Recent Changes in Pubertal Timing in Healthy Danish Boys: Associations with Body Mass Index

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Context: In the 1990s, the American population-based study NHANES III renewed the focus on possible secular trends in male puberty. However, no conclusions could be made on pubertal onset due to the lack of compatible data.

Objective: The aim of the study was to evaluate secular trends in pubertal onset during the recent 15 yr and their relation to body mass index (BMI) in boys.

Design and Setting: We conducted a cross-sectional study in 1991–1993 and a combined cross-sectional and longitudinal study in 2006–2008 (The Copenhagen Puberty Study) at a tertiary center for pediatric endocrinology.

Participants: A total of 1528 boys aged 5.8 to 19.9 yr participated (n = 824 in 1991–1993, and n = 704 in 2006–2008). Genital and pubic hair stages as well as testicular volume by orchidometry were evaluated. Blood samples were analyzed for LH, FSH, testosterone, and SHBG.

Main Outcome Measures: We measured age at onset of pubertal markers.

Results: Onset of puberty, defined as age at attainment of testicular volume above 3 ml, occurred significantly earlier in 2006–2008 [11.66 yr (11.49–11.82); mean (95% confidence interval)] than in 1991–1993 [11.92 yr (11.76–12.08); $P = 0.025$]. Significantly higher LH, but not testosterone, levels were found in the 11- to 16-yr-old boys from 2006–2008 compared to 1991–1993 ($P = 0.020$). BMI Z-score increased significantly from 1991–1993 [0.044 (−0.016 to 0.104)] to 2006–2008 [0.290 (0.219–0.361); $P < 0.001$]. Interestingly, pubertal onset and LH levels were no longer significantly different between study periods after adjustment for BMI.

Conclusions: Estimated mean age at onset of puberty has declined significantly during the recent 15 yr. This decline was associated with the coincident increase in BMI. (***J Clin Endocrinol Metab*** **95: 263–270, 2010)**

Age of sexual maturation is considered a marker of general public health. From the late 19th century to the mid 20th century a gradual decline in age at puberty has been reported in girls (1), after which this trend ceased most likely as a result of increased stability in socioeconomic conditions, nutritional status. and hygiene. However, in the mid 1990s, data from the Third National Health and Nutrition Examination Survey (NHANES III), an American population-based study, revived focus on possible current trends toward earlier age at puberty in both boys and girls (2–6). In NHANES III, boys had a markedly lower age at pubertal onset (2, 3, 5) than previously reported from the United States (4, 7–9). However, due to lack of data on pubertal onset from the previous population-based study [Third National Health Examination Survey (NHES III)] (8), some controversy has ex-

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Abbreviations: BMI, Body mass index; CPP, central precocious puberty; CV, coefficient(s) of variation.

isted as how to interpret the NHANES III findings (2, 4–6). A secular trend analysis between NHES III and NHANES III did not find persuasive evidence in favor of earlier age at puberty, although some indications were present in non-Hispanic white boys (4). An expert panel has subsequently concluded that the currently available data are insufficient in quality and quantity to confirm a recent change in pubertal timing in U.S. boys (1). In addition, the incidence of idiopathic central precocious puberty (CPP) has not been reported to increase in the United States, and lowering the age limit for the diagnosis of CPP has, in contrast to girls, not been recommended in boys (10). Contemporary European studies (11–15) have reported markedly higher ages at pubertal onset in comparison with NHANES III. However, only a limited number of secular trend studies exist from Europe, and those available do not support a secular trend toward earlier age at pubertal onset in boys from the mid 1960s to the late 1990s (11, 13). In accordance, Danish data on the incidence of CPP have recently been shown to be stable (16). However, we recently demonstrated a decline in age at voice break in Danish choir boys over a 10-yr period crossing the millennium (17), indicating a recent trend toward earlier onset or faster progression of puberty.

The current obesity epidemic has received special attention with respect to earlier pubertal development due to the coincident time periods at which these changes have occurred (18–21). Despite increasing evidence in support of a link between increased adiposity and early onset of pubertal markers in girls (7, 18), data in boys are more ambiguous (7, 19, 22, 23). No previous studies have evaluated the influence of adiposity on pubertal onset based on testicular volume, despite being considered a more reliable measure of pubertal timing (1, 24). In addition, the onset of testicular growth is preceded by activation of the hypothalamic-pituitary-gonadal axis in boys (25, 26). Thus, determination of reproductive hormone levels may support the clinical evaluation of puberty. However, previous studies on secular trends in pubertal development in boys did not measure reproductive hormone levels to back up their findings (4, 11).

In the present study, we evaluated pubertal development by visual assessment of genital and pubic hair stages as well as orchidometry-estimated testicular volume in a large cohort of healthy boys to look for secular trends in pubertal onset in the same geographical area between 1991–1993 and 2006–2008. In addition, secular trends in pubertal onset were evaluated in association with body mass index (BMI) and reproductive hormone levels.

