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# Association between pubertal timing and bone and body composition in young adult men

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**Bone and muscle development are important processes in pubertal maturation. The aim of this study was to investigate associations between pubertal timing and bone density and body composition in young adult men. In this observational study, bone and body composition was cross-sectionally assessed by dual-energy X-ray absorptiometry in 2056 healthy young men with median age 19, who retrospectively self-reported if they experienced pubertal changes at an earlier, similar or later age than their peers. Associations between voice break timing and bone and body composition were analyzed by linear regression. Men reporting earlier voice break than their peers (n = 417, 20%) had higher lumbar bone mineral density (BMD) and higher total body BMD. Apart from higher BMI, there were no differences in body composition. Men who reported later voice break (n = 353, 17%) had lower lumbar bone mineral content, bone area and volume, but similar BMD. They had lower BMI, lean mass and fat mass, resulting in a lower fat-to-muscle ratio. In conclusion, even after adult height has been reached, physiological variations in pubertal timing were associated with differences in bone and body composition in young adult men.**

**Keywords** Puberty, Bone density, Body composition, Voice break

Major physical changes occur during puberty, influenced by genetic factors, physical activity, body weight, nutritional status and hormones (sex steroids, growth hormone, insulin-like growth factors)[1–3]. The timing of pubertal onset, growth spurt and physical maturation during puberty varies substantially between individuals and can have a major impact on adult health[4,5]. In women, it is well known that a later menarcheal age is a risk factor for osteoporosis and osteoporotic fractures[5]. Similarly, late-normal pubertal timing has been linked to lower bone mineral density (BMD) in young adult men, with increased fracture risk in adulthood[6,7]. Other studies, however, suggest a catch-up in BMD in young adulthood in men with late-normal pubertal onset[8].

Most larger population-based studies in men use age at peak height velocity as an indirect measure of pubertal status in boys, although this requires detailed growth charts over time[5]. However, similar to menarche in females, voice break is a distinct, although very late milestone occurring several years after pubertal onset. Age at voice break has been used as a marker for pubertal maturation in large cohort studies[9–12]. Despite the fact that voice break is a late pubertal marker[12], genetic variants identified in large genome-wide association studies on age at voice break significantly associate with markers of pubertal onset like age at testicular growth and pubic hair development, suggesting that these events share a genetic architecture across ethnically distinct populations[3]. In the Copenhagen Puberty Study, a cohort study of 730 healthy Danish boys, voice break occurred at a mean age of 13.6 (95% CI 13.5–13.8), with peak height velocity occurring around the same age. Voice break correlated with testicular enlargement and increasing testosterone levels, but there was no clear threshold in serum testosterone levels[10].

Even after linear growth is completed and final height has been reached, skeletal maturation and bone mass accrual continues until peak bone mass (PBM), the maximal bone mass achieved in life, is reached[5]. At the age of 18 years, PBM at the lumbar spine and femoral neck has been reached. Although around 90% of PBM is acquired at this age, bone mass accrual is still ongoing in long bones such as radius and tibia[13]. The final, achieved PBM

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is important for the risk of osteoporotic fractures in later life[1,14]. Moreover, during growth, bone and muscle interact, and mechanical forces from muscle are important for bone strength development. The peak in muscle mass increase in puberty takes place before the peak in bone mass is reached[15].

Timing of pubertal onset is an important determinant for adolescent maturation and biological age. It remains unclear to what extent differences in physical maturation induced by pubertal timing persist in young adulthood. The aim of this study was to investigate the association between pubertal timing and bone and body composition measurements in healthy young men from the general population. In all participants, bone and body composition parameters were measured by dual-energy X-ray absorptiometry (DXA). We hypothesized that pubertal timing, using self-reported voice break as pubertal milestone, is associated with bone and body composition measurements in young men, even after pubertal transition has been completed and adult height has been reached.

## Methods

### Participants: young men

All young Danish men attend a medical examination at age 18–25 before entering military service, except men with severe chronic illness. These men can be considered to represent the general population of healthy young men. During this examination for fitness for military service, men were invited to participate in a cross-sectional observational study about reproductive health. Participation rate was 25%. A detailed description of this observational study and methods has been published previously[16]. Men could participate in the study irrespective of their qualification for military service. Participants were unselected regarding reproductive parameters, pubertal development, hormonal parameters or other factors that could potentially influence bone and muscle maturation.

In this analysis, 2311 men were included between 2012 and 2019. The ethical committee of the Capital region (Copenhagen, Denmark) provided approval (permit number H-KF-289428, date of approval: July 27th 2012). All participants gave written informed consent. Participants were compensated for their time (DKK 500). All research was performed in accordance with local guidelines and regulations and in accordance with the Declaration of Helsinki.

