Maternal Testosterone Concentrations in Third Trimester and Offspring Handgrip Strength at 5 Years: Odense Child Cohort

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**Abstract**

**BACKGROUND:** Maternal testosterone in pregnancy may have conditioning effects on offspring muscle strength.

**PURPOSE:** To investigate possible associations between maternal testosterone concentrations in 3rd trimester and offspring handgrip strength (HGS) at 5 years.

**METHODS:** In the prospective, population-based Odense Child Cohort, total testosterone (TT) at gestational week 27-28 and 5-y HGS were measured in 1,017 mother-child-pairs. TT was measured by liquid chromatography-tandem mass spectrometry and free testosterone (FT) was calculated from TT and SHBG. Multivariable regression analyses were performed with HGS <10th percentile as cut-off for low HGS.

**RESULTS:** Third trimester FT concentration was 0.004 (0.002-0.007) nmol/L, geometric mean (mean - SD; mean +SD). The mean (SD) 5-y HGS was 8.7 (1.8) kg in boys and 8.1 (1.7) kg in girls, p <0.001. Higher FT concentrations were associated with lower HGS (β = -0.186, p =0.048), after adjustment for maternal age, parity, offspring sex, 5-y height and weight. FT >0.004 nmol/L was associated with higher risk of 5-y HGS <10th percentile, odds ratio [95% CI] 1.58 [1.01;2.47], p =0.047 (n =1,017); and 1.69 [1.05; 2.74], p =0.032 after further adjustment for children’s organized sports in subgroup analysis (n =848). Lower HGS in relation to higher FT concentrations was found in all linear models but was not always statistically significant. HGS was not associated with maternal TT and SHBG levels.

**CONCLUSION:** Third trimester FT was inversely associated with offspring muscle strength assessed by HGS at 5 years-of-age, which may suggest a negative effect of maternal FT on offspring muscle strength.

Key words: Muscle strength, handgrip, testosterone, pregnancy, children.
Introduction

Children's muscle strength, power and endurance are positively linked to sex, age, puberty, physical training, bone health and self-esteem among other factors (1–3); and these muscle parameters are inversely correlated with adiposity, metabolic risk factors and later cardiovascular disease among children and teens (1). Indeed, low muscle strength in adolescence independently increases the risk of early adulthood mortality (4).

Maternal lifestyle and nutritional status have direct effects on embryonic development, and there is growing evidence that certain environmental prenatal exposures may have significant consequences for long-term health in agreement with the Developmental Origins of Health and Disease (DOHaD) hypothesis (5). During 1st and 2nd trimester pregnancy, maternal total testosterone (TT) concentrations increase as a result of higher SHBG levels in parallel with high estradiol (6–8); free testosterone (FT) levels are stable within the non-pregnant range until 3rd trimester, where FT levels become significantly higher (7). Maternal testosterone is transferred to the fetus via placenta and maternal testosterone concentrations correlate with fetal testosterone levels in the second half of pregnancy (9–11). In the male fetus, a surge in fetal testicular testosterone production is seen from gestation weeks 10 to 20, initially driven by human chorionic gonadotropin and in gestation weeks 15 to 20 weeks, primarily stimulated by fetal pituitary LH (12–14). In mid-gestation, male fetuses have significantly higher TT compared to female fetuses, but this difference disappears at term due to a decrease of TT in boys and an increase in girls (12,15).

Testosterone is known for its anabolic effects on muscle cells (16). In boys of pubertal age, testosterone is positively linked to increased isometric strength (3). Likewise, adult women with higher testosterone concentrations, as it is seen in polycystic ovary syndrome (PCOS), have higher muscle strength compared to women without PCOS (17,18).
Little is known about genetic and in utero factors to affect muscle strength in children and about factors associated with handgrip strength (HGS) in pre-pubertal children, except for age, sex, height and weight (19–21). We investigated the relationship between maternal testosterone levels in 3rd trimester pregnancy and HGS in offspring at five years of age in a large population cohort.

Methods

Study population

This study was based on data from Odense Child Cohort, a large-scale, ongoing and population-based mother and child cohort. Early pregnant women resident in the Municipality of Odense, Denmark, were recruited between January 1st, 2010, and December 31st, 2012. At the time of inclusion, a maternal blood sample was drawn, and information was collected using questionnaires, maternal journals, and information from the Municipality of Odense. The follow-up of the participant’s children takes place at various time points until the age of 18 years. Design and overall objectives of the Odense Child Cohort are described in detail previously (22).

In this study, we included mother-child pairs with an available maternal 3rd trimester testosterone concentration and a HGS test at the 5-year-examination of the child. Mother-child pairs were excluded in case of multiple pregnancy, very low birth weight (BW < 1500 g), or if the child had a chronic or acute disease with risk of affected HGS performance (Figure 1). Women with a diagnosis of PCOS were included in the study population. All children were pre-pubertal with Tanner stadium I at the time of examination. None of the children had a diagnosis of early adrenarche, early or late onset congenital adrenal hyperplasia or showed any clinical signs of adrenarche at clinical examination. Five male children with early treated or spontaneously resolved cryptorchidism were
included in the study cohort. The mothers of these children all had FT concentrations within the reference limits. The study complied with the STROBE guidelines (23).