Subjects and Methods

Study subjects

A total of 1528 boys aged 5.8 to 19.8 yr participated in the Copenhagen Puberty Study that was conducted at schools in the same geographic area in Copenhagen in two time periods: 1991–1993 and 2006–2008. All boys from the selected schools were invited to participate. To avoid confounding from differences in ethnic makeup over the study period, all participants of non-Caucasian origin were excluded from the analyses. Participants with previous or current medical conditions potentially affecting pubertal timing were likewise excluded. In 1991–1993 (1991-cohort), a total of 824 boys were included for final analyses (overall participation rate, 35%). Other details have previously been published (13, 27, 28). In 2006–2008 (the 2006-cohort), a total of 3101 boys were invited to join the study, of which 767 boys were examined (overall participation rate, 24.7%). Of these, 63 boys were excluded due to disease (n = 1) or non-Caucasian origin (n = 62), leaving 704 boys for the final analyses. Sixty-three of the boys examined in the 2006-cohort were part of a longitudinal study and were examined every 6 months. The number of examinations for these boys ranged from two to four, resulting in a total of 835 observations in the 2006-cohort. All examinations in both cohorts were done using similar methodologies and equipment.

Clinical examination

Pubertal stages were assessed by clinical examination according to the methods by Marshall and Tanner (29). Testicular volume was estimated by palpation to the nearest 1 ml using Prader's orchidometer (30). In case testicular volumes of the two testes were not equal, the larger testis measurement was used. Pubertal onset was defined as a testicular volume of more than 3 ml (1, 9, 15). All evaluations of puberty were done by one of three pediatric endocrinologists, one of whom was involved in both cohorts.

Standing height was measured to the nearest 0.1 cm using a portable stadiometer (Holtain Ltd., Crymych, United Kingdom). Weight was measured on a digital electronic scale (Seca delta, model 707; Seca, Hamburg, Germany) with a precision of 0.1 kg. The children were weighed without shoes, wearing light clothing. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. BMI Z-score was generated from the Centers for Disease Control and Prevention 2000 reference (31). Overweight was defined as BMI from the 85th to below the 95th percentile and obesity as BMI at least in the 95th percentile. Blood samples were withdrawn from an antecubital vein between 0830 and 1300 h and were available in 344 boys in the 1991-cohort and in 621 boys in the 2006-cohort. Blood samples were clotted and centrifuged, and serum was stored at −20 C until hormone analyses were performed.

Laboratory analysis

Serum FSH and LH were measured by time-resolved immunofluorometric assays (Delfia; PerkinElmer, Boston, MA) with detection limits of 0.06 and 0.05 IU/liter for FSH and LH, respectively. Intra- and interassay coefficients of variation (CV) were less than 5% in both gonadotropin assays. Testosterone was measured with the DPC Coat-A-Count RIA kit (Diagnostic Products, Los Angeles, CA). The detection limit was 0.23 nmol/liter, and the intra- and interassay CV were 7.6 and 8.6%, re-

TABLE 1. Estimated mean ages at attainment of pubertal stages in 1528 healthy school boys from the Copenhagen area in two different time periods

	1991–1993			2006–2008			
	Mean	95% CI	95% PI	Mean	95% CI	95% PI	*P*
TV >3 ml	11.92	11.76–12.08	10.25–13.59	11.66	11.49–11.82	9.62–13.70	0.025
G2	11.83	11.66–12.00	9.95–13.71	11.59	11.42–11.76	9.37–13.81	0.052
G3	13.30	13.11–13.49	11.11–15.49	13.13	12.91–13.35	10.81–15.45	0.256
G4	14.31	14.12–14.50	12.21–16.41	13.61	13.39–13.83	11.59–15.63	<0.001
G5	15.40	15.18–15.61	12.89–17.91	14.31	14.08–14.54	12.46–16.15	<0.001
PH2	11.89	11.66–12.07	9.48–14.30	12.38	12.16–12.61	9.51–15.25	0.001
PH3	13.45	13.26–13.64	11.28–15.62	13.25	13.03–13.47	11.01–15.48	0.182
PH4	14.28	14.09–14.48	12.08–16.48	13.67	13.44–13.89	11.56–15.78	<0.001
PH5	15.56	15.34–15.78	12.97–18.15	14.45	14.21–14.68	12.51–16.39	<0.001

Data are presented as mean, 95% confidence intervals (CI), and 95% predictions intervals (PI) for age at attainment of testicular volume above 3 ml (TV >3 ml), entry into genital stages 2–5 (G2–G5), and pubic hair stages 2–5 (PH2–PH5), respectively.

spectively. SHBG was determined by a time-resolved immunofluorescence assay (Delfia; Wallac Oy, Turku, Finland) with a detection limit of 0.20 nmol/liter. Intra- and interassay CV were 5.8 and 6.4%, respectively.