### Assessments

All examinations took place on the same day at the Department of Growth and Reproduction, Rigshospitalet, a major hospital in Copenhagen, Denmark. Study examinations consisted of a physical examination, blood sample, semen sample and completion of a questionnaire concerning general and reproductive health, demographic and lifestyle factors. One part of the questionnaire covered retrospective self-reported data on sexual maturation and pubertal transition, by asking if they experienced signs of adrenarche (pubic hair development) and signs of gonadarche (penile growth, increase in testis size and voice break) at an earlier, similar or later age compared to their peers. The men thus reported how they perceived the timing of this pubertal milestone relative to their peers. Additionally, a recall of the age at which these pubertal milestones occurred was also noted. Height and weight were measured and body mass index (BMI) was calculated. Testis size was assessed using Prader's orchidometer.

### Laboratory measurements

A fasting morning blood sample was drawn. Alkaline phosphatase was measured by enzymatic absorption photometry (Cobas c702, Roche Diagnostics, Basel, Switzerland). Luteinizing hormone (LH), follicle stimulating hormone (FSH), and total estradiol (E2) were measured by an immunofluorometric assay (Wallac, Turku, Finland). Total testosterone (total T), sex hormone-binding globulin (SHBG) and inhibin B were measured by an enzyme-linked immunosorbent assay (Beckman Coulter, Wycombe, UK). Free testosterone (free T) was calculated by the Vermeulen equation[17]. The total T/total E2 ratio was calculated by dividing total T in pmol/L by total E2 in pmol/L.

### Dual-energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy, GE Healthcare, USA) was used to assess bone at the lumbar spine (L1-L4), femoral neck and total body as well as body composition (Software: Prodigy, enCORE 2004, version 8.8; GE Lunar Corp., Madison, WI, USA).

For femoral measurements, the average of the left and right scan was calculated. Calibration was done regularly according to manufacturer guidelines (daily calibrations and weekly spine phantom scans). The precision errors of these measurements were lower than 0.5%. Coefficients of variation were 2% for bone and body fat measurements in humans.

Bone mineral content (BMC, in grams (g)) and bone area ($cm^2$) was measured. From BMC and bone area, areal BMD was calculated ($g/cm^2$). As DXA is a two-dimensional measurement method and BMD depends on the bone volume (in $cm^3$), areal BMD can be underestimated in case of a reduced bone size (such as in children or adults with short stature). To compensate for that, bone mineral apparent density (BMAD, in $g/cm^3$) was calculated to normalize BMD measurements for bone volume (in $cm^3$), that can be estimated from the projected bone area (in $cm^2$). Lumbar BMAD was calculated as $BMC/area^{1.5}$ and femoral neck BMAD as $BMC/area^2$ ref.[18,19]. Muscle mass, bone mass, total fat mass and trunk fat mass were measured. Fat-to-muscle ratio was calculated by dividing fat mass by muscle mass.

### Statistical analysis

Descriptive statistics are presented as median, 5th and 95th percentile for continuous variables and percentage for categorical variables.

As voice break is the most valid parameter to compare with peers, we chose this parameter to stratify men according to earlier, similar or later self-reported timing of puberty. As the age of pubertal transition is variable among adolescents, participants were stratified based on the question in which they indicated when they experienced voice break relative to their peers, so earlier, similar or later timing of the pubertal milestone[20].

One-way analysis of variance was used to compare characteristics of the groups, with Bonferroni correction for multiple-hypothesis testing. Associations between voice break timing (predictor) and DXA measurements (main outcome measures) were assessed by linear regression with 'similar timing' as reference group. Analyses were performed unadjusted and adjusted for age, BMI, smoking (regular/occasional versus non-smokers), alcohol intake (units in the last week) and physical activity (hours of moderate and strenuous activity in the last week) for bone measurements and adjusted for age, smoking, alcohol intake and physical activity for body composition. DXA measurements and residuals of the linear regression models followed a normal distribution. To assess the robustness of results, analyses were repeated according with the other pubertal milestones (pubic hair development, penile growth and increase in testis size) as predictor as well as in the subset of young men with concordant answers on early, similar or late timing across all four pubertal markers.

A p-value < 0.05 was considered statistically significant. Data were analyzed using Stata version 15.1 (StataCorp, College station, TX, USA).

## Results

From the 2311 participating men, men with missing DXA (n = 8) and voice break data (n = 255) were excluded, leaving 2056 men with a median age of 19.0 years (5th-95th percentile interval: 18.4–22.3) in the analytical sample. General characteristics are described in Table 1. 20% of men reported to have experienced an earlier voice break than their peers and 17% later. Height, testis size and (free) testosterone concentrations were similar across the groups. SHBG and inhibin B gradually increased according to age at voice break, whereas concentrations of estradiol showed a decrease. Other hormonal parameters did not differ. Alkaline phosphatase was lower in men who reported earlier voice break and higher when voice break occurred later (Table 1).

