Recent Decline in Age at Breast Development: The Copenhagen Puberty Study
Lise Aksglaede, Kaspar Sørensen, Jørgen H. Petersen, Niels E. Skakkebæk and Anders Juul
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OBJECTIVE. Recent publications showing unexpectedly early breast development in American girls created debate worldwide. However, secular trend analyses are often limited by poor data comparability among studies performed by different researchers in different time periods and populations. Here we present new European data systematically collected from the same region and by 1 research group at the beginning and end of the recent 15-year period.

METHODS. Girls (N = 2095) aged 5.6 to 20.0 years were studied in 1991–1993 (1991 cohort; n = 1100) and 2006–2008 (2006 cohort; n = 995). All girls were evaluated by palpation of glandular breast, measurement of height and weight, and blood sampling (for estradiol, luteinizing hormone, and follicle-stimulating hormone). Age distribution at entering pubertal breast stages 2 through 5, pubic hair stages 2 through 5, and menarche was estimated for the 2 cohorts.

RESULTS. Onset of puberty, defined as mean estimated age at attainment of glandular breast tissue (Tanner breast stage 2+), occurred significantly earlier in the 2006 cohort (estimated mean age: 9.86 years) when compared with the 1991 cohort (estimated mean age: 10.88 years). The difference remained significant after adjustment for BMI. Estimated ages at menarche were 13.42 and 13.13 years in the 1991 and 2006 cohorts, respectively. Serum follicle-stimulating hormone and luteinizing hormone did not differ between the 2 cohorts at any age interval, whereas significantly lower estradiol levels were found in 8- to 10-year-old girls from the 2006 cohort compared with similarly aged girls from the 1991 cohort.

CONCLUSIONS. We found significantly earlier breast development among girls born more recently. Alterations in reproductive hormones and BMI did not explain these marked changes, which suggests that other factors yet to be identified may be involved. Pediatrics 2009;123:e932–e939

ENTERING PUBERTY IS an important milestone in reproductive life, and changes in the timing of puberty have been an area of great research interest for decades. A declining age at menarche was observed in industrialized European countries as well as in the United States over the last 100 years until the middle of the 20th century, when this trend seemed to cease, probably because of increasing stability of socioeconomic conditions, nutritional status, and hygiene (for review see ref 1).

In accordance with these findings, we recently reported a significant downward trend in the timing of puberty as determined by age at peak height velocity in a large cohort of 135 000 schoolgirls born between 1930 and 1969. However, timing of puberty seemed to have been stable for the last 30 to 40 years in industrialized countries until the recent reports of an unexpected further advance in age at breast development in American girls in 2 independent studies, the Pediatric Research in Office Settings study and the population-based National Health and Nutrition Examination Survey published in 1997 and 2002, compared with previous studies. Thus, breast development among American girls seemed to occur 1½ to 2 years earlier than the age expected according to textbooks.

Secular trend analyses, based on existing data for sexual maturation, are often limited by varying data comparability among studies in different populations, different periods of time, and the use of different methods such as...
palpation versus inspection of breast development. In line with this, the 2 American results have been debated, and many controversies have been raised. In the American studies, age at breast development in girls had declined significantly, but a decline of similar magnitude in age at menarche was not reported, suggesting that the duration of the pubertal transition had increased in that same period. However, because discrimination of breast (glandular) tissue from fat may be difficult, especially in overweight or obese girls with excessive subcutaneous fat, the risk of erroneous classification of breast development is significant if palpation is not performed.

Secular trend analyses have been conducted in several countries during recent decades. In Denmark, no downward secular trend in the timing of puberty was detected between 1964 and 1991–1993. However, conclusions vary from country to country, with some studies finding a tendency toward earlier pubertal development in girls and others finding no such trend.

Timing of puberty follows a familial pattern and, therefore, seems to be controlled by strong genetic factors, although environmental factors also must play a role. Thus, nutritional status, chronic diseases, migration to a healthy environment (as with foreign adoption), frequent infectious diseases, pollution, and exposure to chemicals with endocrine-disrupting properties are all likely candidates for influencing the endogenous endocrine milieu and, therefore, affect the differentiation and development of hormone-dependent reproductive tissues such as the breast (for review see ref 1). However, the etiologies of the apparent change in pubertal development found in American girls remain unknown.

We studied the timing of puberty by clinical evaluation of breast development and measurements of reproductive hormones in a large cohort of >2000 healthy European schoolgirls to look for possible secular trends in pubertal development in the period between 1991–1993 and 2006–2008 in the same geographical area of Copenhagen, Denmark.