Statistical analyses

The data were cross-sectional as well as longitudinal in 63 boys participating in the 2006-cohort. The cross-sectional data constituted a so-called current-status design in that each person's age at entry into a certain puberty stage (including whether or not attainment of a testicular volume above 3 ml had occurred) was unknown except for the fact that he 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 he had not yet entered the stage, in which case the current age was a lower bound (right censored). The longitudinal data similarly yielded an upper bound, a lower bound, or an interval that contained the true age of entry (interval-censored data). Only the interval in which the change occurred was included. Thus, no participants were included with multiple measurements. The age distributions at entry into the various stages of puberty were estimated, taking into account the current-status design as well as the prospective follow-up study design. Two analytical approaches were employed. The first assumed a Gaussian distribution for the ages at entry into a puberty stage that allowed using all the data, including the follow-up data, in the estimation and resulting in easily interpretable mean ages of entry into each puberty stage. This approach is also called a Probit analysis. In the other approach, the Gaussian assumption was omitted, and the Turnbull estimator was used to estimate the age distribution function while allowing for interval-, left-, or right-censored data. The Turnbull estimator is a generalization of the Kaplan-Meier estimator and is the natural nonparametric version of the Probit analysis. The two analytical approaches were in agreement, allowing straightforward reporting of the results based on the Gaussain distribution model. Results are presented as mean age at entry into the different pubertal stages along with a 95% confidence and prediction intervals. Reference curves for BMI, testicular volume, and reproductive hormones as a function of age were obtained in both cohorts using locally weighted regression quantiles.

Comparisons of testicular volume, BMI, and hormone levels between the 1991- and 2006-cohorts were done by univariate ANOVAs. LH and testosterone was square-root-transformed, and testicular volume, BMI, FSH, and SHBG were log-transformed to approximate normal Gaussian distribution of the residuals as well as to obtain a residual variance, which did not depend on the level. We divided the analysis into three age groups [below 11.0 yr (n = 403), from 11.0 to 16.0 yr (n = 376), and above 16.0 yr (n = 186)] to look for differences between the two cohorts in hormone levels in these age periods approximately corresponding to the prepubertal, pubertal, and postpubertal periods. All ANOVAs were performed age-adjusted with age included as 1-yr age intervals. Differences in the prevalence of overweight and obesity as well as differences in testosterone levels between the two cohorts were tested by Fisher's exact test.

Ethical considerations

The study was approved by the local ethical committee (KF 01 282214 and V200.1996/90) and was conducted in accordance with the Second Helsinki Declaration. All participants and their parents gave informed consent.

Results

Puberty

Mean estimated ages at attainment of a testicular volume above 3 ml as well as age at entry into genital (G2–G5) and pubic hair (PH2–PH5) stages are presented in Table 1. Pubertal onset based on testicular volume and genital stages declined from the 1991-cohort to the 2006-cohort, although this was only borderline significant for differences in age at entry into G2 (Fig. 1). In contrast, the estimated mean age at entry into PH2 increased over the study period (Table 1). Estimated ages at onset of G2, PH2, and testicular volume greater than 3 ml were all inversely associated with BMI ($P < 0.001$). The between-cohort differences in ages at onset of testicular and genital development were no longer significant ($P = 0.105$ and $P = 0.152$, respectively) after adjustment for BMI. Testicular volumes in relation to age were significantly higher in the 2006-cohort compared with the 1991-cohort (Fig. 2). In addition, the yearly increment in testicular volume

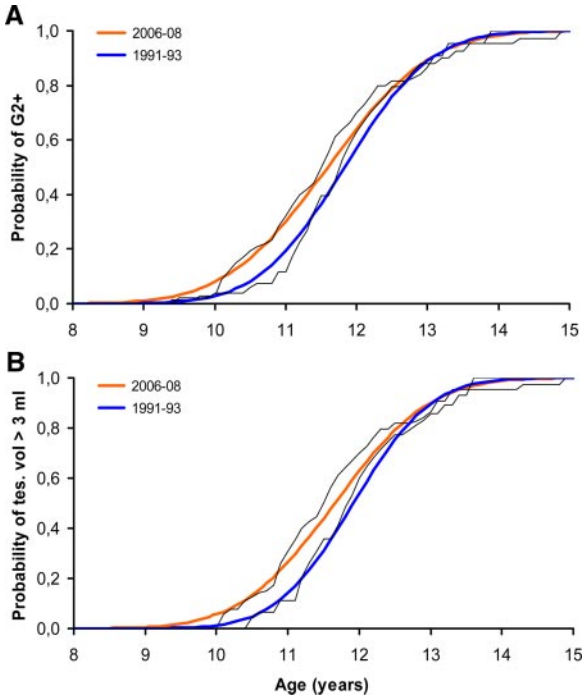

FIG. 1. Pubertal development in healthy boys from the Copenhagen Puberty Study. *Lines* represent the probabilities of observing genital stage 2 or more (G2+) (A) or a testicular volume greater than 3 ml (TV >3) (B) in relation to age in 1528 healthy school boys in two study periods; the 2006–2008 (*orange line*) and the 1991–1993 (*blue line*) study periods. The nonparametric Turnbull estimates are presented as *thin black lines*. Mean age at entry into G2+ and attainment of TV >3 were lower in the 2006-cohort compared with the 1991-cohort ($P = 0.052$ and $P = 0.025$, respectively).

was significantly greater in 2006 [3.8 ml/yr (3.6–4.1) (95% CI)] compared with 1991 [3.7 ml/yr (3.4–4.0), $P < 0.001$] in boys aged 11 to 16 yr.