In the univariate analysis, men who reported an earlier voice break had higher lumbar and femoral BMC, areal BMD, Z-score and BMAD, as well as higher total body BMD and Z-score compared to men who recalled to experience voice break at a similar age as their peers (Suppl Table 1). In the fully adjusted model, only lumbar areal BMD, Z-score and BMAD s, as well as total body BMD and Z-score remained positively associated to self-reported timing of voice break. Men that reported a later voice break had significantly lower lumbar BMC, bone area and bone volume after adjustments, but areal and BMAD were not different (Tables 2, 3, Fig. 1 and Suppl Table 1). When recalled age of voice break was included in the analysis as a continuous variable (available in 915 men), increasing age of voice break was associated with lower lumbar BMAD and higher femoral neck bone area and bone volume (Suppl Table 2).

| | Entire cohort | Early voice break | Similar voice break | Late voice break | P-value |
|---|---|---|---|---|---|
| | (n = 2056) | (n = 417–20%) | (n = 1286–63%) | (n = 353–17%) | |
| Age (years) | 19.0 (18.4; 22.3) | 18.9 (18.4; 21.8) | 19.0 (18.4; 22.5) | 19.0 (18.4; 21.8) | 0.22 |
| Self-reported age of voice break *(n = 915)* | 13 (11; 16) | 12 (11; 14) *(n = 208)* | 13 (12; 15) *(n = 532)* | 15 (13; 16) *(n = 175)* | **< 0.001** |
| Smoking (daily + occasional) n (%) | 1082 (53%) | 220 (53%) | 678 (53%) | 184 (52%) | 0.93 |
| Alcohol (units last week) | 6 (0; 33) | 6 (0; 30) | 6 (0; 33) | 7 (0; 35) | 0.67 |
| Physical activity (hours/week) | 7 (0; 36) | 7.8 (0; 40) | 7.0 (0: 33.5) | 7.5 (0; 33) | **0.010** |
| Mean testis size (mL) | 22 (12.5; 30) | 22.5 (12.5; 30.0) | 22.0 (12.5; 30.0) | 21.3 (12.0; 30.0) | 0.23 |
| Height (cm) | 182.6 (171.9; 193.7) | 181.9 (171.4; 193.5) | 182.8 (172.0; 193.7) | 182.3 (171.6; 194.3) | 0.19 |
| Weight (kg) | 73.7 (58.4; 93.5) | 76.2 (57.9; 95.2) | 73.8 (59.2; 93.4) | 71.0 (55.9; 92.4) | **< 0.001** |
| BMI | 22.1 (18.2; 27.6) | 22.8 (18.4; 27.9) | 22.1 (18.3; 27.7) | 21.0 (17.5; 26.7) | **< 0.001** |
| BMI < 20 – n (%) | 442 (22%) | 71 (17%) | 262 (20%) | 109 (31%) | **0.019** |
| BMI > 25 – n (%) | 325 (16%) | 83 (20%) | 207 (16%) | 35 (10%) | **0.005** |
| FSH (U/L) | 2.6 (1.0; 6.6) | 2.7 (1.0; 6.1) | 2.6 (1.0; 6.6) | 2.8 (1.2; 7.0) | 0.15 |
| LH (U/L) | 3.3 (1.6; 6.4) | 3.3 (1.5; 6.5) | 3.3 (1.6; 6.4) | 3.2 (1.6; 6.2) | 0.51 |
| Total T (nmol/L) | 17.7 (11.0; 27.7) | 17.5 (10.1; 28.1) | 17.8 (11.1; 27.9) | 17.3 (11.0; 26.8) | 0.15 |
| Calculated free T (pmol/L) | 394 (248; 624) | 394 (255; 648) | 401 (248; 630) | 378 (228; 593) | 0.06 |
| SHBG (nmol/L) | 29.0 (14.7; 50.8) | 28.0 (14.0; 48.0) | 29.0 (14.9; 51.0) | 30.9 (15.8; 50.7) | **0.007** |
| Inhibin B (pg/mL) | 174 (87; 282) | 165 (87; 269) | 175 (84; 284) | 182 (94; 287) | **0.001** |
| Total E2 (pmol/L) | 82 (37; 136) | 85 (40; 141) | 82 (39; 138) | 74 (33; 128) | **0.001** |
| Ratio total T/total E2 | 222 (124; 449) | 210 (115; 436) | 222 (125; 443) | 231 (134; 491) | 0.14 |
| Alkaline phosphatase (U/L) | 79 (51; 125) | 73 (51; 113) | 79 (51; 122) | 84 (51; 144) | **< 0.001** |

**Table 1**. General characteristics for the entire cohort of young men and according to self-reported onset of voice break compared to their peers. Median and 5th–95th percentile values for continuous variables and percentage for categorical variables. Physical activity: hours of strenuous and moderate activity per week. One-way analysis of variance was used to compare the three groups. Significant values are in bold.

