Elevated serum levels of free triiodothyronine in adolescent boys with gynaecomastia compared with controls

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Abstract

Objective: Pubertal gynaecomastia is a frequent phenomenon occurring in 20–40% of otherwise healthy adolescent boys. Little is known about the aetiology of pubertal gynaecomastia. Markedly elevated thyroid hormone levels in adults with hyperthyroidism are associated with gynaecomastia.
Design: A cross-sectional examination of 444 healthy boys with and without pubertal gynaecomastia.
Methods: We evaluated TSH, triiodothyronine (T_3), thyroxine (T_4), free T_4 and free T_3 in a cohort of healthy boys with and without pubertal gynaecomastia.
Results: Boys with gynaecomastia had significantly higher serum free T_3, even after correction for age, BMI and pubertal stage. After inclusion of IGF1 in the model the differences disappeared. TSH, T_4, free T_4 and T_3 did not differ between the groups.
Conclusions: We speculate that the GH/IGF1 axis and thyroid hormones interact and influence the development of pubertal gynaecomastia.

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Introduction

Physiologic pubertal gynaecomastia is the most common condition in adolescent boys with breast development. This phenomenon frequently occurs between 13 and 14 years of age, lasts for 6–12 months and then disappears. Other and much more rare conditions to consider in adolescents and young adults with gynaecomastia are Klinefelter's syndrome (1), partial androgen insensitivity syndrome (2) or feminising testicular or adrenal tumors, and hyperthyroidism.

A marked elevation of thyroid hormone levels is associated with gynaecomastia in adults with hyperthyroidism (3, 4, 5, 6, 7, 8, 9, 10, 11, 12). Thus gynaecomastia is observed in 20–40% of men with hyperthyroidism (4, 10) and thyroid hormones are elevated in ~2% of newly referred patients with gynaecomastia (9).

However, a possible association between thyroid hormone levels and development of pubertal gynaecomastia has, to the best of our knowledge, not previously been described. We therefore aimed to compare circulating levels of thyroid hormones in healthy adolescent boys with and without pubertal gynaecomastia.

Subjects and methods

We examined 444 healthy Danish boys as part of the Copenhagen Puberty Study (13, 14) (ClinicalTrials.gov ID: NCT01411527) conducted between 2006 and 2008 (3101 were invited, 767 examined and 279 excluded due to lack of blood samples and 42 subjects due to non-Caucasian origin, in order to reduce the potential confounding

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Table 1 Thyroid hormone levels in all boys included in the study.

	All boys	
Parameters	*n*	Median (25–75% percentiles)
TSH	445	2.59 (1.97–3.61)
T_4	439	107.9 (97.06–118.7)
Free T_4	446	16.58 (15.19–17.74)
T_3	446	2.24 (2.03–2.45)
Free T_3	442	6.18 (5.80–6.64)

influence of ethnicity on risk of pubertal gynaecomastia). Two boys with gynaecomastia have also been excluded, one boy due to lack of pubertal assessment and one prepubertal boy as this study aimed to evaluate pubertal gynaecomastia and its association with thyroid hormones. Other aspects from this study have previously been published (15, 16).

Clinical examination

Pubic hair and genital stages (PH1–6 and G1–5) were assessed by clinical examination according to the methods by Marshall & Tanner (17). Gynaecomastia was evaluated by inspection and palpation of each breast as described by Braunstein (18).

Blood sampling procedure

Blood from the antecubital vein was drawn between 0830 and 1300 h. The blood samples were allowed to clot and subjected to centrifugation, and the serum was separated and stored at −20 °C until hormone analyses were performed, but no later than 12 months after collection.

Serum hormone analyses

Thyroid-stimulating hormone (TSH; with a day-to-day precision of 4–6% and a detection limit of 0.005 mIU/l), thyroxine (T_4; with a day-to-day precision of 7% and a detection limit of 5.4 nmol/l), triiodothyronine (T_3; with a day-to-day precision of 6–10% and a detection limit of 0.3 nmol/l), free T_4 (with a day-to-day precision of 7% and detection limit of 0.3 pmol/l) and free T_3 (with a day-to-day precision of 6–10% and a detection limit of 0.4 pmol/l) were analysed on a Modular ANALYTIC-SP/ISE-E-module system (Roche Diagnostics), using the CFAS-specific Roche calibrators and the Roche Modular reagents for all assays.