Reproductive hormones

Reproductive hormone levels in relation to age in the two cohorts are shown in Fig. 3. In the pubertal period (11 to 16 yr), the age-adjusted LH levels were statistically significantly higher in 2006 compared with 1991 ($P = 0.020$). However, the difference was no longer significant after additional adjustment for BMI ($P = 0.068$). No significant differences were found for age-adjusted FSH, testosterone, or SHBG levels between the two cohorts in this age group. Age-adjusted BMI was positively associated with LH, FSH, and testosterone levels and negatively associated with SHBG (all $P < 0.001$). Due to some testosterone measurements below detection limit in this age group, we analyzed the proportional distribution above and below the median testosterone levels in 2006 but found a similar nonsignificant difference between cohorts. In boys aged 16 yr and above, the testosterone levels were significantly lower in 2006 compared with 1991 ($P < 0.001$), independent of BMI. No differences in other reproductive hormones were found in the above 16-yr-old or below 11-yr-old boys.

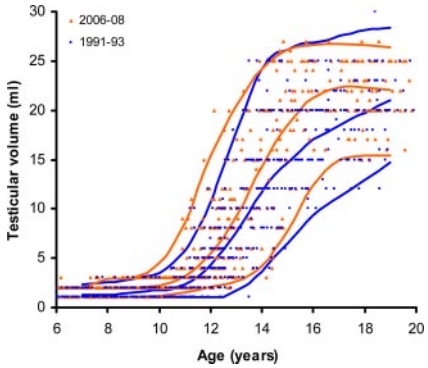

FIG. 2. Testicular volumes (milliliters) in relation to age in 1528 healthy school boys from the Copenhagen area in two different time periods: the 2006–2008 (*orange triangles*) and 1991–1993 (*blue squares*) study periods. Median, 2.5th, and 97.5 percentiles are presented for 2006–2008 (*orange lines*) and 1991–1993 (*blue lines*). Significant larger testicular volumes were found in 2006–2008 compared with 1991–1993 (all included $P < 0.001$ by age-adjusted univariate ANOVA).

BMI

Age-adjusted BMI was significantly higher in the 2006-cohort compared with the 1991-cohort (Fig. 4). The BMI Z-score increased significantly from the 1991-cohort [0.044 (−0.016 to 0.104); mean (95% confidence interval)] to the 2006-cohort [0.290 (0.219–0.361); $P < 0.001$]. The number (prevalence) of overweight and obese boys was statistically significantly higher in the 2006-cohort [68 (9.7%) and 66 (9.4%), respectively] compared with the 1991-cohort [48 (5.8%) and 44 (5.3%), respectively; $P < 0.001$ and $P = 0.003$, respectively].

Discussion

In this large clinical study on pubertal development in healthy Caucasian boys, we found a significant decline in age at pubertal onset based on testicular volume assessment and genital staging during a short period of 15 yr. Furthermore, significantly higher LH levels were found in the 2006-cohort compared with the 1991-cohort in the 11 to 16 yr olds. BMI increased significantly between the study periods, and the significant secular trends toward earlier onset of testicular growth and higher LH levels were no longer present after adjustment for BMI.

In the mid 1990s, data from the American population-based study NHANES III initiated speculations on a current downward secular trend for pubertal development in boys (2, 5, 6). Although, secular trend analysis between NHANES III and population-based data from the late 1960s (NHES III) did not find persuasive evidence for earlier puberty in the United States, some indications were present in white boys (4). However, secular trends in pu-