| | Entire cohort | Early voice break | Similar voice break | Late voice break | P-value |
|---|---|---|---|---|---|
| | (n = 2056) | (n = 417) | (n = 1286) | (n = 353) | |
| L1-L4 BMC (g) | 75.6 (56.7; 99.6) | 77.5 (56.7; 103.1) | 75.8 (57.2; 99.2) | 73.0 (53.9; 98.7) | **< 0.0001** |
| L1-L4 bone area ($cm^2$) | 63.0 (54.1; 73.9) | 63.3 (54.1; 74.4) | 63.3 (54.3; 73.9) | 62.1 (53.1; 72.7) | **0.0020** |
| L1-L4 bone volume ($cm^3$) | 251 (200; 320) | 253 (199; 323) | 253 (201; 319) | 245 (194; 310) | **0.0024** |
| L1-L4 areal BMD ($g/cm^2$) | 1.20 (0.99; 1.44) | 1.22 (1.03; 1.47) | 1.20 (0.99; 1.44) | 1.17 (0.97; 1.39) | **< 0.0001** |
| L1-L4 Z-score | −0.18 (−1.80; 1.68) | 0.02 (−1.50; 1.94) | −0.18 (−1.82; 1.64) | −0.39 (−1.92; 1.37) | **< 0.0001** |
| L1-L4 BMAD ($g/cm^3$) | 0.30 (0.25; 0.36) | 0.31 (0.26; 0.37) | 0.30 (0.25; 0.36) | 0.30 (0.25; 0.35) | **0.0001** |
| Femoral neck BMC (g) | 6.24 (4.77; 7.98) | 6.34 (4.77; 8.19) | 6.23 (4.78; 7.92) | 6.15 (4.70; 7.93) | **0.0038** |
| Femoral neck area ($cm^2$) | 5.42 (4.78; 6.09) | 5.43 (4.74; 6.12) | 5.42 (4.79; 6.06) | 5.40 (4.76; 6.14) | 0.5820 |
| Femoral neck bone volume ($cm^3$) | 29.4 (22.9; 37.1) | 29.5 (22.5; 37.4) | 29.4 (23.0; 36.8) | 29.2 (22.6; 37.8) | 0.5312 |
| Femoral neck areal BMD ($g/cm^2$) | 1.16 (0.92; 1.42) | 1.18 (0.95; 1.44) | 1.16 (0.92; 1.41) | 1.14 (0.90; 1.41) | **0.0093** |
| Femoral neck Z-score | 0.38 (−1.36; 2.35) | 0.51 (−1.15; 2.63) | 0.37 (−1.37; 2.34) | 0.25 (−1.53; 2.32) | **0.0038** |
| Femoral neck BMAD ($g/cm^3$) | 0.21 (0.17; 0.27) | 0.22 (0.18; 0.27) | 0.21 (0.17; 0.27) | 0.21 (0.17; 0.27) | **0.0211** |
| Total body areal BMD ($g/cm^2$) | 1.28 (1.10; 1.49) | 1.32 (1.13; 1.52) | 1.28 (1.10; 1.49) | 1.26 (1.06; 1.46) | **< 0.0001** |
| Total body Z-score | 0.72 (−0.84; 2.43) | 0.99 (−0.58; 2.66) | 0.69 (−0.84; 2.43) | 0.50 (−1.12; 2.25) | **< 0.0001** |
| Muscle mass (kg) | 56.1 (45.2; 67.6) | 56.6 (44.8; 68.8) | 56.3 (45.9; 67.7) | 55.0 (43.9; 66.2) | **0.0002** |
| Bone mass (kg) | 3.1 (2.5; 3.7) | 3.1 (2.5; 3.8) | 3.1 (2.5; 3.7) | 3.0 (2.4; 3.6) | **< 0.0001** |
| Lean mass (muscle + bone) (kg) | 59.1 (47.7; 71.2) | 59.7 (47.3; 72.0) | 59.3 (48.4; 71.3) | 58.1 (46.2; 69.3) | **0.0001** |
| Lean mass (muscle + bone) % | 80.8 (67.9; 87.1) | 79.9 (67.5; 87.3) | 80.6 (68.0; 86.9) | 81.9 (67.6; 87.2) | **0.0003** |
| Fat mass (kg) | 14.1 (8.6; 29.4) | 15.1 (8.6; 28.7) | 14.3 (8.8; 28.3) | 12.5 (8.1; 28.8) | **< 0.0001** |
| Fat mass % | 19.2 (12.9; 32.1) | 20.1 (12.7; 32.5) | 19.4 (13.1; 32.0) | 18.1 (12.8; 32.4) | **0.0003** |
| Fat-to-muscle ratio (FMR) | 0.25 (0.16; 0.50) | 0.27 (0.15; 0.51) | 0.25 (0.16; 0.49) | 0.23 (0.15; 0.51) | **0.0013** |
| Trunk fat mass (kg) | 0.78 (0.34; 2.37) | 0.88 (0.35; 2.48) | 0.79 (0.35; 2.36) | 0.62 (0.30; 2.27) | **< 0.0001** |

**Table 2**. DXA measurements for bone and body composition for the entire cohort of young men and according to self-reported onset of voice break compared to their peers. Median and 5th–95th percentile values for continuous variables and percentage for categorical variables. One-way analysis of variance was used to compare the three groups. Significant values are in bold. *BMC* bone mineral content, *BMD* bone mineral density, *V* volume, *BMAD* bone mineral apparent density.