**Analysis of maternal testosterone and SHBG**

At gestation week 27 to 28, morning fasting venous blood samples were collected, centrifuged for 10 minutes at 3000 revolutions/min., separated, and stored at -80°C degrees until analysis. Plasma TT was analyzed using liquid chromatography-tandem mass spectrometry, and serum SHBG was determined using immunoassay as previously described (24). Analysis quality was ensured through monthly participation in the external quality control program for steroid hormones from The United Kingdom National External Quality Assessment Service (NEQAS, UK). FT concentrations were calculated using the Vermeulen equation (25), assuming a plasma albumin concentration of 42 g/L. Four participants had available TT, but missing SHBG, hence FT could not be calculated.

**Assessment of HGS**

Within a week of their fifth birthday, the children were invited to a clinical examination, including measurement of HGS. The examinations were performed by three trained medical laboratory technicians blinded for questionnaire and laboratory data. HGS was measured using a DHD-1 Digital Hand Dynamometer (CE2195; SAEHN Corporation, Seoul, South Korea). The manometer was held in a 90-degree elbow flexed position with a slight abduction of the shoulder and hand in neutral position. The child was instructed to squeeze as hard as possible for 5 seconds. Each child had three to five measurements on each hand depending on whether the highest HGS was measurement in last try. The method is previously described in details (26). The maximal strength across all measurements regardless of hand side was chosen for further analyses. HGS measurements differ only slightly from gold-standard isokinetic muscle strength testing, which makes hand-held dynamometry a valid indicator of general muscle strength in children (27–29).
Assessment of covariates

The clinical examination of the child at age 5 included measurement of height (to the nearest cm; Seca Corporation, Hamburg, Germany) and weight with underwear (to the nearest 100 grams; Seca Corporation). Skin fold thickness was measured to the nearest 0.1 mm at triceps and subscapular sites (at nondominant side; Harpenden Skinfold Caliper; Pro Terapi A/S, Ballerup, Denmark), and the mean of three measurements was used. Body fat percentage estimates using the skin fold measurements were calculated using Slaughter’s equation as described earlier (26). Body mass index (BMI) was calculated as weight divided by the square of height (kg/m\(^2\)). Information on medical history, demographics and lifestyle was obtained through questionnaires and obstetrical reports. Placenta efficiency was calculated as the ratio between BW and the total placental weight (BW/PW ratio) (30).

Categorical variables included parental education (lower, high school or less; intermediate, high school plus 1 to 3 years; higher, high school plus 4 years or more), smoking during pregnancy (yes or no), preterm birth (yes or no), the child’s physical activity compared with peers (less active; as active; more active), and child participation in organized sport at least once a week for the last year (yes or no).

Statistical analysis

Baseline characteristics were reported by mean and standard derivation (SD) for parametric data, and median and interquartile range [IQR] for non-parametric data. TT and FT were ln-transformed for statistical purposes and hence reported as geometric mean (mean - SD; mean + SD). Categorical data were described in frequency (n) and percentage (%). The distribution of data was investigated with histograms and Skewness/Kurtosis test. All data were evaluated for each sex separately as well as combined. Differences between boys and girls were assessed using the Student t test, Mann-Whitney test, and \( \chi^2 \)-test for parametric, non-parametric and categorical data, respectively. TT and
FT concentrations were skewed to the right and were ln-transformed when used in the models as a continuous variable. FT was investigated as a continuous variable as well as in quartile categories. HGS was used as a continuous variable. HGS was furthermore dichotomized according to the lowest extreme decile within each sex.

Multivariable linear regression models were used to examine the main association between maternal FT (TT) and child HGS. Mandatory covariates with relevance for child HGS were chosen in accordance with literature, and potential confounders regarding maternal testosterone concentrations were tested one at a time for change of the estimate of more than 10%. Model 1 was adjusted for the mandatory (31,32) and highly associated covariates height, weight, and sex of offspring (Supplementary Table 1) (33). Of other potential confounders including maternal age, pregestational BMI, parity, and smoking status, only age and parity changed the estimate more than 10% and were therefore applied to the final Model 2. We examined for interaction by sex by including an interaction term into the model (FT × sex) and performed analyses stratified by sex as well. However, no interaction between FT and sex was detected (p = 0.9).

Sensitivity analyses were performed by exclusion of preterm children or mothers with PCOS, and in subgroups with available data on gestational weight gain (GWG), body fat percentage, and child participation in organized sports. Multivariable logistic regression was used to estimate odds ratios (ORs) for the association between FT and HGS values < 10th vs. ≥ 10th percentile (10p), as well as the risk of offspring HGS < 10p at FT level above the median. In the models where FT was used as a continuous variable, estimates were back-transformed to express percentage change in HGS, or OR of low HGS value associated with a doubling of FT. Secondary analyses included the association between TT and 5-y HGS, and SHBG and 5-y HGS.

Because maternal age and parity may be correlated, we secured correct final model specification using the link test (hatsq p = 0.494). A further Ramsey RESET test did not indicate any mis-specified variables in the model (p = 0.657). The assumptions underlying the linear regression model about
normal distribution of the residuals were inspected visually using quantile-normal plots of the standardized residuals. Model assumptions were met given that scatterplot showed symmetric plotted studentized residuals and fitted values; and that QQ plots pictured final model over studentized residuals linear.