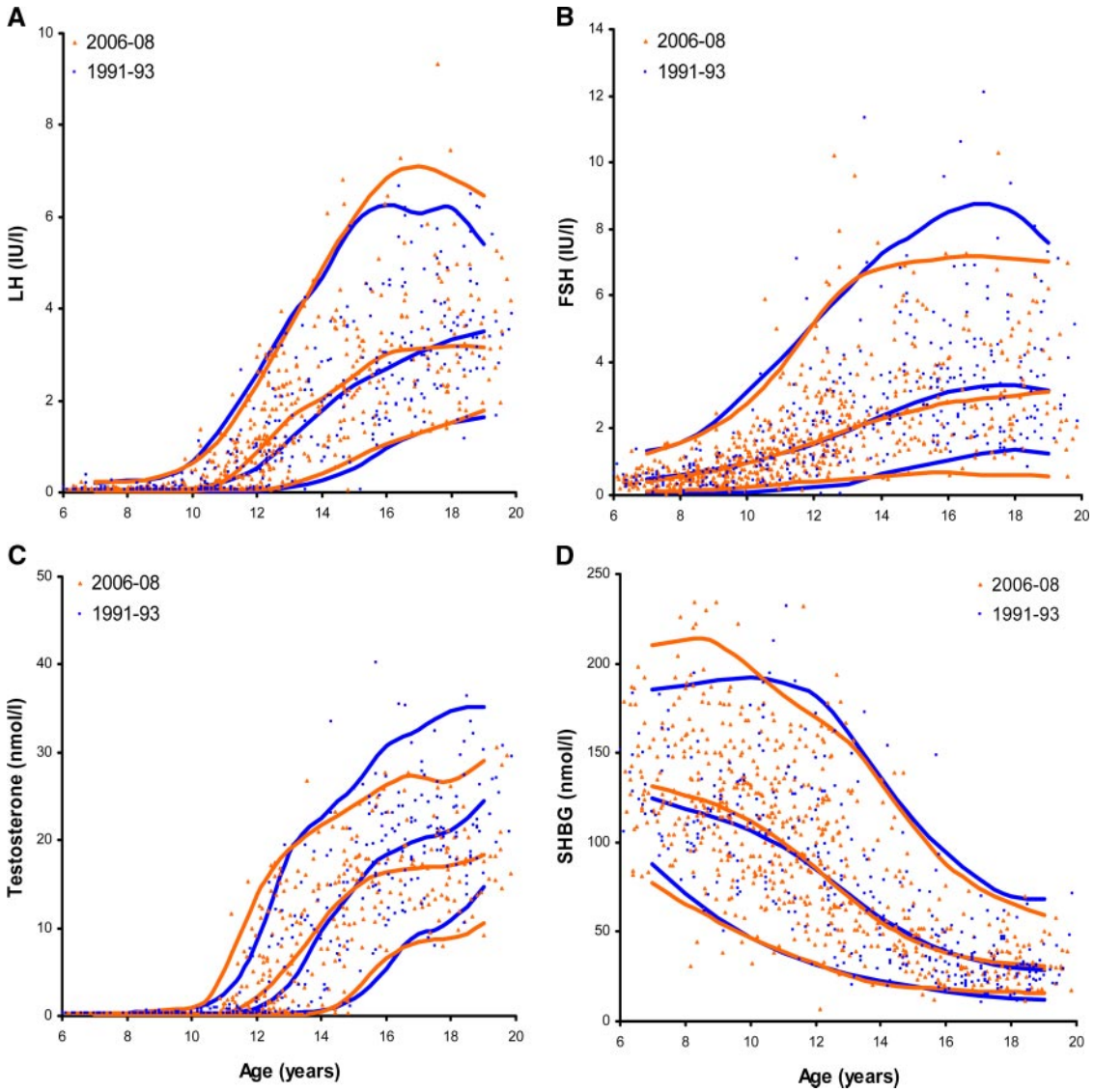

FIG. 3. LH (A), FSH (B), testosterone (C), and SHBG (D) in relation to age in 968 healthy school boys from the Copenhagen area in two different time periods: the 2006–2008 (*orange triangles*) and the 1991–1993 (*blue squares*) study periods. Median, 2.5th, and 97.5th percentiles are presented for 2006–2008 (*orange lines*) and 1991–1993 (*blue lines*). In boys aged 11 to 16 yr, significantly higher LH levels were found in 2006 compared with 1991 ($P = 0.020$ by age-adjusted univariate ANOVA). In the above 16-yr-olds, testosterone was significantly lower in 2006 compared with 1991 ($P < 0.001$). No significant differences were found in other hormones between the two cohorts in either age group.

bertal onset could not be evaluated due to lack of pubertal evaluation before the age of 12 in the NHES III. In Europe, no secular trend in pubertal onset has previously been reported in boys (11). In the present study, pubertal onset defined as mean age at attainment of a testicular volume above 3 ml had declined approximately 3 months between 1991 and 2006. A similar tendency was found for mean age at entry into genital stage 2. The age at pubertal onset found is in agreement with the majority of previous European studies (11, 14, 15), and far higher than the low figures reported in white non-Hispanic boys from the NHANES III (2, 3, 5). A concomitant increase in the incidence of idiopathic central precocious puberty would be expected if pubertal onset were as early as found in NHANES III. However, no such trends have been documented, and the diagnostic age limit recommendation in the United States has accordingly not been decreased from the current 9 yr (10). The incidence and prevalence of precocious puberty in boys does not seem to have increased in Denmark, at least during the narrow time window between 1993 and 2001 (16). However, distinction of idiopathic from organic central precocious puberty was impossible in this study. In the present study, the lower limit of the 95% prediction interval was approximately 9.5 yr, which is still far from the current 9 yr diagnostic age limit for CPP (32).