MATERIALS AND METHODS

Study Subjects
A total of 2095 girls aged 5.6 to 20.0 years participated in the study that was conducted at schools in the Copenhagen area in 1991–1993 and 2006–2008. In 1991–1993, 1100 girls (the 1991 cohort), and in 2006–2008, 995 girls (the 2006 cohort) were examined by using similar methodologies and the same equipment. All girls in the randomly selected schools were invited to participate, resulting in an overall participation rate of ~40% in 1991–1993. Details from the 1991–1993 study have been published. In 2006–2008, the overall participation rate was 35%. However, the distribution of girls was skewed, with the majority of girls being 5.0 to 11.9 years old (n = 660 [43% of those invited]), whereas the remainder were between 12.0 and 20.0 years old (n = 437 [28% of those invited]). Thus, of a total of 3102 invited girls in the 2006–2008 study, 2005 declined to participate, resulting in 1097 girls who were examined. Of these, 102 were excluded from the final analysis because of chronic disease (n = 4) or because 1 or both parents originated from a non-European country (n = 98). Fifty three of the girls examined in 2006–2008 were part of a longitudinal study and were examined every 6 months. The median number of examinations for these girls was 3 (range: 2–4), resulting in a total of 1102 observations in the 2006 study.

Clinical Examination
Pubertal stages were assessed by clinical examination according to the methods of Marshall and Tanner, that is, breast stages (B) 1 through 5 (by palpation) and pubic hair stages (PH) 1 through 5 were evaluated. Standing height was measured to the nearest 0.1 cm by using a portable stadiometer (Holttain Ltd, Crymych, United Kingdom). Weight was measured on a digital electronic scale (model 707 [Seca Delta, Hamburg, Germany]) with a precision of 0.1 kg. Each child was weighed without shoes while wearing light clothing. BMI z scores were calculated by using the data from the 1991 cohort as a reference.

Laboratory Analysis
Blood samples were withdrawn from an antecubital vein between 8:30 AM and 1:00 PM and were available from 424 (estradiol was only available in 405) girls in the 1991 cohort and from 871 girls in the 2006 cohort. Sixty girls were excluded from the hormonal analyses because of intake of the contraceptive pill, leaving 811 girls from the 2006 cohort. There was no difference in height, weight, or ages at reaching B2 or menarche between the girls who had a blood sample taken and those who did not.

Blood samples were clotted and centrifuged, and serum was stored at −20°C until hormone analyses were performed. All samples were analyzed within a period of 3 weeks by using the same batch for each analysis. Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured by time-resolved immunofluorometric assays (Delfia [PerkinElmer, Boston, MA]) with detection limits of 0.06 and 0.05 IU/L for FSH and LH, respectively. Intra-assay and interassay coefficients of variation were <5% in both gonadotropin assays. Estradiol was measured by radioimmunoassay (Pantex, Santa Monica, CA [before 1998 distributed by Immuno Diagnostic Systems, Boldon, United Kingdom]). The detection limit was 18 pmol/L, and the intra-assay and interassay coefficients of variation were <8% and 13%, respectively.

Statistical Analyses
The data were cross-sectional, and we had prospective follow-up data for the 53 girls who participated in the longitudinal follow-up study in the 2006 cohort.

The cross-sectional data constituted a so-called current-status design in which each person’s age at entry into a certain puberty stage (including whether menarche had occurred) was unknown except for the fact that she had either already entered the stage, in which case the current age at examination was known to be an
upper bound for the true age at entry (left censored), or she had not yet entered the stage, in which case the current age was a lower bound (right censored). The follow-up data similarly yielded an upper bound, a lower bound, or an interval in which the true age of entry is contained (interval-censored data).

The age distributions at entry into the various stages of puberty were estimated by taking into account the current-status design as well as the prospective follow-up study design. See the article by Keiding et al for a description of the analysis of current-status data.

Two approaches were taken. With 1 approach we assumed a Gaussian distribution for the ages at entry into a puberty stage for the girls. This approach allowed using all the data, including the follow-up data, in the estimation and resulted in easily interpretable mean ages of entry into each puberty stage. With the other approach, the Gaussian assumption was omitted and the Turnbull estimator (a generalization of the Kaplan-Meier estimator) was used to estimate the age distribution function while allowing for interval-, left-, or right-censored data. The 2 approaches were in agreement, which allows for straightforward reporting of the results.