Finally, we examined possible differences between participants and non-participants by Student t test, Mann-Whitney U test or \( \chi^2 \) tests, where appropriate. An \textit{a priori} power calculation showed, given \( n = 1000 \), that the study was able to detect a true difference in HGS of 0.6 kg for every 0.0001 nmol/L increase in p-FT (\( \alpha = 0.05; \beta = 0.20; \text{FT SD} 0.0055 \text{ nmol/L}; \text{HGS SD} 1.7 \text{ kg})

All data were analyzed using STATA/IC (version 16.0, StataCorp LP). Two-sided p-values < 0.05 were considered statistically significant.

\textbf{Ethics}

This study was conducted in accordance with the Helsinki II declaration and was approved by the Regional Scientific Ethical Committee, no. s-20090130, and the Danish Data Protection Agency no. 19/46601. HGS examination was an approved part of the standard 5-y examination in Odense Child Cohort. The children were free to refuse any part of the examinations. All parents received written and oral information and provided written consent for participation in the cohort.

\textbf{Results}

\textit{Population characteristics}

From the Odense Child Cohort, we included 1,017 mother-child pairs with available 3\textsuperscript{rd} trimester plasma testosterone samples and offspring 5-y HGS performance. The mothers had a mean age of 30 years and 60 women were diagnosed with PCOS. The children were on average 5.02 years on examination and 515 (51 \%) were boys. Boys and girls had comparable gestational age (GA) and
placenta weight. Preterm birth was seen in 35 pregnancies with a median (range) GA of 251 days (221-258 days); BW 2615 g (1605-3520 g). Participant characteristics by child’s sex are shown in Table 1.

The geometric mean (mean – SD; mean + SD) of TT was 1.97 (1.17-3.32) nmol/L; FT 0.004 (0.002-0.007) nmol/L. No difference in TT, or FT, split by offspring sex was seen. SHBG had median [p25; p75] of 464.5 [393.3; 543.8] nmol/L.

As expected, boys had higher mean (SD) HGS than girls, 8.7 (1.8) kg vs. 8.1 (1.7) kg, p < 0.001. The HGS < 10\textsuperscript{th} percentile cut-off was 6.4 kg in boys (n = 45) and 6.0 kg in girls (n = 50). Mothers with PCOS had a median (range) TT of 3.83 (0.87-8.97) nmol/L; FT 0.006 (0.002-0.031) nmol/L, and SHBG of 3.83 (0.87-8.97) nmol/L. The HGSs of their offspring were evenly distributed and represented in all HGS quartiles.

** FT and HGS associations**

In univariate regression analysis, 3\textsuperscript{rd} trimester FT was not associated with 5-y offspring HGS (Table 2). However, a weak trend was observed towards an inverse association between the log-transformed FT and HGS in the model adjusted for sex, 5-y height, and 5-y weight (Model 1, Table 2). Final adjustment for maternal age and parity resulted in a significant, inverse association between \text{lnFT} and HGS (Model 2). A doubling in back-transformed FT was associated with a decrease in HGS of 0.12 (95% CI 0.01; 0.27) kg. When running the model with FT categorized in quartiles, a similar, but not statistically significant inverse association was found (Table 2).

In the univariate logistic regression analysis, we found a trend towards increased risk of HGS < 10p with a doubling of FT (Table 3). The multivariable logistic regression Model 1 showed the same trend, driven by a significant association for girls. In the final Model 2, a doubling in FT was
associated with a 14 % increased odds of 5-y-offspring HGS < 10p, driven by the strong association in girls. The results remained significant when FT were categorized into quartiles with a 1.24 increase in OR for HGS < 10p for each quartile increase. FT above the median level increased the risk of having offspring HGS < 10p with 58 %, driven by a significant association in girls. The sex-difference, however, was not statistically significant (interaction p = 0.9).

We investigated the potential role of BW and BW/PW in the observed FT-HGS association. Plasma FT was inversely correlated with BW, and BW was positively associated with HGS (p < 0.001 for both). FT was likewise inversely correlated with the BW/PW ratio (Supplementary Table 2) (34). Adjusting Model 2 further with either BW or BW/PW did, however, only changed the β-coefficients marginally (absolute change 0.003, and 0.0003, respectively).

**TT, SHBG and HGS associations**

When examining the association between TT and HGS, an inverse association was observed in the adjusted Model 2 (Table 4). After back-transforming lnTT, a doubling in TT indicated decrease in HGS of 0.05 kg (95% CI -0.11; 0.07). In both univariate and adjusted analyses, TT tended to be associated with higher odds for HGS < 10p for girls, but not in boys (p-interaction > 0.05). None of the associations were however statistically significant. Furthermore, no association was found between HGS and maternal SHBG concentrations.