Differences in study design may explain some of the disparity between NHANES III and the present study. The strengths of our study rely on the fact that all pubertal examinations were done by one of three pediatric endo-

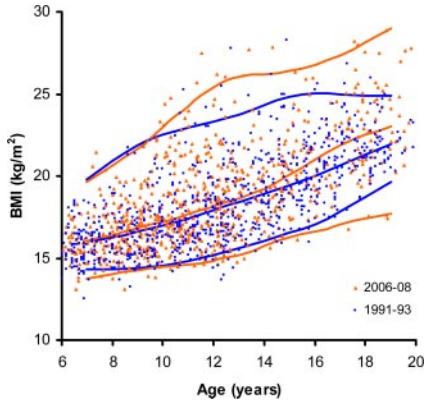

FIG. 4. BMI (kilograms per meter^2) in relation to age in 1528 healthy school boys from the Copenhagen area in two different time periods; the 2006–2008 (*orange triangles*) and the 1991–1993 (*blue squares*) study periods. Significantly higher BMI was found in the 2006–2008 compared with 1991–1993 (all included, $P < 0.001$ by age-adjusted univariate ANOVA).

crinologists, one of whom participated in both cohorts to ensure consensus of the evaluations. Examination included orchidometry by palpation that, in contrast to visual grading of genital stages (33), has a lower inter- and intraobserver variability (24). In addition, palpation is strongly correlated to testicular volume measurements by water displacement and ultrasonography (34). Thus, quantification of testicular volume will improve reproducibility and reliability in the determination of pubertal onset (1). In the NHANES III, pubertal development was classified solely by visual grading of genital stages by multiple examiners. Thus, the low age at entry into genital stage 2 in the NHANES III may be related to a possible overclassification of prepubertal boys as being early pubertal. In accordance, Biro *et al.* (9) found that age at pubertal onset based on longitudinal estimations of testicular volume in a mixed cohort of black and white American boys was in accordance with most contemporary European studies. In addition, age at onset of pubic hair development in the NHANES III (5) was not earlier than contemporary European studies (11, 13, 15). Surprisingly, we found that the age at onset of pubic hair was delayed approximately 5 months in 2006 compared with our findings from 1991, indicating that the time interval from onset of testicular growth to onset of pubic hair development has lengthened between the two study periods. In addition, we found that age at entry into genital as well as pubic hair stages 4 and 5 was reached significantly earlier in 2006 compared with 1991 and that these differences seem to be more pronounced as puberty progressed. In accordance, age-adjusted testicular volume, which is likely a more precise estimate of progression of puberty, was found to be higher and to increase faster in 2006 compared with 1991. This was in contrast to the findings of slower progression from genital stage 3 to 5 in white boys between the recent American population-based studies (4). Importantly, one should have in mind that time intervals between occurrences of different pubertal markers as well as progression of puberty should be interpreted with great caution in cross-sectional studies like our present study.

The onset of pubertal testicular growth is preceded by central activation of the hypothalamic-pituitary-gonadal axis (25, 26). If centrally mediated, secular trend in pubertal timing should be paralleled by changes in gonadotropin levels. In accordance, we demonstrated significantly higher LH levels in the 11- to 16-yr-old boys in 2006 compared with 1991. Although testosterone levels seemed to increase at an earlier age in 2006 compared with 1991, mean levels were not significantly different. Participation rates for blood sampling were much higher in 2006 than 1991, but the age and BMI distribution between those with and without blood samples did not differ, making it unlikely to have influenced the results.

The worldwide obesity epidemic has received special attention in relation to the recent changes in pubertal development (18–21). In the present study, we found a significant secular trend toward higher BMI. In addition, prevalence of overweight and obesity nearly doubled between the two study periods. However, the degree of adiposity was still markedly lower than reported from the NHANES III (35). Despite the convincing evidence for a relationship between the coincident changing trends toward higher adiposity and earlier pubertal timing in girls, this relationship remains controversial in boys (18). The highest prevalence of overweight and obesity has been associated with late (19) as well as with early sexual maturation in boys (23). However, none of these studies used testicular volume estimations for pubertal timing. In the present study, age-adjusted BMI was negatively associated with age at onset of testicular growth as well as of genital and pubic hair development. Thus, the higher the BMI for a given age, the more likely a boy would have entered puberty. In accordance, the differences in age at onset of genital and testicular growth between the study periods became less pronounced and statistically nonsignificant after adjustment for BMI. Importantly, due to the primarily cross-sectional design, the temporal cause-and-effect relationship could not be determined from these data. However, consistent evidence from longitudinal studies indicates that higher childhood BMI is associated with earlier timing of late pubertal markers such as peak height velocity (36) and voice-break (17), indicating that higher childhood adiposity may be implicated in earlier onset or faster progression of puberty. Thus, our present data support the hypothesis that increasing adiposity in the general population may positively influence pubertal onset in boys through earlier activation of the hypothalamic-pituitary-

gonadal axis. However, to which extent obesity *per se* is involved in this trend remains unknown. Most studies showing positive associations between childhood BMI and early pubertal timing have been performed in nonobese subjects (17, 36). In addition, hormonal factors associated with increased adiposity such as insulin and leptin act in permissive manners to allow puberty to progress if fat and energy stores are adequate but may by themselves be insufficient to initiate sexual maturation (21, 37, 38). Thus, the observed secular trend in pubertal onset may theoretically be more strongly correlated to a shift toward fewer lean rather than more overweight and obese adolescents in the population. In addition, the influence of adiposity may influence different pubertal markers differently. The secular trend toward higher age at onset of pubic hair development seems discordant, taking into account the negative association found to BMI in the entire cohort. However, high adiposity has been associated with attenuated steroidogenesis in the testes as well as with increased aromatization of androgens to estrogens (39, 40). Thus, some of the explanation for the apparent secular trend toward higher age at pubic hair development may be related to a higher percentage of overweight and obese in 2006 compared with 1991. However, adiposity is most likely not the only explanation. Other nonadiposity factors need to be considered.