Serum insulin-like growth factor 1 (IGF1) and IGFBP3 levels were determined by immunoassay (IMMULITE 2000, Siemens Healthcare Diagnostics, Los Angeles, CA, USA), with detection limits of 20 and 100 ng/ml respectively. Intra- and interassay coefficient of variation values were <4 and $<9\%$ respectively.

Statistical analyses

To allow for an age-adjusted comparison between boys with and without gynaecomastia, we composed a subset of the controls who were in the same age range as the boys with gynaecomastia (aged 11.2–17.4). The homogeneity of variance between the two age-matched groups was tested with Levene's test ($P=0.344$). To minimise differences in hormone levels due to changes after pubertal onset, only boys who had entered puberty (defined as G>1 or PH>1) were included in this subgroup ($n=100$).

Thyroid hormone levels were presented as medians and ranges (minimum–maximum). The possible association between levels of thyroid hormones and the presence/absence of gynaecomastia was evaluated using nonparametric analyses (Mann–Whitney *U*-tests). The influence of hormone levels, pubertal stages, and body composition on presence of gynaecomastia was evaluated using binary logistic regression. Statistical significance was accepted at P value <0.05. Furthermore the relationship between levels of IGF1 and thyroid hormones was investigated using Spearman's ρ.

Clinical Trial Registration Number: NCT01411527.

Table 2 Thyroid hormone levels in an age-matched group (age 11.2–17.4) of boys entered puberty (G2–G5 or PH2–PH6), including a Mann–Whitney (M–W) *U*-test for comparison between boys with and without pubertal gynaecomastia.

	Gynaecomastia		Controls		M–W
Parameters	*n*	Median (25–75% percentiles)	*n*	Median (25–75% percentiles)	*P* value
Age	28	12.92 (12.38–14.70)	99	13.14 (12.25–14.97)	0.859
TSH	28	2.12 (1.84–3.35)	99	2.59 (1.99–3.39)	0.615
T_4	28	98.14 (82.33–114.8)	97	103.8 (92.61–114.5)	0.156
Free T_4	27	15.41 (14.75–16.76)	100	16.15 (14.95–17.27)	0.167
T_3	28	2.31 (2.09–2.55)	100	2.19 (1.97–2.45)	0.122
Free T_3	27	6.97 (6.15–7.30)	98	6.12 (5.83–6.66)	0.001*