Men with an early self-reported voice break had higher BMI, whereas men with a late voice break had a lower body weight and BMI. Of note, 31% of these men had a BMI < 20 $kg/m^2$, compared to 17% of men with early voice break (p-value 0.019), whereas the percentage of overweight men was higher in the early voice break group (20%) (Tables 1–3) compared to similar (17%) and late puberty (10%) (p-value 0.005). Body composition measurements showed lower muscle and lean mass as well as lower total fat mass and trunk fat mass in men with later puberty (Table 3, Fig. 2, Suppl Table 1). In relative terms, lean mass percentage was higher and fat mass percentage was lower in these men compared to the reference group, resulting in lower fat-to-muscle ratio (Table 3, Suppl Table 1). Apart from a slightly higher trunk fat mass, there were no differences in body composition measurements for men that experienced an earlier voice break (Table 3, Suppl Table 1). When the analysis was repeated with recalled age of voice break as a continuous variable, this showed similar results for the body composition parameters as the observations made when men were categorized in early, similar, late voice break. Increasing age of voice break is associated with higher lean mass percentage and lower fat mass measurements (Suppl Table 2).

Analyses were also repeated with the other pubertal milestones as predictor, showing overall similar results (Suppl Tables 3–5). Regarding the four pubertal signs (voice break, pubic hair, penile growth, testicular growth), 59.5% (n = 1224) of men answered all four questions. 56% of these men (n = 685) gave the same answer to all 4 questions. Of those, 17% (115 men) reported an early pubertal timing, 69% (n = 476) a similar timed puberty compared to their peers and 14% (n = 94) a late puberty. When repeating the analysis in men who reported to have an early, similar or late puberty on all four questions about occurrence of pubertal signs, we found similar results for bone and body composition (Suppl Table 6).

## Discussion

In this large cohort of healthy young Danish men, we observed that men who reported to have their pubertal voice break at a later time than their peers have a lower BMC as well as bone volume at the lumbar spine at a median age of 19, whereas femoral and total body measurements were similar. Importantly, also muscle mass and lean body mass were lower, as well as BMI and total and trunk fat mass. Overall, this was associated with a healthier body composition with lower body fat percentage, higher lean mass percentage and lower fat-to-muscle ratio. In men with an earlier voice break, lumbar BMD, BMAD and total body BMD were higher, but apart from a higher BMI and slightly higher trunk fat mass, there were no differences in body composition parameters.

| | Early onset | Similar onset | Late onset |
|---|---|---|---|
| Bone | | | |
| L1-L4 BMC (g) | 1.37 (−0.05; 2.78) | ref | **−2.04 (−3.54; −0.53)**** |
| L1-L4 bone area ($cm^2$) | 0.09 (−0.59; 0.76) | ref | **−1.04 (−1.75; −0.32)**** |
| L1-L4 bone volume ($cm^3$) | 0.57 (−3.50; 4.64) | ref | **−6.13 (−10.5; −1.80)**** |
| L1-L4 areal BMD ($g/cm^2$) | **0.02 (0.01; 0.04)*** | ref | −0.01 (−0.03; 0.002) |
| L1-L4 Z-score | **0.14 (0.03; 0.25)*** | ref | −0.12 (−0.24; 0.0003) |
| L1-L4 BMAD ($g/cm^3$) | **0.004 (0.001; 0.008)*** | ref | −0.001 (−0.005; 0.003) |
| Femoral neck BMC (g) | 0.08 (−0.02; 0.18) | ref | 0.02 (−0.09; 0.13) |
| Femoral neck area ($cm^2$) | 0.002 (−0.048; 0.052) | ref | −0.0002 (−0.054; 0.053) |
| Femoral neck bone volume ($cm^3$) | 0.06 (−0.47; 0.58) | ref | 0.007 (−0.56; 0.57) |
| Femoral neck areal BMD ($g/cm^2$) | 0.011 (−0.003; 0.03) | ref | 0.002 (−0.015; 0.020) |
| Femoral neck Z-score | 0.10 (−0.02; 0.22) | ref | 0.01 (−0.12; 0.14) |
| Femoral neck BMAD ($g/cm^3$) | 0.003 (−0.0002; 0.007) | ref | 0.001 (−0.003; 0.005) |
| Total body areal BMD ($g/cm^2$) | **0.02 (0.01; 0.03)***** | ref | −0.01 (−0.02; 0.003) |
| Total body Z-score | **0.15 (0.05; 0.25)**** | ref | −0.08 (−0.18; 0.03) |
| Alkaline phosphatase (U/L) | **−5.70 (−8.35; -3.06)***** | ref | **8.56 (5.75; 11.37)***** |
| Body composition | | | |
| Height (cm) | −0.62 (−1.35; 0.11) | ref | −0.08 (−0.86; 0.69) |
| Weight (kg) | 1.05 (−0.14; 2.24) | ref | **−3.06 (−4.32; −1.79)***** |
| BMI | **0.45 (0.13; 0.77)**** | ref | **−0.90 (−1.24; −0.56)***** |
| Muscle mass (kg) | 0.34 (−0.40; 1.07) | ref | **−1.56 (−2.34; −0.78)***** |
| Bone mass (kg) | **0.05 (0.01; 0.09)*** | ref | **−0.08 (−0.12; −0.04)***** |
| Lean mass (muscle + bone) (kg) | 0.39 (−0.38; 1.15) | ref | **−1.63 (−2.46; −0.82)***** |
| Lean mass (muscle + bone) % | −0.60 (−1.26; 0.06) | ref | **1.15 (0.45; 1.85)**** |
| Fat mass (kg) | 0.65 (−0.07; 1.37) | ref | **−1.46 (−2.22; −0.70)***** |
| Fat mass % | 0.60 (−0.06; 1.26) | ref | **−1.15 (−1.85; −0.45)**** |
| Fat-to-muscle ratio (FMR) | 0.01 (−0.002; 0.02) | ref | **−0.02 (−0.03; −0.01)**** |
| Trunk fat mass (kg) | **0.09 (0.01; 0.17)*** | ref | **-0.14 (−0.22; −0.06)**** |