**Sensitivity analyses**

In a subgroup analysis excluding preterm birth (n=35), the β-coefficient (95% CI) in the FT-HGS association analysis Model 2 changed from -0.186 (-0.371; -0.001) to -0.191 (-0.379; -0.003). Other subgroup analyses were hampered by low number of participants. Excluding the 60 women with PCOS changes the β-coefficient in Model 2 to -0.1821 (-0.3763; 0.012). For those with available data on GWG (n=543), adjustment for GWG changed the β-coefficient from -0.205 (-0.463; 0.052) to -0.220 (95% CI -0.479; 0.039). For those with available body fat percentage data (n=973), adjustment
for body fat percentage instead of weight gave a $\beta$-coefficient of -0.152 (-0.341; 0.038) compared to -0.171 (-0.356; 0.015). For those with data on child participation in organized sports (n=848), addition of organized sport to the model changed the $\beta$-coefficient from -0.146 (-0.345; 0.053) to -0.142 (-0.341; 0.057) (Table 5). Significant FT associations to HGS <10th percentile were largely unchanged. FT above the median was associated with an OR of 5-y HGS below the 10th percentile of 1.69 [1.05; 2.74], $p = 0.032$ compared to 1.69 [1.05; 2.74], $p = 0.032$ (n=848) (no changes).

**Participants vs non-participants**

In tests for differences between participants and non-participants, non-participant mothers were less educated ($p=0.005$) and were more likely to smoke during pregnancy compared to participants ($p<0.001$) (Supplementary Table 3) (35).

**Discussion**

In this population-based cohort, we observed a novel, inverse association between maternal 3rd trimester FT and offspring 5-y HGS. Each doubling in FT was associated with a decrease in 5-y HGS of 0.12 kg. Furthermore, mothers with FT above the median level had 58 % increased risk of having offspring with HGS < 10p, and 69 % increased risk after further adjustment for children’s organized sports in subgroup analysis.

The present study is the first to investigate maternal testosterone levels and offspring muscle strength. In general, the literature on maternal testosterone exposure and predictors for offspring muscular strength is very sparse. In one paper, a low digit ratio (2D:4D), indicating high prenatal testosterone exposure, has been associated to high sprinting speed, endurance and HGS in boys (36). However, the 2D:4D method is not validated in children, and the study participants were both pre- and post-pubertal (36).
Sex differences

In our study, the inverse association between 3rd trimester FT and HGS < 10p was significant in girls only, albeit the sex difference did not reaching statistical significance. This may reflect a relatively higher contribution of maternal FT to the fetal FT concentrations in females compared to males. Moreover, testosterone may be more potent in the female placentae due to testosterone’s more potent inhibition of explant cytokine production from female placentae; and due to the greater expression of 5a-reductase protein in female placentae at term (37).

We did not detect any offspring sex-dependent associations to maternal TT or FT. A fetal sex-dependent difference has been reported in one very small study (n=37) (38), but not in other, larger studies (7,39,40).

Total testosterone and SHBG

TT showed an inverse trend association to 5-y HGS, whereas SHBG showed no associations with HGS in our cohort. A small decrease in SHBG with higher TT may have been enough to enhance FT concentrations to allow for influence on the FT-HGS association. SHBG increases 3-5-fold during normal pregnancy, but decreases in conditions with insulin resistance, including obesity, type 2 diabetes and PCOS (41). Indeed, our previous PCOS studies within Odense Child Cohort showed both higher TT and FT in PCOS vs controls (24,42). The SHBG decrease with increasing TT were seemingly of minor importance in our generally healthy, non-obese study population. In keeping, pre-gestational BMI did not affect our FT-HGS association.
The link between maternal testosterone and offspring HGS

Biological mechanisms to explain the observed inverse FT-HGS association may include an impact of maternal testosterone on placenta function, as well as fetal muscle mass and fetal pituitary LH.

Regarding the action of testosterone on placenta, animal research showed increased expression of hypoxia and inflammatory markers in prenatal testosterone-treated placentomes (43). Maternal testosterone levels may therefore modify placenta function and reduce transport capacity of nutrients or gonadotropins to the fetus, leading to reduced fetal growth. In our cohort, both BW and the BW/PW ratios (a marker of placental efficiency) were reduced with higher FT in our cohort (Supplementary Table 2) (34). We could not, however, detect mediating effects of BW, or the BW/PW ratio, on our FT-HGS association, suggesting that an impact of testosterone on placenta was not an explanatory factor for the FT-HGS association.

In another of our studies in Odense Child Cohort, mothers with PCOS had higher median FT than controls (0.005 vs. 0.004 nmol/L), but the offspring BW was comparable between the groups (42). This may be ascribed to the relatively low number of mothers with PCOS. In both animal and other human studies (44,45), inverse associations between maternal testosterone and offspring BW have been found, and mothers with PCOS have in other settings a higher prevalence of small for gestational age children compared with controls, ascribed to higher androgen levels (18).

In only one other study addressing the possible link between BW and later HGS, a direct association between BW and HGS in 7- to 10-y-old Brazilian children was found. This study, however, included many children with low BW and gave no data on gestational age, preterm birth, nutritional status, or puberty stage (46).

Another possible biological explanation is that maternal testosterone via lower fetal nutrient supply leads to reduced muscle mass per se, rather than low BW. Indeed, BW and muscle mass at birth are
not closely related measures (47). The lower muscle mass may be tracking into childhood as seen for low BW children born at term, who have lower fat-free mass tracking even into their adulthood (48).