Results are presented as mean age at entry into the different pubertal stages along with a 95% prediction interval (PI) or confidence interval (CI). Standard likelihood theory in the Gaussian-based approach was valid and allowed computing P values for comparisons of the mean ages of various stages of puberty between the 2006 and 1991 cohorts.

In addition, for girls who progressed from stage B1 to B2 or more during follow-up, analysis results were verified by estimating the mean age at B2+ using for each girl the age between 2 examinations where breast development had started. Thus, the longitudinal data were used to the fullest rather than selecting a random sample of the actual data.

To assess whether the changes found in distributions of the ages at entry into the puberty stages between the 2006 and 1991 cohorts were not simply attributable to a changing BMI distribution for the 2 cohorts of girls, supplementary analyses were conducted including BMI as a covariate.

Reference curves for BMI and the reproductive hormones as a function of age were obtained for the girls examined in the 1991 cohort by using a local linear regression smoother. The data for BMI and estradiol were log-transformed, whereas LH and FSH were square root-transformed, to obtain normally distributed errors, and the mean curve of variance was estimated by smoothing of the observations and residuals, respectively. After calculation of the mean and SD on the transformed data, the data were back-transformed, which resulted in geometric means and the corresponding intervals corresponding to a ~95% PI (±2 SD).

Age-stratified comparisons of BMI and serum estradiol, LH, and FSH between the 1991 and 2006 cohorts were performed by Mann-Whitney tests.

### Ethical Considerations

The study was approved by the local ethical committee (KF 01 282214 and V200.1996/90) and conducted in accordance with the second Helsinki Declaration. All children and parents received written information and were invited to an information meeting. The study was presented as a study on growth and puberty timing. All participants and their parents gave informed consent.

### RESULTS

#### Pubertal Development

Estimated mean age at onset of breast and pubic hair development and mean age at menarche are presented in Table 1. Age at entry into pubertal stage B2 was significantly lower in the 2006 cohort compared with the 1991 cohort (P < .0001) (Table 1 and Fig 1). This difference remained significant (P < .0001) after adjustment for BMI. On average, breast development occurred 1.02 years (95% CI: 0.78 to 1.26) earlier in the 2006 cohort as compared with those in the 1991 cohort (P < .0001). PH2 and menarche occurred 0.20 years (95% CI: −0.013 to 0.42) (P = .066) and 0.29 years (95% CI: 0.04 to 0.55) (P = .023) earlier in the girls examined in the 2006 cohort compared with those in the 1991 cohort, respectively. Adjustment for BMI did not change these results.

<table>
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<tbody>
<tr>
<td>B2a,b</td>
<td>10.88 10.69–11.06</td>
<td>9.86 9.70–10.11</td>
</tr>
<tr>
<td>B3</td>
<td>12.40 12.23–12.56</td>
<td>10.97 10.82–11.12</td>
</tr>
<tr>
<td>B4+a</td>
<td>13.54 13.38–13.71</td>
<td>12.29 12.13–12.44</td>
</tr>
<tr>
<td>PH2e,f</td>
<td>11.29 11.13–11.46</td>
<td>11.09 10.95–11.23</td>
</tr>
<tr>
<td>PH3+e</td>
<td>12.39 12.23–12.55</td>
<td>11.74 11.59–11.89</td>
</tr>
<tr>
<td>PH4+a</td>
<td>13.51 13.34–13.68</td>
<td>12.50 12.32–12.67</td>
</tr>
</tbody>
</table>

a P < .001.
b P < .0001 after adjustment for BMI.
c P = .023.
d P = .1272 after adjustment for BMI.
e P = .066.
f P = .10 after adjustment for BMI.
The decline in age at PH2 and menarche was not as pronounced as the decline in age at breast development, and the time interval between B2 and menarche increased from 2.54 to 3.38 years.

**Reproductive Hormones**

Serum values of estradiol, FSH, and LH are shown in Fig 2 and Table 2. There was no difference between serum levels of estradiol between the 2 cohorts in the age intervals <8 years and between 10 and 12 years. In girls aged 8 to 10 years and >12 years, serum estradiol levels were significantly lower in the 2006 cohort (both P < .0001). When dividing the 2006 cohort into groups of prepubertal (B1) and pubertal (B2+) girls, we found higher serum estradiol levels in the pubertal girls in all age intervals, although this only reached statistical significance in the interval between 10 and 12 years (P < .0001) (Fig 3). All girls older than 12 years had reached puberty in the 2006 cohort. Serum FSH and LH levels did not differ in any of the above-mentioned age intervals.

