predict menarche occurrence than WHR.

In addition, our results confirm that accumulation of fat in lower body region antecedes accumulation of fat in upper body region. While changes in lower body part (hips circumference and pelvis breadth) tended to peak in the year preceding first menstruation, changes in upper body part (triceps and subscapular skinfold thickness) peaked in the year following first menstruation occurrence. This indicates that although fat distribution changes around menarche in general, it occurs first mainly in the lower part of the body.

Results obtained in our study are in agreement with results noted by other authors. Van’t Hof and Roede (1977) compared correlation coefficients for nine anthropometric traits measured at menarche in a large cohort of Dutch girls with correlation coefficients obtained from Monte Carlo statistical model where age of menarche was randomly redistributed over the sample. Differences between actual and random coefficients indicated that skeletal age, height and sitting height was critically associated while pelvis breadth moderately associated with age of menarche.

Similarly, Ellison (1982) on the longitudinal data of 67 girls measured semiannually from 8.0 to 18.0 years showed that menarche is associated with attaining appropriate skeletal maturation indicated by height velocity approaching menarche. He also found relatively smaller contribution of fat accumulation indicated by relative weight as a factor influencing menarcheal age. He demonstrated that menarche in this sample occurred at the average pelvis breadth of 26.2 cm (SD=1.7 cm). This value closely covers result obtained in our sample (25.7 cm, SD=1.43), although is slightly higher.

In contrast, recent results obtained by Lassek and Gaulin (2007) questioned skeletal maturation as an important factor associated with menarche occurrence. Using American data from the third National Health and Nutrition Examination Survey (NHANES III) these authors showed that fat accumulation in lower body part is more closely related to menarche than skeletal pelvis maturation. However, as pointed by authors themselves, their results should be interpreted with caution because data used in their study was cross-sectional. They also demonstrated general independence
of menarche from total body fat estimated based on subscapular and triceps skinfold thickness. This observation is in agreement with our previous (Koziel & Malina 2005) and current results indicating relatively smaller effect of changes in upper body skinfold thickness on menarche occurrence.

Although “critical threshold” hypothesis as proposed by Frisch and Revelle (1970) is not supported anymore (Bilewicz et al. 1976, Crawford & Osler 1975, Ellison 1981, Scott & Johnston 1982, Sherar et al. 2007), adipose tissue plays important role in reproductive process (Schneider 2004, Wade et al. 1996) and certain amount of fat might be necessary to support reproductive function in females. However, physical constrains must be overcome before energetic issues become relevant (Ellison 1982). Several studies demonstrated increased risk of birth complication, obstructed labors and stillbirth for teenage mothers due to fetopelvic disproportion (Clark et al. 1987, Hulka & Schaaf 1964, Lenhard et al. 2010, Malabarey et al. 2012). These evidences suggest that attaining certain level of pelvis development is crucial for the successful outcome of the reproductive event. We should thus expect that evolution would favor females that synchronize moments of skeletal maturation with sexual maturation (Ellison 1982).

While the presented hypothesis explains why pelvis development should antecede body fat storage, they do not explain why fat storage occurs mostly in hips and tights area (i.e. gluteofemoral fat tissue). As pointed by Pawlowski (2001) the use of fat stored at hips, as a metabolic fuel is very inefficient. Rate of lipolysis in this area is significantly lower than in omentum or even breasts (Arn er 1995). Thus it has to be other reason why fat is stored in such an amount in this region. He suggested that storing fat in this area is a way of counterbalancing the additional weight that is created by developing fetus and after the pregnancy by carried infant. It helps to keep low body mass center and prevent frequent falls that could be dangerous both for mother and her infant during the critical periods of reproduction (biomechanical hypothesis) (Pawlowski 2001).

These three evolutionary reasons that are not mutually exclusive, namely the need for adequate pelvic maturation, the need to secure energetic resources to allow successful reproduction and the need to establish mechanical balance during reproduction, can be responsible for almost parallel increase in pelvic breadth and lower body fat increase observed in our study.

Among limitation of our study, first imprecise moment of taking the measurements of body anthropometrics should be indicated. Although we knew the exact day of menarche (self-reported by the participants), we could not collect body measurements from this day. Thus the ratios (WHR and HCPBR) that we used in our study as one year before menarche (year –1) were in fact calculated based on the measurements taken somewhere between days –364 to –1 before the menarche. The same is true for the ratios for year 0 that can reflect anthropometry in the time range from day +1 to +364 after the menarche. However on average, the menarche took place in the midpoint of the period between the last measurement taken year before menarche (year –1) and the first measurement taken year after menarche (year 0). This means that we cannot be completely sure about HCPBR linear increase in the year period when menarche occurred. Al-
though HCPBR did not stop to increase after menarche, it is possible that it did not increase up to menarche and started to increase only when reaching menarche. In that case significant increase in HCPBR value would not be a predictor of menarche but rather the consequence of it. It is however unlikely as other studies independently demonstrated stable linear growth of pelvis bones (Volgyi et al. 2010) and decrease in WHR in the year preceding menarche (Codner et al. 2004).

In conclusion, our longitudinal study demonstrated parallel changes in skeletal growth of the pelvis and in lower body fat deposition that were almost equally predictive for menarche occurrence in young girls. We confirmed that skeletal maturation of pelvis antecedent deposition of gluteofemoral fat tissue. We also showed that hips circumference to pelvis breadth ratio could be used as a reliable predictor of upcoming menarche due to its specific pattern of change in the period around menarche. More investigation is however needed to confirm pattern of change in HCPBR observed in our study in other populations of adolescent girls.

Acknowledgement

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Authors’ Contributions

AZ drafted the manuscript, interpreted the results, carried out the statistical analysis; SK revised the manuscript and carried out the statistical analysis.

Conflict of interest

The authors declare that there is no conflict of interest.

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References


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