A third biological explanation for the FT-HGS relation may be the direct action of placenta-transferred maternal testosterone on the fetus. In male fetus, the testes become fetal LH-dependent about halfway through the gestation (14), and the Leydig cell function is most likely dependent on fetal LH in late gestation (13,49). Higher maternal testosterone concentrations may inhibit fetal pituitary LH secretion in the 3rd trimester, leading to a negative programming effect on the fetus’ own ability to produce pituitary sex hormones, potentially persisting into postnatal life. As an important long-gestation animal model, fetal exposure to excess testosterone in sheep leads to an enhanced negative feedback at the pituitary level and suppressed Leydig cell function in male offspring (50). Taken together, we suggest an effect of maternal testosterone on offspring handgrip strength through an impact on reduced fetal muscle mass and fetal pituitary LH secretion, leading to persistent lower muscle mass and strength in the offspring. Aspects of this hypothesis could be evaluated in future studies by associating FT with offspring anogenital distance, penile length, cryptorchism, testosterone concentrations in the minipuberty of infancy, lean body mass, and puberty.

**Strengths and limitations**

*Strengths of this study include the population-based, prospective cohort design;* the relatively large study number; the use of blinded trained examiners and the use of standardized HGS protocol applicable to 5-year-old children; and the abundant background data allowing for test of several potential confounding factors. We used calculated FT as our primary exposure. Substitution of FT with bioavailable testosterone showed the same results (data not shown). Furthermore, maternal SHBG was not associated with 5y-HGS, excluding SHBG as an explanation behind the testosterone-HGS associations found by use of FT.
Several limitations need to be acknowledged. These include the observational nature of the study making inference of causality more difficult; minor differences between the cohort participants and the background population (22), as well as between participants and non-participants within Odense Child Cohort for the present study. Muscle strength is in general assumed to be related to training level (51,52), but high-quality data on physical activity could not be achieved at the age of 5 years, hence valid measures of physical activity was not included in the final model. Likewise, data on lean body mass were not available for the present study. Although we adjusted for several potential confounders, residual confounding cannot be excluded. We were not able to study genetic factors affecting the complex development of fetal gonads and regulation of fetal sex hormone production, such as variants in the CYP19A1 gene, which may affect the aromatase enzyme activity and hence alter the sex steroid balance (53).

In conclusion, higher levels of 3\textsuperscript{rd} trimester free testosterone were associated with a lower HGS in the 5-y-old offspring. Further studies are needed to determine the relationship between maternal testosterone levels and offspring muscle strength, including the potential negative effect of maternal testosterone on fetal LH production and offspring muscle mass with resultant lower muscle strength.

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Data availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.
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Figure Legend

Figure 1: Participant inclusion flowchart.
### Table 1: Characteristics of 1,017 included mother-child pairs from Odense Child Cohort according to child sex

|                          | Boys   | Girls  | Total  | P-value \(^a\) |
|--------------------------|--------|--------|--------|---------------|
|                          | n=515 (51%) | n=502 (49%) | n=1,017 |               |
| Maternal age, y, n=1,017 | 30.5 (4.5) | 30.3 (4.3) | 30.4 (4.4) | 0.389         |
| TT, nmol/L, n=1,017      | 1.97 (1.16-3.33) | 1.98 (1.18-3.30) | 1.97 (1.17-3.32) | 0.847         |
| FT, nmol/L, n=1,013      | 0.004 (0.002-0.007) | 0.004 (0.002-0.007) | 0.004 (0.002-0.007) | 0.916         |
| SHBG, nmol/L, n=1,013    | 465.1 [156.4] | 464.2 [142.0] | 464.5 [150.5] | 0.581         |
| Pregestational BMI, kg/m\(^2\), n=1,016 | 23.4 [5.3] | 23.6 [5.1] | 23.5 [5.3] | 0.987         |
| Smoking during pregnancy, n=1,008 |                |                |                |               |
| Yes                      | 16 (3.2%) | 13 (2.6%) | 29 (2.9%) |               |
| No                       | 490 (96.8%) | 489 (97.4%) | 979 (97.1%) | 0.583         |
| Parity n=1,016           |        |        |        |               |
| 1                        | 281 (54.7%) | 290 (57.9%) | 571 (56.2%) | 0.306         |
| >1                       | 233 (45.3%) | 212 (42.1%) | 445 (43.8%) |               |
| Maternal GWG, kg, n=557  | 14.9 (8.0) | 14.4 (5.8) | 14.7 (7.0) | 0.397         |
| Parents’ education n=1,005 |        |        |        |               |
| Lower \(^b\)             | 157 (30.8%) | 123 (24.8%) | 280 (27.8%) | 0.097         |
| Intermediate \(^c\)      | 253 (49.6%) | 262 (52.8%) | 515 (51.2%) |               |
| Higher \(^d\)            | 100 (19.6%) | 110 (22.4%) | 210 (20.9%) |               |
| Age at 5-y exam, y, n=1,017 | 5.02 [0.09] | 5.01 [0.10] | 5.02 [0.09] | 0.897         |
| HGS, kg, n=1,017         | 8.7 (1.8) | 8.1 (1.7) | 8.41 (1.8) | \(<0.001\)   |
| Height, cm, n=1,016      | 112.7 (4.4) | 111.7 (4.4) | 112.3 (4.4) | \(<0.001\)   |
| Weight, kg, n=997        | 19.4 (2.3) | 19.2 (2.3) | 19.3 (2.3) | 0.177         |
| BMI, kg/m\(^2\), n=997   | 15.20 (1.18) | 15.32 (1.18) | 15.26 (1.18) | 0.109         |
| BW, g, n=1,015           | 3,616 (521) | 3,483 (479) | 3,550 (505) | \(<0.001\)   |
| PW, g, n=1,005           | 632 (143) | 625 (125) | 629 (134) | 0.371         |
| BW/PW ratio, g\(^{-1}\), n=1,004 | 5.88 (1.00) | 5.70 (0.89) | 5.79 (0.95) | \(0.002\)    |
| Gestational age, days, n=1,017 | 281 [14] | 282 [12] | 281 [12] | 0.990         |
| Preterm birth (<259 days), n=1,017 |        |        |        |               |
| Yes                      | 18 (3.5%) | 17 (3.4%) | 35 (3.4%) | 0.924         |
| No                       | 497 (96.5%) | 485 (96.6%) | 982 (96.6%) |               |
| Triceps skinfold, mm, n=985 | 8.2 [2.5] | 9.7 [2.8] | 9.0 [2.9] | \(<0.001\)   |
| Subscapular skinfold, mm, n=975 | 4.9 [1.2] | 5.6 [1.5] | 5.2 [1.5] | \(<0.001\)   |
| Body fat, %, n=973       | 12.9 [3.4] | 14.9 [3.8] | 13.8 [3.9] | \(<0.001\)   |
| Physical activity \(^e\), n=863 |        |        |        |               |
| Less active              | 9 (2.1%) | 8 (1.8%) | 17 (2.0%) | 0.510         |
| As active                | 306 (72.5%) | 335 (76.0%) | 641 (74.3%) |               |
| More active              | 107 (25.4%) | 98 (22.2%) | 205 (23.8%) |               |