In summary, ages at pubertal onset based on testicular volume assessments and genital staging in healthy Caucasian boys have declined by approximately 3 months from 1991 to 2006. In addition, BMI increased significantly during the study period. Importantly, the earlier age at onset of pubertal testicular growth in 2006 compared with 1991 was no longer significant after adjustment for BMI, indicating that BMI, at least partly, explained these findings. The existing data on the association between adiposity and pubertal timing in boys remain unresolved and still merit further attention.

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References

1. **Euling SY, Herman-Giddens ME, Lee PA, Selevan SG, Juul A, Sorensen TI, Dunkel L, Himes JH, Teilmann G, Swan SH** 2008 Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. Pediatrics 121(Suppl 3):S172–S191
2. **Karpati AM, Rubin CH, Kieszak SM, Marcus M, Troiano RP** 2002 Stature and pubertal stage assessment in American boys: the 1988–1994 Third National Health and Nutrition Examination Survey. J Adolesc Health 30:205–212
3. **Sun SS, Schubert CM, Chumlea WC, Roche AF, Kulin HE, Lee PA, Himes JH, Ryan AS** 2002 National estimates of the timing of sexual maturation and racial differences among US children. Pediatrics 110:911–919
4. **Sun SS, Schubert CM, Liang R, Roche AF, Kulin HE, Lee PA, Himes JH, Chumlea WC** 2005 Is sexual maturity occurring earlier among U.S. children? J Adolesc Health 37:345–355
5. **Herman-Giddens ME, Wang L, Koch G** 2001 Secondary sexual characteristics in boys: estimates from the National Health and Nutrition Examination Survey III, 1988–1994. Arch Pediatr Adolesc Med 155:1022–1028
6. **Herman-Giddens ME** 2006 Recent data on pubertal milestones in United States children: the secular trend toward earlier development. Int J Androl 29:241–246; discussion 286–290
7. **Foster TA, Voors AW, Webber LS, Frerichs RR, Berenson GS** 1977 Anthropometric and maturation measurements of children, ages 5 to 14 years, in a biracial community—the Bogalusa Heart Study. Am J Clin Nutr 30:582–591
8. **Harlan WR, Grillo GP, Cornoni-Huntley J, Leaverton PE** 1979 Secondary sex characteristics of boys 12 to 17 years of age: the U.S. Health Examination Survey. J Pediatr 95:293–297
9. **Biro FM, Lucky AW, Huster GA, Morrison JA** 1995 Pubertal staging in boys. J Pediatr 127:100–102
10. **Kaplowitz PB, Oberfield SE** 1999 Reexamination of the age limit for defining when puberty is precocious in girls in the United States: implications for evaluation and treatment. Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society. Pediatrics 104:936–941
11. **Mul D, Fredriks AM, van Buuren S, Oostdijk W, Verloove-Vanhorick SP, Wit JM** 2001 Pubertal development in The Netherlands 1965–1997. Pediatr Res 50:479–486
12. **Sørensen K, Nielsen J, Busch P, Hamborg-Petersen B, Hansen E, Lassen LB, Nielsen K, Poulsen JP, Rossen K, Schaap SK, Taudorf K, Thelle T, Vestermark S, Vrang C** 1979 [Measurement of testicular volume with Prader's orchiometer in 1,389 boys 5–15 ½ years old]. Ugeskr Laeger 141:915–918
13. **Juul A, Teilmann G, Scheike T, Hertel NT, Holm K, Laursen EM, Main KM, Skakkebaek NE** 2006 Pubertal development in Danish children: comparison of recent European and US data. Int J Androl 29:247–255; discussion 286–290
14. **Lindgren G** 1996 Pubertal stages 1980 of Stockholm schoolchildren. Acta Paediatr 85:1365–1367
15. **Largo RH, Prader A** 1983 Pubertal development in Swiss boys. Helv Paediatr Acta 38:211–228
16. **Teilmann G, Pedersen CB, Jensen TK, Skakkebaek NE, Juul A** 2005 Prevalence and incidence of precocious pubertal development in Denmark: an epidemiologic study based on national registries. Pediatrics 116:1323–1328
17. **Juul A, Magnusdottir S, Scheike T, Prytz S, Skakkebaek NE** 2007 Age at voice break in Danish boys: effects of pre-pubertal body mass index and secular trend. Int J Androl 30:537–542
18. **Kaplowitz PB** 2008 Link between body fat and the timing of puberty. Pediatrics 121(Suppl 3):S208–S217
19. **Wang Y** 2002 Is obesity associated with early sexual maturation? A comparison of the association in American boys versus girls. Pediatrics 110:903–910
20. **Kaplowitz PB, Slora EJ, Wasserman RC, Pedlow SE, Herman-**