**Table 3**. Association between voice break timing of young men and bone and body composition measurements. Reported as β-coefficient and 95% CI. For bone measurements, analysis was adjusted for age, BMI, smoking, alcohol intake (units in the last week), physical activity (hours/week). For body composition measurements, analysis was adjusted for age, smoking, alcohol intake (units in the last week), physical activity (hours/week). Results from univariate models can be found in Supplementary Table 1. Significant values are in bold. *BMC* bone mineral content, *BMD* bone mineral density, *BMAD* bone mineral apparent density, *BMI* body mass index. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Age at menarche is a distinct and late pubertal milestone in girls and related to years of endometrial exposure to estrogens. Voice break is a distinct and similarly late event in pubertal development in boys, occurring between Tanner stages 3 and 4[12]. As it is comparable among peers, it has been used as an indicator for pubertal maturation in observational and epidemiological studies[9–11].

In the Copenhagen Puberty Study, pubertal maturation was assessed in detail in 730 healthy Danish boys with clinical examination of pubertal milestones and growth charts. The objective of this study was to assess the temporal relation of several milestones in male puberty, and to evaluate in detail how clinical measures of pubertal development (testicular enlargement, growth, …) correlate with measures that are frequently used in large epidemiological studies (such as voice break). Voice break occurred at a mean age of 13.6 (95% CI 13.5–13.8), with peak height velocity occurring around the same age, confirming voice break as a late pubertal milestone. Voice break correlated with timing of other pubertal milestones, such as pubic hair growth, testicular enlargement and increasing testosterone levels, but it was not dependent on a threshold for serum testosterone levels[10]. In a study of Danish choir boys with detailed and frequent voice assessments, the median age at voice break was 14.0 years[21]. We found a median self-reported age at voice break of 13 years, however, at age 19 years, only 44.5% of men in our study could recall the exact age at which their voice changed in addition to the reporting of timing relative to peers, and the age was given without decimals. This is in accordance with the well-known recall bias and uncertainty of this pubertal milestone. The proportion of men reporting voice break earlier or later than peers was overall similar. Differences between the observations when voice break was stratified in three groups versus when the recalled age was used, could be due to the lower number of participants that could recall their exact age when voice break occurred.

Pubertal timing and pubertal hormone changes are important determinants for attainment of peak bone mass and adult BMD. In women, menarcheal age above the median has been associated with lower BMD and an increased fracture risk[5]. Men with a history of constitutionally delayed puberty have lower areal BMD in