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|     | Yes   | 304 (71.2%) | 356 (80.5%) | 660 (75.9%) | **0.001** |
|-----|-------|-------------|-------------|-------------|-----------|
| No  | 123 (28.8%) | 86 (19.5%) | 209 (24.0%) |

Values presented as mean (SD), median [IQR] or n (%). TT and FT were ln-transformed, geometric mean (mean – SD; mean + SD). Significant p-values in bold. Percentages may not add up due to rounding.

Abbreviations: TT; total testosterone, FT; free testosterone, BMI; body mass index, GWG; gestational weight gain, HGS; handgrip strength, BW; birth weight, PW; placental weight.

* Difference between boys and girls analyzed by Student t test or Mann-Whitney U test where appropriate; \( \chi^2 \) test for categorical data. \(^b\) High school or less. \(^c\) High school plus 1 to 3 years. \(^d\) High school plus 4 years or more. \(^e\) Compared with peers. \(^f\) At least once a week for the last year.
Table 2: Change in 5-y handgrip strength according to increasing maternal free testosterone concentration, stratified by sex among 1,017 mother-child pair in the Odense Child Cohort

|                      | Boys                      |                    | Girls                      |                    | Total                      |                    |
|----------------------|---------------------------|--------------------|---------------------------|--------------------|---------------------------|--------------------|
|                      | \( \beta \) [95\% CI]     | P-value            | \( \beta \) [95\% CI]    | P-value            | \( \beta \) [95\% CI]    | P-value            |
| **Univariate regression model** |                           |                    |                           |                    |                           |                    |
| FT and HGS, kg       |                           |                    |                           |                    |                           |                    |
| Ln FT, continuous    | -0.062                    | 0.677              | -0.072                    | 0.609              | -0.063                    | 0.542              |
|                      | [-0.352; 0.229]           |                    | [-0.348; 0.204]           |                    | [-0.266; 0.140]           |                    |
| FT, quartile trend   | -0.023                    | 0.753              | -0.028                    | 0.677              | -0.026                    | 0.604              |
|                      | [-0.167; 0.121]           |                    | [-0.162; 0.106]           |                    | [-0.126; 0.073]           |                    |
| **Multivariable regression models** |                           |                    |                           |                    |                           |                    |
| Model 1              |                           |                    |                           |                    |                           |                    |
| Ln FT, continuous    | -0.171                    | 0.175              | -0.125                    | 0.320              | -0.145                    | 0.103              |
|                      | [-0.419; 0.077]           |                    | [-0.370; 0.121]           |                    | [-0.320; 0.029]           |                    |
| FT, quartile trend   | -0.084                    | 0.179              | -0.040                    | 0.513              | -0.060                    | 0.172              |
|                      | [-0.207; 0.039]           |                    | [-0.159; 0.080]           |                    | [-0.145; 0.026]           |                    |
| Model 2              |                           |                    |                           |                    |                           |                    |
| Ln FT, continuous    | -0.221                    | 0.097              | -0.150                    | 0.262              | -0.186                    | 0.048              |
|                      | [-0.483; 0.040]           |                    | [-0.412; 0.112]           |                    | [-0.371; -0.001]          |                    |
| FT, quartile trend   | -0.108                    | 0.101              | -0.049                    | 0.451              | -0.078                    | 0.090              |
|                      | [-0.237; 0.021]           |                    | [-0.176; 0.078]           |                    | [-0.168; 0.012]           |                    |

Significant p-values in bold. Model 1 adjusted for 5-year height and weight, sex. Model 2 adjusted for 5-year height and weight, sex, maternal age, and parity. Model 2 \( r^2 = 0.27 \). Q1 was used as reference in the quartile trend analyses.