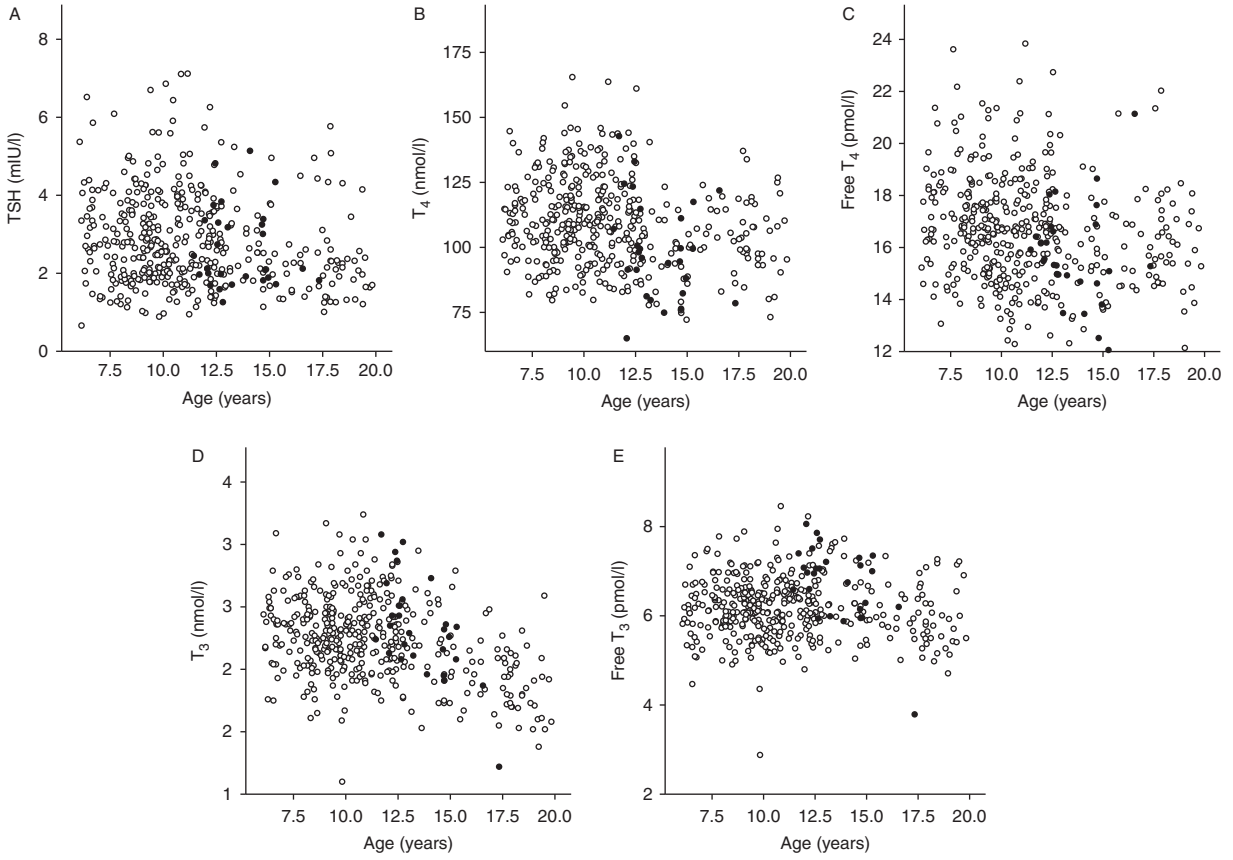

Figure 1

TSH (A), T_4 (B), free T_4 (C), T_3 (D) and free T_3 (E) levels according to age in Danish healthy school boys with (closed circle) and without gynaecomastia (open circle).

Results

Boys with gynaecomastia ($n=27$) had significantly higher serum concentrations of free T_3, whereas serum concentrations of TSH, T_4, free T_4 and T_3 did not differ significantly compared with a group of age-matched pubertal boys (Tables 1 and 2). Scatter plots for TSH, T_4, free T_4, T_3 and free T_3 according to age of boys with and without gynaecomastia (all ages) are displayed in Fig. 1.

Free T_3 levels were positively correlated (Spearman's ρ) with IGF1 levels ($r_s=0.219$, $P<0.001$), whereas TSH levels were negatively associated with IGF1 ($r_s=-0.016$, $P=0.014$), T_4 ($r_s=-0.220$, $P<0.001$), free T_4 ($r_s=-0.149$, $P=0.002$) and T_3 ($r_s=-0.112$, $P=0.018$) in all prepubertal and pubertal boys.

Differences in free T_3 levels between boys with and without gynaecomastia remained statistically significant even after additional correcting for age ($P=0.003$), genital stage ($P=0.003$) and BMI ($P=0.003$) respectively (Table 3). However, this difference did not remain significant when adjusting for IGF1 ($P=0.065$). In an attempt to take into account the possible interactions between these confounders, we included them in a combined model, which did not change the significance of free T_3 ($P=0.121$). It is, however, important to notice the rather small number of events per variable in the latter model.

Discussion

In the present population study of healthy Danish school boys, we demonstrate significantly higher free T_3 serum levels in boys with pubertal gynaecomastia compared with boys in the subset of control. This may suggest that thyroid hormones affect, directly or indirectly, the development of glandular breast tissue in adolescent boys.

Excess thyroid hormones may theoretically stimulate glandular breast tissue growth by direct effects of thyroid hormones on breast tissue. The presence of thyroid hormone receptor alpha (THRα) has been demonstrated

Table 3 Logistic regression models with the presence/absence of pubertal gynaecomastia as the outcome variable and free T_3, BMI, age, genital stage and IGF1 as explanatory variables.