Giddens ME 2001 Earlier onset of puberty in girls: relation to increased body mass index and race. Pediatrics 108:347–353
21. **Slyper AH** 2006 The pubertal timing controversy in the USA, and a review of possible causative factors for the advance in timing of onset of puberty. Clin Endocrinol (Oxf) 65:1–8
22. **Rosenfield RL, Lipton RB, Drum ML** 2009 Thelarche, pubarche, and menarche attainment in children with normal and elevated body mass index. Pediatrics 123:84–88
23. **Ribeiro J, Santos P, Duarte J, Mota J** 2006 Association between overweight and early sexual maturation in Portuguese boys and girls. Ann Hum Biol 33:55–63
24. **Karaman MI, Kaya C, Caskurlu T, Guney S, Ergenekon E** 2005 Measurement of pediatric testicular volume with Prader orchidometer: comparison of different hands. Pediatr Surg Int 21:517–520
25. **Albertsson-Wikland K, Rosberg S, Lannering B, Dunkel L, Selstam G, Norjavaara E** 1997 Twenty-four-hour profiles of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol levels: a semilongitudinal study throughout puberty in healthy boys. J Clin Endocrinol Metab 82:541–549
26. **Mitamura R, Yano K, Suzuki N, Ito Y, Makita Y, Okuno A** 1999 Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, and testosterone secretion before the onset of male puberty. J Clin Endocrinol Metab 84:29–37
27. **Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jørgensen K, Müller J, Hall K, Skakkebaek NE** 1994 Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. J Clin Endocrinol Metab 78:744–752
28. **Main K, Philips M, Jørgensen M, Skakkebaek NE** 1991 Urinary growth hormone excretion in 657 healthy children and adults: normal values, inter- and intraindividual variations. Horm Res 36:174–182
29. **Marshall WA, Tanner JM** 1970 Variations in the pattern of pubertal changes in boys. Arch Dis Child 45:13–23
30. **Zachmann M, Prader A, Kind HP, Häfliger H, Budliger H** 1974 Testicular volume during adolescence. Cross-sectional and longitudinal studies. Helv Paediatr Acta 29:61–72
31. **Ogden CL, Kuczmarski RJ, Flegal KM, Mei Z, Guo S, Wei R, Grummer-Strawn LM, Curtin LR, Roche AF, Johnson CL** 2002 Centers for Disease Control and Prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. Pediatrics 109:45–60
32. **Carel JC, Eugster EA, Rogol A, Ghizzoni L, Palmert MR, Antoniazzi F, Berenbaum S, Bourguignon JP, Chrousos GP, Coste J, Deal S, de Vries L, Foster C, Heger S, Holland J, Jahnukainen K, Juul A, Kaplowitz P, Lahlou N, Lee MM, Lee P, Merke DP, Neely EK, Oostdijk W, Phillip M, Rosenfield RL, Shulman D, Styne D, Tauber M, Wit JM** 2009 Consensus statement on the use of gonadotropin-releasing hormone analogs in children. Pediatrics 123:e752–e762
33. **Carlsen E, Andersen AG, Buchreitz L, Jørgensen N, Magnus O, Matulevicuus V, Nermoen I, Petersen JH, Punab M, Suominen J, Zilaitiene B, Giwercman A** 2000 Inter-observer variation in the results of the clinical andrological examination including estimation of testicular size. Int J Androl 23:248–253
34. **Sakamoto H, Saito K, Oohta M, Inoue K, Ogawa Y, Yoshida H** 2007 Testicular volume measurement: comparison of ultrasonography, orchidometry, and water displacement. Urology 69:152–157
35. **Troiano RP, Flegal KM** 1998 Overweight children and adolescents: description, epidemiology, and demographics. Pediatrics 101:497–504
36. **Kindblom JM, Lorentzon M, Norjavaara E, Lönn L, Brandberg J, Angelhed JE, Hellqvist A, Nilsson S, Ohlsson C** 2006 Pubertal timing is an independent predictor of central adiposity in young adult males: the Gothenburg osteoporosis and obesity determinants study. Diabetes 55:3047–3052
37. **Ebling FJ** 2005 The neuroendocrine timing of puberty. Reproduction 129:675–683
38. **Gamba M, Pralong FP** 2006 Control of GnRH neuronal activity by metabolic factors: the role of leptin and insulin. Mol Cell Endocrinol 254–255:133–139
39. **Pitteloud N, Hardin M, Dwyer AA, Valassi E, Yialamas M, Elahi D, Hayes FJ** 2005 Increasing insulin resistance is associated with a decrease in Leydig cell testosterone secretion in men. J Clin Endocrinol Metab 90:2636–2641
40. **Isidori AM, Caprio M, Strollo F, Moretti C, Frajese G, Isidori A, Fabbri A** 1999 Leptin and androgens in male obesity: evidence for leptin contribution to reduced androgen levels. J Clin Endocrinol Metab 84:3673–3680