Abbreviations: FT; free testosterone, HGS; handgrip strength.
Table 3: Odds ratios of low handgrip strength according to free testosterone concentration, stratified by sex among 1,017 mother-child pair in the Odense Child Cohort

|                | Boys                        | Girls                       | Total                       |
|----------------|-----------------------------|-----------------------------|-----------------------------|
|                | OR [95%CI]                  | P-value                     | OR [95%CI]                  | P-value                     | OR [95%CI]                  | P-value                     |
| Univariate     |                             |                             |                             |                             |                             |                             |
| regression     |                             |                             |                             |                             |                             |                             |
| model          |                             |                             |                             |                             |                             |                             |
| FT, continuous| 0.99 [0.83; 1.18]           | 0.869                       | 1.15 [1.00; 1.32]           | 0.052                       | 1.08 [0.97; 1.20]           | 0.162                       |
| FT, quartile   | 1.00 [0.75; 1.34]           | 0.982                       | 1.24 [0.99; 1.57]           | 0.066                       | 1.14 [0.96; 1.37]           | 0.142                       |
| trend          |                             |                             |                             |                             |                             |                             |
| Multivariable  |                             |                             |                             |                             |                             |                             |
| regression     |                             |                             |                             |                             |                             |                             |
| Model 1        |                             |                             |                             |                             |                             |                             |
| FT, continuous| 1.00 [0.83; 1.21]           | 0.994                       | 1.17 [1.02; 1.35]           | 0.030                       | 1.11 [0.99; 1.24]           | 0.076                       |
| FT, quartile   | 1.03 [0.76; 1.41]           | 0.837                       | 1.27 [1.00; 1.62]           | 0.052                       | 1.18 [0.98; 1.43]           | 0.087                       |
| trend          |                             |                             |                             |                             |                             |                             |
| Model 2        |                             |                             |                             |                             |                             |                             |
| FT, continuous| 1.06 [0.87; 1.29]           | 0.578                       | 1.18 [1.01; 1.37]           | 0.035                       | 1.14 [1.01; 1.28]           | 0.034                       |
| FT, quartile   | 1.13 [0.81; 1.57]           | 0.462                       | 1.28 [0.98; 1.67]           | 0.063                       | 1.24 [1.01; 1.52]           | 0.039                       |
| trend          |                             |                             |                             |                             |                             |                             |
| FT > median    | 1.13 [0.58; 2.30]           | 0.731                       | 1.87 [1.03; 3.38]           | 0.039                       | 1.58 [1.01; 2.47]           | 0.047                       |

Significant p-values in bold. Continuous FT results are back-transformed from ln-scale and indicated as OR of HGS < 10p associated with a doubling of FT. Low HGS defined as HGS < 10 percentile.

Abbreviations: FT; free testosterone, HGS; handgrip strength.
Table 4: Change in 5-y handgrip strength/odds ratios of low handgrip strength according to increasing maternal total testosterone or SHBG concentrations, stratified by sex among 1,017 mother-child pair in the Odense Child Cohort