**BMI**

There was no difference between BMI in the 2 cohorts when categorizing the girls into age intervals (<8, 8–10, 10–12, and >12 years) (Fig 2). BMI z scores were equal in the 2 cohorts (median BMI z scores: −0.06 and 0.02, respectively; P = .16).

**DISCUSSION**

In our large study on puberty development in Danish girls we found a substantial decline in the estimated age at onset of puberty during a recent 15-year period. Thus, the estimated mean age from which glandular breast tissue could be palpated decreased from 10.9 to 9.9 years in the 2006 cohort when compared with the 1991 cohort from the same geographic area. The estimated ages at first signs of pubic hair development and menarche also declined, although to a lesser extent than did the age at first sign of breast development. As a result of the uneven changes in early and late markers of puberty, the length of puberty seemed to have increased. A similar finding in a recent study of American girls caused considerable controversy.3–5 Interestingly, among the Danish girls, earlier breast development was not associated with higher levels of gonadotropins as a sign of earlier pubertal activation of the pituitary-gonadal axis. In contrast, there was a slight but significant decrease in estradiol levels among 8- to 10-year-old girls in the 2006 cohort compared with similarly aged girls from the 1991 cohort. This result suggests that our observations may not reflect earlier activation of the pituitary-gonadal axis but, rather, gonadotropin-independent estrogenic actions at the level of the breast. Because of the cross-sectional nature of our study, we cannot determine at what speed puberty will progress in each individual girl who presents with early breast development.

A crucial question is whether the early breast development could be a result of the current worldwide epidemic of obesity10,24,25 because there have been studies that showed that overweight children tend to undergo sexual maturation earlier than leaner children.26,27 In Denmark, the prevalence of childhood overweight and obesity has also increased.28–30 However, we did not find any difference between the BMI of the 2 cohorts of girls, and adjusting our data on puberty timing for BMI did not change the results. Therefore, we do not believe that the increasing incidence of obesity among children can explain our findings.

Another possibility is that increased exposure to endocrine-disrupting chemicals (EDCs) from modern lifestyle may be involved in the observed trends. Several studies have suggested that EDCs from the environment can influence puberty timing in experimental and wild animals.31 However, little is known about the possible role of EDCs in the timing and progression of puberty in humans. It is, however, a fact that children and adolescents are exposed to a large number of chemicals with endocrine-disrupting properties, and these have been demonstrated in fluids and tissues from children and adolescents.32–34 Theoretically, chemical agents could have either accelerating or decelerating effects on puberty. Several agents with estrogenic action (eg, bisphenol A), used in the manufacture of many industrial products including the interior lining of food and beverage cans, has been shown to alter mammary gland mor-
Exposure to such agents has also been shown to advance pubertal development in animals. However, the BPA area of research is very controversial, and other investigators have reported negative findings. Furthermore, the estrogenic potency of “natural” phytoestrogens present in our food (e.g., genistein from soy) is higher than most industrial chemicals. Exposure of prepubertal mice to phytoestrogens at levels reported in humans led to accelerated mammary gland development, and the effects were largest when the exposure happened close to the time when the mice were expected to...
enter puberty. However, it remains to be seen whether current exposure of humans to phytoestrogens such as genistein can induce breast development in prepubertal girls.

Humans are exposed to a high number of different endocrine-disrupting chemicals and often in tiny concentrations. The majority of hormone-disrupting chemicals seem to mimic sex steroids’ action or interfere with their metabolism. Thus, prepubertal gynecomastia in boys caused by the estrogenic and antiandrogenic activities of lavender and tea tree oils has been reported. However, other environmental agents such as polychlorinated aromatic hydrocarbons may, in contrast, slow pubertal development. The net result of exposure to a “cocktail” of various agents, therefore, is difficult to predict. However, animal data have suggested that there could be direct or indirect effects on breast physiology without a concomitant maturation of the pituitary resulting in a surge in gonadotropin levels. Another challenge is that we have limited knowledge on the importance of timing of exposure for effects on pubertal development. Recent data suggested that there are critical windows for effects during prenatal, perinatal, and pubertal periods. One reason for the increased sensitivity to estrogens in prepubertal girls may be that they are primed with low levels of endogenous estrogen. Theoretically, even the small addition of an exogenous compound with sex steroid activity, even at minute levels, may have significant effects.