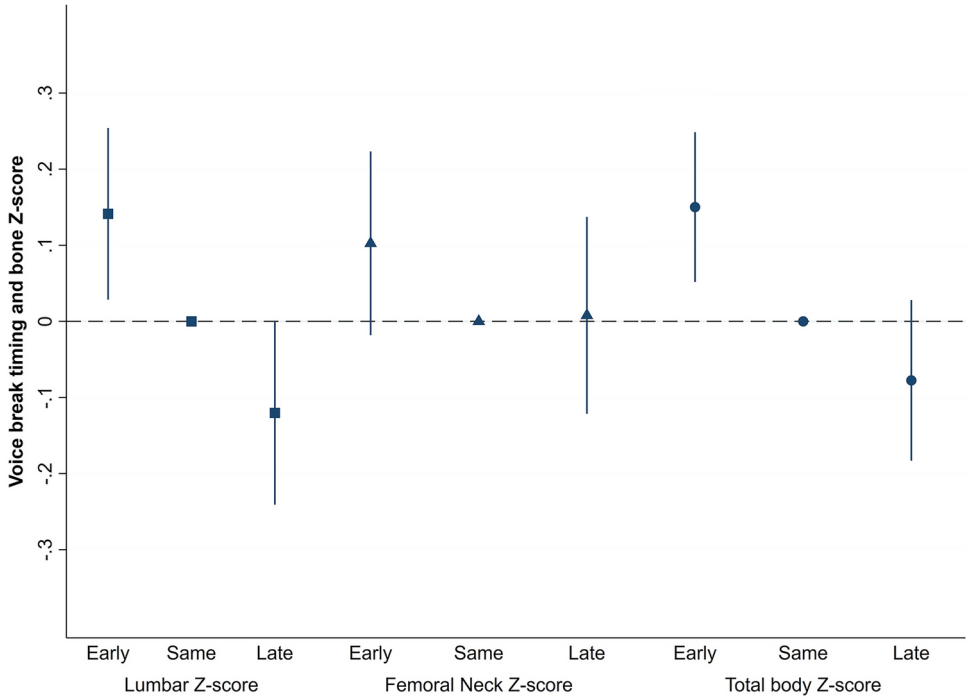


**Fig. 1**. Association between voice break timing of young men and bone parameters. Linear regression was used to assess the association between voice break timing and lumbar (squares), femoral neck (triangles) and total body (circles) Z-scores. Men with similar voice break timing compared to their peers were used as the reference group. Data are reported as β-coefficient and 95% CI, with adjustments for age, BMI, smoking, alcohol intake (units in the last week), physical activity (hours/week).

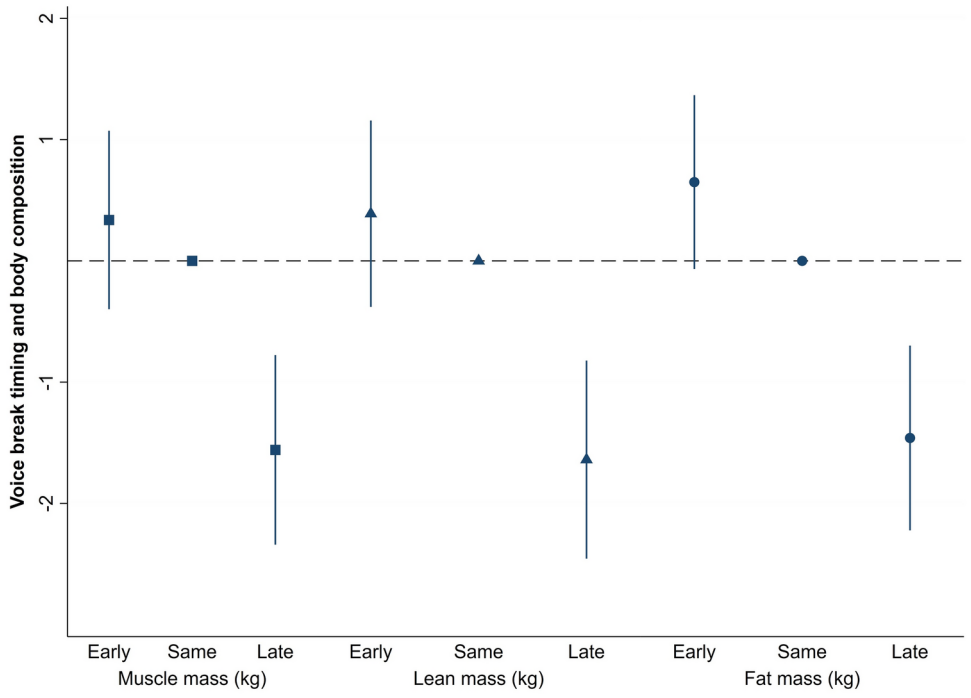


**Fig. 2**. Association between voice break timing of young men and body composition parameters. Linear regression was used to assess the association between voice break timing and muscle mass (squares), lean mass (triangles) and fat mass (circles). Men with similar voice break timing compared to their peers were used as the reference group. Data are reported as β-coefficient and 95% CI, with adjustments for age, smoking, alcohol intake (units in the last week), physical activity (hours/week).