|                | Boys          |                | Girls         |                | Total          |                |
|----------------|---------------|---------------|---------------|---------------|----------------|---------------|
|                | β/OR [95%CI]  | P-value       | β/OR [95%CI]  | P-value       | β/OR [95%CI]  | P-value       |
| **Total testosterone** |               |               |               |               |               |               |
| Univariate regression model |               |               |               |               |               |               |
| HGS, kg        | 0.033         | 0.831         | -0.155        | 0.297         | -0.060         | 0.580         |
|                | [-0.270; 0.336] |               | [-0.448; 0.137] |               | [-0.273; 0.153] |               |
| HGS, <10p      | 0.92          | 0.359         | 1.15          | 0.056         | 1.06           | 0.357         |
|                | [0.76; 1.10] |               | [1.00; 1.34]  |               | [0.94; 1.18]  |               |
| Multivariable regression models |               |               |               |               |               |               |
| Model 1        |               |               |               |               |               |               |
| HGS, kg        | -0.123        | 0.353         | -0.142        | 0.286         | -0.124         | 0.185         |
|                | [-0.382; 0.136] |               | [-0.403; 0.119] |               | [-0.308; 0.060] |               |
| HGS, <10p      | 0.94          | 0.551         | 1.15          | 0.074         | 1.06           | 0.299         |
|                | [0.77; 1.15] |               | [0.99; 1.33]  |               | [0.95; 1.20]  |               |
| Model 2        |               |               |               |               |               |               |
| HGS, kg        | -0.182        | 0.202         | -0.181        | 0.213         | -0.176         | 0.082         |
|                | [-0.462; 0.098] |               | [-0.465; 0.104] |               | [-0.375; 0.023] |               |
| HGS, <10p      | 1.00          | 0.976         | 1.16          | 0.075         | 1.10           | 0.145         |
|                | [0.81; 1.24] |               | [0.99; 1.36]  |               | [0.97; 1.25]  |               |
| **SHBG**       |               |               |               |               |               |               |
| Univariate regression model |               |               |               |               |               |               |
| HGS, kg        | 0.0010        | 0.151         | -0.0005       | 0.516         | 0.0003         | 0.566         |
|                | [-0.0004; 0.0024] |               | [-0.0018; 0.0009] |               | [-0.0007; 0.0013] |               |
| HGS, <10p      | 1.00          | 0.102         | 1.00          | 0.488         | 1.00           | 0.128         |
|                | [1.00; 1.00] |               | [1.00; 1.00]  |               | [1.00; 1.00]  |               |
| Multivariable regression models |               |               |               |               |               |               |
| Model 1        |               |               |               |               |               |               |
| HGS, kg        | 0.0006        | 0.333         | 0.0002        | 0.774         | 0.0004         | 0.319         |
|                | [-0.0006; 0.0018] |               | [-0.0011; 0.0014] |               | [-0.0004; 0.0013] |               |
| HGS, <10p      | 1.00          | 1.87          | 1.00          | 1.73          | 1.00           | 0.042         |
|                | [1.00; 1.00] |               | [1.00; 1.00]  |               | [1.00; 1.00]  |               |
| Model 2        |               |               |               |               |               |               |
| HGS, kg        | 0.0006        | 0.359         | 0.0002        | 0.778         | 0.0004         | 0.348         |
|                | [-0.0006; 0.0017] |               | [-0.0011; 0.0014] |               | [-0.0004; 0.0013] |               |
| HGS, <10p      | 1.00          | 0.247         | 1.00          | 0.150         | 1.00           | 0.045         |
|                | [1.00; 1.00] |               | [1.00; 1.00]  |               | [1.00; 1.00]  |               |

Significant p-values in bold.
HGS, kg: Linear regression, indicated as change in HGS with every increase in lnTT
HGS < 10p vs ≥ 10p: Logistic regression, indicated as OR of HGS < 10p associated with a doubling of TT.
Model 1 adjusted for 5-year height and weight, sex. Model 2 adjusted for 5-year height and weight, sex, maternal age, and parity. Low HGS defined as HGS < 10 percentile.
Abbreviations: HGS; handgrip strength, TT; total testosterone.
Table 5: Change in 5-y handgrip strength according to increasing maternal free testosterone concentration, and odds ratios of low handgrip strength according to free testosterone concentration, stratified by sex among 848 mother-child pair in the Odense Child Cohort with data on organized sport activities at 5 years.

| Subgroup analysis, Model 3, β-values            | Boys                | Girls               | Total               |
|------------------------------------------------|---------------------|---------------------|---------------------|
| Ln FT, continuous                               | -0.043 [-0.128; 0.041] | -0.041 [-0.127; 0.044] | -0.043 [-0.103; 0.017] |
| FT, quartile trend                              | -0.052 0.464        | -0.061 0.371        | -0.054 0.268        |
| trend [-0.191; 0.087]                            |                     |                     |                     |

Subgroup analysis, Model 3, OR for HGS < 10p

|                       | Boys                | Girls               | Total               |
|-----------------------|---------------------|---------------------|---------------------|
| FT, continuous        | 1.07 0.496          | 1.19 0.066          | 1.14 0.046          |
| FT, quartile trend    | 1.17 0.367          | 1.34 0.049          | 1.28 0.025          |
| trend                 | 0.83; 1.64          | 1.00; 1.79          | 1.03; 1.60          |
| FT > median           | 1.24 0.569          | 2.06 0.032          | 1.69 0.032          |
|                       | [0.59; 2.58]        | [1.07; 3.97]        | [1.05; 2.74]        |

In subgroup analysis Model 3, Model 2 was further adjusted for children’s organized sport (at least once a week for the last year, yes or no) at 5 years. Model 2 $r^2= 0.26$. Q1 was used as reference in the quartile trend analyses. Significant p-values in bold. Continuous FT results are back-transformed from ln-scale and indicated as OR of HGS < 10p associated with a doubling of FT. Low HGS defined as HGS < 10 percentile.

Abbreviations: FT; free testosterone, HGS; handgrip strength.
Figure 1: Participant inclusion flowchart.

- Included in Odense Child Cohort n=2,874 (100%)
  - No maternal testosterone status n=1,292 (46%)
  - Multiple birth n=54 (2%)
  - Children with very low birth weight (<1500 g) n=1 (<1%)
- Children with maternal testosterone status n=1,525 (53%)
  - Chronic or acute disease n=9 (<1%)
- Eligible for inclusion in study n=1,516 (53%)
  - No HGS at 5-year n=499 (17%)
- Main study population: All available HGS and maternal testosterone status n=1,017 (35%)

Percentages may not add up due to rounding. Abbreviations: HGS; handgrip strength.

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