The strength of our investigation is that the whole study was performed under leadership of the same pediatric research group, and identical methods and study designs were used in both the 1991 and 2006 cohorts. In addition, only European girls were included in this analysis, excluding confounding resulting from ethnic differences. Furthermore, all hormone analyses were conducted in the same laboratory and using the same hormone assays. Discrimination of breast (glandular) tissue from fat may be difficult, and palpation is necessary, especially in overweight or obese girls with excessive subcutaneous fat. Therefore, evaluation of breast development was performed by both inspection and palpation, which diminished the risk of error in distinguishing between breast and fat tissue. A potential limitation of our study was that not all children in the participating schools volunteered. However, for a study of this nature (including blood sampling), the participation rate was rather high (~35%) and was similar in the 1991 and 2006 cohorts. Furthermore, the pubertal changes associated with B2 are so subtle that knowledge by the girls

![FIGURE 3](image-url)  
Serum estradiol in relation to chronological age and pubertal stage in healthy schoolgirls. Prepubertal girls, orange dots; pubertal girls (B2 or higher), green dots.

### Table 2: Reproductive Hormone Levels in Girls Examined in 1991–1993 and 2006–2008

<table>
<thead>
<tr>
<th>Year</th>
<th>Age Group</th>
<th>n</th>
<th>% in B2+</th>
<th>Estradiol, Median (Range), pmol/L</th>
<th>FSH, Median (Range), IU/L</th>
<th>LH, Median (Range), IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991–1993</td>
<td>&lt; 8 y</td>
<td>50</td>
<td>0</td>
<td>18 (18–30)</td>
<td>1.06 (0.29–2.41)</td>
<td>0.05 (0.05–0.48)</td>
</tr>
<tr>
<td></td>
<td>8–9.9 y</td>
<td>60</td>
<td>2.3</td>
<td>24 (18–63)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.53 (0.28–4.44)</td>
<td>0.05 (0.05–0.23)</td>
</tr>
<tr>
<td></td>
<td>10–11.9 y</td>
<td>71</td>
<td>48.4</td>
<td>37 (18–1379)</td>
<td>2.66 (0.36–8.79)</td>
<td>0.13 (0.05–5.38)</td>
</tr>
<tr>
<td></td>
<td>&gt; 12 y</td>
<td>243</td>
<td>99.5</td>
<td>166 (18–1442)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.62 (0.06–12.69)</td>
<td>3.65 (0.05–24.48)</td>
</tr>
<tr>
<td>2006–2008</td>
<td>&lt; 8 y</td>
<td>119</td>
<td>3.4</td>
<td>18 (18–53)</td>
<td>1.09 (0.12–3.60)</td>
<td>0.05 (0.05–0.41)</td>
</tr>
<tr>
<td></td>
<td>8–9.9 y</td>
<td>182</td>
<td>24.4</td>
<td>18 (18–229)</td>
<td>1.39 (0.29–8.18)</td>
<td>0.05 (0.05–7.88)</td>
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<tr>
<td></td>
<td>10–11.9 y</td>
<td>223</td>
<td>75.1</td>
<td>31 (18–388)</td>
<td>2.80 (0.27–9.22)</td>
<td>0.19 (0.05–19.00)</td>
</tr>
<tr>
<td></td>
<td>&gt; 12 y</td>
<td>287</td>
<td>100.0</td>
<td>130 (18–1346)</td>
<td>4.32 (0.06–12.40)</td>
<td>3.7 (0.05–26.30)</td>
</tr>
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</table>

<sup>a</sup> P < .0001.
themselves or their parents about puberty development most likely did not influence the girls’ choice about participation. Finally, all participants were from areas in Copenhagen with similar social and economic backgrounds.

CONCLUSIONS
During an observation period of the recent 15 years we found significantly earlier breast development among girls living in Copenhagen. Reproductive hormones and BMI were similar and did not explain these marked changes, which suggests that other factors yet to be identified are involved.

ACKNOWLEDGMENTS
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REFERENCES


42. Sharpe RM. Perinatal determinants of adult testis size and function. *J Clin Endocrinol Metab.* 2006;91(7):2503–2505


44. Klein KO, Baron J, Barnes KM, Pescevitz OH, Cutler GB Jr. Use of an ultrasensitive recombinant cell bioassay to determine estrogen levels in girls with precocious puberty treated with a luteinizing hormone-releasing hormone agonist. *J Clin Endocrinol Metab.* 1998;83(7):2387–2389


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