				95% CI for Exp (*B*)	
	Exp (*B*) (S.E.M.)	*P* value	Exp (*B*)	Lower	Higher
Model 1					
BMI	−0.078 (0.102)	0.442	0.925	0.757	1.129
Free T_3	0.948 (0.321)	0.003	2.580	1.376	4.839
Model 2					
Age	0.093 (0.150)	0.533	1.098	0.819	1.472
Free T_3	0.985 (0.331)	0.003	2.677	1.400	5.118
Model 3					
Genital stage	0.222 (0.176)	0.209	1.248	0.883	1.764
Free T_3	0.953 (0.321)	0.003	2.593	1.384	4.860
Model 4					
IGF1	0.005 (0.002)	0.016	1.005	1.001	1.008
Free T_3	0.626 (0.339)	0.065	1.871	0.962	3.638

in glandular and myoepithelial cells in the normal human breast (http://www.proteinatlas.org/ENSG00000126351/normal/breast, page accessed on the 07.10.2013). Furthermore, thyroid hormones exert effects on breast cancer cells *in vitro* (19, 20). In accordance, a very recent study has demonstrated the presence of both THRα and THRβ in breast cancer cells (21).

Elevated thyroid hormone levels may affect the pituitary–gonadal axis and the metabolism of sex steroid hormones, and thereby the oestrogen/androgen action at the breast tissue level. Hyperthyroid men are reported to have: i) increased follicle-stimulating hormone and luteinising hormone levels (22), increased testosterone (23) and reduced clearance of testosterone (24); ii) increased peripheral aromatase activity resulting in an unfavourable oestradiol (E_2):testosterone ratio (9); and iii) increased serum sex hormone-binding globulin (SHBG) (9, 11, 25), which results in lower free testosterone. A British case study suggests an association between hyperthyroidism, hypogonadism and gynaecomastia, hypothesising that the male breast may be more susceptible to subtle change in oestrogen/androgen balance in the presence of both hypogonadism and hyperthyroidism (26). However, in this study the boys with gynaecomastia did not have impaired testicular function based on testicular volume, gonadotropins, SHBG or sex hormone levels (15).

The pituitary–thyroid axis is affected by growth hormone (GH). GH-deficient children treated with GH are at risk of unmasking latent hypothyroidism (27) due to effects of GH on the peripheral deiodination of T_4. On the other hand, T_3 replacement in hypothyroid patients rapidly stimulates the synthesis and subsequent increase in serum levels of GH (28). In addition, elevated thyroid hormones may stimulate serum IGF1 levels (29) despite controversial reports. In accordance, antithyroid treatment significantly decreases serum IGF1 levels in adults with hyperthyroidism (30). Furthermore, an *in vitro* study has reported autocrine secretion of IGF1 by human thyroid follicular cells, which was stimulated by TSH and GH (31).

Thus, multiple interactions between the GH/IGF1 axis and thyroid hormones have been reported by several authors (31, 32, 33).

In our recent report, based on data from the present study, we have found no differences in testosterone, E_2, testosterone:E_2 ratio or SHBG levels between boys with and without gynaecomastia (15). Obviously, the lack of association between circulating hormones and their ratio does not exclude the possibility of altered local aromatase activity being involved in the differences observed in boys who develop gynaecomastia and those who do not. However, we did find increased IGF1 serum levels in the boys presented with gynaecomastia (15), and when correcting for IGF1 the observed statistical differences in free T_3 levels between boys with and without gynaecomastia disappeared. Altogether these findings suggest that both IGF1 and thyroid hormones interact and theoretically both may be involved in the development of gynaecomastia. The exact mechanism behind this interaction at this point is unclear, but it seems plausible that the growth of breast tissue in the boys who develop pubertal gynaecomastia could be due to the stimulatory effect of IGF1, with a possible additional effect of T_3. However, further studies are required to elucidate this hypothesis.

In conclusion, we found that our healthy Danish boys with pubertal gynaecomastia had significantly higher serum levels of free T_3 when compared with age- and puberty-matched boys without gynaecomastia at the time

of examination. However, this result did not remain statistically significant after correction for IGF1. We speculate that the GH/IGF1 axis and thyroid hormones interact and may both influence the development of pubertal gynaecomastia.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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