their late twenties[22,23]. Likewise, adult men with congenital hypogonadotropic hypogonadism have reduced BMD despite years of androgen supplementation[24]. Also men who reached peak height velocity at a later age, indicating late normal pubertal timing, have lower BMD as young adults[6,7]. However, we only observed lower lumbar BMC and bone volume in men with late voice break, but no differences in lumbar areal BMD or BMAD, nor femoral measurements. Also in the Gothenburg Osteoporosis and Obesity Determinants (GOOD) Study, peak BMD had been reached at the lumbar spine, femoral neck and total body at age 19[13]. Interestingly, in the GOOD study, peak BMD at radius and tibia was not yet achieved at age 19[13]. Ongoing periosteal bone formation after epiphyseal closure at these sites can explain our observation of higher alkaline phosphatase levels in men with a late voice break, thus suggestive of ongoing bone maturation. A longitudinal study in 501 men did suggest a catch-up in BMD by the age of 24 in men with late-normal pubertal onset[8]. However, in the longitudinal 1946 British birth cohort study, later puberty was associated with lower trabecular volumetric BMD at age 60–64[25], and a large Swedish cohort study showed that men with peak height velocity at a later age had an increased risk of fractures in adulthood[7].

Androgens and estrogens are important for trabecular bone development and bone mass accrual[5,26]. This is in contrast to longitudinal bone growth, which is mainly influenced by growth hormone levels. Of note, we did not observe any differences in testosterone levels across the groups. However, estradiol levels were highest in men with an early voice break and lowest in men with late voice break, which could be related to differences in BMI and body fat mass.

Although men who reported to have an earlier voice break had higher BMI, body composition measurements were not significantly different compared to men with voice break timing similar to peers. In contrast, men with a late voice break showed distinct differences in body composition parameters. Both absolute muscle mass, lean mass and fat mass were lower, whereas in relative terms lean mass percentage was higher, with lower fat mass percentage and lower fat-to-muscle ratio. Notably, 31% of men with a late voice break had a BMI < 20 kg/m$^2$, compared to only 17% of men with early voice break. This suggests that changes in body composition occur relatively late in puberty. Similar to ongoing periosteal bone formation, our findings suggest that men with late puberty have not reached their adult body weight, muscle mass and body composition yet, despite reaching adult height.

Multiple studies investigated how body composition and BMI influence pubertal timing. In girls, there are consistent associations between higher BMI and earlier pubertal onset[27]. Data in boys are less consistent, but point towards an inverse association between BMI and pubertal onset[10,28]. Pubertal timing and progression can also influence body composition in (young) adult men. Smaller studies, such as the Amsterdam Growth and Health Study and the Leuven Growth Study, showed higher BMI in boys with early puberty assessed by peak height velocity[29,30]. Men with earlier pubertal onset have a higher central fat mass in adulthood and a higher risk of developing type 2 diabetes later in life[31,32]. Data from the longitudinal 1946 British Birth Cohort Study indicated that men with complete voice break at age 14 have a higher BMI throughout their adult life compared to men with no voice break or only starting to break at age 14. Body composition measured by DXA at age 60–64 years was also different in these men, with higher lean mass and abdominal fat mass in men reporting earlier voice break[9]. Recent UK Biobank data associated earlier voice break with adverse metabolic and cardiovascular outcomes, whereas voice break at a later age appeared metabolically protective, but was associated with lower overall health. Of note, there was no association between voice break and osteoporosis[33].

Main strengths of the study are the large number of healthy young men that were recruited from the general population. This allows us to associate differences in physiological timing of puberty with bone and body composition measurements in early adulthood. Furthermore, DXA was used for a detailed assessment of both bone and body composition parameters in all participants. Although there are several cohort studies reporting on the association between pubertal timing and bone at a young adult age[6–8], there are no data from large cohort studies reporting on body composition parameters measured by DXA in men.

The major limitation is the cross-sectional and observational nature of the data. Self-reported retrospective recall of voice break is used to stratify men across early, similar and late puberty compared to their peers. Although there was accordance with other questions about pubertal timing, uncertainty in recalling the timing of pubertal events cannot be excluded. Voice break was used as a proxy for pubertal timing, based on data from cohort studies that have shown there is a correlation with clinically measured pubertal milestones, such as testicular enlargement and peak height velocity[10]. As the study population is predominantly Caucasian, our findings cannot be extrapolated to other ethnicities.

## Conclusion

Physiological variations in pubertal timing were associated with differences in bone and body composition in healthy men at a median age of 19. Young adult men who recalled having an earlier voice break have a higher lumbar BMD, whereas bone measurements do not differ in men with late voice break compared to men with a similar voice break compared to their peers. However, later than average pubertal transition was clearly associated with body composition in young adulthood, with a lower BMI and fat-to-muscle ratio.

## Data availability

Data used in the present study is maintained centrally at Rigshospitalet and data access is regulated by national data protection rules. Thus, data are not publicly available and anonymized data can only be accessed after approval by relevant authorities.

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## Author contributions

Research design and data acquisition: LP, SAH, LN, AKB, NJ. Data analysis and interpretation, drafting of the manuscript: LA, LP, AJ, DV, NJ. All authors critically reviewed the manuscript and approved the final version.

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## Declarations

## Competing interests
The authors declare no competing interests.

## Additional information
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